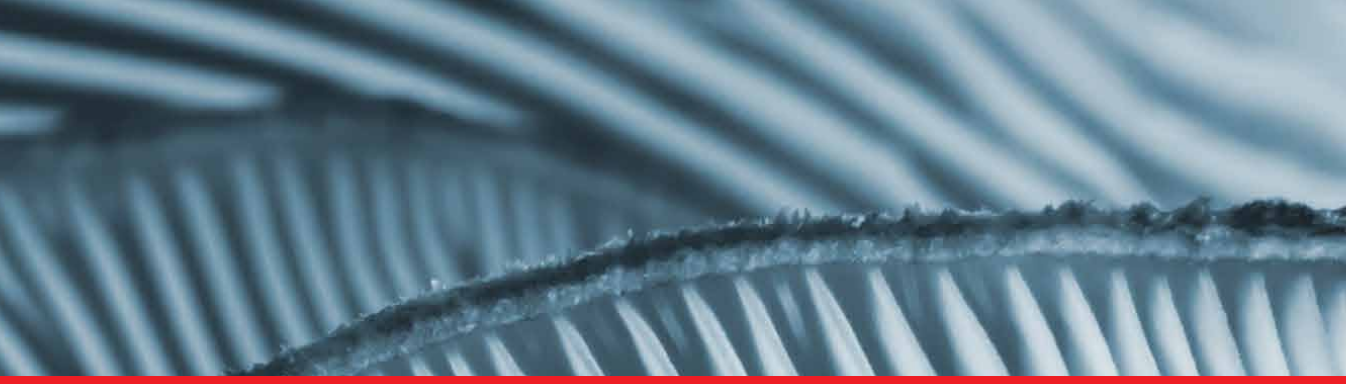

IntechOpen

IntechOpen Series
Food Science and Nutrition, Volume 16

Fruit Crops Science

Ecophysiological and Horticultural Perspectives

Edited by Mateus Pereira Gonzatto and Júlia Scherer Santos

Fruit Crops Science - Ecophysiological and Horticultural Perspectives

Edited by Mateus Pereira Gonzatto and Júlia Scherer Santos

Published in London, United Kingdom

Fruit Crops Science – Ecophysiological and Horticultural Perspectives
http://dx.doi.org/10.5772/intechopen.1006017
Edited by Mateus Pereira Gonzatto and Júlia Scherer Santos

Contributors
André Luis B.S. Barreiros, Carina Gutierrez-Flores, Caroline Esperança, Damini Singh, Danilo Força Baroni, Diego Ismael Rocha, Eduardo Cuevas, Elias Fernandes de Sousa, Eliemar Campostrini, Ellen de Moura Vale, Francisco de Assis Lopes, Gaurav Kumar, Gerson Nestor Boettcher, Gilberto Manzo-Sánchez, Guilherme Augusto Rodrigues de Souza, Ishani Sharma, Jayesh Garg, Josimar dos Santos Ladeira, José Joaquín Velázquez-Monreal, José Luiz Petri, José Ángel Mercado-Hornos, João Victor Baptista Silveira, Judith Georgette Alcalde Mosqueira, Júlia Scherer Santos, Karina de la Paz García-Mariscal, Lorena Aleman-Figueroa, Lucilene Silva de Oliveira, Luís Henrique Bueno, Manuel de Jesús Bermúdez-Guzmán, Marciano Manuel Robles-González, Maribel López-Atanacio, Mario Orozco-Santos, Marizeth Libório Barreiros, Mateus Pereira Gonzatto, Miguel Ángel Manzanilla-Ramírez, Miroslava Rakočević, Otalicio Damasio da Costa Junior, Rodrigo Lucas-García, Sabrina Raquel Griebeler, Sara Posé, Sergio Francisco Schwarz, Shiv Kumar Shivandu, Sérgio Yoshimitsu Motoike, Thamires Fernanda Gomes, Tsylla Assis Santos Ferreira, Víctor Rosas-Guerrero, Wallace de Paula Bernado, Weverton Pereira Rodrigues

© The Editor(s) and the Author(s) 2025
The rights of the editor(s) and the author(s) have been asserted in accordance with the Copyright, Designs and Patents Act 1988. All rights to the book as a whole are reserved by INTECHOPEN LIMITED. The book as a whole (compilation) cannot be reproduced, distributed or used for commercial or non-commercial purposes without INTECHOPEN LIMITED's written permission. Enquiries concerning the use of the book should be directed to INTECHOPEN LIMITED rights and permissions department (permissions@intechopen.com) Violations are liable to prosecution under the governing Copyright Law.

Individual chapters of this publication are distributed under the terms of the Creative Commons Attribution 4.0 License which permits commercial use, distribution and reproduction of the individual chapters, provided the original author(s) and source publication are appropriately acknowledged. If so indicated, certain images may not be included under the Creative Commons license. In such cases users will need to obtain permission from the license holder to reproduce the material. More details and guidelines concerning content reuse and adaptation can be found at http://www.intechopen.com/copyright-policy.html.

Notice
Statements and opinions expressed in the chapters are these of the individual contributors and not necessarily those of the editors or publisher. No responsibility is accepted for the accuracy of information contained in the published chapters. The publisher assumes no responsibility for any damage or injury to persons or property arising out of the use of any materials, instructions, methods or ideas contained in the book.

First published in London, United Kingdom, 2025 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 167-169 Great Portland Street, London, W1W 5PF, United Kingdom

For EU product safety concerns: IN TECH d.o.o., Prolaz Marije Krucifikse Kozulić 3, 51000 Rijeka, Croatia, info@intechopen.com or visit our website at intechopen.com.

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Fruit Crops Science – Ecophysiological and Horticultural Perspectives
Edited by Mateus Pereira Gonzatto and Júlia Scherer Santos
p. cm.

This title is part of the Food Science and Nutrition Book Series, Volume 16
Topic: Food Technology
Series Editor: Maria Rosário Bronze
Topic Editor: Carlos Dias Pereira

Print ISBN 978-1-83634-419-3
Online ISBN 978-1-83634-418-6
eBook (PDF) ISBN 978-1-83634-420-9
ISSN 2977-8174

If disposing of this product, please recycle the paper responsibly.

IntechOpen
intechopen.com

Built by scientists, for scientists

Explore all IntechOpen books

IntechOpen Book Series

Food Science and Nutrition

Volume 16

Aims and Scope of the Series

The significance of food is undeniable, especially in light of the impending challenge facing humanity: ensuring there will be enough food to meet the basic needs of a population expected to reach approximately 10 billion by 2050. These food-related challenges align with some of the United Nations' sustainable development goals, with a target to achieve them by 2030. One thing is certain: food should be not only nourishing and safe but also tailored to the diverse needs of individuals throughout their lifetimes, all while meeting consumers' sensory expectations. Understanding the diverse chemical composition of food, often referred to as biodiversity, and how these components can contribute to human health by considering factors like bioaccessibility, bioavailability, and bioactivity at the organ level, is crucial for grasping and promoting a healthy diet. Thanks to the continuous evolution of analytical methods and interdisciplinary research, significant strides have been made in the field of food science and nutrition.

Meet the Series Editor

Maria Rosário Bronze has been working in Analytical Chemistry since 1986. Her Ph.D. in 1999 contributed to the study of food products using capillary electrophoresis. The main goal of her research since 1999 has been focused on Analytical Chemistry applied mainly to the analysis of foods and by-products of food industry. She conducted research in collaboration with national and international research groups, at iBET and ITQB Technology Division. From 2017 until 2021 she was head of Food & Health Division at iBET and head of the Food Functionality and Bioactives Laboratory. MR Bronze has been an Associate Professor at the Pharmacy Faculty of Lisbon University and head of the Structural Analysis Laboratory since 2012. As a researcher, MR Bronze is a Senior Scientific Advisor at Food & Health Division at iBET and Head of Food Functionality and Bioactives Laboratory at the same Institute, Collaborator at iMED and Researcher at ITQB NOVA. Her current research is focused on quality and beneficial health effects of food components. Gas and liquid chromatography associated with mass spectrometry are used by MR Bronze in the characterization of samples. Sensory evaluation is also an important area of her research. The main food products studied by her are olive tree products (olive, olive oil, leaves), cereals such as maize, legumes (faba bean, pea, chickpea, lentils) fruits (apple, grapes, opuntia ficus), fruit juices and wine, among others. More recently her interests have also involved biodiversity, bioaccessibility, and bioavailability studies on food products and their components, mainly phytochemicals as phenolic compounds, using different analytical tools such as mass spectrometry. As a senior scientific advisor at Food & Health Division at iBET she is involved in different areas: (i) isolation, characterization and formulation of bioactive and functional compounds or extracts from natural sources and wastes from food and other related industries; (ii) pre-clinical assays to provide support to understand health claims related with the beneficial effects of food nutrients/bioactive components; (iii) establishment of analytical methodologies including mass spectrometry state-of-the-art to fully characterize different matrices, from food products, natural extracts or biological fluids (Food Functionality and Bioactives Laboratory).

Meet the Volume Editors

Dr. Mateus Pereira Gonzatto is a Professor of Citrus and Mango Ecophysiology in the Department of Agronomy at the Federal University of Viçosa, Brazil. He works as an advisor in the post-graduate course of Plant Science. He is the director of representations and publications of the Brazilian Fruit Crops Society (SBF). He researches topics such as citriculture, scion/interstock/rootstock interactions, plant growth regulators, biostimulants, fruit thinning, fruit maturation, abiotic and biotic stresses in fruit production, and orchard management.

Júlia Scherer Santos is a pharmacist with a Ph.D. in Pharmaceutical Nanotechnology from the Federal University of Rio Grande do Sul, Brazil. She is a specialist in cosmetology and in aesthetic health. She works in the areas of cosmetology, nanotechnology, pharmaceutical technology, beauty, and bioactive compounds of plant origin. Dr. Santos also has experience as a professor of graduate courses.

Contents

Preface

Fruits are cultivated worldwide, and their consumption is linked to their nutraceutical properties. To ensure a fruit supply chain to the consumer market, there must be suitable production management of these fruits. Hence, this book presents recent contributions and the current state of knowledge on ecophysiological aspects and horticultural practices related to different fruit crops. It is divided into three sections: *Fruit Ecophysiology and Production, Tropical and Subtropical Fruit Crops*, and *Temperate Fruit Crops*.

Section 1, *Fruit Ecophysiology and Production*, presents overall aspects of fruit production. The introductory chapter compiles recent information on global fruit production and research. In chapter two, aspects related to the status of water in the soil-plant-atmosphere system and the effects of water stress on fruit production are presented. The last chapter of this section discusses the role of plant growth regulators in fruit crops, including the effects on flowering, delaying senescence, and reducing drought stress.

Section 2, titled *Tropical and Subtropical Fruit Crops*, explores the physiological aspects and management production strategies for tropical and subtropical fruits, including citrus, mango, and palm trees. Chapter 4 discusses the use of plant growth regulators and biostimulants in mandarins, focusing on the reproductive phase. Chapter 5 presents details on Mexican lime production. Chapter 6 discusses aspects of the reproductive biology of the 'Ataulfo' mango. Chapter 7 presents advances in the propagation and conservation of the following palm tree crops: macaw palm, coconut palm, oil palm, juçara palm, and açaí palm. However, the chemical composition, nutritional value, and antioxidant activity of 22 native and cultivated species of Brazilian fruit crops are presented in Chapter 8.

Section 3, *Temperate Fruit Crop*s, approaches apples and strawberries. Chapter 9 demonstrates the use of plant growth regulators, specifically thiadizuron, prohexadione-Ca, and aminoethoxyvinylglycine, to enhance fruit set in apples, thereby contributing to increased productivity. Finally, Chapter 10 presents the current challenges of strawberry production in Spain, including cultivation systems, effects of climate change, and the proposal for more sustainable alternatives in strawberry production.

Mateus Pereira Gonzatto
Department of Agronomy,
Federal University of Viçosa,
Viçosa, Brazil

Júlia Scherer Santos
Department of Pharmacology,
Institute of Biological Sciences,
Federal University of Juiz de Fora,
Juiz de Fora, Brazil

Section 1

Fruit Ecophysiology and Production

Chapter 1

Introductory Chapter: Worldwide Fruit Crops Production and Research

Mateus Pereira Gonzatto and Júlia Scherer Santos

1. Introduction

All over the world, a large number of fruits are produced despite a fairly limited number of fruit species having significant economic importance. Although there is not a standard definition of cultivated fruit crops, they are horticultural plants, including woody trees or herbaceous perennials, shrubs, or lianas with an edible portion [1]. Also, they are identified as "edible fruit-bearing plants" [2], in which the edible portion can be true fruits, pseudofruits, or having structures parts close to the fruits [1, 2]. In turn, nuts are generally grouped as fruit crops or as "fruit and nut crops," mainly because they are woody perennial trees, and they are approached within the area named as "fruticulture" in some countries [3]. On the other hand, short-cycle plants with vegetative growth, including strawberry, watermelon, and melon, which are annually cultivated, can also be understood as fruit crops [1]. Nevertheless, these plants are generally approached in olericulture, another horticulture area.

Fruit crops can be classified according to their preferred cultivation climate as tropical, subtropical, and temperate climates [4]. Among the main fruit crops of tropical climates, there are bananas and plantains (*Musa* spp.), mangoes (*Mangifera indica*), pineapples (*Ananas comosus*), papayas (*Carica papaya*), guavas (*Psidium guajava*), passion fruit (*Passiflora* spp.), and dragon fruits (*Selenicereu*s spp.). Regarding subtropical climate fruits, we can mention citrus fruits (mainly *Citrus* spp.), avocado (*Persea* spp.), persimmons (*Dyospyrus kaki*), figs (*Ficus carica*), loquat (*Eriobotrya japonica*), litchi (*Litchi chinensis*), and several plants of the Myrtaceae family, including jabuticaba (*Plinia trunciflora*). Apples (*Malus domestica*), pears (*Pyru*s spp.), grapes (*Vitis* spp.), stone fruits (*Prunus* spp.), strawberries (*Fragaria* × *ananassa*), other berries (such as blueberry, blackberry, raspberry), kiwifruits (*Actinidia* spp.), olive (*Olea europea*), and pistachio (*Pistacia vera*) can be classified as temperate fruits.

2. World fruit production and research

The global production of primary crops in 2022 reached 9.6 billion tons which is equivalent to USD 2.9 trillion. Fruit production is 10% of the world's production of primary crops in volume. This production represents 17% of production value, ranking just behind cereals (29%) and vegetables (19%) [5].

World fruit production has grown linearly in the last 10 years, as shown in **Figure 1** [6]. The most recent data from the year 2023 show that the annual world

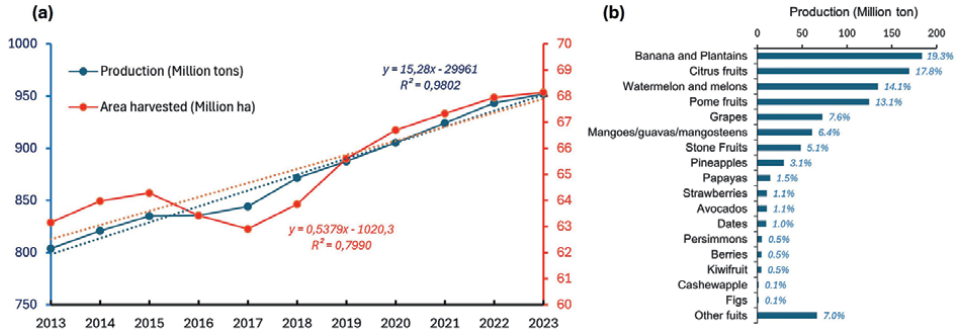

Figure 1.
(a) World production of fruit crops between 2013 and 2023, and (b) the production of different fruit crops in 2023. Data were retrieved from searching aggregate item "fruit primary" in Food and Agriculture Organization of the United Nations [6].

fruit production is 951.51 million tons in about 68.10 million hectares, with an average fruit yield of 13.97 tons ha^{-1}. The production increase is around 15 million tons $year^{-1}$. The harvested area remained between 63 and 64 million hectares between 2013 and 2018, with subsequent growth to 68 million hectares in the following years, denoting an increased production of around 500 thousand ha $year^{-1}$ in the 10-year period analyzed (**Figure 1a**).

Among the main groups of fruits, the most produced ones are bananas and plantains, with 183.67 million tons produced, accounting for 19.3% of world production. The second most important fruit category is citrus (which includes oranges, tangerines, limes, and lemons), with 169.39 million tons, and represents 17.8% of world production. Watermelons and melons account for 14.1% of world production. Diversely, pome fruits (apples, pears, quinces, among others) account for 13.1% of world production. Moreover, grapes and stone fruits are responsible for 7.6% and 5.1% of world production, respectively. On the other hand, mangoes, guavas, and mangosteens production of 6.4%, while pineapples have 3.1%.

Papayas, strawberries, avocados, and dates have production ranging from 1.0% to a maximum of 1.5%. Last, less representative fruits (< 1%) are persimmons, berries, kiwifruit, cashew apples, and figs (**Figure 1b**) [6].

Besides, fruits were produced all over the world. In 2023, Asia countries are the most important producers, accounting for 58.7% of production and 52.5% of the harvested area. The American countries are the second largest fruit producers, producing 17.8% of world production in 14.1% of the harvested area. Subsequently, with 14.4% of world production and 22.3% of the harvested area, is the African continent. The European continent is responsible for 8.2% of fruit production and 10.2% of the harvested area. The least relevant continent is Oceania, where 0.9% of fruit production occurred in 0.9% of the harvested area [6].

Further, the main fruit-producing country in the world is China, which alone is responsible for 28.3% of world fruit production (269.33 million tons) and 23.3% of the harvested area (15.81 million hectares). India appears as the second largest fruit producer, representing 12% of world fruit production and 11.1% of the harvested area. Brazil is the third-largest producer, responsible for 4.5% of world production and 3.5% of the harvested area. Turkey, Mexico, Indonesia, the United States of America (USA), the Philippines, Spain, Iran, Egypt, Nigeria, Italy, Vietnam, and Colombia are smaller producers, with production between 1.2 and 2.9% (**Figure 2a**) [6].

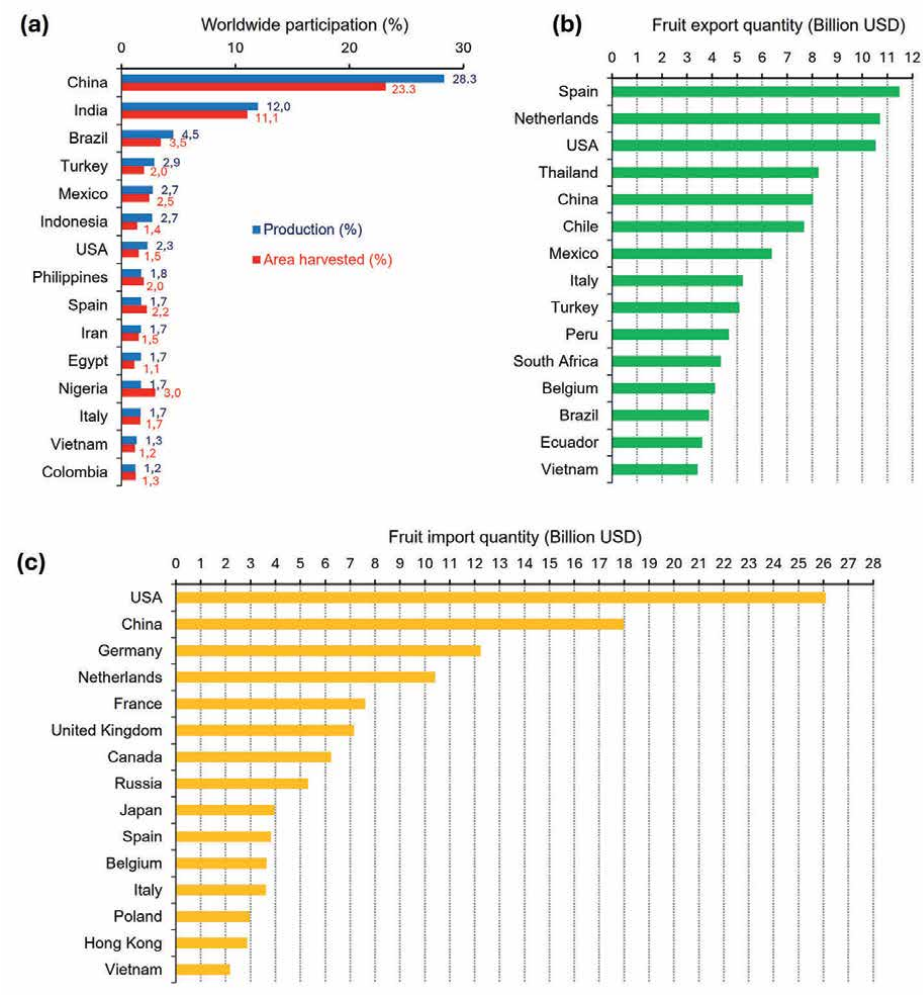

Figure 2.
Fifteen main fruit-producing countries (a) in percentage of area and quantity produced were retrieved by searching the aggregate item "fruit primary" (b) in fruit exports and (c) in fruit imports in 2023 obtained when the item "fruit" was searched in Food and Agriculture Organization of the United Nations [6].

International trade in fruit crops generates a large amount of money due to a worldwide distribution flow of fruits despite their production being limited to certain climates. The export of fruit crop products, which include fresh and dried fruits and fruit juices, is around USD 149.85 billion. Spain is the main exporter country, accounting for 11.5 billion USD, with 32.46% of this export due to the export of fresh citrus fruits. The Netherlands is the second main exporter, with USD 10.72 billion in exports, with emphasis on fruit juices, bananas, and grapes. The USA is the third-largest exporter, with USD 10.54 billion in exports, with 22.59% of this value referring to pistachios in shell (**Figure 2b**) [6].

Yet, the following countries export less than 10 billion USD: Thailand, China, Mexico, Italy, Turkey, Peru, South Africa, Belgium, and Brazil (**Figure 2b**). Thailand exports mainly tropical fruits, while China exports fruit prepared[1], apples, man-

[1] FAO item "Fruit prepared n.e.c.". Refers to prepared or preserved fruits not specified in other categories [6].

darins, and grapes. Chile exports cherries and grapes, and Mexico exports fruit prepared, berries, and limes. Turkey and South Africa export fresh citrus fruits. Brazil mainly exports orange juice, mangoes, and melons [3]. Belgium exports fruit prepared, bananas, and kiwifruits, and Ecuador exports bananas and plantains. In turn, Vietnam is highlighted by the exportation of tropical fruits [6].

Moreover, import of fresh, dried, and processed fruits is estimated to be around USD 163 billion [6]. The main importer is the USA with a value of USD 26.1 billion imported in 2023, whose main products (60%) are fruit prepared, bananas, grapes, raspberries, blueberries, and strawberries. China is the second most importer country with an import value of USD 17.98 billion, mainly related to the following tropical fruits: cherries, fruit prepared, and bananas. The third-largest importer is Germany, mainly related to fruit prepared, citrus fruits, bananas, and grapes. The Netherlands is the fourth largest importer of mainly bananas, grapes, fruit prepared, oranges, and cranberries (**Figure 2c**).

As also shown in **Figure 2c**, France, the United Kingdom, Canada, Russia, Japan, Spain, Belgium, and Italy are the ones that import less than USD 10 billion. Citrus fruits are imported mostly by Russia, Canada, the United Kingdom, Spain, Italy, Poland, Hong Kong, Vietnam, and Russia. Differently, bananas are mostly imported by the United Kingdom, Canada, Japan, Spain, Belgium, and Italy. Fruit prepared is mostly imported by Canada, Japan, Spain, Belgium, and Poland. Grapes are the most imported fruits by the United Kingdom, Canada, and Vietnam.

Scientific research is essential to enable and optimize the production and commercialization of fruits and plays an important role in the development of technological innovations which enable a globalized fruit distribution chain. Scopus database search on fruit crops shows that there is a number of publications ranging from 30,000 to 35,000 from 2020 to 2024 (**Figure 3a**). The most important countries in terms of publication on fruit crops are China, India, the USA, Brazil, Italy, Spain, Indonesia, Iran, the United Kingdom, Turkey, Germany, Malaysia, Australia, South Korea, and Japan (**Figure 3b**). Among these, nine are among the largest fruit producers in the world: China, India, Brazil, Turkey, Indonesia, the USA, Spain, Iran, and Italy (**Figure 2a**). The largest publishing country is China, with over 46,000 publications, representing 25.7% of the total publications. India had almost 20,000 publications, representing 11.1% of the total, while the USA was responsible for around 18,500 publications. Next is the fourth most publisher, Brazil, which had over 10,000 publications. It is also worth mentioning Italy, Spain, Indonesia, Iran, and the United Kingdom, with more than 5000 publications each. Meanwhile, Turkey, Germany, Malaysia, Australia, South Korea, and Japan had between 4000 and 5000 publications (**Figure 3b**) [7].

The areas of knowledge covering fruits are shown in **Figure 3c**, of which 52.6% are associated with the Agricultural and Biological Sciences area, 18.1% are associated with Biochemistry, Genetics, and Molecular Biology, while 14.2% are associated with Environmental Science. Also, there were a significant number of publications addressing fruit crops in other areas: Medicine; Chemistry; Engineering Chemical Engineering; Computer Science; Material Science; Pharmacology, Toxicology and Pharmaceutics; and Physics and Astronomy [7]. The wide range of fruit applications in different areas of knowledge denotes the relevance of research on fruits.

The consumption of fresh fruits is encouraged due to their health benefits, and strategies to ensure the post-harvest quality of fruits allow them to reach non-producing countries of these fruits. These fruits processing to obtain juice, or byproducts show applications in the food, pharmaceutical, cosmetic, and materials

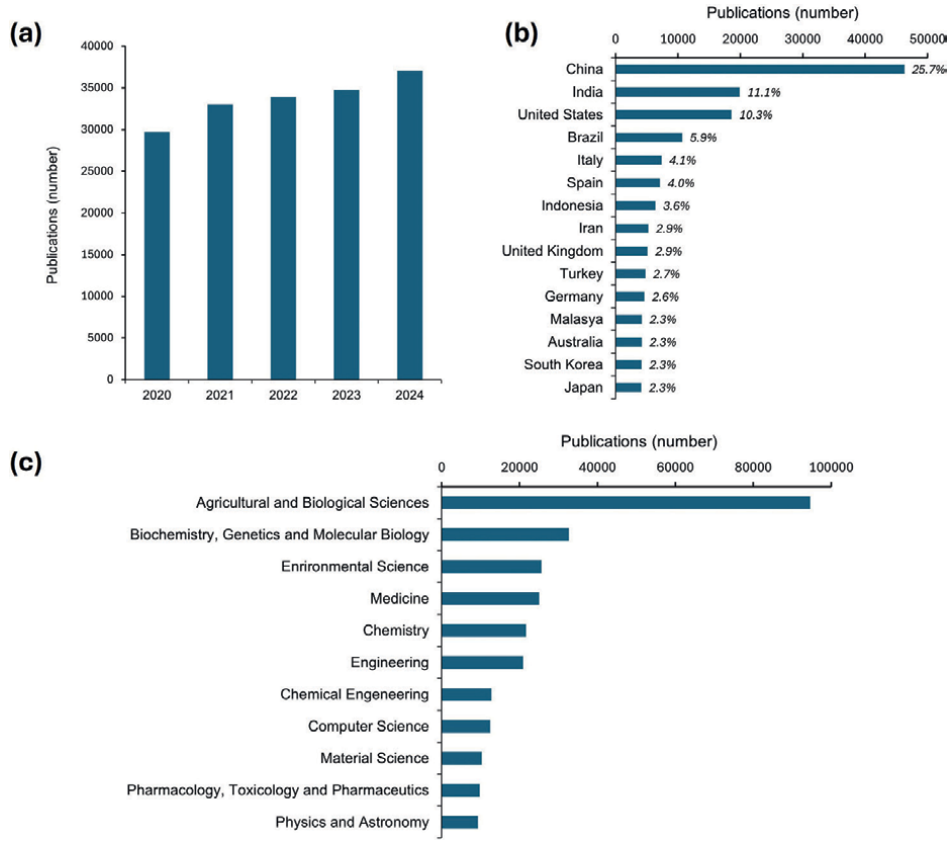

Figure 3.
(a) Number of publications, (b) countries with the highest number of publications, and (c) main areas of knowledge retrieved by the search of the terms "fruit" or "fruits" in the title, abstract, or keywords in the Scopus database from year 2020 until April 20, 2025.

science industries. A suitable quality of fresh fruits and their derivatives is achieved by well-conducted scientific research which ultimately allows to obtain better commercial products. The data on import, export, and production of fruits presented in this chapter show their impact on the global economic aspect, therefore denoting their commercial importance. Likewise, the significant number of publications shows current research interest in fruit crops.

Author details

Mateus Pereira Gonzatto[1]* and Júlia Scherer Santos[2]

1 Department of Agronomy, Federal University of Viçosa, Viçosa, Brazil

2 Institute of Biological Sciences, Department of Pharmacology, Federal University of Juiz de Fora, Juiz de Fora, Brazil

*Address all correspondence to: mateus.gonzatto@ufv.br

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Schaffer B, Andersen PC. In: Schaffer B, Andersen PC, editors. Handbook of Environmental Physiology of Fruit Crops. Boca Raton; London; New York; Washington: CRC Press; 1994. 367 p. DOI: 10.1201/9780203719299

[2] dos Santos LVB, Monteiro DP, do Nascimento ALB, de Barros RFM. The traditional knowledge about the biodiversity of edible Brazilian fruits and their pollinators: An integrative review. Journal of Ethnobiology and Ethnomedicine. 2025;**21**(1):1-16. DOI: 10.1186/s13002-025-00769-1

[3] Petry HB, Baratti ACC, Brighenti AF, Gonzatto MP, Citadin I, Souza ALK. Brazilian fruit agribusiness. Fruit Crop Science Journal. 2025;**1**:1-14. Available from: https://fruitcsjournal.online/index.php/fruit/article/view/1

[4] Sherman WB, Beckman TG. Climatic adaption in fruit crops. Acta Horticulturae. 2003;**622**:411-428. DOI: 10.17660/ActaHortic.2003.622.43

[5] FAO. World Food and Agriculture – Statistical Yearbook 2024. Rome: FAO; 2024. DOI: 10.4060/cd2971en [Accessed: April 27, 2025]

[6] FAO. Food and agriculture organizations of the United Nations. In: Fruit Primary. [Internet]. FAO; 2025. Available from: http://www.fao.org/faostat/en/#data [Accessed: April 20, 2025]

[7] SCOPUS. Scopus database [Internet]. 2025. Available from: http://www.scopus.com [Accessed: April 15, 2025]

Chapter 2

Water Relations in Fruit Trees: Knowing for Better Irrigation Management

Guilherme Augusto Rodrigues de Souza, Ellen de Moura Vale, Wallace de Paula Bernado, Danilo Força Baroni, Elias Fernandes de Sousa, Miroslava Rakočević, Weverton Pereira Rodrigues and Eliemar Campostrini

Abstract

This book chapter initially shows the actions of anthropogenic activities on climate change and how this future scenario can cause water stress in fruit plants, notably through the reduction in water availability caused by drought. Thus, only through advanced knowledge of fruit tree physiology is it possible to apply water in a controlled manner, lowering costs and increasing yield, thereby reducing the water footprint (L per kg of fruit) and improving agronomic and economic water use efficiency, as well as fruit quality. After the introductory part, the basic concepts of water availability in soil, plants and air are shown, as well as methodologies for measuring water status in the soil–plant-atmosphere system. Furthermore, the chapter addresses the effects of water limitation associated with reduced availability of water in the soil and air on the photosynthetic process, the phytohormonal imbalance associated with abscisic acid (ABA), and hydraulic conductivity on the productivity and quality of fruit plants.

Keywords: plant-water balance, stomatal conductance, water potential, water stress, sap flow

1. Introduction

Anthropogenic activities have increased temperatures globally by more than 1°C since the pre-industrial period. In the next 20 years, an increase of up to 1.5°C is expected due to rising greenhouse gas emissions, primarily CO_2 [1]. Indeed, from the pre-industrial era until 2013, anthropogenic activities increased CO_2 concentration [CO_2] from 280 to 400 μL L^{-1}, reaching 423.6 ± 0.5 μL L^{-1}, rising by approximately 2 μL L^{-1} per year (Available from: https://www.co$_2$.earth/daily-co$_2$). Based on this

information, models predict that, by 2100, atmospheric [CO_2] could reach between 800 and 1150 μL L^{-1}. According to the Intergovernmental Panel on Climate Change [1], in tropical regions for the same period, the rise in [CO_2] could lead to a 1.2 to 6°C increase in temperature. This could cause extreme atmospheric pressure variations, leading to changes in air mass movement, which may result in zones of extreme water scarcity as well as areas with high rainfall.

Ortiz-Bobea et al. [2] showed that anthropogenic activities since 1960 have reduced agricultural yields by 21%, equivalent to a loss of seven years of productivity growth. The effects will be substantially more severe (a decline between 34 and 36%) in regions with higher temperatures such as Africa, Latin America, and the Caribbean. Thus, scientists are nearly unanimous in reporting that tropical regions are the first affected by global climate change [3, 4]. This is due to poor economic conditions, high dependence on natural resources, and small temperature variations. Like all cultivable species, fruit trees are dependent on climate and are and would be significantly impacted by climate change. In tropical countries such as Brazil, these crops are socially and economically relevant, evident in Brazil's ranking as the third largest fruit producing country in the world [5].

Among the environmental stresses expected to increase in the coming decades, water stress is a severe and frequently occurring abiotic stress that significantly restricts global agricultural production by reducing crop growth and yields [6–8]. Drought is the main effect of climate change, and tropical fruit plants will certainly be subjected to and compromised by this climatic factor. This may lead scientists to intensify their studies on the effects of water limitation on the photosynthesis, growth, and development of tropical fruit trees.

Given that, in fruit-bearing plants, the commercially valuable product mostly comes from reproductive organs, studying the effects of water scarcity in soil and air, which will be drastically changed by global climate change, will be crucial in sustaining fruit production in Brazil. One of the primary lines of research will be to attempt to reduce water application in plants without compromising the photosynthetic and reproductive processes, thereby minimizing the effects on final crop yield [9, 10]. This could save time, space, and resources. However, along with the attempt to reduce water application in fruit-bearing plants to save water, moderate water stress may not be as harmful in these plant classes since fruit quality is more important than quantity *("less can be more"),* unlike in grains (corn, wheat, rice, etc.) and oils (soybean, sunflower, etc.) [11].

In certain situations, moderate soil water stress may significantly increase the concentration of important phytochemicals in fruits (phenolic compounds, sugars, vitamin C, carotenoids, volatile compounds, etc.), which are important for human and animal health [11, 12]. Furthermore, mineral nutrient concentrations in fruits can also be altered by climate change [13]. This situation may shift the focus of scientists toward the idea that, in a future scenario of reduced water availability, "*less can be more*".

Another aspect is that excess water in the soil may have serious effects on fruit tree growth and development, due to the significant changes in O_2 availability in the rhizosphere [14]. Thus, irrigation management in these plant classes is extremely important. Only through advanced knowledge of fruit tree physiology is it possible to apply water in a controlled manner, lowering costs and increasing yield, thereby reducing the water footprint (L per kg of fruit) and improving agronomic and economic water use efficiency, as well as fruit quality. These measures can lead to sustainable water resource management.

1.1 Water potential

Water molecules move in the soil–plant-atmosphere continuum (SPAC) due to differences in the free energy (energy available to do work) of water between different parts of this continuum. This free energy can be quantified by a variable called water potential (Ψ), defined as the chemical potential of water in any system compared to the free energy of pure water under the same temperature and pressure conditions [15]. The potential of pure water is considered to be zero, so Ψ is usually negative in all parts of the SPAC. This generally results in water moving from the soil to plant regions with more negative values, that is, from soil to the atmosphere around the leaf [16]. However, there may be a reverse route driven by water absorption through the leaf, defined as an atmosphere-plant–soil (APS) route [17, 18]. Indeed, under low temperature conditions with high relative humidity (fog), when soil water availability is low, water can enter the leaf through the cuticle, accounting for about 42% of leaf water content. This water is transported through the leaf cuticle to the xylem, and then the roots, which increases stomatal opening (raising stomatal conductance) and CO_2 assimilation, with positive effects on plant growth [19]. Water absorption *via* fog is an important mechanism for plant water acquisition and can mitigate the harmful effects of drought. In this condition, when compared to leaf water potential (Ψ_{leaf}), Ψ is higher in the air around the leaf.

The water potential (Ψ) inside a cell or in a soil solution may be influenced by solute concentration and/or pressure. Slatyer [15] reports that ψ is measured in megapascals (MPa) and can generally be simplified by Eq. (1):

$$\Psi = \Psi_s + \Psi_p \tag{1}$$

where ψ is the water potential, ψ_s is the osmotic potential, and ψ_p is the pressure potential (sometimes known as matric potential (Ψ_m)) [20].

Osmotic potential (ψ_s) is always negative, or zero for pure water. The negative value occurs because water in the system is less able to perform work than pure water. The presence of solutes in water contributes to reducing the water potential. However, the pressure potential (Ψ_p) can have a negative, zero, or positive value. Positive pressure results from an increase in pressure in the system, whereas tension results in negative pressure. As such, Ψ can have negative or zero values, since pressure can be positive and high, equaling the negative values of Ψs. For example, in the sieve elements of phloem in source leaves, Ψ can be −1.1 MPa, derived from the sum of the two components Ψ_s = −1.7 MPa and Ψ_p = +0.6 MPa, while in the xylem vessel elements adjacent to the phloem in the same source, Ψ is −0.8 MPa, resulting from the sum of Ψ_s = −0.1 MPa and Ψ_p = −0.7 MPa [21].

Several species from arid and semi-arid regions have a high capacity for osmotic adjustment (OA) (decline in osmotic potential due to the accumulation of osmotically active solutes in the cell in response to water stress [22]). This adjustment allows the plant to maintain high transpiration rates despite lower soil water availability. In this condition, leaf water potential (Ψ_{leaf}) may decline well before it affects Ψ_p. This decrease in Ψ_{leaf} without affecting Ψ_p allows the leaf to maintain a constant water potential gradient between itself and the soil (Eq. (2)):

$$\Delta\Psi = \Psi_{soil} - \Psi_{leaf} \tag{2}$$

where E_{plant} = (Ψ_{soil} - Ψ_{leaf})/R_{plant}. Here, E_{plant} represents plant transpiration and R_{plant} the plant hydraulic resistance [23]. Under conditions of soil water limitation,

OA enables the plant to maintain constant transpiration. In plants with greater OA capacity compared to those without it, embolism/cavitation occurs only under very intense soil water potential conditions (very dry soils). Thus, in plants with OA, longer intervals between the irrigation periods would be allowed.

In the case of woody fruit plants, during intense soil water scarcity, $\Delta\Psi$ may increase substantially, intensifying water stress in the xylem (very negative Ψ_{xylem} values). This increase in $\Delta\Psi$ may result in embolism/cavitation in a large number of xylem vessels, thereby increasing R_{plant}. Greater hydraulic resistance causes stomatal closure, reduced transpiration, higher leaf temperatures, and lower CO_2 assimilation, with a negative effect on fruit plant growth and yield.

1.1.1 Soil water

In most non-saline soils, defined that have bulk electrical conductivity (ECe) lower than 2 dS m^{-1} [24], water potential is primarily influenced by negative pressure potential ($\Psi_p < 0$), and Ψ_m represents the negative hydrostatic pressure [20]. In these soils, the value of Ψ_s becomes insignificant. Soil particles retain water with sufficient force to keep it tensioned and attached to the particles. For a plant to absorb water from the soil, the root system needs a force greater than the attraction force between water molecules and soil particles, which depends on the soil type. Normally, a plant can extract water attached to soil particles with a force greater than −1.5 MPa (−1500 kPa). Thus, in general, for agronomically important plants such as fruit trees, −1.5 MPa can be considered the soil water potential that causes the permanent wilting point (PWP) in the plant. At PWP, the plant does not recover leaf turgor even with stomatal closure, and nocturnal temperature (< air vapor pressure deficit, VPD_{air}) decreases. However, in fruit tree irrigation management systems, ψ_p values between 0 and 0.03 MPa (−30 kPa) are considered suitable for cultivation. In young papaya plants (103 days old) grown in 30 L pots, a decline in pressure potential from −10 to −50 kPa decreased stomatal conductance (g_s) (increased stomatal closure) from 0.7 to 0.1 mol m^{-2} s^{-1} (80% reduction in g_s) [25, 26]. In the same species, in 6-month-old plants under field conditions, CO_2 assimilation declined by 50% (from 20 to 10 μmol m^{-2} s^{-1}) when water potential fell from −20 to −68 kPa. Soil water potential, assessed through the Ψ_p component, is commonly measured using tensiometers, vapor pressure methods, and sensors with ceramic plates or gypsum blocks (**Figure 1**).

Tensiometers (**Figure 1A**) operate in a range from 0 to −0.2 MPa (−200 kPa). However, most cavitate at around −0.08 MPa (−80 kPa). The vapor pressure method (**Figure 1B**) has a working range from approximately −0.1 to −300 MPa (−300,000 kPa). Gypsum sensors work in a range of 0 to −0.2 MPa (−200 kPa), while ceramic sensors operate in a broader and more suitable range from −5 to −100 MPa (−100,000 kPa).

Soil water content can also be assessed by the amount and not the energy with which water is attached to soil particles [27]. This methodology tends to be easier to measure and can include different techniques such as thermogravimetry (obtained through the weight change of samples dried in ovens at 105°C for 24 h, that is, the ratio between soil water content and soil dry weight) and the dielectric, resistivity, neutron probe and thermal properties of soil methods [27]. However, for proper assessment of plant water availability, the soil water retention curve (SWRC) (**Figure 2**), a key hydraulic property, is widely used in irrigation management in different soil types. This curve describes the functional relationships between soil water content and soil water potential controlled by pressure potential under equilibrium conditions [29].

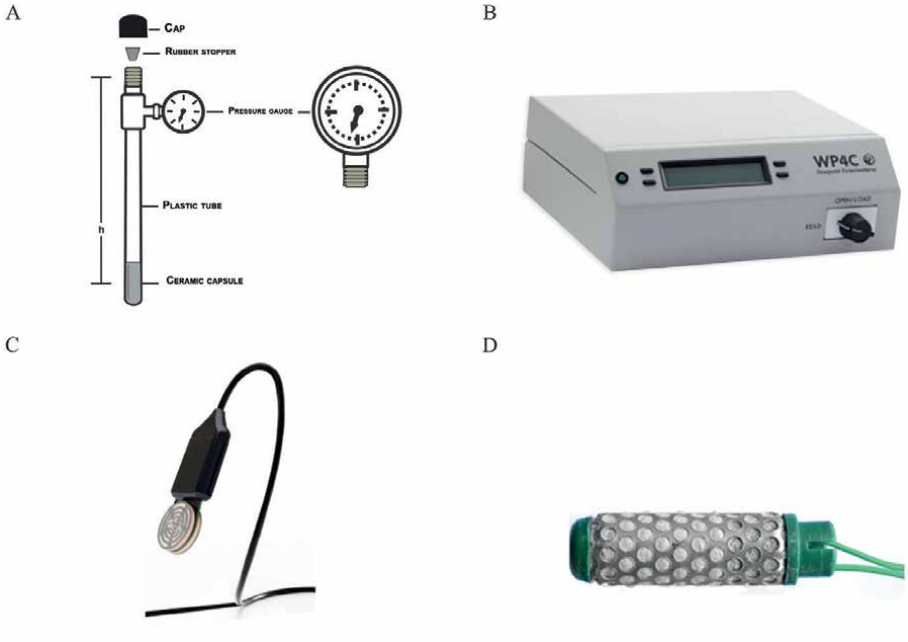

Figure 1.
Porous plate tensiometer (A), WP4C dew point hygrometer (Meter Environment, USA) (B), Teros 21 ceramic sensor (Meter Environment, USA) (C), and gypsum porous sensor (D).

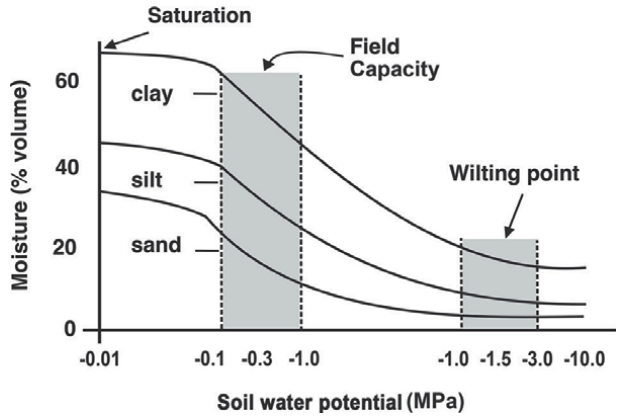

Figure 2.
Soil water potential (MPa). Adapted from [28].

In the SPAC, Ψ can be measured in the soil, root, stem xylem, leaf xylem, and air, in order to understand water movement throughout the continuum.

1.2 Water in the plant

As occurs in the soil, a plant's water status can be assessed by the water molecule concentration in the evaluated plant tissue (relative water content, RWC) or the water molecule energy assessed by the water potential (Ψ).

1.2.1 Relative water content in the plant

The water status assessed by the water molecule concentration is typically measured in the leaf, using RWC [30]. RWC compares the leaf water content to the maximum amount of water when the leaf is fully saturated (turgid) [31]. This characteristic is obtained by weighing the leaf or leaf discs without the main vein immediately after removing the leaf from the plant (leaf or disc fresh weight, $FW_{L/D}$). After weighing, the leaf or discs are saturated with water. The leaves are placed on the lower shelf of a refrigerator, and the petiole was immersed in water at 10°C (turgid leaf or disc weight, $TW_{L/D}$). The leaf discs are placed in a container with deionized water [32]. Next, the leaf or discs are placed in an oven at 80°C for 24 hours (leaf or disk dry weight, $DW_{L/D}$). RWC can be obtained from these variables using the Eq. (3):

$$RWC(\%) = (FW_{L/D} - DW_{L/D}) / (TW_{L/D} - DW_{L/D}) \times 100 \quad (3)$$

where RWC is the relative water content (%), $FW_{L/D}$ is the leaf or disc fresh weight (g), $DW_{L/D}$ is leaf or disc dry weight (g), and $TW_{L/D}$is leaf or disc turgid weight (g).

Although assessing RWC in leaf tissue is suitable for measuring water status in terms of the physiological consequences of cellular water stress, the methodology requires equipment such as precision balances and ovens, making it prone to errors, particularly in obtaining leaf/disc turgid weight. Furthermore, RWC does not show the effect of OA on maintaining the water status of plant tissues. OA is a physiological mechanism associated with the synthesis of osmotically active solutes by plant cells, primarily associated with the synthesis of proline, glycine betaine, mannitol, and sorbitol [33]. These compounds interact with water molecules, reducing their free energy and thus lowering the osmotic potential and consequently the water potential of the cell. As such, plants exhibiting OA as a response mechanism to water stress may show very low RWC values due to overestimation of turgid weight [34].

However, care should be taken to avoid overestimating turgid weight, as leaf or disc samples may absorb water beyond their physiological capacity if soaked for prolonged periods. Hydration should ideally be conducted in distilled water at a controlled temperature (4–10°C) and low light, conditions which limit metabolic responses and prevent excess water uptake [30, 35]. Additionally, the use of distilled or deionized water ensures accurate osmotic balance, essential for reliable RWC measurements [35].

RWC decreases with increasing water stress, especially in plants considered anisohydric. In these plants, as soil water availability decreases, both leaf water potential (Ψ_{leaf}) and RWC decrease. Anisohydric plants exhibit compromised stomatal sensitivity to stomatal closure [36]. By contrast, isohydric plants display the opposite response, that is, with decreasing soil water availability, stomata respond rapidly by closing, thereby reducing transpiration and conserving water in the leaf, evident in the stable RWC and Ψ_{leaf}. In 'Red Lady' papaya genotype, isohydric response was observed in young plants after irrigation suspension, where soil water potential started at −1 kPa and reached maximum water stress at −60 kPa [37]. Under these conditions, RWC remained stable, indicating a certain tolerance of papaya to soil water limitation [37]. However, Torres Netto [38], working with 'Golden' and 'Hybrid UENF/Caliman 01' genotypes, observed anisohydric responses. When plants were subjected to water stress, there was an increase in abscisic acid (ABA) biosynthesis in the roots [39]. In some plants, this phytohormone is transported to the shoots *via* the xylem, promoting stomatal closure. In some situations, ABA synthesis/translocation

is observed even before any effect on leaf water potential occurs [40]. According to Gomes [41], the effects of water stress on ABA content in papaya is evident 24 h after the onset of stress. This demonstrates that this phytohormone may be involved in decreasing stomatal conductance (g_s) in plants exposed to water limitation in the substrate. Indeed, in papaya plants submitted to water stress (10% soil moisture compared to 24% in controls 40 days after treatment (DAT) in 30 L pots), Mahouachi et al. [42] demonstrated that ABA concentration increased continuously in leaves and roots, with maximum values at peak stress day (40 DAT) of 225 ng g^{-1} and 65 ng g^{-1} in leaf and root dry weight, respectively. In citrus plants, drought also caused an increase in ABA concentration in leaves, roots, and fruitlets [43]. This increase in ABA is responsible for promoting stomatal closure, reducing transpiration, and mitigating water stress damage by inducing genes associated with osmolyte synthesis [44]. These responses may contribute to the plant's ability to cope with water stress [44].

RWC remains the most suitable variable for evaluating plant water status [30]. However, the need for an analytical balance, along with the use of ovens and a system for complete leaf and leaf disc hydration, makes using this variable for irrigation management impractical.

1.2.2 Water potential in plants

Plant water status is closely linked to growth, fruit size, and yields. Thus, a number of related variables are used as indicators of water stress for fruit plant irrigation management [23]. Water potential quantifies the free energy of water in plant tissues, measured in MPa, and is the main force driving water movement from roots to leaves and from leaves to the surrounding air. During leaf transpiration (with stomata open), there is a gradient of water vapor concentration and water potential between the leaf and the air, as well as a reduction in water concentration in the air spaces near the sub-stomatal cavity and between cells due to leaf water loss [21]. Given the equilibrium between liquid water in the cell wall and water vapor in the intercellular spaces, liquid water from the mesophyll cell walls evaporates. During water evaporation from the cell walls, and due to hydrogen bonding between molecules, tension is formed and distributed throughout the water column between the soil and the leaf, allowing water to rise to the leaf [21]. This tension is characterized as the component $\Psi_p = -2\ T/r$, where T is the surface tension of water = 7.28×10^{-8} MPa and r the radius of the water meniscus formed between cellulose microfibrils in the cell wall. Since lower amounts of solutes are present in the xylem sap (more diluted sap) compared to the phloem sap, the osmotic potential of the former is less negative (closer to zero), making Ψ_s less important in the final calculation of Ψ_w in the xylem. Thus, Ψ_w in the xylem is practically defined by Ψ_p.

There are several techniques for measuring water potential in the leaf, the most widely used organ for assessing plant water status. These include the dye method (Chardakov's method) [45], equilibrium between tissue volume and a solution of known water potential [45], the psychrometric method [46], and the pressure chamber method [47]. Among these, the pressure chamber method is the most widely used due to its practicality and large-scale application under field conditions. To evaluate leaf water potential using the pressure chamber method, a mature leaf grown in full sun is removed from the plant using a razor blade and placed inside the pressure chamber (**Figure 3**). It is important not to re-cut the petiole of the leaf or, if using branches, to not re-cut them when inserting the samples into the pressure chamber (**Figure 3A**).

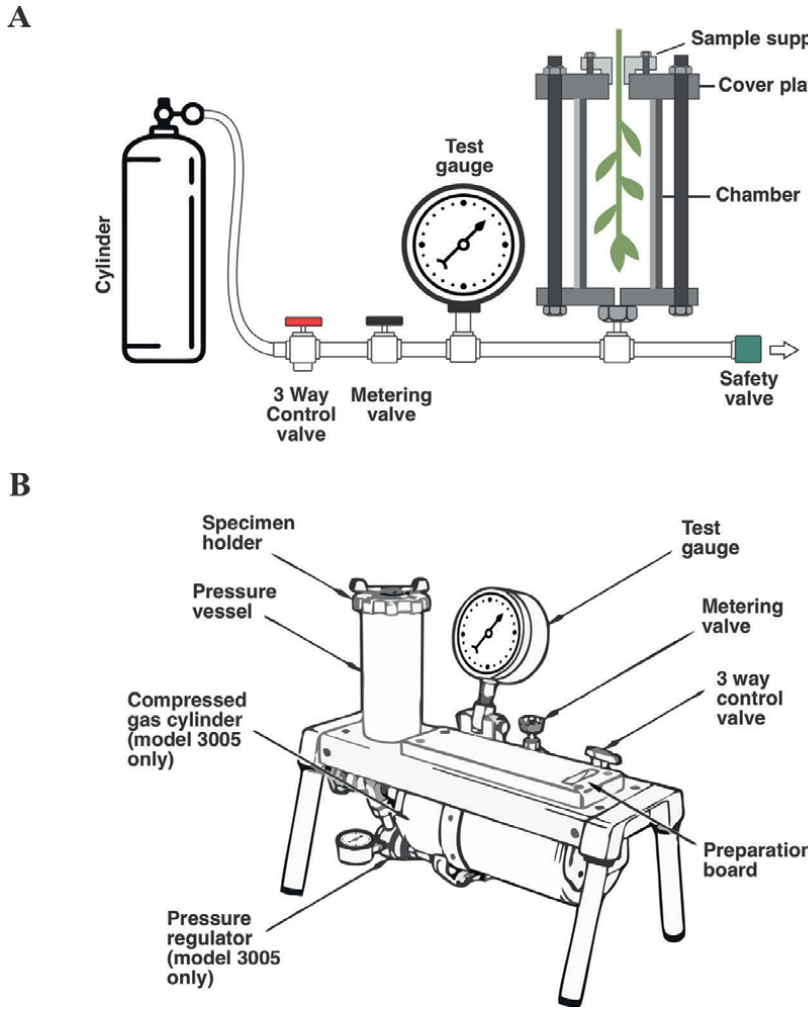

Figure 3.
(A). Pressure chamber to measure leaf water potential. Training Course in Plant Ecophysiology, USP/ESALQ. Prof. Sergio Roberto Sigrist. 2014. (B). Commercially available pressure chamber, the Plant Water Status Console, from Soilmoisture Equipment Corp. A gas cylinder is attached to the bottom of the wooden base, making the unit self-contained.

Pressure is applied by slowly injecting N_2 at 0.01 MPa s^{-1} or 0.1 bar s^{-1}, distributing it throughout the leaf inside the chamber, until xylem sap exudation begins at the petiole cut region. The pressure at which xylem sap exudation starts is recorded, and this value multiplied by –1 corresponds to the water tension in the xylem (MPa). It is important to note that if the plant material (leaf or branch) is under intense water stress, the pressure inside the chamber can be increased by 0.05 MPa s^{-1} (0.5 bar s^{-1}) [23]. Additionally, due to hydraulic resistance, the author reports that higher applied pressures increase the temperature inside the chamber and lead to reading errors, thereby increasing the time between pressure increments and the appearance of xylem sap at the cut point of the sample (leaf or branch). Pressure control is essential for accurately determining the point at which xylem sap appears at the cut point of the leaf/branch. Fernández [23] also observed that air bubbles within the xylem sap at the cut point of the leaf/branch indicate that the pressure inside the chamber was excessive. Since xylem is distributed

throughout the leaf, this negative pressure refers to leaf pressure potential ($-\Psi_p$), which corresponds to the leaf water potential, as the value of xylem Ψ_s is negligible [47].

Leaf water potential is highly variable due to variations in environmental conditions surrounding the leaf [48]. For example, at midday, with increased atmospheric water demand, leaf water potential can be significantly lower when compared to the root system, reflecting leaf water stress not experienced by the root system, given that the soil may be at field capacity. Thus, assessing predawn leaf water potential (Ψ_{am}) may mitigate the effects of intense variations in the atmosphere around the leaf during periods of high temperature and relative humidity (> VPD_{air}), making Ψ_{am} assessment more related to soil and root water status. Ψ_{am} is not influenced by the isohydric or anisohydric response of a genotype or daily adjustment of Ψ_s, unlike midday Ψ assessment. Thus, according to Fernández [23], midday ψ_{leaf} measurements are not sensitive for assessing the water status of an isohydric genotype. In isohydric plants, g_s may be more sensitive to water limitation because stomata respond more rapidly to decreasing soil moisture. In anisohydric plants, midday Ψ is a more sensitive indicator, unlike g_s. With respect to Ψ_{am}, in terms of practical irrigation management, using predawn leaf water potential before sunrise is challenging due to working hours.

Ψ_{am} assessments indicate overnight water potential through equilibrium with the soil, due to nocturnal stomatal closure and lower atmospheric water demand during this period. By contrast, measuring branch/trunk water potential (Ψ_{branch}) in woody plants, such as most fruit trees, is more related to overall plant water status and more appropriate for assessing the hydration status of fruit trees [48, 49]. Early studies on plant irrigation management indicators used Ψ_{branch} as a better indicator of plant water status when compared to Ψ_{leaf} [50].

To measure branch/trunk water potential, the leaf should be bagged while still on the plant using an aluminized polyethylene bag or aluminum foil (to prevent excessive leaf heating), approximately 4 to 5 h [49, 51] or 1 to 2 h [52, 53] before Ψ_{branch} assessments. The plastic bag prevents transpiration, as the stomata are closed and the relative humidity around the leaf is nearly 100%, thereby eliminating the water potential gradient between the leaf and the air. When the leaf is bagged and remains attached to the plant, leaf water potential reaches equilibrium with the stem/trunk water potential, thus maintaining water balance with the entire plant [48]. As such, Ψ_{branch} is less influenced by environmental variables surrounding the leaf when compared to Ψ_{leaf}. Indeed, in apple trees (*Malus domestica*) [54], nectarines (*Prunus persica var. nucipersica*) [55], lychee (*Litchi chinensis*) [56], pears (*Pyrus communis*) [49], mangoes *(Mangifera indica*) [57], citrus [58], and grapevines (*Vitis vinifera*) [59, 60], Ψ_{branch} has been used to assess water status. Naor [53] also reported greater Ψ_{branch} sensitivity measured at midday compared to Ψ_{leaf} measured at the same time.

During water potential assessments, the pressure chamber (**Figure 3**) can be taken to the plant in the field or greenhouse, or the detached leaf can be brought to the pressure chamber. Ψ_{leaf} and Ψ_{branch} should be assessed immediately after removing the leaf from the plant.

When transporting the leaf to the pressure chamber, it should be placed inside an opaque container with a moist paper towel to prevent it from heating up. Under these dark conditions and lower VPD_{air} inside the container, the leaf water potential remains unchanged [23]. For example, the midday leaf water potential (Ψ_{leaf_12h}) of grapevines between −0.8 and −1.1 MPa, respectively, pre- and post-veraison, is considered critical for initiating irrigation in this species [61]. In young citrus leaves, the leaf water potential at stomatal closure is (Ψ_{leaf} = −1.6 MPa), which coincides with turgor potential (Ψ_{p_leaf}) being zero [62].

2. Water in the air

Water limitation in plants may be caused by the decline in water molecule concentration and energy in both soil and air. Air water limitation is assessed through VPD_{air}, which is the difference between saturation vapor pressure (e_s) and actual vapor pressure (e_a) [63]. The variable e_s depends on temperature and represents the maximum amount of water that can be placed in a volume of air at a given temperature. The variable e_a also depends on relative humidity, used to calculate VPD_{air} (Eq. 4):

$$VPD_{air} = e_s - \left(RH \times e_s / 100\right) \quad (4)$$

where RH is the relative humidity. VPD_{air} measures the atmosphere's drying force [63] and is responsible for significant reductions in crop yields [64, 65]. In other words, it quantifies the air's capacity to remove water from a surface. With climate change, VPD_{air} values are expected to increase due to rising temperatures and lower relative humidity [66]. VPD_{air} is positively related to the air water potential (Ψ_{air}, Eq. 5):

$$\Psi_{air} = RT / V \ln\left(e_a / e_s\right) \quad (5)$$

where R is the gas constant (0.0831 kg bar^{-1} mol^{-1} K^{-1}), T is the absolute temperature (K), V is the molar volume of water (18 cm^3 mol^{-1}), and e/e_s is the relative humidity [35]. Ψ_{air} measures the energy of water molecules in the air capable of performing work at a specific temperature and relative humidity. As Ψ_{air} declines logarithmically with temperature and relative humidity, under certain conditions during summer, in unventilated greenhouses or field settings during the hottest times of the day, Ψ_{air} can reach around −300 MPa. This value is extremely negative and characterizes air with high water demand (**Figure 4**).

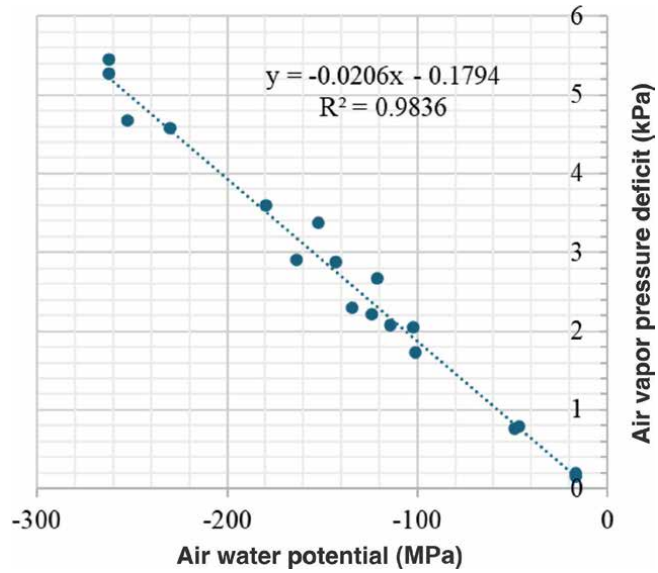

Figure 4.
Relationship between VPD_{air} and air water potential (Ψ_{air}). Adapted from Binks et al. [67].

In general, physiological processes such as leaf gas exchanges, growth, and mineral nutrient absorption are not affected when plants are cultivated at VPD_{air} values between 0.5 to 1 kPa [68, 69]. Values below 0.5 kPa can reduce transpiration and cause nutrient absorption problems, particularly affecting metabolic processes related to Ca++ [68]. Values above 1 kPa lead to reduced gas exchanges through decreased g_s and CO_2 assimilation (A) [70, 71].

Plant scientists are interested in the direct effects of VPD_{air} on leaf gas exchanges and thus prefer using $VPD_{leaf\text{-}air}$, as this variable may provide a more complete assessment. According to Grossiord et al. [63], $VPD_{leaf\text{-}air}$ is an accurate variable for evaluating leaf water balance (and possibly that of the entire plant and even at canopy level). This variable is calculated as the difference between the water vapor pressure in the stomatal pore (100% RH) (e_{sleaf}) and that in the air surrounding the leaf (e_{aair}) [72]. Thus, $VPD_{leaf\text{-}air}$ is highly dependent on leaf temperature. Rapid increases in $VPD_{leaf\text{-}air}$ cause a decrease in stomatal opening expressed in g_s [73]. Stomatal sensitivity to $VPD_{leaf\text{-}air}$ varies significantly among species [74, 75]. For example, in papaya trees, values above 2.5/3.0 kPa already prompt stomatal closure [76–78].

According to Flore and Lakso [79], leaves and roots are further apart in fruit plants, due to their greater height when compared to their herbaceous counterparts, resulting in higher stomatal sensitivity to increases in VPD_{air} and $VPD_{leaf\text{-}air}$. This sensitivity is attributed to greater hydraulic resistance [79]. The increased leaf-to-root distance allows leaf water potential (Ψ_{leaf}) and leaf hydraulic conductance (K_{leaf}) to be the major determinants of how epidermal water potential and guard cell turgor respond to changes in VPD_{air} and $VPD_{leaf\text{-}air}$, thereby controlling stomatal responses to these changes [74]. In fruit-bearing plants, higher hydraulic resistance in the entire plant (leaves, roots and trunk), primarily in leaves [80], is associated with greater g_s reductions at midday (when atmospheric water demand is highest > VPD_{air} or > $VPD_{leaf\text{-}air}$). This decrease in CO_2 assimilation during the period of the highest temperature and radiation is known as the midday depression of photosynthesis [81] and can significantly reduce fruit production. Understanding the effects of VPD_{air} on leaf gas exchange is crucial for agricultural zoning of fruit-bearing plants and for controlling this variable in protected cultivation. For example, in papaya trees, Salinas et al. [82] showed that, in summer conditions in Southern Spain, maintaining relative humidity above 60% through canopy misting with microsprinklers at a flow rate of 1 L m^{-2} h^{-1} favored growth, flowering, fruit set, and consequently increased fruit weight. However, misting to reduce VPD_{air} and $VPD_{leaf\text{-}air}$ (<60% RH) did not improve fruit quality in terms of external skin appearance, acidity, and °Brix. Moreover, in field-grown papaya plants, applying water during peak temperature and solar radiation (midday) to reduce $VPD_{leaf\text{-}air}$ resulted in an 18% decline in $VPD_{leaf\text{-}air}$, a 24% increase in stomatal opening and a 13% rise in CO_2 assimilation [83]. This misting technique applied over the canopy for 20 weeks of harvesting cooled the leaves, significantly increasing fruit yield by six fruits per plant [84].

Another strategy to reduce $VPD_{leaf\text{-}air}$ is the application of processed kaolinite particle films (PKPF) on the leaves of fruit-bearing plants [85, 86]. After these films were applied, PKPF increased solar radiation reflection [76, 85, 87], thereby lowering leaf temperature (T_{leaf}). This decline in T_{leaf} can be between 3 and 5°C [86]. Wand et al. [88] reported that applying PKPF decreased apple leaf temperature by 20% and significantly reduced fruit sunburn, or even increased coffee bean size 16 by 50% and increased total productivity [87].

In plants without PKPF on the leaves, solar radiation can elevate leaf temperature and increase $VPD_{leaf\text{-}air}$ values [89]. Indeed, in citrus leaves (*Citrus paradisi*) treated

with PKPF, solar radiation reflectance increased, leaf temperature fell, and $VPD_{leaf\text{-}air}$ declined [89]. In addition, in citrus plants, PKPF on the leaves increased g_s, A, and water use efficiency (WUE). In two years of research on these citrus plants, production increased in plants with 3% PKPF on the leaves. Similarly, in peach plants, Glenn et al. [90] demonstrated that using PKPF lowered canopy temperature and did not affect plant growth.

In grapevine plants, applying 6% PKPF enhanced maturation, increased berry sugar content, and decreased acidity. The concentration of secondary metabolites, mainly phenolic compounds, was elevated. Anthocyanin content was higher in plants with 6% PKPF on fruit and leaf surfaces due to lower berry temperature [91]. In mango plants, leaf coverage with PKPF protected against excessive solar radiation, reduced leaf temperature and $VPD_{leaf\text{-}air}$, and resulted in higher g_s and A. PKPF increased the number of fruits and total fruit weight by 41 and 44%, respectively [92].

3. Assessing the water status of fruit-bearing plants

With increased biological understanding of plant water relationships, and knowledge of water dynamics in soil and air, as well as technological advances in electronics, precision irrigation has attracted significant interest. It aims to efficiently use irrigation systems by more accurately determining and quantifying crop water needs and applying water as accurately as possible [93]. Thus, advanced scientific analysis of one part of the SPAC, such as the plant component, is essential for the success of accurate irrigation, saving space, time, and resources. In addition to assessing water potential and relative water content, other methodologies for evaluating the water status of fruit-bearing plants, some of which can be automated, are of utmost importance for decision-making. These methodologies include stomatal conductance assessment, infrared thermometry (thermography), near-infrared spectroscopy, xylem sap flow, branch and fruit dendrometry and leaf turgor pressure assessment.

3.1 Stomatal conductance

Gas exchange control between the leaf and the surrounding atmosphere is governed by stomata, making them crucial for plant transpiration, photosynthesis, and yield [94]. These structures are mostly located on the abaxial surface of the leaf, although many species have stomata on both surfaces. In general, woody fruit-bearing plants have stomata only on the abaxial surface. These include mango [95], citrus [96], grapevine [97], papaya [77], and coffee plants [98]. However, in banana plants, stomata are distributed on both leaf surfaces [99].

Almost all water leaving the plant passes through stomata. Thus, assessing the extent of stomatal opening is fundamental for estimating the leaf water exit capacity and relating it to the plant's water status. Under suitable light (800 $\mu mol\ m^{-2}\ s^{-1}$), CO_2 concentration (400 $\mu L\ L^{-1}$), and air humidity ($VPD_{air} < 1$ kPa), greater stomatal opening is associated with better leaf and plant hydration.

Stomatal opening is assessed through g_s ($mol\ H_2O\ m^{-2}\ s^{-1}$), which is inversely proportional to the resistance offered by stomata to the water exit rate and CO_2 entry into the leaf. Stomatal conductance is influenced by light quality and intensity, CO_2 concentration, leaf temperature, and primarily by water availability in soil and air [94]. As such, g_s can be considered one of the most effective indicators of precocity and sensitivity for water stress [100].

Porometers or portable gas exchange measurement systems (InfraRed Gas Analyzers, IRGA) (**Figures 5** and **6**) measure g_s and can be easily used in field conditions. Measurements are taken based on a specific leaf area inside each chamber. This area can vary from 0.31 cm^2 to 9 cm^2.

Adequate measurement of leaf temperature is important for correct assessment of g_s, as the equation for calculating g_s is defined as $g_s = E/VPD_{leaf\text{-}air}$, where E is leaf transpiration and $VPD_{leaf\text{-}air}$ the difference between the saturation vapor pressure inside the leaf and the vapor pressure of the air around the leaf ($e_{sleaf} - e_{air}$) [101]. Thus, when analyzing the equation, g_s can be interpreted as the amount of water leaving the leaf through the stomata *via* transpiration, in relation to the amount of water inside the leaf, at a given leaf temperature. Under conditions where soil water potential is near field capacity, VPD_{air} varies throughout the day and can cause

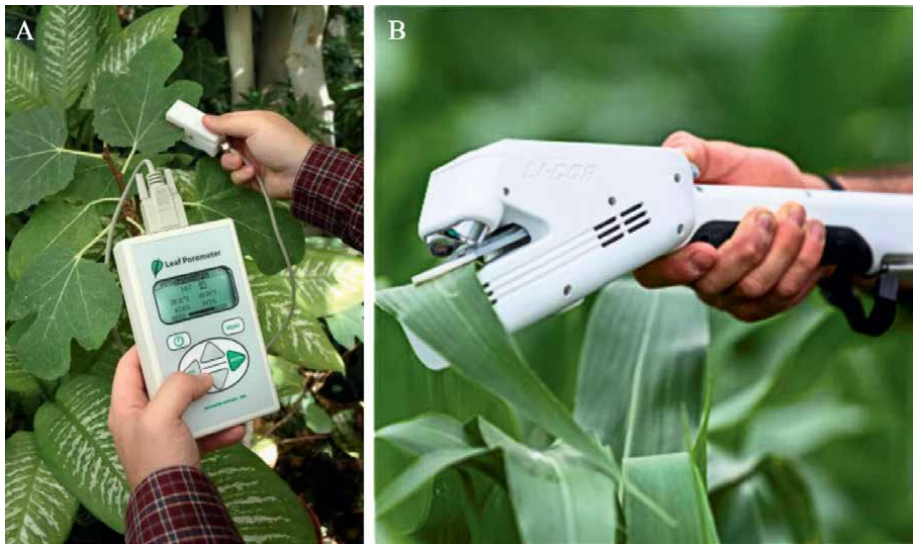

Figure 5.
Porometers (A: Meter Group; B: LiCor Environmental).

Figure 6.
Infrared Gas Analyzers (IRGA) (A: Licor, USA) (B: CiD. Inc., USA).

variations in g_s, with lower values typically occurring between 12 and 3 p.m., due to high solar radiation intensity. The maximum stomatal aperture value (maximum stomatal conductance, g_{smax}) is used as a good indicator of water stress [23, 102], and this variable is often measured in the early hours after sunrise when there is adequate radiation and suitable air and leaf temperatures. However, a daily curve can be generated to determine the diurnal time when g_{smax} occurs [103].

Although g_s determination is a good indicator of plant water stress, its use in irrigation management is still limited because measurements are typically taken manually, which restricts its application in precision irrigation. However, in fruit-bearing plants using automated systems, Hernandez-Santana et al. [104] indirectly estimated g_s based on xylem sap flow and VPD_{air}. Another limitation in using g_s for irrigation management is that g_s value may be reduced even when the soil is at field capacity, because increased hydraulic resistance in fruit-bearing plants, along with higher evaporative demand of the air, causes stomatal closure without correlating directly with soil water potential (field capacity soil). In fruit-bearing plants, especially under low wind speed and due to their great height, stomatal closure is much more effective in reducing E when compared to smaller plants, such as herbaceous crops of agronomic interest [105].

In grapevines, using g_s as an integrative variable to assess plant water stress, three phases of photosynthetic response to water limitation have been defined: mild (g_s between 0.7 to 0.15 mol H_2O m^{-2} s^{-1}), moderate (0.15 > g_s > 0.05 mol H_2O m^{-2} s^{-1}), and severe water stress (g_s < 0.05 mol H_2O m^{-2} s^{-1}) [100]. In papaya plants, it seems that 0.4 mol H_2O m^{-2} s^{-1} is a critical g_s value for irrigation management, given that values below this threshold have been shown to reduce CO_2 assimilation and growth [26].

3.2 Infrared thermometry

Leaf or canopy temperature exhibits a strong and positive correlation with transpiration and can be used as an indicator of g_s [106]. This suggests that leaf/canopy temperature can be applied in irrigation management [107, 108]. Thus, under soil water limitation conditions, stomatal close, decreasing transpiration, and leaf/canopy temperature increases, characterizing thermal stress associated with water stress. As such, leaf/canopy temperature may indicate the plant's water status.

Based on leaf temperature, Jackson et al. [109] and Idso et al. [110] developed the crop water stress index (CWSI, Eq. (6)). Jones et al. [108] developed an index associated with stomatal conductance denominated I_G (Eq. (7)):

$$\mathrm{CWSI} = \frac{(\mathrm{Tcanopy} - \mathrm{Tmin})}{(\mathrm{Tmax} - \mathrm{Tmin})} \tag{6}$$

$$\mathrm{I_G} = \frac{(\mathrm{Tmax} - \mathrm{Tcanopy})}{(\mathrm{Tcanopy} - \mathrm{Tmin})} \tag{7}$$

Both indices use canopy temperature (T_{canopy}), minimum temperature (T_{min}), and maximum temperature (T_{max}). T_{canopy} refers to the representative canopy leaf temperature obtained at the time of assessment; T_{min} the temperature of a leaf dipped in water to minimize leaf temperature due to water evaporation from the leaf surface; and T_{max} the temperature of a leaf with Vaseline applied to the abaxial surface (both sides for amphistomatous leaves), providing the maximum leaf temperature

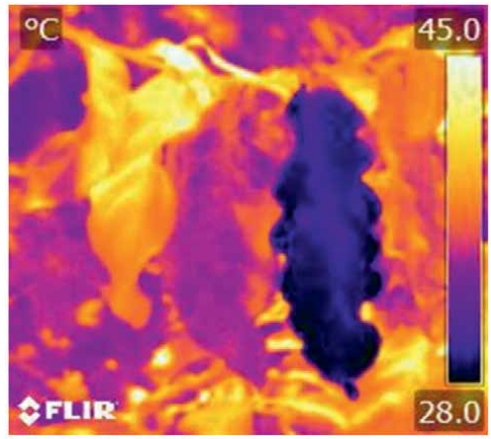

Figure 7.
Three coffee leaves representing, from left to right, T_{max}, T_{canopy}, and T_{min}. Adapted from Abreu [111].

during solar radiation exposure. Vaseline on the abaxial surface blocks transpiration (closes stomata), thereby achieving T_{max} (**Figure 7**).

With technological advances, small cameras with considerable capacity of data storage, processing capabilities, and high-quality image capture have been developed [112]. These cameras have been installed in unmanned vehicles, improving thermal stress assessment in crops associated with water stress [113], as well as through satellite imagery [114]. However, it is important to underscore that leaf (T_{leaf}) and canopy temperature (T_{canopy}), in addition to being influenced by soil water status, are also affected by other factors such as solar radiation, CO_2 concentration, air temperature, relative humidity, and wind speed. Moreover, the time of day when thermal images are captured may impact the information obtained, since stomatal aperture varies during the day, affecting transpiration and temperature. As such, Ben-Gal et al. [115] recommend that thermal image assessments be conducted during periods of maximum stress, typically occurring between 12 and 3 p.m.

Thermal imagery has been used in both monitoring water status and its relationship with photosynthesis in papaya [116], irrigation management in grapevines [117, 118], olive trees [115], and citrus [119]. In papaya, Lima et al. [116] used the difference between leaf temperature (T_{leaf}) and air temperature (T_{air}) ($\Delta T_{leaf\text{-}air} = T_{leaf} - T_{air}$) as an indicator of water stress. They reported that a value of −2 was considered a critical value. $\Delta T_{leaf\text{-}air}$ values greater than −2, that is, between −2 and 0, indicated severely compromised CO_2 assimilation.

In grapevines, Grant et al. [118] demonstrated that measuring the temperature of several canopy leaves could be a better way to assess thermal stress associated with water limitation in the plant, as this reduces the effect of leaf angle on readings. The CWSI was considered sufficient for the relative detection of thermal stress for potential use in irrigation monitoring in this crop. In grapevines, Bellvert et al. [120] showed the possibility of indirectly obtaining plant water potential (Ψ_{leaf}) and CWSI through thermal images installed on an aerial vehicle. This study proved to be important in assisting irrigation management in grapevines under a regulated irrigation system. However, the authors made several observations: (i) the importance of obtaining high spatial resolution images, especially in the early stages of the crop; (ii) avoiding thermal image acquisition on days following rain; and (iii) on days the

authors considered to exhibit lower vapor pressure deficit (VPD < 2.3 kPa) values, negatively influencing remote Ψ_{leaf} estimation and the CWSI value. Matese et al. [121] demonstrated that CWSI values in grapevines derived from remote and proximal sensors are suitable indicators for assessing the spatial variability of water status in plants of this species in the Mediterranean region of Italy. The study found a strong negative correlation (−0.80) between CWSI and CO_2 assimilation.

For *Citrus sinensis* L, the temperature difference between canopy (T_{canopy}) and air temperature (T_{air}) ($\Delta T_{canopy\text{-}air} = T_{canopy} - T_{air}$) proved to be a valuable characteristic for monitoring the water status. Plants with $\Delta T_{canopy\text{-}air}$ values between 6 and 7 exhibited increased stomatal closure and Ψ_{branch} values lower than −2.4 MPa [62].

3.3 Near infrared spectroscopy (NIR) in detection of plant water status

Within the radiation spectrum, two regions can be used to detect plant water stress [122]: one from 700 to 1300 nm and another from 1300 to 3000 nm. Additionally, there are absorptions at 760 nm and between 950 and 970 nm [123]. Spectral reflectance of leaves at 400–1100 nm (near infrared) and 1100–2500 nm can reveal the water status of this organ [23]. Carter [124] reported that the highest sensitivity for estimating leaf water concentration is in the 1300 to 2500 nm range. Peñuelas et al. [122] and Peñuelas and Filella [123] reported a ratio between reflectance at 970 and 900 nm, known as the water index (WI) (R_{970}/R_{900} or R_{900}/R_{970}).

Because water molecules absorb strongly in the NIR region of the electromagnetic spectrum, plants with adequate water availability exhibit higher absorbance in 950 and 970 nm when compared to those under water stress. In assessing canopy water status, Rollin and Milton [125] reported that photon reflection at a wavelength of 1150 nm showed a strong positive correlation with relative water content. Gutiérrez et al. [126] used a non-invasive NIR device that allows spectrum scanning between 1600 and 2400 nm on the adaxial part of grapevine leaves. These authors demonstrated the potential of this technique to assess the water status of this species, using mathematical models. In the same species, Bei et al. [127] reported the use of NIR (970 and 1400–1450 nm) through two leaf spectrophotometers as a non-destructive analysis under field conditions for irrigation management. The authors showed that Ψ_{leaf} and Ψ_{branch} could be measured using NIR, considering reference values for Ψ_{branch} as non-stressed plants ($\Psi_{branch} > -1$ MPa), moderate stress between Ψ_{branch} (−1 and − 1.2 MPa) and severe stress between Ψ_{branch} (−1.2 and −1.5 MPa) [100, 128]. According to Fernández et al. [103], limitations in precision irrigation using NIR are related to equipment and data acquisition costs (requiring vehicles to cross plantations) and data processing time.

3.4 Xylem sap flow (XSF)

Transpiration can be estimated *via* techniques that quantify xylem sap flow, using heat as a marker of sap movement in the xylem [129]. The method most widely used involves inserting internal probes into the stem through drilled holes located in the active xylem region (**Figure 8**) [131]. The active xylem area can be estimated using dyes, where several stems of different diameters are cut and immersed in a solution containing a dye (Safranin, methylene blue). Next, the stems are removed, and active xylem area, proportional to the colored area of the vessels, is determined. In this experiment, a mathematical relationship between stem diameter and active xylem area can be indirectly obtained without destroying the plants [132].

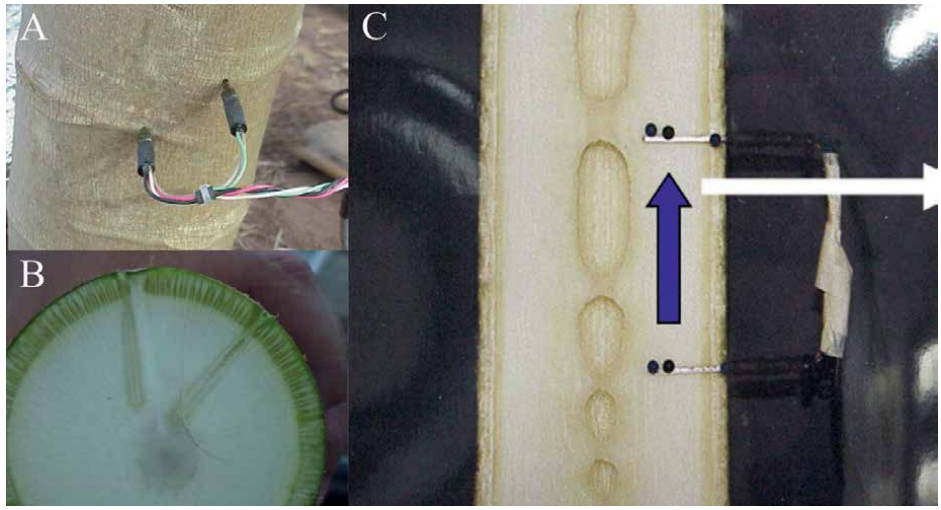

Figure 8.
Probes for measuring xylem sap flow in papaya. (A): the upper probe has a constant heat source, and the lower is not heated. (B): cross-section indicating the positioning of the probes in the papaya stem. (C): the arrow indicates the direction of upward movement of xylem sap, consisting of water, mineral nutrients, and phytohormones [84, 130].

Measuring XSF offers several advantages, such as direct measurement of xylem sap flow (water + mineral nutrients + phytohormones + amino acids) through the plant stem, high repeatability, continuous monitoring, and long-term non-destructive analysis of shoot or root transpiration. Three general types of probes are available: thermal dissipation, heat balance, and heat ratio [129, 133, 134]. The thermal dissipation method reported by Granier [134] determines xylem sap velocity through the temperature difference between the heated and unheated probe inserted radially into the active xylem region of the stem (**Figure 8**). With a rise in plant transpiration, heat dissipation from the upper probe increases due to the heat carried by xylem sap movement, resulting in a lower temperature difference between the two probes (heated and unheated). However, when transpiration decreases, the opposite occurs [135]. Calibration is subsequently needed between xylem sap flow and the temperature difference between probes. In papaya, this calibration was performed using balloons to quantify whole-plant transpiration [136].

A major advantage of XSF technique is the possibility of automating irrigation systems based on plant transpiration [137–139]. An adaptation of the method involves using heat pulse rather than constant heat [129]. However, Smith and Allen [129] report that heat pulse needs to be calibrated for each species.

Using calibrations with water injection under pressure in papaya, Reis et al. [132] adjusted an exponential model ($u = 0.5511 * K^{1.9104}$) for the relationship between K value (thermal coefficient obtained from temperature differences between probes) (**Figure 8**) and sap flow density (u) in L h^{-1} m^{-2} of the active xylem area. For the same K value, this mathematical model [132] differed from, and overestimated the model proposed by Granier [134]. Reis et al. [132] found that xylem sap flow in L h^{-1} m^{-2} expressed per leaf area showed a positive correlation with a net photosynthetic rate up to 0.28 L h^{-1} m^{-2}, demonstrating that management practices promoting increased water movement through the plant stem (optimizing leaf water status) can raise carbon gain in *Carica papaya* L. (papaya). For more accurate estimation of

transpiration in this species using the constant heat technique (**Figure 8**) (thermal dissipation probes), it was possible to establish a correlation between probe temperature difference (K value; $K = [\Delta T_{max}/\Delta T_{min})-1]$) and actual whole-plant transpiration, using transparent balloons ($K = 0.48 X + 0.16$) [136]. X = whole-canopy transpiration ($L\ m^{-2}\ h^{-1}$).

Schmid and Bettner [140] adapted the heat dissipation method proposed by Granier [134] to measure xylem sap flow in 10-year-old grapevines with leaf areas between 3.19 and 4.90 m^2. These measurements were compared with whole-plant gas exchange measurements taken on the same plants using an infrared gas analyzer. The authors obtained maximum xylem sap flow density of 0.09 L m^{-2} leaf area h^{-1} (0.06 L cm^{-2} active xylem h^{-1}) and xylem sap flow velocities between 8.4 and 12.4 m h^{-1}. They observed that high xylem sap flow rates were always correlated with high rates of net photosynthesis. In their study, water consumption in plants with leaf areas of 3.19 and 4.90 m^2 over a 94-day period was 169 and 287 L per plant, respectively.

Under field conditions over a five-day period, Dragoni et al. [141] worked with apple trees ('Empire'/M.9), demonstrating a combined technique for measuring transpiration rates. These authors related the transpiration rate determined by heat pulse method of XSF, with simultaneous measurements of whole-plant transpiration assessed using transparent balloons mounted on the plants. Maximum transpiration rates determined in whole plants varied between 3 and 4 L h^{-1} $plant^{-1}$, while those estimated by XSF ranged from 3 to 9 L h^{-1} $plant^{-1}$. Regression analysis revealed a high correlation ($R^2 = 0.94$) between the two techniques, justifying the use of xylem sap flow assessments *via* probes inserted into the trunk to estimate whole-plant transpiration rates, following calibration of probe temperatures with whole-plant gas exchange measurements.

To perform the accurate analyses, it is important to relate that larger trunk diameters require more thermal probes to assess xylem sap flow, because larger trunk diameters exhibit greater azimuthal variability in sap flow in different parts of the trunk.

4. Water limitation and fruit plant physiology

4.1 Impact of water limitation in leaf and plants

In general, plants develop various strategies to cope with water stress, as are the responses of evasion, escape, tolerance, and recovery, all of which play an important part in the plant's capacity to tolerate and adapt to water scarcity [142]. Those strategies are species-dependent, but mechanisms of responses are also related to intensity and duration of water stress [143]. In addition to these stress management strategies, plants may develop mechanisms that allow them to recognize a re-exposure to a similar stressor and respond more effectively, where plants may "remember" the stressor, and defend themselves more effectively relative to the first time/stressful event, a process known as stress memory. Understanding the mechanisms involved in responses to water stress and how they relate to different environments is vital in optimizing management strategies and guiding plant breeding aimed at developing genotypes with better drought resistance For the fruit plants, the resistance to water stress means not only the ability to cope with a stress factor but also the achievement of a stable yield and good fruit quality [144].

In general, one of the initial responses of fruit plants to water stress is stomatal closure to decrease water loss, which may result in reduced CO_2 availability at the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) carboxylation sites, thereby decreasing photosynthesis. As soil water availability decreases, leaf water potential becomes more negative, triggering changes in hydraulic and biochemical processes that reduce g_s – stomatal closure [145, 146]. However, stomatal sensitivity varies between species and cultivars of the same species. For example, under water stress conditions, at (pre-dawn) leaf water potentials to −1 MPa, the Grenache grape cultivar showed the fastest decline and highest g_s sensitivity when compared to Semillon and Syrah grapes, and below this value, these cultivars exhibited similar sensitivity [145].

Studies on fruit species have demonstrated that the decline in stomatal opening predominantly occurs *via* a so-called hydraulic mechanism, that is, a decrease in g_s associated with an increase in ABA concentration in the leaf [147]. ABA acts on the regulation of ion channels in the membrane, as well as changes in electrogenic pumps associated with the formation of proton gradients in the plasma membranes of guard cells. These actions promote changes in osmotic and water potential in the cytosol, which controls water outflow and causes stomatal closure [94]. Indeed, decreased soil water availability leads to higher ABA concentration in leaves, which has been reported in grapevines [146], citrus [43], mango [148], apple [149], and papaya [42], among others.

Less soil water availability increases ABA biosynthesis in the roots, which is transported to the leaves *via* transpirational flow, where it acts on stomatal closure [150]. Recent studies have demonstrated that ABA biosynthesis can also occur in the leaf, especially under more stressful leaf conditions [151] resulting in loss of turgor and a decline in mesophyll cell volume. Speirs et al. [152] found an increase in ABA concentration in the roots, xylem, and leaves, primarily during the later stages of water stress. However, in the Cabernet Sauvignon grape cultivar, gene expression involved in ABA biosynthesis (VviNCED1 and VviNCED2) remained stable in the leaves but increased in the roots, indicating that the root may be a primary site of ABA production.

Under conditions of water limitation, increased ABA production by the roots has been used as an irrigation strategy to optimize water use in fruit species. Partial root-zone drying (PRD) and deficit irrigation strategies have been used. Deficit irrigation involves reducing the amount of water applied at specific crop stages, whereas PRD also alters the irrigated side of the root system, that is, while one part of the root is irrigated, the other part is exposed to dry soil, inducing the root in the latter part to produce ABA [116, 153, 154]. ABA is transported to the leaves through transpirational flow, inducing partial stomatal closure without significantly affecting photosynthesis. This response effectively increases water use efficiency, primarily in fruit species that exhibit "luxury" stomatal behavior, such as papaya [77]. Indeed, this technique has significantly reduced water use (increased water productivity, reduced water footprint) in a number of fruit crops. For example, a 50% decrease in water applied after veraison in grapevines [154, 155] and 35% in Tainung 01 papaya of the Formosa group [9, 10] under field conditions did not affect fruit yield or quality. As such, this approach can be used in commercial cultivation as a strategy to conserve water and mitigate water stress.

Stomata play a key role in plant responses to water stress, as under increased $VPD_{leaf\text{-}air}$, reduced g_s minimizes water loss through transpiration. On contrast, rapid water loss through the leaf could lead to a significant drop in leaf water potential,

given that each species has an optimal range of Ψ_{leaf} for leaf metabolism. For instance, in irrigated grape orchards, a typical safety margin in stem water potential (Ψ_{stem}) is around −1.5 MPa, which may result in turgor loss or cavitation [156]. Changes in Ψ_{leaf} and/or hydraulic conductivity affect guard cell turgor and consequently g_s. Hydraulic conductivity changes primarily stem from aquaporins, integral proteins found in the plasma membrane or tonoplast that facilitate water and small molecule transport across membranes [157, 158]. These proteins encompass a large gene family whose regulation is complex, and many studies have demonstrated their involvement in stomatal regulation [159]. Several studies have shown that ABA acts in regulating their activity, as well as the transcription of various aquaporin isoforms in leaves, roots, and guard cells [160, 161]. Changes in Ψ_{leaf} are partly modulated by changes in leaf hydraulic conductivity, which, in turn, is modulated by ABA, creating an interdependency between these factors. However, studies on grapevines have shown that ABA increased only when g_s declined drastically ($\leq$50 mmol m^{-2} s^{-1}), suggesting that initial stomatal closure was driven by hydraulic signals rather than ABA [162–164]. Contrasting stomatal responses in two strawberry cultivars under osmotic stress may partly be attributed to jasmonic acid pathway, rather than to abscisic acid [165].

As water deficit increases, tension in the water column within xylem vessels also rises, potentially leading to embolism and cavitation. These processes decrease xylem vessel hydraulic conductivity and can ultimately result in hydraulic failure (interruption of transpirational flow). Vulnerability to embolism depends on a number of anatomical and physiological factors that vary between species and varieties in the same species. For example, grapevines with larger diameter xylem vessels are generally more susceptible to embolism than their smaller diameter counterparts, due to the larger surface area of pit membranes and a greater number of pits in vessel cells [166].

Soil water scarcity or high VPD_{air} can cause rapid leaf dehydration, reducing cell turgor loss. On contrast, solute concentration in cell cytoplasm enables leaves to maintain turgor pressure even under negative Ψ_{leaf} [167]. Thus, Ψ_{leaf} at which cells lose turgor (turgor loss point) has been used as a drought tolerance characteristic in several species, including fruit-bearing varieties [164]. The turgor loss point is correlated with stomatal closure [80] and declining leaf hydraulic conductivity [168, 169]. Thus, genotypes with leaves losing turgor at more negative leaf water potentials may sustain hydraulic and stomatal conductance, as well as growth and yield under drier conditions [170]. To reduce the water potential at which turgor loss occurs, genotypes undergo osmotic adjustment by increasing ion transport and synthesizing osmotically active compounds in cells, such as sucrose, sugar alcohols (for example, raffinose and trehalose), proline, glycine-betaine, etc. [171]. In addition to these functions, under water stress, some compounds such as carbohydrates can act as osmoprotectants by stabilizing membranes, while osmoprotectants such as the amino acid proline can act as antioxidants in the cell [172, 173].

Osmotic adjustment in the root system is a trait that can help genotypes successfully overcome dry periods and can therefore be used as a strategy in rootstock selection [174, 175]. For example, in a study with different rootstocks, where the Cleopatra mandarin was compared to the Rangpur lime under drought conditions, the former exhibited higher ABA concentrations and high levels of sugars such as sucrose, raffinose and trehalose, which helped in cell protection, especially in maintaining turgor [173]. Similarly, under water stress conditions compared to controls, Swingle citrumelo rootstocks, modified to increase proline synthesis, showed higher photosynthetic rates and transpiration, which were associated with higher leaf RWC

in these plants [176]. However, an increase in constitutive proline synthesis may alter metabolic pathways and metabolic profiles, and reduce sucrose availability [177], potentially increasing competition for carbon skeletons with primary N metabolism [178, 179]. Thus, these strategies seem to be more appropriate for prolonged drought conditions, where accumulation of osmotically active compounds such as proline, through induced mechanisms (see example in Farinacio et al. [180]), could result in better agronomic performance under no or moderate water stress. An increase in osmotically active compounds such as dehydrins, glycine betaine and osmotin also played an important role in drought tolerance in 110R grape rootstock [181].

In addition to osmotic adjustment, other traits relevant to rootstock performance under water-limiting conditions are associated with root system architecture and hydraulics. Several studies have shown that apple rootstocks that induce greater assimilate partitioning for root growth and explore deeper and larger soil volumes demonstrated greater drought tolerance [149, 182]. However, a deep root system does not always guarantee efficient water uptake. Other characteristics such as the number of fine roots and hydraulic properties are of utmost importance [183]. Yıldırım et al. [181] reported that drought-resistant 110R grape rootstocks had less impact on root architecture characteristics, such as root area and length, and number of root emissions and ramifications. Interestingly, these responses are associated with increased carbohydrate and nitrogen translocation, as well as greater expression of membrane transporters for sugars and proteins such as Sweet and NRT1/PTR. In the 110R grape genotype, under severe water demand, another reported mechanism suggests a relationship between suberization and root hydraulic conductivity, whereby suberization creates a barrier to apoplastic water movement from roots and predicts the flow of this molecule from the roots to the soil [181, 184].

Root hydraulic conductivity has been the subject of many studies aimed at understanding rootstock function in drought tolerance. In apple rootstocks, drought resistance has been attributed to increased root hydraulic conductivity, as well as greater xylem embolism recovery and changes in assimilate partitioning for root growth [182, 185, 186]. However, under prolonged stress conditions, increased root and leaf hydraulic conductivity maximizes stomatal opening and may result in faster soil water depletion. In this case, close coordination between root and leaf hydraulic conductivity, associated with higher stomatal sensitivity, may reduce plant transpiration (greater water use efficiency) and conserve water for longer in the soil. In this respect, it is important to underscore that individual responses observed in rootstocks cannot be used as a rule, since the interaction between graft and rootstock is highly variable, depending on the genotypes used [149, 174, 187]. Another important point is the graft-to-rootstock connections, which may become areas of greater resistance to water transport through healing processes and may reduce plant hydraulic conductivity. For example, the Flying Dragon rootstock (trifoliate orange), which has fewer vessels per xylem cross-section, increasing resistance to water and nutrient flow, may result in lower leaf water potential under high or even moderate atmospheric demand [188, 189]. This situation may be exacerbated when using the interstock technique to solve incompatibility problems, since it raises the number of connections and consequently hydraulic resistance [189].

The most recent studies with various fruit species grown under conditions of water limitation have demonstrated that plants respond differently to the occurrence of single or recurrent water deficit events [190]. In plants subjected to a single water stress event, acute responses are observed, such as a reduction in photosynthetic rate, reduction in leaf water potential, wilting and leaf senescence, in addition to high

oxidative damage [191]. In *Citrus* spp. seedlings, the exposure to successive events of dehydration/rehydration makes the citrus seedlings more resistant to future exposures to water stress [192]. Plants subjected to recurrent water stress events appear to acclimatize to the preceding stress conditions, through "stress memory" mechanisms, causing these plants to more quickly activate strategies to mitigate damage resulting from stress, from adjustments physiological and biochemical adjustments to molecular adjustments. Stress memory depends on the intensity of the priming event, as well as on genetic characteristics that influence stress tolerance [193]. In fruit species, 'memory' responses are observed, among other species, in field grown *Coffea arabica* [194, 195]; in four-year-old Sangiovese and Montepulciano vines (*Vitis vinifera*) where previous water stress would led to less conservative plant strategy toward water loss and decreased water use efficiency [196]; in adult olives that had gone through various drought exposures, had improved stress tolerance mechanisms and overall improved growth and survival rates [197]. After the passage of recurrent water stresses, the fruit plant responses seem to be coordinated by the stomatal response to decreasing water potential, causing a reduction of xylem hydraulic safety margin.

4.2 Impact of water limitation on fruit production and quality

Under conditions of water limitation, reduced photosynthesis due to stomatal closure causes a decrease in the primary metabolite supply to fruits [198]. However, an accumulation of sugars is commonly found in several fruit species under water stress, usually due to lower fruit weight, which increases soluble solid concentration and sugar remobilization from starch breakdown [12]. In grapevines, improved fruit quality is attributed to an increase in the berry skin-to-pulp ratio, given that many quality-related metabolites (phenolic compounds) are synthesized in the skin. However, under prolonged water stress, a decline in leaf sugar content may occur due to decreased photosynthetic rates and increased sugar remobilization to other plant organs aimed at survival [12]. Decreased fruit weight results in lower yield per area, which may show a significant correlation with water potential. In grapevines, a 0.2 MPa decrease in water potential resulted in yield losses of about 10% [199], although these changes depend on the phenological stage at which stress occurs and its duration, with pre-veraison stages the most sensitive.

In general, water stress increases the concentration of osmotically active compounds in fruits, such as amino acids (proline, leucine, isoleucine, and valine), organic acids, sugars, and potassium, as a means of promoting osmotic adjustment [12, 200, 201]. The accumulation of these compounds depends on the stage at which the stress occurs. For example, under water stress conditions, organic acid content declined in the later stages of grape development, which may be associated with less availability of citric acid cycle intermediates, while amino acid content rose [202–205].

In fruits, secondary metabolite-derived compounds are responsible for color, aroma, and nutritional characteristics [204]. Under water stress, an increase in the concentration of these compounds is expected, which may result in better fruit quality [206]. Grapevine cultivars show a significant increase in anthocyanin content in fruits, thereby raising berry color intensity [199, 207], which is generally associated with increased sugar concentration. Sugar concentration promotes the expression of enzymes involved in anthocyanin synthesis [205]. Water stress increases ABA synthesis, which participates in fruit ripening. Thus, ABA changes the metabolic

composition of the fruit, particularly sugar and anthocyanin content [208]. However, increased anthocyanin concentration is not a universal response, since some studies have shown no change or even a reduction in anthocyanin concentration due to a decrease in phenylalanine ammonia lyase activity [204]. Variations in anthocyanin content depend on climatic conditions, variety, and the magnitude and duration of stress [199]. Under water stress conditions, ascorbic acid (or Vitamin C) is another antioxidant compound that has increased fruit concentration, which can promote drought tolerance [209].

In fruits, water stress increases the concentration of flavonols, another important class of flavonoids [12], especially during their ripening phase [210]. However, Savoi et al. [200] showed no water stress effect on these compounds. These contradictory responses may be associated with the effect of water stress on canopy growth/development, that is, flavonol biosynthesis is stimulated under light and ultraviolet radiation conditions. Thus, different water stress conditions can have a different impact on canopy growth/development [199]. In terms of fruit quality, carotenoids are other important compounds derived from secondary metabolism. Under water stress conditions, some studies have shown an increase in the concentration of certain carotenoids (violaxanthin and beta-carotene), for example, in grapevines and mangoes [211, 212].

Volatile compounds are key to fruit aroma, with terpenoids (monoterpenes, sesquiterpenes, and C13-norisoprenoids) representing the largest classes. Under water stress conditions, several studies found an increase in the concentration of these compounds due to higher gene expression in the methylerythritol 4-phosphate pathway [210], while others have also reported decreases in terpene concentration under these same conditions [213].

In general, water stress can enhance fruit quality at the expense of quantity (yield) due to the accumulation of primary and secondary metabolites. Thus, using partial root-zone irrigation may save water (greater agronomic water use efficiency) and improve fruit quality. However, the actual impact will depend on the duration and intensity of the stress, as well as the specific species and cultivars in question [12]. As such, studies should aim to determine when (phenological stage) to apply water deficit, its duration, and the amount of water reduction needed to enhance fruit quality.

5. Conclusions

Soil and air water limitations significantly compromise the metabolism of fruit-bearing plants. The effects of this abiotic factor will be exacerbated by climate change, which has already begun and is expected to worsen in the near future. As such, understanding how to quantify water and the duration of its application in the soil–plant-atmosphere system, as well as advanced knowledge of water status in plant tissues, is essential for correct irrigation management of these plant classes. Applying water in appropriate level will increase photosynthetic rates, growth, yield and fruit quality, in addition to improving water use efficiency, reducing costs and promoting environmental sustainability.

Author details

Guilherme Augusto Rodrigues de Souza[1], Ellen de Moura Vale[1], Wallace de Paula Bernado[1], Danilo Força Baroni[1], Elias Fernandes de Sousa[2], Miroslava Rakočević[1], Weverton Pereira Rodrigues[3] and Eliemar Campostrini[1]*

1 Plant Physiology Department, LMGV, Agricultural Science and Technology Center, State University of North Fluminense, Campos dos Goytacazes, RJ, Brazil

2 Irrigation Department, LEAG, Agricultural Science and Technology Center, State University of North Fluminense, Campos dos Goytacazes, RJ, Brazil

3 Center of Agricultural, Natural and Literary Sciences, State University of the Tocantina Region of Maranhão (UEMASUL), Estreito, Maranhão, Brazil

*Address all correspondence to: campostrini@uenf.br

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

[1] IPCC. IPCC Climate Change (2021) - The Physical Science Basis, Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. In: Masson-Delmotte V, Zhai P, Pirani A, Connors SL, Péan C, Berger S, et al., editors. Cambridge University Press; 2021

[2] Ortiz-Bobea A, Ault TR, Carrillo CM, Chambers RG, Lobell DB. Anthropogenic climate change has slowed global agricultural productivity growth. Nature Climate Change. 2021;**11**(4):306-312

[3] Edenhofer O, Pichs-Madruga R, Sokona Y, Farahani E, Kadner S, Seyboth K, et al., editors. Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom, and New York, NY, USA: Cambridge University Press; 2014

[4] Sheldon KS. Climate change in the tropics: Ecological and evolutionary responses at low latitudes. Annual Review of Ecology, Evolution, Systematics. 2019;**50**(1):303-333

[5] Bornal DR, Silvestrini MM, Pio LAS, Costa AC, Peche PM, Ramos MCP. Brazilian position in the international fresh fruit trade network. Revista Brasileira de Fruticultura. 2021;**43**:e-021

[6] Shao H-B, Chu L-Y, Jaleel CA, Zhao C-X. Water-deficit stress-induced anatomical changes in higher plants. Comptes Rendus Biologies. 2008;**331**(3):215-225

[7] Evans JR, Lawson T. From green to gold: Agricultural revolution for food security. Journal of Experimental Botany. 2020;**71**(7):2211-2215

[8] Gupta A, Rico-Medina A, Caño-Delgado AI. The Physiology of Plant Responses to Drought. 2020;**368**(6488):266-269

[9] Santos DL, Coelho EF, da Cunha FF, Donato SLR, de Paula BW, Rodrigues WP, et al. Partial root-zone drying in field-grown papaya: Gas exchange, yield, and water use efficiency. Agricultural Water Management. 2021;**243**:106421

[10] Santos DL, Coelho EF, de Oliveira RA, Filgueiras R, da Silva AM, Rodrigues WP, et al. Impact of soil water regimes and partial root-zone drying in field-grown papaya in semi-arid conditions. Scientific Reports. 2021;**11**(1):10638

[11] Ripoll J, Urban L, Staudt M, Lopez-Lauri F, Bidel LPR, Bertin N. Water shortage and quality of fleshy fruits—Making the most of the unavoidable. Journal of Experimental Botany. 2014;**65**(15):4097-4117

[12] Romero H, Pott DM, Vallarino JG, Osorio S. Metabolomics-based evaluation of crop quality changes as a consequence of climate change. Metabolites. 2021;**11**(7):461

[13] Dong J, Gruda N, Lam SK, Li X, Duan Z. Effects of elevated CO2 on nutritional quality of vegetables: A review. Front. Plant Science. 2018;**9**(924):1-11

[14] Schaffer B, Andersen PC, Ploetz R. Responses of fruit crops to flooding. Horticultural Reviews. 1992;**13**:257-313

[15] Slatyer RO. Plant Water Relationships. New York: Academic Press; 1967. p. 366

[16] Fitter A. Environmental Physiology of Plants. Environmental Stress. New York: Academic Press; 1981. pp. 73-119

[17] Gregory RG. Changing directions: The atmosphere–plant–soil continuum. The New Phytologist. 2013;**199**(1):4-6

[18] Boanares D, Oliveira RS, Isaias RMS, França MGC, Peñuelas J. The neglected reverse water pathway: Atmosphere–plant–soil continuum. Trends in Plant Science. 2020;**25**(11):1073-1075

[19] Eller CB, Lima AL, Oliveira RS. Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (W interaceae). New Phytologist. 2013;**199**(1):151-162

[20] Passioura JB. The meaning of matric potential. Journal of Experimental Botany. 1980;**31**(4):1161-1169

[21] Nobel PS. Physicochemical and Environmental Plant Physiology. Amsterdam: Academic Press; 2009

[22] Girma FS, Krieg DR. Osmotic adjustment in sorghum: I. Mechanisms of diurnal osmotic potential changes. Plant Physiology. 1992;**99**(2):577-582

[23] Fernández JE. Plant-based methods for irrigation scheduling of woody crops. Horticulturae. 2017;**3**(2):35

[24] Shahid SA, Zaman M, Heng L. Introduction to soil salinity, sodicity and diagnostics techniques. In: Zaman M, Shahid SA, Heng L, editors. Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques. Cham: Springer International Publishing; 2018. pp. 1-42

[25] Lima RSND. Partial Rootzone Drying (PRD) and Regulated Deficit Irrigation (RDI) in *Carica papaya* L.: Photosynthetic Capacity, Growth and Water Use Efficiency. Campos dos Goytacazes, RJ: UENF; 2014

[26] Lima RSNd, de Assis FAMM, Martins AO, de Deus BCdS, Ferraz TM, de Assis Gomes MdM, et al. Partial rootzone drying (PRD) and regulated deficit irrigation (RDI) effects on stomatal conductance, growth, photosynthetic capacity, and water-use efficiency of papaya. Scientia Horticulturae 2015;183:13-22

[27] Bittelli M. Measuring soil water content: A review. HortTechnology hortte. 2011;**21**(3):293-300

[28] Ward AD, Trimble SW, Burckhard SR, Lyon JG. Environmental Hydrology. 3rd ed. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2016. 652 p

[29] Al Jabri SA, Al Yahyai RA. Plant-soil approach for irrigation scheduling of mango trees in the Sultanate of Oman. Acta Horticulturae. 2017:153-158

[30] Barrs H, Weatherley P. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences. 1962;**15**(3):413-428

[31] Ihuoma SO, Madramootoo CA. Recent advances in crop water stress detection. Computers and Electronics in Agriculture. 2017;**141**:267-275

[32] Arndt SK, Irawan A, Sanders GJ. Apoplastic water fraction and rehydration techniques introduce significant errors in measurements of relative water content and osmotic potential in plant leaves. Physiol Plant. 2015;**155**(4):355-368

[33] Blum A. Stress, strain, signaling, and adaptation –not just a matter of

definition. Journal of Experimental Botany. 2016;**67**(3):562-565

[34] Boyer JS, James RA, Munns R, Condon T, Passioura JB. Osmotic adjustment leads to anomalously low estimates of relative water content in wheat and barley. Functional Plant Biology. 2008;**35**(11):1172-1182

[35] Turner NC. Techniques and experimental approaches for the measurement of plant water status. Plant and Soil. 1981;**58**(1):339-366

[36] Parkash V, Singh S. A review on potential plant-based water stress indicators for vegetable crops. Sustainability. 2020;**12**(10):3945

[37] Marler TE, George AP, Nissen RJ, Andersen PC. Miscellaneous tropical fruits. In: Schaffer B, Andersen PC, editors. Handbook of Environmental Physiology of Fruits Crops VII, Subtropical and Tropical Crops. Florida: CRC Press; 1994. p. 199e224

[38] Torres NA. Atributos fisiológicos e relações hídricas em genótipos de mamoeiro (*Carica papaya* L.) na fase juvenil. Campos dos Goytacazes, RJ: UENF; 2005

[39] Zhang J, Davies WJ. Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. Plant, Cell & Environment. 1990;**13**(3):277-285

[40] Davies WJ, Zhang J. Root signals and the regulation of growth and development of plants in drying soil. Annual Review of Plant Biology. 1991;**42**(1):55-76

[41] Gomes MA, Ferraz TM, Siqueira LN, Campostrini E, Chiquieri TB, Bortot PA, et al. Trocas gasosas e conteúdo de ácido abscísico em mamoeiro submetido à secagem parcial do sistema radicular (split-root). In: X Congresso Brasileiro de Fisiologia Vegetal/XII Congresso Latino Americano de Fisiologia Vegetal. Recife-Pernambuco: Brazilian Society of Plant Physiology; 2005

[42] Mahouachi J, Arbona V, Gómez-Cadenas A. Hormonal changes in papaya seedlings subjected to progressive water stress and re-watering. Plant Growth Regulation. 2007;**53**(1):43-51

[43] Gomez-Cadenas A, Tadeo FR, Talon M, Primo-Millo E. Leaf abscission induced by ethylene in water-stressed intact seedlings of Cleopatra mandarin requires previous abscisic acid accumulation in roots. Plant Physiology. 1996;**112**(1):401-408

[44] Bray EA. Abscisic acid regulation of gene expression during water-deficit stress in the era of the Arabidopsis genome. Plant, Cell & Environment. 2002;**25**(2):153-161

[45] Slavík B, Jarvis MS. Methods of Studying Plant Water Relations. Heidelberg, Berlin: Academia Publishing House of the Czechoslovak Academy of Sciences: Springer; 1974. 472 p

[46] Boyer JS, Knipling EB. Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proceedings of the National Academy of Sciences of the United States of America. 1965;**54**(4):1044

[47] Scholander PF, Bradstreet ED, Hemmingsen E, Hammel H. Sap pressure in vascular plants: Negative hydrostatic pressure can be measured in plants. Science. 1965;**148**(3668):339-346

[48] McCutchan H, Shackel K. Stem-water potential as a sensitive indicator of water stress in prune trees (*Prunus*

domestica L. cv. French). Journal of the American Society for Horticultural Science. 1992;**117**(4):607-611

[49] Shackel KA, Ahmadi H, Biasi W, Buchner R, Goldhamer D, Gurusinghe S, et al. Plant water status as an index of irrigation need in deciduous fruit trees. HortTechnology hortte. 1997;**7**:23-29

[50] Garnier E, Berger A. Testing water potential in peach trees as an indicator of water stress. Journal of Horticultural Science. 1985;**60**(1):47-56

[51] Barry GH, Castle WS, Davies FS. Rootstocks and plant water relations affect sugar accumulation of citrus fruit via osmotic adjustment. Journal of the American Society for Horticultural Science. 2004;**129**(6):881-889

[52] Naor A. Irrigation scheduling and evaluation of tree water status in deciduous orchards. Horticultural reviews. 2006;**32**:111-165

[53] Naor A. Midday stem water potential as a plant water stress indicator for irrigation scheduling in fruit trees. III International Symposium on Irrigation of Horticultural Crops. 1999:447-454

[54] Naor A, Klein I, Doron I. Stem water potential and apple size. Journal of the American Society for Horticultural Science. 1995;**120**(4):577-582

[55] Naor A, Hupert H, Greenblat Y, Peres M, Kaufman A, Klein I. The response of nectarine fruit size and midday stem water potential to irrigation level in stage III and crop load. Journal of the American Society for Horticultural Science. 2001;**126**(1):140-143

[56] Stern R, Meron M, Naor A, Wallach R, Bravdo B, Gazit S. Effect of fall irrigation level in'Mauritius' and'Floridian'lychee on soil and plant water status, flowering intensity, and yield. Journal of the American Society for Horticultural Science. 1998;**123**(1):150-155

[57] Nordey T, Léchaudel M, Génard M. The decline in xylem flow to mango fruit at the end of its development is related to the appearance of embolism in the fruit pedicel. Functional Plant Biology. 2015;**42**(7):668-675

[58] Sdoodee S, Somjun J, Technology. Measurement of stem water potential as a sensitive indicator of water stress in neck orange (*Citrus reticulata* Blanco). Science. 2008;**30**(5):561-564

[59] Gálvez R, Callejas R, Reginato G, Peppi M. Irrigation schedule on table grapes by stem water potential and vapor pressure deficit allows to optimize water use. Ciência e Técnica Vitivinícola. 2014;**29**(2):60-70

[60] Rienth M, Scholasch T. State-of-the-art of tools and methods to assess vine water status. OENO one. 2019;**4**:619-637

[61] Mabrouk H. The use of water potentials in irrigation management of table grape grown under semiarid climate in Tunisia. OENO one. 2014;**48**(3):123-133

[62] Syvertsen JP. Minimum leaf water potential and stomatal closure in citrus leaves of different ages*. Annals of Botany. 1982;**49**(6):827-834

[63] Grossiord C, Buckley TN, Cernusak LA, Novick KA, Poulter B, Siegwolf RTW, et al. Plant responses to rising vapor pressure deficit. New phytologist. 2020;**226**(6):1550-1566

[64] Asseng S, Ewert F, Martre P, Rötter RP, Lobell DB, Cammarano D, et al. Rising temperatures reduce global

wheat production. Nature Climate Change. 2015;**5**(2):143-147

[65] Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, et al. Temperature increase reduces global yields of major crops in four independent estimates. Proceedings of the National Academy of Sciences. 2017;**114**(35):9326-9331

[66] Byrne MP, O'Gorman PA. Land–Ocean warming contrast over a wide range of climates. Convective Quasi-Equilibrium Theory and Idealized Simulations Journal of Climate. 2013;**26**(12):4000-4016

[67] Binks O, Coughlin I, Mencuccini M, Meir P. Equivalence of foliar water uptake and stomatal conductance? Plant, Cell Environmental and Experimental Botany. 2020;**43**(2):524-528

[68] Grange RI, Hand DW. A review of the effects of atmospheric humidity on the growth of horticultural crops. Journal of Horticultural Science. 1987;**62**(2):125-134

[69] Bakker JC. Leaf conductance of four glasshouse vegetable crops as affected by air humidity. Agricultural and Forest Meteorology. 1991;**55**(1):23-36

[70] Mott KA. Leaf hydraulic conductivity and stomatal responses to humidity in amphistomatous leaves. Plant, Cell and Environment. 2007;**30**(11):1444-1449

[71] Damour G, Simonneau T, Cochard H, Urban L. An overview of models of stomatal conductance at the leaf level. Plant, Cell and Environment. 2010;**33**(9):1419-1438

[72] Marchin RM, Broadhead AA, Bostic LE, Dunn RR, Hoffmann WA. Stomatal acclimation to vapour pressure deficit doubles transpiration of small tree seedlings with warming. Plant, Cell & Environment. 2016;**39**(10):2221-2234

[73] Buckley TN, Sack L, Gilbert ME. The role of bundle sheath extensions and life form in stomatal responses to leaf water status. Plant Physiology. 2011;**156**(2):962-973

[74] Franks P, Farquhar G. A relationship between humidity response, growth form and photosynthetic operating point in C3 plants. Plant, Cell & Environment. 1999;**22**(11):1337-1349

[75] Gao J, Zhao P, Shen W, Niu J, Zhu L, Ni G. Biophysical limits to responses of water flux to vapor pressure deficit in seven tree species with contrasting land use regimes. Agricultural and Forest Meteorology. 2015;**200**:258-269

[76] Campostrini E, Glenn D, Yamanishi OJ. Papaya: Ecophysiology of Growth and Production. NY: Eottc NSP; 2010. pp. 287-307

[77] Campostrini E, Schaffer B, Ramalho JDC, González JC, Rodrigues WP, da Silva JR, et al. Chapter 19 - Environmental Factors Controlling Carbon Assimilation, Growth, and Yield of Papaya (*Carica papaya* L.) Under Water-Scarcity Scenarios. In: García Tejero IF, Durán Zuazo VH, editors. Water Scarcity and Sustainable Agriculture in Semiarid Environment. Cambridge, Massachusetts: Academic Press; 2018. pp. 481-505

[78] Ruas KF, Baroni DF, de Souza GAR, Bernado WP, Paixão JS, dos Santos GM, et al. A Carica papaya L. genotype with low leaf chlorophyll concentration copes successfully with soil water stress in the field. Scientia Horticulturae. 2022;**293**:110722

[79] Flore J, Lakso ANJH. Environmental and Physiological Regulation of

Photosynthesis in Fruit Crops. 1989;**11**:111-157

[80] Brodribb TJ, Jordan GJ. Internal coordination between hydraulics and stomatal control in leaves. Plant, Cell & Environment. 2008;**31**(11):1557-1564

[81] Grassi G, Ripullone F, Borghetti M, Raddi S, Magnani F. Contribution of diffusional and non-diffusional limitations to midday depression of photosynthesis in *Arbutus unedo* L. Trees. 2009;**23**(6):1149-1161

[82] Salinas I, Hueso JJ, Cuevas J. Active control of greenhouse climate enhances papaya growth and yield at an affordable cost. Agronomy. 2021;**11**(2):378

[83] Reis FO, Campostrini E. Microaspersão de água sobre a copa: um estudo relacionado às trocas gasosas e à eficiência fotoquímica em plantas de mamoeiro. Revista Brasileira de Agrociencia. 2011;**17**(3):284-295

[84] Reis F. Microaspersão sobrecopa em mamoeiro 'Golden': um estudo relacionado às trocas gasosas, à eficiência fotoquímica e ao fluxo de seiva xilemática. Campos do Goytacazes: UENF; 2007

[85] Glenn DM, Puterka GJ. Particle films: A new technology for agriculture. In: Janick J, editor. Horticultural Reviews. Vol. 31. Hoboken, New Jersey; 2004. pp. 1-44

[86] Sharma RR, Vijay Rakesh Reddy S, Datta SC. Particle films and their applications in horticultural crops. Applied Clay Science. 2015;**116**:54-68

[87] Abreu DP, Roda NM, Krohling CA, Campostrini E, Rakocevic M. Kaolin particle film mitigates supra-optimal temperature stress effects at leaf scale and increases bean size and productivity of *Coffea canephora*. Experimental Agriculture. 2023;**59**:e13

[88] Wand SJE, Theron KI, Ackerman J, Marais SJS. Harvest and post-harvest apple fruit quality following applications of kaolin particle film in south African orchards. Scientia Horticulturae. 2006;**107**(3):271-276

[89] Jifon JL, Syvertsen JP. Kaolin particle film applications can increase photosynthesis and water use efficiency of ruby Red'Grapefruit leaves. Journal-American Society for Horticultural Science. 2003;**128**(1):107-112

[90] Glenn DM, Puterka G, Vanderzwet T, Byers R, Feldhake C. Hydrophobic particle films: A new paradigm for suppression of arthropod pests and plant diseases. Journal of Economic Entomology. 1999;**92**(4):759-771

[91] Wang Y, Xue T, Han X, Guan L, Zhang L, Wang H, et al. Kaolin particle film affects grapevine berry quality in cv. Meili in humid climate conditions. HortScience. 2020;**55**(12):1987-2000

[92] Chamchaiyaporn T, Jutamanee K, Kasemsap P, Vaithanomsat P. Selection of the most appropriate coating particle film for improving photosynthesis in mango. Agriculture Natural Resources. 2013;**47**(3):323-332

[93] Adeyemi O, Grove I, Peets S, Norton T. Advanced monitoring and Management Systems for Improving Sustainability in precision irrigation. Sustainability. 2017;**9**(3):353

[94] Lawson T, Matthews J. Guard cell metabolism and stomatal function. Annual Review of Plant Biology. 2020;**71**:273-302

[95] Ganogpichayagrai A, Rungsihirunrat K, Palanuvej C,

Ruangrungsi N. Characterization of Mangifera indica cultivars in Thailand based on macroscopic, microscopic, and genetic characters. Journal of Advanced Pharmaceutical Technology Research. 2016;**7**(4):127-133

[96] Ogundare C, Saheed S. Foliar epidermal characters and petiole anatomy of four species of *citrus* L.(Rutaceae) in South-Western Nigeria. Bangladesh Journal of Plant Taxonomy. 2012;**19**(1):25

[97] Rogiers SY, Hardie WJ, Smith JP. Stomatal density of grapevine leaves (*Vitis vinifera* L.) responds to soil temperature and atmospheric carbon dioxide. Australian Journal of Grape and Wine Research. 2011;**17**(2):147-152

[98] Bernado WP, Rakocevic M, Santos AR, Ruas KF, Baroni DF, Abraham AC, et al. Biomass and leaf acclimations to ultraviolet solar radiation in juvenile plants of *Coffea arabica* and *C. Canephora*. Plants. 2021;**10**(4):640

[99] Auliya I, Hapsari L, Azrianingsih R. Comparative study of leaf stomata profiles among different ploidy levels and genomic groups of bananas (*Musa* L.). In: IOP Conference Series: Earth and Environmental Science. Purwokerto, Indonesia: IOP Publishing; 2019. p. 012037

[100] Cifre J, Bota J, Escalona JM, Medrano H, Flexas J. Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera* L.): An open gate to improve water-use efficiency? Agriculture, Ecosystems and Environment. 2005;**106**(2):159-170

[101] Long SP, Hallgren JE. CHAPTER 6 - Measurement of CO_2 assimilation by plants in the field and the laboratory. In: Coombs J, Hall DO, Long SP, Scurlock JMO, editors. Techniques in Bioproductivity and Photosynthesis 2nd ed. Kidlington, Oxford: Pergamon; 1985. p. 62-94

[102] Linhart L, Moretti B, Herrera JC, Forneck A. Maximum stomatal conductance rather than stomatal sensitivity to drought differentiates the PIWI grapevine cultivar Souvignier gris from Muscaris and Donauriesling. OENO one. 2023;**57**(2):129-138

[103] Fernández JE, Moreno F, Girón IF, Blázquez OM. Stomatal control of water use in olive tree leaves. Plant and Soil. 1997;**190**(2):179-192

[104] Hernandez-Santana V, Fernández JE, Rodriguez-Dominguez CM, Romero R, Diaz-Espejo A. The dynamics of radial sap flux density reflects changes in stomatal conductance in response to soil and air water deficit. Agricultural and Forest Meteorology. 2016;**218-219**:92-101

[105] Fereres E, Goldhamer DA, Parsons LR. Irrigation water management of horticultural crops. HortScience. 2003;**38**(5):1036-1042

[106] Sinclair TR, Tanner C, Bennett J. Water-use efficiency in crop production. Bioscience. 1984;**34**(1):36-40

[107] Jones HG. Thermal imaging and infrared sensing in plant ecophysiology. In: Sánchez-Moreiras AM, Reigosa MJ, editors. Advances in Plant Ecophysiology Techniques. Cham: Springer International Publishing; 2018. pp. 135-151

[108] Jones HG, Stoll M, Santos T, Sousa C, Chaves MM, Grant OM. Use of infrared thermography for monitoring stomatal closure in the field: Application to grapevine. Journal of Experimental Botany. 2002;**53**(378):2249-2260

[109] Jackson RD, Idso SB, Reginato RJ, Pinter PJ Jr. Canopy temperature as

a crop water stress indicator. Water Resources Research. 1981;**17**(4):1133-1138

[110] Idso SB, Jackson RD, Pinter PJ, Reginato RJ, Hatfield JL. Normalizing the stress-degree-day parameter for environmental variability. Agricultural Meteorology. 1981;**24**:45-55

[111] Abreu DP. Aplicação de filme de partículas de caulinita processada (FFCP) sobre o dosse de *Coffea sp*.: efeitos no metabolismo fotoquímico, produtividade e qualidade do fruto. Campos dos Goytacazes, RJ: UENF; 2018

[112] Bellvert J, Marsal J, Girona J, Gonzalez-Dugo V, Fereres E, Ustin SL, et al. Airborne thermal imagery to detect the seasonal evolution of crop water status in peach, nectarine and Saturn peach orchards. Remote Sensing. 2016;**8**(1):39

[113] Gago J, Douthe C, Coopman RE, Gallego PP, Ribas-Carbo M, Flexas J, et al. UAVs challenge to assess water stress for sustainable agriculture. Agricultural Water Management. 2015;**153**:9-19

[114] Ha W, Gowda PH, Howell TA. A review of potential image fusion methods for remote sensing-based irrigation management: Part II. Irrigation Science. 2013;**31**:851-869

[115] Ben-Gal A, Agam N, Alchanatis V, Cohen Y, Yermiyahu U, Zipori I, et al. Evaluating water stress in irrigated olives: Correlation of soil water status, tree water status, and thermal imagery. Irrigation Science. 2009;**27**(5):367-376

[116] Lima RSN, García-Tejero I, Lopes TS, Costa JM, Vaz M, Durán-Zuazo VH, et al. Linking thermal imaging to physiological indicators in Carica papaya L. under different watering regimes. Agricultural Water Management. 2016;**164**:148-157

[117] Möller M, Alchanatis V, Cohen Y, Meron M, Tsipris J, Naor A, et al. Use of thermal and visible imagery for estimating crop water status of irrigated grapevine*. Journal of Experimental Botany. 2006;**58**(4):827-838

[118] Grant OM, Tronina Ł, Jones HG, Chaves MM. Exploring thermal imaging variables for the detection of stress responses in grapevine under different irrigation regimes. Journal of Experimental Botany. 2006;**58**(4):815-825

[119] García-Tejero IF, Durán-Zuazo VH, Muriel-Fernández JL, Jiménez-Bocanegra JA. Linking canopy temperature and trunk diameter fluctuations with other physiological water status tools for water stress management in citrus orchards. Functional Plant Biology. 2011;**38**(2):106-117

[120] Bellvert J, Marsal J, Girona J, Zarco-Tejada PJ. Seasonal evolution of crop water stress index in grapevine varieties determined with high-resolution remote sensing thermal imagery. Irrigation Science. 2015;**33**(2):81-93

[121] Matese A, Baraldi R, Berton A, Cesaraccio C, Di Gennaro SF, Duce P, et al. Estimation of water stress in grapevines using proximal and remote sensing methods. Remote Sensing. 2018;**10**(1):114

[122] Peñuelas J, Filella I, Biel C, Serrano L, Savé R. The reflectance at the 950-970 nm region as an indicator of plant water status. International Journal of Remote Sensing. 1993;**14**(10):1887-1905

[123] Peñuelas J, Filella I. Visible and near-infrared reflectance techniques for diagnosing plant physiological status. Trends in Plant Science. 1998;**3**(4):151-156

[124] Carter GA. Primary and secondary effects of water content on the spectral

reflectance of leaves. American Journal of Botany. 1991;**78**(7):916-924

[125] Rollin EM, Milton EJ. Processing of high spectral resolution reflectance data for the retrieval of canopy water content information. Remote Sensing of Environment. 1998;**65**(1):86-92

[126] Gutiérrez S, Tardaguila J, Fernández-Novales J, Diago MP. Data mining and NIR spectroscopy in viticulture: Applications for plant phenotyping under field conditions. Sensors. 2016;**16**(2):236

[127] Bei R, Cozzolino D, Sullivan W, Cynkar W, Fuentes S, Dambergs R, et al. Non-destructive measurement of grapevine water potential using near infrared spectroscopy. Australian Journal of Grape and Wine Research. 2011;**17**(1):62-71

[128] Sibille I, Ojeda H, Prieto J, Maldonado S, Lacapere J-N, Carbonneau A. Relation between the values of three pressure chamber modalities (midday leaf, midday stem and predawn water potential) of 4 grapevine cultivars in drought situation of the southern of France. Applications for the irrigation control. In: Proceedings of the 15th International Symposium GESCO 2007; 20-23 June. Porec, Croatia: Institute of Agriculture and Tourism; 2007. pp. 830-840

[129] Smith DM, Allen SJ. Measurement of sap flow in plant stems. Journal of Experimental Botany. 1996;**47**(12): 1833-1844

[130] Ferraz TM. Trocas gasosas em mamoeiro Golden: relações entre plantas inteiras e folhas individuais. Campos dos Goytacazes, RJ: UENF; 2008

[131] Wullschleger SD, Meinzer FC, Vertessy RA. A review of whole-plant water use studies in tree. Tree Physiology. 1998;**18**(8-9):499-512

[132] Reis FO, Campostrini E, de Sousa EF, MGE S. Sap flow in papaya plants: Laboratory calibrations and relationships with gas exchanges under field conditions. Scientia Horticulturae. 2006;**110**(3):254-259

[133] Sakuratani T. A heat balance method for measuring water flux in the stem of intact plants. Journal of Agricultural Meteorology. 1981;**37**(1):9-17

[134] Granier A, editor. Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. In: Annales des Sciences forestières. EDP Sciences; 1985. pp. 193-200

[135] Clearwater MJ, Meinzer FC, Andrade JL, Goldstein G, Holbrook NM. Potential errors in measurement of nonuniform sap flow using heat dissipation probes. Tree Physiology. 1999;**19**(10):681-687

[136] Ferraz TM, Netto AT, De Oliveira RF, Peçanha AL, De Sousa EF, Filho JAM, et al. Relationships between sap-flow measurements, whole-canopy transpiration and reference evapotranspiration in field-grown papaya (Carica papaya L.). Theoretical and Experimental Plant Physiology. 2015;**27**(3):251-262

[137] Van Bavel CH, van Bavel MG, Lascano RJ, Camp C. Automatic irrigation based on monitoring plant transpiration. In: Proceedings of the ASAE International Conference; San Antonio, Texas. 1996. pp. 1088-1092

[138] Nadezhdina N, Cermak J. Automatic Control Unit for Irrigation Systems Based on Sensing the Plant Water Status. Lisboa, Portugal: Anais do Instituto Superior de Agronomia; 1997. pp. 149-157

[139] Fernández J, Romero R, Montaño J, Diaz-Espejo A, Muriel J, Cuevas M, et al. Design and testing of an automatic irrigation controller for fruit tree orchards, based on sap flow measurements. Australian Journal of Agricultural Research. 2008;**59**(7):589-598

[140] Schmid J, Bettner W. Experience with granier-type sensors for sap flow measurements in grapevines. In: I ISHS Workshop on Water Relations of Grapevines. 1998. pp. 177-186

[141] Dragoni D, Lakso AN, Piccioni RM. Transpiration of apple trees in a humid climate using heat pulse sap flow gauges calibrated with whole-canopy gas exchange chambers. Agricultural and Forest Meteorology. 2005;**130**(1):85-94

[142] Fang Y, Xiong L. General mechanisms of drought response and their application in drought resistance improvement in plants. Cellular and Molecular Life Sciences. 2015;**72**(4):673-689

[143] Sun Y, Wang C, Chen HYH, Ruan H. Response of plants to water stress: A meta-analysis. Frontiers in Plant Science. 2020;**11**(198):1-10

[144] Bandurska H. Drought stress responses: Coping strategy and resistance. Plants. 2022;**11**(7):922

[145] Dayer S, Herrera JC, Dai Z, Burlett R, Lamarque LJ, Delzon S, et al. The sequence and thresholds of leaf hydraulic traits underlying grapevine varietal differences in drought tolerance. Journal of Experimental Botany. 2020;**71**(14):4333-4344

[146] Marusig D, Tombesi S. Abscisic acid mediates drought and salt stress responses in Vitis vinifera—A review. International Journal of Molecular Sciences. 2020;**21**(22):8648

[147] McAdam SAM, Brodribb TJ. Ancestral stomatal control results in a canalization of fern and lycophyte adaptation to drought. New Phytologist. 2013;**198**(2):429-441

[148] Helaly MN, El-Hoseiny H, El-Sheery NI, Rastogi A, Kalaji HM. Regulation and physiological role of silicon in alleviating drought stress of mango. Plant Physiology and Biochemistry. 2017;**118**:31-44

[149] Tworkoski T, Fazio G, Glenn DM. Apple rootstock resistance to drought. Scientia Horticulturae. 2016;**204**:70-78

[150] Soar CJ, Speirs J, Maffei SM, Penrose AB, Mccarthy MG, Loveys BR. Grape vine varieties shiraz and Grenache differ in their stomatal response to VPD: Apparent links with ABA physiology and gene expression in leaf tissue. Australian Journal of Grape and Wine Research. 2006;**12**(1):2-12

[151] McAdam SAM, Brodribb TJ. Mesophyll cells are the Main site of abscisic acid biosynthesis in water-stressed leaves. Plant Physiology. 2018;**177**(3):911-917

[152] Speirs J, Binney A, Collins M, Edwards E, Loveys B. Expression of ABA synthesis and metabolism genes under different irrigation strategies and atmospheric VPDs is associated with stomatal conductance in grapevine (*Vitis vinifera* L. cv cabernet sauvignon). Journal of Experimental Botany. 2013;**64**(7):1907-1916

[153] Lamaoui M, Chakhchar A, El Kharrassi Y, Wahbi S, El Modafar C. Morphological, physiological, and biochemical responses to water stress in melon (*Cucumis melo*) subjected to regulated deficit irrigation (RDI) and partial Rootzone drying (PRD). Journal of Crop Science and Biotechnology. 2018;**21**(4):407-416

[154] Silva JR, Rodrigues WP, Ferreira LS, Bernado WP, Paixão JS, Patterson AE, et al. Deficit irrigation and transparent plastic covers can save water and improve grapevine cultivation in the tropics. Agricultural Water Management. 2018;**202**:66-80

[155] Howell C, Myburgh PJSAJE. Using grapevine water status measurements for irrigation scheduling of table grapes-a review. Viticulture. 2023;**44**(1):75-80

[156] Charrier G, Delzon S, Domec J-C, Zhang L, Delmas CEL, Merlin I, et al. Drought will not leave your glass empty: Low risk of hydraulic failure revealed by long-term drought observations in world's top wine regions. Science. 2018;**4**(1):eaao6969

[157] Maurel C. Aquaporins and water permeability of plant membranes. Annual Review of Plant Biology. 1997;**48**:399-429

[158] Maurel C, Prado K. Aquaporins and leaf water relations. In: Chaumont F, Tyerman SD, editors. Plant Aquaporins: From Transport to Signaling. Cham: Springer International Publishing; 2017. pp. 155-165

[159] Coupel-Ledru A, Tyerman SD, Masclef D, Lebon E, Christophe A, Edwards EJ, et al. Abscisic acid Down-regulates hydraulic conductance of grapevine leaves in Isohydric genotypes only. Plant Physiology. 2017;**175**(3):1121-1134

[160] Alexandersson E, Fraysse L, Sjövall-Larsen S, Gustavsson S, Fellert M, Karlsson M, et al. Whole gene family expression and drought stress regulation of Aquaporins. Plant Molecular Biology. 2005;**59**(3):469-484

[161] Zhu C, Schraut D, Hartung W, Schäffner AR. Differential responses of maize MIP genes to salt stress and ABA. Journal of Experimental Botany. 2005;**56**(421):2971-2981

[162] Tombesi S, Nardini A, Frioni T, Soccolini M, Zadra C, Farinelli D, et al. Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. Scientific Reports. 2015;**5**(1):12449

[163] Degu A, Hochberg U, Wong DCJ, Alberti G, Lazarovitch N, Peterlunger E, et al. Swift metabolite changes and leaf shedding are milestones in the acclimation process of grapevine under prolonged water stress. BMC Plant Biology. 2019;**19**(1):69

[164] Gambetta GA, Herrera JC, Dayer S, Feng Q, Hochberg U, Castellarin SD. The physiology of drought stress in grapevine: Towards an integrative definition of drought tolerance. Journal of Experimental Botany. 2020;**71**(16):4658-4676

[165] Merlaen B, De Keyser E, Van Labeke M-C. The jasmonic acid pathway, rather than abscisic acid, may partly explain contrasting stomatal responses in two strawberry cultivars under osmotic stress. Plant Physiology and Biochemistry. 2020;**151**:21-33

[166] Wheeler JK, Sperry JS, Hacke UG, Hoang N. Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: A basis for a safety versus efficiency trade-off in xylem transport. Plant Cell Environ. 2005;**28**(6):800-812

[167] Hsiao TC, Acevedo E, Fereres E, Henderson D. Water stress, growth and osmotic adjustment. Philosophical Transactions of the Royal Society of London B, Biological Sciences. 1976;**273**(927):479-500

[168] Scoffoni C, Rawls M, McKown A, Cochard H, Sack L. Decline of leaf

hydraulic conductance with dehydration: Relationship to leaf size and venation architecture. Plant Physiology. 2011;**156**(2):832-843

[169] Trifiló P, Raimondo F, Savi T, Lo Gullo MA, Nardini A. The contribution of vascular and extra-vascular water pathways to drought-induced decline of leaf hydraulic conductance. Journal of Experimental Botany. 2016;**67**(17):5029-5039

[170] Bartlett MK, Scoffoni C, Sack L. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: A global meta-analysis. Ecology Letters. 2012;**15**(5):393-405

[171] Blum A. Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant, Cell and Environment. 2017;**40**(1):4-10

[172] Molinari HBC, Marur CJ, Filho JCB, Kobayashi AK, Pileggi M, Júnior RPL, et al. Osmotic adjustment in transgenic citrus rootstock Carrizo citrange (*Citrus sinensis* Osb. X *Poncirus trifoliata* L. Raf.) overproducing proline. Plant Science. 2004;**167**(6):1375-1381

[173] Santana-Vieira DDS, Freschi L, Almeida LAH, Moraes DHS, Neves DM, Santos LM, et al. Survival strategies of citrus rootstocks subjected to drought. Scientific Reports. 2016;**6**(1):38775

[174] Rodríguez-Gamir J, Primo-Millo E, Forner JB, Forner-Giner MA. Citrus rootstock responses to water stress. Scientia Horticulturae. 2010;**126**(2):95-102

[175] Kumar P, Rouphael Y, Cardarelli M, Colla G. Vegetable grafting as a tool to improve drought resistance and water use efficiency. Frontiers in Plant Science. 2017;**8**:1-9

[176] Barichello D, Farinacio R, dos Santos CCN, Andrade GA, Nagashima GT, Garbúglio DD, et al. Drought-tolerant transgenic Swingle Citrumelo controls accumulation of proline modulating the expression of key genes of the proline metabolism. Citrus Research Technology. 2017;**38**(2):1-12

[177] Oliveira CS, Carlos EF, Vieira LGE, Lião LM, Alcantara GB. HR-MAS NMR metabolomics of 'Swingle' citrumelo rootstock genetically modified to overproduce proline. Magnetic Resonance in Chemistry. 2014;**52**(8):422-429

[178] Yamada M, Morishita H, Urano K, Shiozaki N, Yamaguchi-Shinozaki K, Shinozaki K, et al. Effects of free proline accumulation in petunias under drought stress. Journal of Experimental Botany. 2005;**56**(417):1975-1981

[179] Araújo WL, Martins AO, Fernie AR, Tohge T. 2-Oxoglutarate: Linking TCA cycle function with amino acid, glucosinolate, flavonoid, alkaloid, and gibberellin biosynthesis. Frontiers in Plant Science. 2014:5

[180] Farinacio R, Barichello D, Andrade GA, Leite DAC, dos Santos CCN, Felicio MS, et al. Enhanced drought tolerance in transgenic *Swingle citrumelo* overexpressing VaP5CS-F129A under the control of stress induced RD29A promoter. Citrus Research Technology. 2017;**38**(1):1-13

[181] Yıldırım K, Yağcı A, Sucu S, Tunç S. Responses of grapevine rootstocks to drought through altered root system architecture and root transcriptomic regulations. Plant Physiology and Biochemistry. 2018;**127**:256-268

[182] Bauerle TL, Centinari M, Bauerle WL. Shifts in xylem vessel diameter and embolisms in grafted apple trees of differing rootstock growth

potential in response to drought. Planta. 2011;**234**(5):1045-1054

[183] Padgett-Johnson M, Williams L, Walker M. Vine water relations, gas exchange, and vegetative growth of seventeen Vitis species grown under irrigated and nonirrigated conditions in California. Journal of the American Society for Horticultural Science. 2003;**128**(2):269-276

[184] Barrios-Masias FH, Knipfer T, McElrone AJ. Differential responses of grapevine rootstocks to water stress are associated with adjustments in fine root hydraulic physiology and suberization. Journal of Experimental Botany. 2015;**66**(19):6069-6078

[185] Atkinson CJ, Policarpo M, Webster AD, Kuden AM. Drought tolerance of apple rootstocks: Production and partitioning of dry matter. Plant and Soil. 1999;**206**(2):223-235

[186] Cohen S, Naor A, Bennink J, Grava A, Tyree M. Hydraulic resistance components of mature apple trees on rootstocks of different vigours. Journal of Experimental Botany. 2007;**58**(15-16):4213-4224

[187] Prinsi B, Simeoni F, Galbiati M, Meggio F, Tonelli C, Scienza A, et al. Grapevine rootstocks differently affect physiological and molecular responses of the Scion under water deficit condition. Agronomy. 2021;**11**(2):289

[188] Martínez-Alcántara B, Rodriguez-Gamir J, Martínez-Cuenca MR, Iglesias DJ, Primo-Millo E, Forner-Giner MA. Relationship between hydraulic conductance and citrus dwarfing by the flying dragon rootstock (*Poncirus trifoliata* L. Raft var. monstruosa). Trees. 2013;**27**(3):629-638

[189] Sousa MC, Marinho CS, Silva MPS, Carvalho WSG, Amaral BD, Assis-Gomes MM, et al. Effects of grafting and gradual rootstock substitution on gas exchanges of orange seedlings under high atmospheric evaporative demand. Scientia Horticulturae. 2019;**247**:67-74

[190] Menezes-Silva PE, Sanglard LMVP, Ávila RT, Morais LE, Martins SCV, Nobres P, et al. Photosynthetic and metabolic acclimation to repeated drought events play key roles in drought tolerance in coffee. Journal of Experimental Botany. 2017;**68**(15):4309-4322

[191] Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, et al. Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants. 2021;**10**(2):259

[192] Toro-Herrera MA, Vieira DA, Alvarenga JP, Silva L, Mendonça AMC, Ferreira EA, et al. Drought/rewatering cycles on the vegetative growth of citrus seedlings. Pesquisa Agropecuária Brasileira. 2023;**58**:e03331

[193] Nteve G-M, Kostas S, Polidoros AN, Madesis P, Nianiou-Obeidat I. Adaptation mechanisms of olive tree under drought stress: The potential of modern omics approaches. Agriculture. 2024;**14**(4):579

[194] Rakocevic M, Costes E, Campostrini E, Ramalho JC, Ribeiro RV. Drought responses in *Coffea arabica* as affected by genotype and phenophase. II – Photosynthesis at leaf and plant scales. Experimental Agriculture. 2024;**60**:e22

[195] Rakocevic M, Matsunaga FT, Pazianotto RAA, Ramalho JC, Costes E, Ribeiro RV. Drought responses in *Coffea arabica* as affected by genotype and phenophase. I – Leaf distribution and branching. Experimental Agriculture. 2024;**60**:e7

[196] Tombesi S, Frioni T, Poni S, Palliotti A. Effect of water stress "memory" on plant behavior during subsequent drought stress. Environmental Experimental Botany. 2018;**150**:106-114

[197] Ben Abdallah M, Methenni K, Nouairi I, Zarrouk M, Youssef NB. Drought priming improves subsequent more severe drought in a drought-sensitive cultivar of olive cv. Chétoui. Scientia Horticulturae. 2017;**221**:43-52

[198] Ripoll J, Urban L, Bertin N. The potential of the MAGIC TOM parental accessions to explore the genetic variability in tomato acclimation to repeated cycles of water deficit and recovery. Frontiers in Plant Science. 2016;**6**(1172):1-15

[199] Gambetta JM, Romat V, Schmidtke LM, Holzapfel BP. Secondary metabolites Coordinately protect grapes from excessive light and sunburn damage during development. Biomolecules. 2021;**12**(1):42

[200] Savoi S, Wong DCJ, Degu A, Herrera JC, Bucchetti B, Peterlunger E, et al. Multi-omics and integrated network analyses reveal new insights into the systems relationships between metabolites, structural genes, and transcriptional regulators in developing grape berries (*Vitis vinifera* L.) exposed to water deficit. Frontiers in Plant Science. 2017;**8**(1124):1-19

[201] Canoura C, Kelly MT, Ojeda H. Effect of irrigation and timing and type of nitrogen application on the biochemical composition of *Vitis vinifera* L. cv. Chardonnay and Syrah grapeberries. Food Chemistry. 2018;**241**:171-181

[202] Sanchez DH, Siahpoosh MR, Roessner U, Udvardi M, Kopka J. Plant metabolomics reveals conserved and divergent metabolic responses to salinity. Physiologia Plantarum. 2008;**132**(2):209-219

[203] Hochberg U, Degu A, Cramer GR, Rachmilevitch S, Fait A. Cultivar specific metabolic changes in grapevines berry skins in relation to deficit irrigation and hydraulic behavior. Plant Physiology and Biochemistry. 2015;**88**:42-52

[204] Torres N, Hilbert G, Luquin J, Goicoechea N, Antolín MC. Flavonoid and amino acid profiling on *Vitis vinifera* L. cv Tempranillo subjected to deficit irrigation under elevated temperatures. Journal of Food Composition and Analysis. 2017;**62**:51-62

[205] Yang B, He S, Liu Y, Liu B, Ju Y, Kang D, et al. Transcriptomics integrated with metabolomics reveals the effect of regulated deficit irrigation on anthocyanin biosynthesis in cabernet sauvignon grape berries. Food Chemistry. 2020;**314**:126170

[206] Berenguer MJ, Vossen PM, Grattan SR, Connell JH, Polito VS. Tree irrigation levels for optimum chemical and sensory properties of olive oil. HortScience. 2006;**41**(2):427-432

[207] Salama A-M, Abdelsalam MA, Rehan M, Elansary M, El-Shereif A. Anthocyanin accumulation and its corresponding gene expression, Total phenol, antioxidant capacity, and fruit quality of 'crimson seedless' grapevine (*Vitis vinifera* L.) in response to grafting and pre-harvest applications. Horticulturae. 2023;**9**(9):1001

[208] Perin EC, da Silva MR, Borowski JM, Crizel RL, Schott IB, Carvalho IR, et al. ABA-dependent salt and drought stress improve strawberry fruit quality. Food Chemistry. 2019;**271**:516-526

[209] Kopta T, Sekara A, Pokluda R, Ferby V, Caruso G. Screening of

chilli pepper genotypes as a source of Capsaicinoids and antioxidants under conditions of simulated drought stress. Plants. 2020;**9**(3):364

[210] Savoi S, Wong DCJ, Arapitsas P, Miculan M, Bucchetti B, Peterlunger E, et al. Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (*Vitis vinifera* L.). BMC Plant Biology. 2016;**16**(1):67

[211] Deluc LG, Quilici DR, Decendit A, Grimplet J, Wheatley MD, Schlauch KA, et al. Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of cabernet sauvignon and chardonnay. BMC Genomics. 2009;**10**(1):212

[212] Zuazo VHD, García-Tejero IF, Rodríguez BC, Tarifa DF, Ruiz BG, Sacristán PC. Deficit irrigation strategies for subtropical mango farming. A review. Agronomy for Sustainable Development. 2021;**41**(1):13

[213] Brillante L, Martínez-Lüscher J, Kurtural SK. Applied water and mechanical canopy management affect berry and wine phenolic and aroma composition of grapevine (*Vitis vinifera* L., cv. Syrah) in Central California. Scientia Horticulturae. 2018;**227**:261-271

Chapter 3

Plant Growth Regulators: Key Drivers of Fruit Crop Productivity

Shiv Kumar Shivandu, Damini Singh, Gaurav Kumar, Ishani Sharma and Jayesh Garg

Abstract

Plant growth regulators (PGRs) play a pivotal role in enhancing yield and quality in fruit crops by influencing the biosynthesis, metabolism, and translocation of plant hormones. These exogenous applications modulate hormonal balance by stimulating or inhibiting the production of specific hormones, thereby altering growth patterns and developmental processes. The strategic use of PGRs has led to significant advancements in fruit production, contributing to superior growth, quality, and yield. PGRs elicit various physiological responses, such as promoting or suppressing growth, inducing flowering or thinning excess blooms, and mitigating biotic and abiotic stresses. They also reduce preharvest fruit drop, improve fruit size, enhance color development, and synchronize fruit maturity. Additionally, PGRs support root system development, boosting nutrient uptake and stress tolerance. In high-value perennial fruit trees, which exhibit intricate physiological interactions, PGRs offer precise and efficient interventions to optimize productivity. Remarkably effective at low concentrations, these compounds enable significant improvements in fruit quality and yield, underscoring their critical role as drivers of sustainable fruit crop management.

Keywords: auxins, gibberellins, productivity, quality, fruits

1. Introduction

In the quest to meet the growing global demand for food and enhance the sustainability of agricultural systems, plant growth regulators (PGRs) have become essential tools for improving fruit crop productivity. Defined as natural or synthetic compounds that influence plant development and metabolism at low doses, PGRs are widely used to modify growth processes such as branching, shoot suppression, flower regulation [1–7], fruit thinning [8], and adjusting the timing of fruit maturity [9]. The efficacy of PGRs depends on various factors, including the plant's vigor, age, cultivar, dose, timing of application, and prevailing weather conditions during their application. To meet the annual global food demand of approximately 3.7 billion metric tons, chemical fertilizers have traditionally been employed to boost productivity [10]. However, their excessive use has disrupted ecosystems, posing a threat to

biodiversity and destabilizing natural processes. This has led to a growing emphasis on sustainable agricultural practices, including environmentally friendly approaches like integrating biofertilizers and PGRs to reduce environmental pollution while maintaining high crop yields.

Fruit crops, being highly sensitive to both biotic and abiotic stresses [11], can be benefited significantly from the application of PGRs. These regulators help mitigate the adverse effects of climate variability, pests, and diseases, thereby stabilizing yields and improving fruit quality. PGR is a very broad term that includes growth promoters and growth retardants. The main classes of PGRs—auxins, gibberellins, cytokinins, ethylene, and abscisic acid—each play distinct roles in plant development. For instance, auxins control cell elongation [12] and root development [13], while gibberellins are critical for stem elongation [14] and breaking dormancy in fruit crops [15]. Cytokinins promote cell division, ethylene regulates ripening, and abscisic acid manages responses to stress, particularly drought.

With technological advances in agriculture, the development of novel PGR formulations and their precise application through modern farming techniques have resulted in a paradigm shift in how PGRs can be used in integrated fruit crop management. The controlled use of these regulators has not only improved the quantity and quality of fruit harvests but also contributed to the economic viability of fruit production by enhancing efficiency, reducing waste, and ensuring consistency in fruit size, taste, and appearance.

This chapter delves into the science behind plant growth regulators, exploring their modes of action, interactions with plant hormones, and the specific effects on fruit crop physiology. By examining real-world applications and case studies, it provides a comprehensive understanding of how PGRs can be leveraged to drive fruit crop productivity, improve quality, and contribute to sustainable agricultural practices.

2. What are plant growth regulators?

PGRs: These are the organic chemical compounds that are naturally and synthetically synthesized, which can alter or regulate the various physiological processes appreciably in a plant when used in minute quantity.

2.1 Overview of plant growth regulators (PGRs)

Plant growth regulators (PGRs) can be broadly categorized into natural and synthetic types, each with distinct characteristics and applications (**Table 1**).

Type of PGRs	Description	Example	Role in fruit crops	References
Natural PGRs	Naturally produced hormones in plants that regulate growth and development	IAA, IBA, ethylene, GA_3, ABA	Control cell division, flowering, fruit ripening, stress response	[2, 3, 11, 12]
Synthetic PGRs	Artificially formulated chemicals that mimic or alter natural hormone actions for specific outcomes	IBA, NAA, GA_3, Progibb paclobutrazol, daminozide	Promote rooting, enhance fruit size, control growth, regulate ripening	[16–20]

Table 1.
Classification of PGRs on the basis of origin.

3. Physiological role of PGRs in fruit crops

Auxins: Auxins are a group of PGRs that plays a pivotal role in various stages of fruit development. Auxins, produced primarily in the shoot, root apices, coleoptile tips, and leaves, play a pivotal role in enhancing fruit productivity. These hormones are transported in an acropetal manner (from the tip toward the base) to zones of action, where they regulate critical growth processes. By activating enzymes like expansins, auxins loosen cell walls, enabling cell enlargement and development of larger and healthier fruits. In fruit development, auxins are indispensable for fruit set, stimulating the transformation of the ovary into fruit after pollination. Their ability to maintain hormonal balance prevents early fruit drop, ensuring a higher yield. External application of auxins can further enhance productivity by inducing parthenocarpy in many crops like grapes, strawberry, and oranges leading to the production of seedless fruits, which are often preferred in the market. The polar transport of auxins creates a regulated concentration gradient across plant tissues, promoting uniform and coordinated growth of fruits. Auxins also interact synergistically with other plant hormones like gibberellins (GA) and cytokinins to regulate cell division and fruit expansion, directly influencing fruit size, structure, and quality. During the later stages of development, a controlled reduction in auxin levels promotes ripening while preventing premature fruit detachment, ensuring a better harvest. This comprehensive role of auxins in fruit set, growth, and ripening highlights their critical contribution to improving both the quantity and quality of fruit production.

Gibberellins: Gibberellins (GA) play a crucial role in cell elongation and breaking dormancy, leading to an increase in fruit size, weight, and volume. For instance, in *Phyllanthus emblica* (aonla), spraying with 100 ppm of GA_3 significantly improved fruit length, width, weight, and volume, while also enhancing qualitative parameters such as ascorbic acid levels and total soluble solids and reducing fruit acidity [10]. In crops like Ber, GA_3 reduced the abscission layer, preventing premature fruit and flower drop while improving fruit set [21]. Similarly, in table grapes, GA_3 application induced seedlessness, which is a desirable trait for commercial production [22]. Furthermore, GA_3 has been shown to enhance chlorophyll synthesis in papaya [23] and protein production in wax apple [24], supporting overall plant growth and fruit development [25]. Fruit as a structural entity that arise out of ovary or parts surrounding the ovary and GA_3 application boosts the initial growth of ovaries [26]. While these effects vary across species, they underscore the versatility and significance of GA_3 in improving fruit yield and quality.

Cytokinins: These are a chemically diverse class of PGRs, which have a role in plant growth and development. Cytokinins (CKs) play a fundamental role in plant growth and development. Cytokinins are found to have an influence in developmental stage from embryogenesis till senescence in all type of plants. They are involved in multiple physiological and biochemical processes of different functions throughout the plant life cycle. CKs actively regulate important processes such as seed germination (radicle and plumule initiation), shoot elongation, and proliferation, as well as the induction of flowering, fruit formation, and seed set. Additionally, they also have an important role in delaying senescence, alongside reducing fruit ripening and preventing premature defoliation [1–7]. These functions are closely linked to their ability to release buds from apical dominance, thereby promoting lateral growth and overall plant vigor. Moreover, CKs contribute significantly to enhancing plant stress tolerance, equipping plants with the resilience needed to withstand adverse environmental conditions [9].

Ethylene: It is well known as a ripening hormone, and its major function in plants is to induce ripening [3]. Ethylene is produced as a signal to induce ripening in fruits as they mature. The level of ethylene in a fruit varies by variety, and some fruits, like McIntosh apples, produce a lot of ethylene. Another role of ethylene is in shelf life: in order to extend the shelf life of the fruit, the levels of ethylene have to be kept under control [27]. For this, the filters or sachets that absorb ethylene can be used. Fruits can also be sprayed with a light mist and kept at a cool temperature. Ethylene also plays a role in other plant processes, such as seed germination, root development, and stress responses [13].

Abscisic Acid: This hormone has an alternate name, which is stress hormone where the presence of this hormone regulates gene expression as ABA binds to receptors, which activates a series of actions in turn regulating the gene expression [28]. These genes have multiple functions like modification of a fruit's color, texture, flavor, and aroma. The other functions involve promotion of photosynthate unloading, and this includes translocation of photosynthate from phloem to developing fruits [29, 30]. The application of ABA or use of compounds/methods to increase the levels of ABA within plant system can result in enhanced sugar utilization [31]; for example, the amount of sugar that is taken up into vacuoles in the flesh of an apple fruit can help in improving the sweetness of apples. ABA is also found involved in regulation of the accumulated pigments are responsible for changing the fruit's color during ripening. The aroma of fruits and fruit softening is caused by changes in the structure and

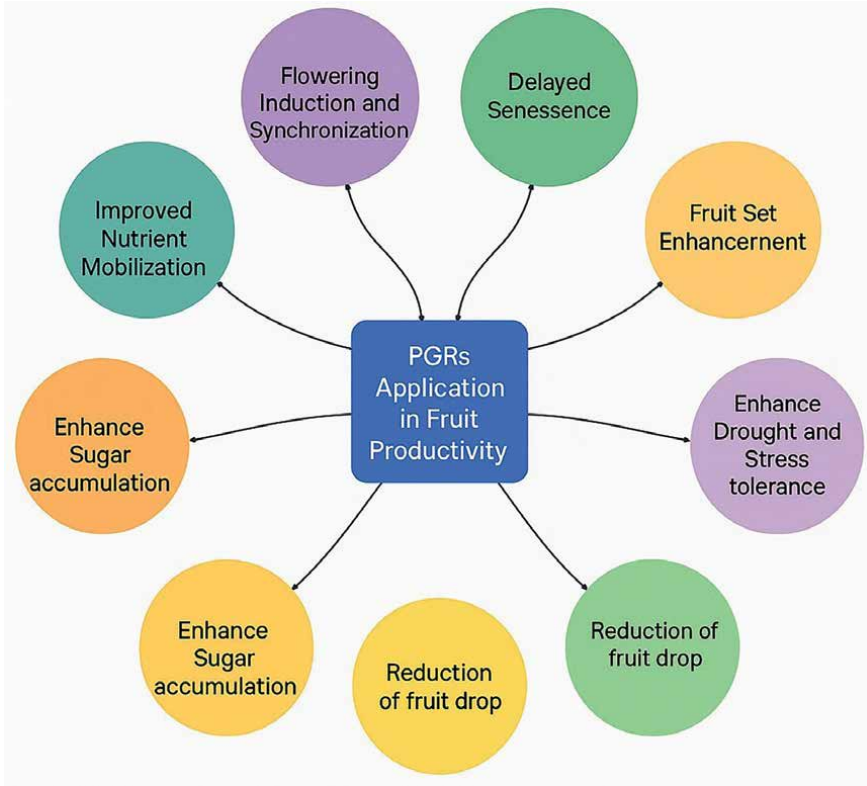

Figure 1.
Mechanisms through which PGRs enhance fruit productivity.

composition of cell walls [32]. ABA levels generally increase during the ripening process in most fruits. ABA interacts with other phytohormones and environmental factors in a complex network [33]. For example, ABA and ethylene interact in a complex network, and their concentrations change in response to each other [34].

4. Mechanisms of action of PGRs in fruit crop productivity

Figure 1 brings out a clear image of the impact of PGR application on diversified responses in fruit productivity. Few of the effects are directly associated with enhancement of the fruit quality while others are indirectly associated. The application of PGRs results in hormonal changes, that is, either increase or decrease in hormonal levels within the plant system. This can be explained on the basis of PGRs and hormonal interactions: applied plant growth regulators interact with the plant's internal hormonal levels, initiating multiple complex physiological processes that include seed germination, flowering, fruit development, and response to environmental stresses.

These interactions can be synergistic, where two or more hormones work together to amplify an effect, or antagonistic, where one hormone counteracts the effect of another. The five major categories of PGRs—auxins, cytokinins, gibberellins, abscisic acid (ABA), and ethylene—all have a unique function; however, their interplay results in the regulation of plant growth.

5. Role of PGRs in enhancing fruit productivity

Role of Auxin in fruit productivity: Auxins, primarily in fruit formation, promote fruit set by stimulating cell division and expansion in the ovary. Even without pollination, auxins can produce fruits through parthenocarpy, seedless fruits. As the fruit grows, auxin regulates cell enlargement and coordinates with hormones like gibberellins and cytokinins, affecting size and shape while guiding vascular tissue development to support the growing fruit. Hormonal interaction with ethylene is vital for regulating ripening. As it promotes early growth, auxin applied at later stages delays ripening by suppressing ethylene production, extending fruit storage life and shelf stability. Auxins are applied at lower concentrations to prevent premature fruit drop, especially in crops like apples and citrus, helping maintain yield. Time of application along with concentration plays an important role in defining the actual role. In seeded fruits, auxin from developing seeds signals the surrounding tissue to grow, which explains why seedless fruits tend to be smaller. Synthetic auxins, such as NAA and 2,4-D, are widely used in horticulture to control fruit set, prevent fruit drop, and enhance the size and quality of fruits, making auxin invaluable for optimizing fruit production.

Role of Gibberellins in fruit productivity: GAs work antagonistically to ABA in regulating dormancy—while ABA induces and maintains dormancy, gibberellins stimulate seed germination by breaking down the starch stored in seeds and converting it into energy. In addition to their interactions with ABA, gibberellins also work alongside auxins to promote cell elongation and fruit development. In many crops, the application of GAs can increase fruit size and improve overall yield.

Role of Cytokinins in fruit productivity: Cytokinins are known for promoting cell division and delaying leaf senescence and often counterbalance the effects of other PGRs like ABA. They counteract ABA and delay stress responses and encourage growth, often acting against ABA's inhibitory effects on germination. The antagonism

between cytokinins and ABA becomes particularly important during periods of environmental stress, such as drought. However, when favorable conditions return, these help to reinitiate growth and promote recovery.

Role of Ethylene in fruit productivity: This often plays a key role in fruit ripening, leaf abscission, and the plant's response to stress. Ethylene's interaction with auxins is especially important in regulating leaf and fruit drop. It is useful for growth restriction and fruit thinning practices. Ethylene also works synergistically with ABA in promoting plant senescence and stress responses but can interact with cytokinins to delay senescence in certain circumstances, adding another layer of complexity to its role in plant development.

Role of ABA in fruit productivity: ABA within the plant system that resulted in restriction of vegetative growth reduces the dependence of plant on food reserves,

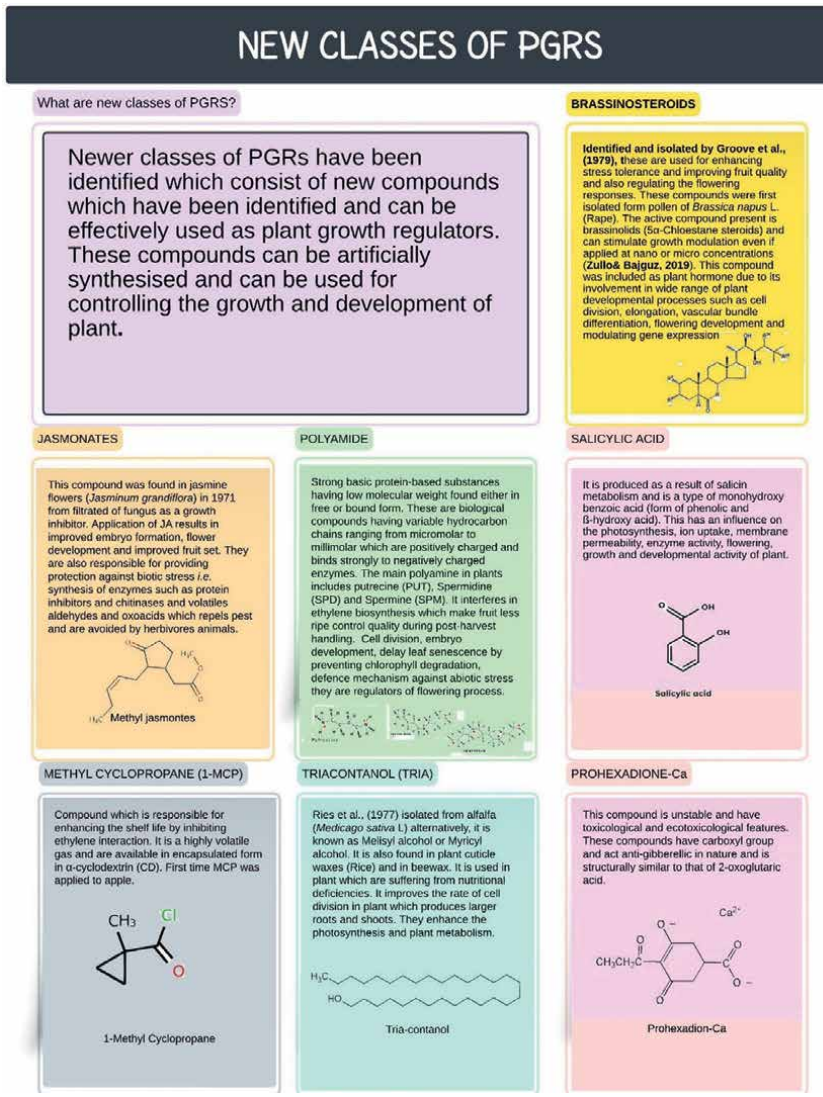

Figure 2.
Brief introduction about newly added classes of hormone.

PGR	Function/Effect on plant	References
Brassinosteroids	Promotion of cell wall expansion and cell elongation. Seed germination, reduce stress effects	
Jasmonates	Root growth, inhibit germination, secondary metabolite productions, stimulate leaf senescence, wound response, pollen development, enhance photosynthetic ability, ion uptake, membrane permeability, enzyme activity, flowering, growth and development of plant. Improve shelf life of fruits, chilling injury protection, firm fruit, and improve TSS.	[35]
Polyamides	Cell division, embryo development, flower development, regulate ripening, reduce physiological weight loss, anti-senescence agent, protection from abiotic stress.	[36]
Salicylic acid	Photosynthesis, ion uptake, membrane permeability, enzyme activity, flowering, growth and developmental activity of plant. Initiates SAR (systematic acquired resistance). Under SAR, if a plant is under attack of pathogen in one part, the other part automatically develops resistance.	[37–39]
Methyl cyclopropane (1-MCP)	Interacts with ethylene-sensitive sites, reduces respiration, delays senescence, development of color, reduces chlorophyll degradation, fruit softening. Post-harvest shelf life is increased prolonging the flavors for extended duration.	[25, 40]
Triacontanol	Improves root/shoot length, fresh and dry weight, increases leaf area per plant, better photosynthetic ability, nutrient accumulation, increases yield and yield attributes, increases nitrogen fixation and enhanced translocation and sugar accumulation.	[41]
Prohexadione-Ca	Inhibits the production of ethylene, checks vegetative growth, reduction in longitudinal shoot growth by blocking dioxygenases, and can be used as a substitute to paclobutrazol. Controls alternate bearing.	[42]

Table 2.
*Impact of newer class of PGRs (***Figure 2***) on plant function.*

which helps the pant to divert the food material toward the growing fruits and overall contribute to fruit quality and productivity. Also, at the time of fruit maturation, ABA levels rise, triggering physiological changes that lead to ripening by promoting color development, sugar accumulation, and flavor enhancement. ABA also interacts with ethylene, the main ripening hormone in climacteric fruits, helping to fine-tune the timing and quality of ripening. Furthermore, ABA is crucial in managing plant responses to stress, especially water stress, by regulating stomatal closure, thus helping fruits maintain water content during dry periods. In relation to fruit drop, ABA can signal the abscission of fruits under unfavorable conditions, effectively reducing the load on the plant to support only well-developed fruits (**Figure 2**, **Table 2**).

6. Application of PGRs in fruit crop management

PGRs are applied through various methods, which include foliar sprays, soil application, and drenching. Foliar spraying involves preparing a careful concentration of the compound to be applied and the solution is directly sprayed onto the plant's leaves, allowing for quick absorption and fast results, making it ideal for targeted

PGR	Dosage (ppm)		Time of application
Naphthalic acetic acid	10–15	Apple	Any time between full bloom to 4 weeks after petal fall
2,4-Dichlorophenoxy acetic acid	2–10		Any time between full bloom till petal fall
2,4,5-Tri-chloroacetic acid	2–25		Any time between full bloom till petal fall
Naphthalic acetic acid	10–50	Stone Fruits	Any time between petal fall to pit hardening
2,4-Dichlorophenoxy acetic acid	2–15		Any time between full bloom to petal fall
2,4,5-Tri-chloroacetic acid	2–25		Any time between full bloom to petal fall
Ethephon	100–500		Any time between petal fall to pit hardening
NAA	10–20	Sweet oranges	Fruit development stage
2,4-D	10–15	Mandarins	4–8 weeks after fruit set
GA_3+ NAA	20–40	Grapes	Berry shatter stage, early fruit set
NAA	10–15	Litchi	Early fruit development stage

Table 3.
Timing and Dosage of PGRs for fruit thinning [8].

applications like correcting nutrient deficiencies or promoting flowering. However, it must be kept in mind that quick absorption of inaccurate dose can also lead to toxicity and result in the development of phytotoxicity. Soil application, on the other hand, delivers PGRs to the plant's root zone, providing a slower, more sustained release that supports root health and plant vigor. Drenching involves saturating the plant's root zone with a solution, ensuring direct uptake by the roots for long-lasting effects. This method is particularly effective for larger plants and trees, especially when applying root-active PGRs or addressing stress-related issues.

Table 3 provides precise information about the experimentally verified doses of various growth regulators in different fruit crops.

7. The role of PGRs in flowering response

The onset of flowering is a crucial precursor to fruit setting, and PGRs are instrumental in influencing this phase. Growth retardants, like paclobutrazol (PBZ), are known to promote flowering, while growth promoters may sometimes suppress it. The effect of PGRs on flowering largely depends on the timing and rate of application, with growth retardants generally enhancing flowering when applied at specific developmental stages. Additionally, certain PGRs can be used to delay flowering, allowing plants to avoid adverse weather conditions that might otherwise damage early blooms. For example, NAA (200–800 ppm) has been shown to delay flowering by 1–2 weeks in apple [16], cherry [17], pear [18], peach [19], and plums [43]. By delaying flowering, PGRs can help protect sensitive stages of fruit crops from spring frosts or other harsh conditions. Certain PGRs are also species-specific in their effectiveness. Daminozide (succinic acid-2,2-dimethyl hydrazide) promotes flowering in

crops like apple, pear, peach, and blueberry, while CCC (cycocel) increases flowering in grapes and lemons. However, in other crops, such as strawberry, peach, plum, and cherry, GA applications support increased fruit set, highlighting the nuanced role of PGRs across various fruit species.

PGRs in Flower and Fruit Thinning for Optimized Yield: To achieve optimal yield and fruit quality, flower and fruit thinning practices are essential. High yields in one year may lead to reduced yields in subsequent years due to biennial bearing—a common issue in many fruit crops. PGRs can assist in thinning flowers and fruit to balance load and ensure the development of high-quality produce, leading to better returns and avoiding the stress of overbearing on plants. Prebloom GA applications are effective for inducing fruit set while also helping to create loosely clustered and visually appealing grape bunches. In peaches, NAA at 5–10 ppm is effective for fruit thinning, while in mango, PBZ (5 g active ingredient per plant) optimizes yield by managing fruit set. Additionally, Promalin (a combination of GA_{4+7} and BA) is widely used as a fruit thinner, helping to maintain desired crop load and fruit quality [44]. NAA @ 800 ppm resulted in deblossoming of guava flower [45]. Interestingly, GA_3 has a specific role in inhibiting flower bud differentiation [46]. Therefore, inhibiting GA production can also serve as a method for inducing flowering, as GA restriction helps support flower formation in certain fruit crops. This controlled manipulation of flowering through PGRs allows for improved management of fruit crops, ultimately aligning with the goals of sustainable productivity and market-quality yields.

8. Impact of PGRs on fruit yield and quality

PGRs have revolutionized the productivity of fruit crops by modulating key physiological processes involved in growth, flowering, fruit development, and stress resilience. Through precise applications, PGRs can strategically support fruit set, enhance fruit size, improve biochemical composition, and adapt plants to environmental challenges, enabling growers to meet evolving market demands. This section explores the diverse roles of PGRs in fruit crops, examining how compounds such as gibberellins, auxins, cytokinins, and ethylene inhibitors contribute to yield improvements, fruit quality, and enhanced post-harvest stability.

The strategic application of PGRs can significantly boost yield and fruit size—two core parameters for productive fruit cultivation. PGRs work by modifying growth stages by stimulating cell division or by reducing the number of fruits to achieve optimal fruit size, set, and retention, thus directly impacting productivity. For instance, auxins have demonstrated great success in improving fruit size in crops like plums, cherries, and apricots [16]. When applied post-bloom, auxins support enhanced fruit set and stability, reducing premature fruit drop [47]. In addition, the use of gibberellins (GA_3), specifically as a 50–100 ppm solution, has proven effective for enlarging berry size in grapes, leading to bigger, elongated fruit that meets market standards [48]. In apples, foliar applications of GA_3 (20–40 ppm) combined with NAA (25–50 ppm) improve fruit retention and overall yield [49]. Similarly, in apricot, the application of Prohexadione-Ca and NAA has demonstrated enhanced fruit weight and yield efficiency [42]. Singh et al., [50] used similar concentration in Ber that resulted in the production of better fruit size and yield. Cytokinins, another PGR group, stimulate cell division in developing fruits, thereby contributing to a larger, fuller fruit profile. Collectively, PGRs allow for targeted growth management, adapting crops to seasonal variations and supporting consistent fruit yield across cycles.

9. Quality improvement through PGRs

Beyond yield, PGRs play a pivotal role in refining fruit quality, which encompasses attributes like sweetness, color, texture, and shelf life—all of which influence market value. By regulating the plant's sugar metabolism, GA_{4+7} @ 1.25 nM increased sucrose content of Jinxiu peach. It was found that GA_{4+7} upregulated the sucrose phosphate synthase gene expression when applied during final stage of ripening [51], and application of GA_3 and NAA improved physiological features of Washington navel oranges, enhancing fruit sweetness and flavor [52]. They also help develop uniform fruit shapes, desirable peel thickness, and favorable pulp-to-stone ratios, essential for consumer appeal and processing needs. Importantly, some PGRs, such as paclobutrazol (PBZ), can increase antioxidant activity in pomegranate [20], phenolic content in strawberry and mango [53, 54], and vitamin C levels in mango [55], leading to brighter fruit coloration, firmer texture, and enhanced fungal resistance. Ethylene regulation further aids in extending shelf life in kiwi [27], particularly when inhibitors like 1-Methylcyclopropene (MCP) in strawberry [25]. MCP, for instance, maintains firmness, acidity, and color in crops like plums [40], enabling extended storage and transport without compromising quality.

10. PGR applications in harvesting and post-harvest quality

PGRs are also employed to facilitate efficient harvesting and improve post-harvest handling. Ethephon, a well-known ripening agent, is widely used for chemical harvesting and dehulling across several crops, including walnut, pecan, olive, apricot, cherry, date, and grapes [8]. Its ability to promote synchronized fruit drop simplifies harvesting and processing operations. Moreover, in walnut cultivation, ethephon aids in efficient dehulling, making it a practical choice for commercial growers. An alternative approach to managing ethylene levels is through diazocyclopentadiene (DCAP), an ethylene inhibitor effectively used to delay ripening in crops such as tomatoes and apples [56], allowing for a prolonged shelf life and extended market availability. Furthermore, the application of PBZ has been linked to reductions in physiological disorders like bitter pit [57], cork spot, and senescent breakdown, commonly observed in apples [19]. This makes PBZ not only a growth regulator but also a contributor to fruit quality preservation, particularly under storage.

11. Challenges and limitations of using PGRs

The application of PGRs in agriculture, while advantageous, also presents significant challenges tied to environmental impact, crop health, and regulatory compliance, all of which demand careful management.

Environmental and Safety Concerns: PGR residues can accumulate in soil and water, potentially harming beneficial organisms and disrupting local ecosystems. Some synthetic PGRs may contribute to soil and water pollution, raising concerns about long-term environmental sustainability. Additionally, PGR residues on fruits can pose food safety issues, especially where consumer preferences are increasingly aligned with organic and natural produce. Excessive or mismanaged use of PGRs can negatively impact crop health, causing issues such as phytotoxicity, inhibited root development, and nutrient imbalances. Chronic overuse may result in weaker plants with reduced resilience against

pests and diseases, leading to diminished productivity over time. Mismanagement, including incorrect dosages or poorly timed applications, can lead to uneven fruit quality, suppressed growth, or even crop losses. Strict regulatory guidelines govern the use of PGRs to ensure food safety and environmental protection, but adhering to these standards can be challenging. Regulations often limit the allowable concentrations and types of PGRs, mandating strict residue testing and monitoring, which increases costs and administrative burden for growers. Safe use practices, including correct timing and dosage, are essential but require specialized knowledge and training, as well as investment in equipment for precise application. To balance the benefits and risks of PGRs, growers are encouraged to follow best practices that prioritize environmental safety and crop health. This includes adhering to regulatory guidelines, optimizing application protocols, and considering sustainable alternatives, like organic amendments, to maintain soil health and plant vitality while enhancing fruit yield and quality [58].

12. Key PGRs currently in market

The application of PGRs to different plant parts has provided crucial insights into modern agricultural requirements and helped bridge existing market gaps. The most widely registered and highly demanded PGRs include trinexapac-ethyl (TE), chlormequat (CCC), ethephon (ETH), paclobutrazol (PBZ), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 6-benzyladenine (BA), kinetin, 1-methylcyclopropene (1-MCP), gibberellic acid (GA), thidiazuron (TDZ), Flumetralin, abscisic acid (ABA), jasmonic acid (JA), strigolactones, daminozide, maleic hydrazide (MH), prohexadione, Forchlorfenuron (FCF), salicylic acid (SA), zeatin, triacontanol, polyamines

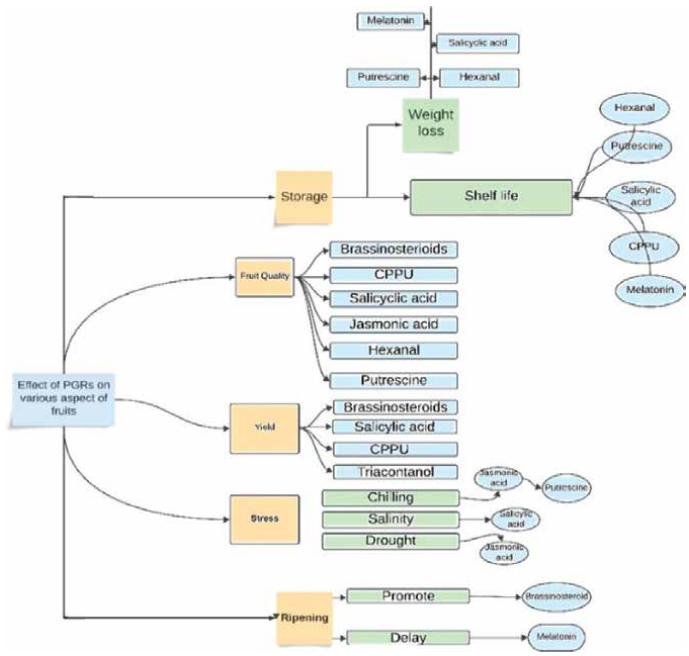

Figure 3.
Visuals presenting information about how PGRs impacts various aspects of fruit production like ripening, stress, Yield, Quality and shelf life. Further highlighting which hormone is effective on particular character.

(putrescine, spermidine, and spermine), brassinolide, and mepiquat chloride (MC). PGRs have extensive utility in fruit. Stimulating fruit growth, enhance it quality, improved fruit size and weight ultimately increasing yields. PGRs like PBZ and BA improve fruit development by promoting the cell division and elongation. Further, ETH and TDZ are responsible for promoting fruit ripening and senescence, alternatively 1-MCP is responsible for delaying fruit growth and ripening extending the fruit's shelf life over the time. Compounds like TE can increase chlorophyll content, eventually increasing photosynthetic efficiency and carbon fixation capacity of the plant. However, ABA and ETH are responsible for inducing senescence and abscission in plants that allow prioritizing the assimilate partitioning to young and growing plant parts at the required time. Under stress condition, the abscisic acid application helps stomatal closure and reduces leaf transpiration rate. **Figure 3** presents a brief idea about how different categories act on different plant parts; the application of PGRs in leaves contributes to adaptation of plants to different growth conditions and environmental stresses.

13. Conclusion

The future of PGRs in fruit crop productivity is intertwined with sustainability, technological innovation, and market dynamics. By integrating modern tools and eco-friendly practices, PGRs will not only enhance yields but also contribute to a more resilient and sustainable fruit production system.

A recently introduced term is precision agriculture technologies, and in precision agriculture, the application of PGRs will become increasingly targeted and efficient. Tools such as drones, remote sensors, and IoT devices can be used to monitor crop health and developmental stages in real time, allowing for precise, need-based PGR application. This minimizes waste, reduces environmental impact, and maximizes the effectiveness of treatments. AI-driven platforms and decision support systems are emerging as essential tools for PGR management. These systems can analyze environmental data, crop conditions, and historical trends to recommend optimal PGR application schedules, ensuring maximum productivity with minimal input.

As climate variability continues to affect fruit production, PGRs will be critical in mitigating stress caused by extreme temperatures, drought, and irregular rainfall patterns. For instance, antitranspirants and growth retardants can help crops adapt to water scarcity, while gibberellins and cytokinins can counteract temperature-induced flowering delays. Moreover, convergence of PGR use with advancements in genomics and molecular breeding offers promising opportunities. Understanding the genetic mechanisms influenced by PGRs can enable breeders to develop fruit crops that are more responsive to specific regulators, enhancing their efficiency and reducing the need for repeated applications.

Consumer preferences for high-quality fruits with superior taste, color, and nutritional value are driving the use of PGRs to enhance these attributes. Regulators like ethylene inhibitors can extend shelf life, while others like auxins and cytokinins can improve size and uniformity. PGRs will play a vital role in meeting production goals while maintaining profitability. Cost-effective and scalable solutions will drive the adoption of PGRs in both smallholder and large-scale farming operations. PGRs have immense hidden potential yet to be explored. Till now, there is lack of awareness and knowledge on using PGRs sustainably and on using PGRs at the right time, in the right amount, and through the right method, which can result in harnessing the best outputs from fruit production.

Ethylene plays a vital role in regulating blueberry fruit ripening by triggering a series of physiological and molecular changes. One of its key functions is the downregulation of photosynthesis-related genes, marking a transition from energy production to the metabolic activities essential for ripening. In addition to this, ethylene influences the complex network of phytohormone metabolism and signaling by promoting the accumulation of abscisic acid (ABA), a hormone known to accelerate ripening, while simultaneously reducing jasmonic acid (JA) levels, which are often associated with delayed maturation and defense responses. This delicate hormonal balance ensures a well-coordinated progression through various ripening stages. Rather than acting alone, ethylene interacts with multiple phytohormones, establishing itself as the central regulator of the ripening process. By orchestrating these intricate biochemical and genetic mechanisms, ethylene ultimately shapes the texture, color, and overall quality of the blueberry fruit.

Auxin, primarily synthesized in the achenes of strawberries, plays a crucial role in promoting receptacle expansion during fruit development. However, as the fruit ripens, auxin levels gradually decline, signaling the transition to maturation. In grapes, the external application of abscisic acid (ABA) after véraison enhances color development but has no significant impact on fruit firmness, pH, total soluble solids (TSS), or titratable acidity (TA). Transcriptome analysis following ABA treatment in grapes reveals its role in downregulating photosynthesis, triggering autocatalytic ABA synthesis, and stimulating pigment biosynthesis, further supporting its involvement in fruit coloration.

Similarly, ABA plays a key role in strawberry ripening by inducing the expression of genes responsible for cell wall degradation, contributing to fruit softening. In climacteric fruits such as tomatoes, ABA levels increase during ripening, preceding the climacteric surge in ethylene production. Moreover, ABA application accelerates ripening and upregulates key ethylene biosynthesis genes, including 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO). This suggests that ABA functions as an upstream regulator of ethylene, orchestrating the complex hormonal interactions that drive the ripening process across different fruit types (**Tables 4** and **5**).

Merits	Demerits
1. Regulation of Plant Growth and Development PGRs serve as essential tools for controlling plant growth, improving productivity, and enhancing the quality of horticultural crops. They regulate key physiological processes such as cell elongation, fruit setting, ripening, and stress tolerance, ultimately leading to optimized growth and development.	1. Stage- and Dose-Specific Application The effectiveness of PGRs is highly dependent on the developmental stage of the plant and the applied concentration. Incorrect timing or excessive dosage can lead to growth abnormalities, reduced yield, or even plant damage.
2. Broad-Spectrum Applications PGRs are versatile and can be applied at different growth stages for varied purposes, including: • Increasing and synchronizing flowering and fruiting • Enhancing fruit set, size, and quality • Prolonging the shelf life of harvested produce • Preventing premature fruit drop Inducing resistance to abiotic and biotic stressors such as drought, salinity, pests, and diseases	2. Technical Expertise and Precision Required The application of PGRs requires skilled personnel who can: • Accurately measure small quantities (milligrams or micrograms) • Select the appropriate solvent for dissolving the PGRs (e.g., gibberellins require ethyl alcohol) Follow strict guidelines for dosage and timing to prevent negative effects

3. Enhanced Crop Performance and Genetic Improvement PGRs play a crucial role in crop breeding, through either conventional methods or genetic engineering. By modulating hormone levels, they help in the selection of desirable traits such as improved fruit quality, better yield potential, and enhanced tolerance to adverse environmental conditions.	3. Complex Interactions and Unpredictable Outcomes The interplay between different PGRs is not fully understood, and when used in combinations, they may result in unexpected synergistic or antagonistic effects. Extensive research and trials are needed to establish the best PGR combinations for different crops.
4. Economic and Resource Efficiency The use of PGRs significantly reduces production costs by: • Decreasing labor-intensive activities like pruning and thinning • Reducing water and nutrient requirements • Enhancing disease resistance, thereby minimizing the need for chemical pesticides • Improving plant architecture for better space utilization in high-density planting systems	4. Potential Side Effects of High Doses • Auxins: Excess application can lead to excessive ethylene production, causing growth inhibition, leaf abscission, and plant death. • Gibberellins: Overuse can lead to excessive elongation of seedlings, causing weak and leggy plants. • Cytokinins: High concentrations may cause excessive callusing in tissue cultures, leading to poor shoot initiation, reduced root formation, and abnormal plant development. • Ethylene: Overexposure can accelerate senescence, cause leaf yellowing, promote abscission, and induce epinasty (stem bending). • Abscisic acid (ABA): Excessive ABA can delay seed germination, reduce stomatal conductance, and negatively affect photosynthesis.
5. Optimized Growth Control PGRs help greenhouse and nursery growers achieve controlled plant growth by applying low-dose treatments to regulate height, branching, and overall canopy structure. This enables efficient space management and reduces overcrowding in controlled environments.	5. Environmental and Residue Concerns Some synthetic PGRs may leave residues in fruits and vegetables, raising concerns about food safety. Additionally, their application in open fields may lead to unintended environmental impacts, such as changes in soil microbiota or effects on non-target plants.
6. Commercial Utility in Horticulture PGRs have diverse commercial applications in fruit crop production, including: • Flowering initiation: Ethylene application in pineapple • Parthenocarpy induction: GA application in apple, pear, peach, apricot, almond, fig, and grape • Fruit set enhancement: Applied in strawberry, peach, plum, and cherry • Fruit thinning: Helps manage fruit load for better quality and uniformity • Improvement of post-harvest quality: Delaying senescence and extending shelf life • Stress tolerance induction: Enhancing plant survival under drought, heat, and cold conditions	6. Limited Research and Crop-Specific Understanding Despite their potential, the use of PGRs in horticulture still lacks a comprehensive understanding. Further research is needed to refine their application in different crops, optimize their use under varying climatic conditions, and develop environmentally friendly alternatives.

Table 4.
Merits and de- merits of using plant growth regulators.

PGR Used	Crop	Part of plant applied	Type of application	Effect	Region of research	References
Brassinosteroid	Mango	Fruit	Post-harvest	Improved color and Softened fruit	Australia	[59]
	Strawberry	Leave, fruit	Foliar application	Early ripening	Spain	[60]
		Fruits	Injection	Fruit ripening	Brazil	[61]
		Fruits	Spray	Enhanced cell division during early fruit growth stages	China	[62]
	Papaya	Fruits	Spray	Increased senescence	Brazil	[63]
	Grapes	Berries	Foliar application	Enhanced ripening	USA	[64]
	Sweet cherry	Vegetative parts	Spray	High TA and TSS, increased firmness, improved anthocyanins, peel color, ascorbic acid, organic acids, increased shelf life	Iran	[65]
		Vegetative parts	Spray	Early maturity, improved peel color, and firm fruit	USA	[66]
	Litchi	Leaves and fruits	Spray	Increased firmness and reduced fruit drop	China	[67]
		Leaves and fruits	Twice (before and 15 days after anthesis)	Reduced cracking and improved physiochemical characters	India	[68]
	Apple	Fruit	Preharvest	Improved L:D ratio weight, fruit firmness, total sugars, and color	Egypt	[69]
Hexanal	Guava	Leaves fruits	Preharvest	Reduced decay, improved firmness, pectin and phenol content	India	[70]
	Apple	Foliar and fruit	Fruit set and Preharvest	Reduce bitter pit, enhance post-harvest storage	—	[71]

PGR Used	Crop	Part of plant applied	Type of application	Effect	Region of research	References
Triacontanol	Strawberry	Leaves	Spray	Improved fruit set	India	[41]
Melatonin	Pomegranate	Fruits	Exogenous application	Improve color and anthocyanin content	Iran	[72]
Putrescine	Peach	Leaves and fruits	Foliar application	Reduced chilling injury, improved fruit quality, and improved shelf life	Pakistan	[73]
Jasmonic acid	Banana	Fruits	Exogenous application	Increased chilling tolerance	China	[35]
	Peach	Leaves		Improve drought tolerance	China	[74]
		Fruit		Improve sugar content	China	[75]
	Apple	Branches	Spray	Improved low temperature tolerance	Japan	[76]
	Pomegranate	Fruits	Exogenous application	Reduced chilling injury	Spain	[37]
Salicylic acid	Strawberry	Fruit and leaves	Foliar application	Improved growth and fruit yield	Egypt	[38]
	Guava	Branch, fruit	Spray	Increase fruit set and yield	India	[77]
	Banana	Fruit	Post-harvest application	Reduced chilling injury and preserve quality during cold storage	Iran	[78]
	Citrus	Fruit	Soaked	Reduced decay and weight loss, preserved TSS acid content, and firmness	Egypt	[39]
	Peach	Fruit	Dipped	Reduced weight loss, increasing flesh stiffness, higher TA	Pakistan	[79]
CPPU	Pomegranate	Leaves and fruits	Spray	Improved TSS and total sugar content	India	[80]
	Guava	Vegetative parts and fruits	Spray	Improve TSS, ascorbic acid and total acid content, fruit firmness	Iran	[81]
	Kiwi	Fruit	Dipped	Increased fruit weight, quality, and yield	India	[82]

Table 5.
Uses of different plant growth regulators being used for direct or indirect improvement of fruit quality.

Acknowledgements

The authors acknowledge the usage of ChatGPT for language polishing of the manuscript.

Conflict of interest

On behalf of all the authors, the corresponding author declares no conflict of interest.

Author details

Shiv Kumar Shivandu[1*], Damini Singh[2], Gaurav Kumar[3], Ishani Sharma[1] and Jayesh Garg[4]

1 Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni Solan, India

2 Indian Agriculture Research Institute, New Delhi, India

3 Career Point University, Hamirpur, India

4 RIMT University Sirhind Side, Mandi Gobindgarh, Punjab, India

*Address all correspondence to: marginshiv05@gmail.com

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. (cc) BY

References

[1] Jameson PE, Song J. Cytokinin: A key driver of seed yield. Journal of Experimental Botany. 2016;**67**(3):593-606. DOI: 10.1093/jxb/erv461

[2] Kinet J, Lejeune P, Bernier G. Shoot-root interactions during floral transition: A possible role for cytokinins. Environmental and Experimental Botany. 1993;**33**:459-469. DOI: 10.1016/0098-8472(93)90019-C

[3] Kumar R, Khurana A, Sharma AK. Role of plant hormones and their interplay in development and ripening of fleshy fruits. Journal of Experimental Botany. 2013;**65**:4561-4575. DOI: 10.1093/jxb/eru277

[4] Kunikowska A, Byczkowska A, Doniak M, Kaźmierczak A. Cytokinins résumé: Their signaling and role in programmed cell death in plants. Plant Cell Reports. 2013;**32**:771-780. DOI: 10.1007/s00299-013-1436

[5] Schmülling T. New insights into the functions of cytokinins in plant development. Journal of Plant Growth Regulation. 2002;**21**:40-49. DOI: 10.1007/s003440010046

[6] Šmehilová M, Spíchal L. The Plant Plasma Membrane. Vol. 22. Berlin, Germany: Springer Science and Business Media LLC; 2013. The biotechnological potential of cytokinin status manipulation; pp. 103-130

[7] Rademacher W. Plant growth regulators: Backgrounds and uses in plant production. Journal of Plant Growth Regulation. 2015;**34**:845-872. DOI: 10.1007/s00344-015-9541-6

[8] Khalil A, Banday FA, Bhat MY, Nazir N. Crop Regulation through Growth Regulators in Temperate Fruit Crops. Banday: FA and Sharma, MK Advances in Temperate Fruit Production; 2010. pp. 177-186

[9] Aremu AO, Fawole OA, Makunga NP, Masondo NA, Moyo M, Buthelezi NM, et al. Applications of cytokinins in horticultural fruit crops: Trends and future prospects. Biomolecules. 2020;**10**(9):1222. DOI: 10.3390/biom10091222

[10] Chaudhary M, Singh DP, Singh JP, Mishra KK. Effect of foliar application of PGRs and mineral nutrients on fruiting behaviour, growth and yield of Aonla CV. NA-7. Journal of Pharmacognosy and Phytochemistry. 2018;**7**(4):2714-2718

[11] Parmar N, Singh KH, Sharma D, Singh L, Kumar P, Nanjundan J, et al. Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: A comprehensive review. 3 Biotech. 2017;**7**:1-35

[12] Velasquez SM, Barbez E, Kleine-Vehn J, Estevez JM. Auxin and cellular elongation. Plant Physiology. 2016;**170**:1206-1215

[13] Wang F, Cui X, Sun Y, Dong CH. Ethylene signaling and regulation in plant growth and stress responses. Plant Cell Reports. 2013;**32**:1099-1109

[14] Jones RL, Kaufman PB. The role of gibberellins in plant cell elongation. Critical Reviews in Plant Sciences. 1983;**1**:23-47

[15] Chen S-Y, Chien C-T, Chung J-D, Yang Y-S, Kuo S-R. Dormancy-break and germination in seeds of *Prunus campanulata* (Rosaceae): Role of covering

layers and changes in concentration of abscisic acid and gibberellins. Seed Science Research. 2007;**17**(1):21-32

[16] Lurie S. Manipulating fruit development and storage quality using growth regulators. In: Plant Growth Regulators in Agriculture and Horticulture. 1st ed. CRC Press; 2024. pp. 175-196. ISBN:9781003578505

[17] Guerriero R, Loreti F, Vitagliano C. The effects of growth regulators on peach flowering date. Revista della ortoflorofruitticoltura Italiana. 1970;**54**:580-596

[18] Maksymiuk J, Grochowska JM, Knzewinska D. Effect of successively applied four growth regulators on shoot elongation, flowering and fruiting of apple cu1tivar Fantazia. Fruit Science Reports. 1986;**13**(4):149-160

[19] Mclaughin JM, Greene DU. Fruit and hormones influence flowering of apple. II. Effect of hormones. Journal of American society for Horticultural Science. 1991;**116**:450-453

[20] Moradi S, Baninasab B, Gholami M, Ghobadi C. Paclobutrazol application enhances antioxidant enzyme activities in pomegranate plants affected by cold stress. The Journal of Horticultural Science and Biotechnology. 2017;**92**(1):65-71. DOI: 10.1080/14620316.2016.1224605

[21] Yadav S, Singh JP, Gupta S, Yadav JS. A study on foliar feeding of GA3 and NAA on fruit drop, retention, yield and quality of Ber fruit (*Ziziphus mauritiana Lamk*.) cv. "Banarasi karaka". Biological Forum–An International Journal. 2021;**13**(3):608-612

[22] Cheng C, Xu X, Singer SD, Li J, Zhang H, Gao M, et al. Effect of GA3 treatment on seed development and seed-related gene expression in grape. PLoS One. 2013;**8**(11):e80044

[23] Ramteke V, Paithankar DH, Baghel MM, Kurrey VK. Impact of GA3 and propagation media on growth rate and leaf chlorophyll content of papaya seedlings. Research Journal of Agricultural Sciences. 2016;**7**(1):169-171

[24] Khandaker MM, Majrashi A, Boyce AN. The influence of gibberellic acid on the chlorophyll fluorescence, protein content and PAL activity of wax apple ('Syzygium samarangense'var. jambu madu) fruits. Australian Journal of Crop Science (Lismore, N.S.W.: Southern Cross Publishers). Dec 2015;**9**(12):1221-1227. ISSN (print): 1835-2693

[25] Tian MS, Prakash S, Elgar HJ, Young H, Burmeister DM, Ross GS. Responses of strawberry fruit to 1-methylcyclopropene (1-MCP) and ethylene. Plant Growth Regulation. 2000;**32**:83-90

[26] Ozga JA, Reinecke DM. Hormonal interactions in fruit development. Journal of Plant Growth Regulation. 2003;**22**:73-81

[27] Hu B, Sun DW, Pu H, Wei Q. Recent advances in detecting and regulating ethylene concentrations for shelf-life extension and maturity control of fruit: A review. Trends in Food Science and Technology. 2019;**91**:66-82. DOI: 10.1016/j.tifs.2019.06.010

[28] Verma V, Ravindran P, Kumar PP. Plant hormone-mediated regulation of stress responses. BMC Plant Biology. 2016;**16**:1-0

[29] Sha J, Wang F, Chen Q, Jia Z, Du X, Ge S, et al. Characteristics of photoassimilates transportation and distribution to the fruits from leaves at different branch positions in apple. Journal of Plant Growth Regulation. 2021;**40**:1222-1232

[30] Müller M, Munné-Bosch S. Hormonal impact on photosynthesis and photoprotection in plants. Plant Physiology. 2021;**185**(4):1500-1522. DOI: 10.1093/plphys/kiaa119

[31] Balestrini R, Chitarra W, Antoniou C, Ruocco M, Fotopoulos V. Improvement of plant performance under water deficit with the employment of biological and chemical priming agents. The Journal of Agricultural Science. 2018;**156**(5):680-688. DOI: 10.1017/S0021859618000126

[32] Goulao LF, Oliveira CM. Cell wall modifications during fruit ripening: When a fruit is not the fruit. Trends in Food Science and Technology. 2008;**19**(1):4-25. DOI: 10.1016/j.tifs.2007.07.002

[33] Parwez R, Aftab T, Gill SS, Naeem M. Abscisic acid signaling and crosstalk with phytohormones in regulation of environmental stress responses. Environmental and Experimental Botany. 2022;**199**:104885. DOI: 10.1016/j.envexpbot.2022.104885

[34] Müller M. Foes or friends: ABA and ethylene interaction under abiotic stress. Plants. 2021;**10**(3):448. DOI: 10.3390/plants10030448

[35] Zhao Y, Song C, Brummell DA, Qi S, Lin Q, Duan Y. Jasmonic acid treatment alleviates chilling injury in peach fruit by promoting sugar and ethylene metabolism. Food Chemistry. 2021;**338**:128005

[36] Shargal AS, Golobovich Z, Yablovich LA, Shlizerman RA, Stern G, Grafi S, et al. Synthetic cytokinins extend the phase of division of parenchyma cells in developing pear (*Pyrus communis* L.) fruits. The Journal of Horticultural Science and Biotechnology. 2006;**5**:915-920

[37] Sayyari M, Babalar M, Kalantari S, Martínez-Romero D, Guillén F, Serrano M, et al. Vapour treatments with methyl salicylate or methyl jasmonate alleviated chilling injury and enhanced antioxidant potential during postharvest storage of pomegranates. Food Chemistry. 2011;**124**(3):964-970

[38] Mohamed RA, Abdelbaset AK, Abd-Elkader DY. Salicylic acid effects on growth, yield, and fruit quality of strawberry cultivars. Journal of Medicinally Active Plants. 2018;**6**(1-4):1-11

[39] Ennab HA, El-Shemy MA, Alam-Eldein SM. Salicylic acid and putrescine to reduce post-harvest storage problems and maintain quality of murcott mandarin fruit. Agronomy. 2020;**10**(1):115

[40] Ha NT, Pham TT, Mac HX, Nguyen LL, Horváth-Mezofi Z, Göb M, et al. Evaluation of the effect of 1-MCP treatment on flesh and surface color of selected European and Asian plum fruit using machine vision. Horticulturae. 2023;**9**(3):341

[41] Baba TR, Ali A, Kumar A, Husain M. Effect of exogenous application of salicylic acid and triacontanol on growth characters and yield of strawberry. The Pharma Innovation Journal. 2017;**6**(11):274-279

[42] Li D, Yang J, Dai Z, Chen Y, Shao Z, Wang C, et al. Prohexadione-calcium improves grape quality by regulating endogenous hormones, sugar and acid metabolism and related enzyme activities in grape berries. BMC Plant Biology. 19 Feb 2024;**24**(1):122

[43] Wani AW, Hassan GI, Dar SQ, Baba TA, Dar MI, Amir S, et al. Influence of different phytohormones and nutrients on fruit set and chemometric attributes of apple. Vegetos. 2017;**30**(2):492-498

[44] Vogel AR, Phillips B, Clavet CD, Kon TM. Leader bagging improves lateral branching and cropping potential of 'Gala'and 'WA 38'Apple during orchard establishment. HortScience. 2024;**59**(6):833-839. DOI: 10.21273/HORTSCI17480-23

[45] Singh G, Saha P, Naveen VS, Singh J, Singh J. Systems of crop regulation in guava and their assessment. International Journal of Advanced Biochemistry Research. 2024;**8**(3):877-885. DOI: 10.33545/26174693.2024.v8.i3j.942

[46] Girardi F, Canton M, Populin F, Tijero V, Bettio G, Munné-Bosch S, et al. A gibberellin-assisted study of the transcriptional and hormonal changes occurring at floral transition in peach buds (*Prunus persica L. Batsch*). BMC Plant Biology. 2024;**24**(1):643. DOI: 10.1186/s12870-024-05360-6

[47] Sanaullah M. Controlling stress responses In fruit crops through the influence of plant hormones. Journal of Physical, Biomedical and Biological Sciences. 2024;**2024**(1):18

[48] El-Boray MS, Shalan AM, Ghany AE, Rizk MM. Employing leaf removal and spraying Gibberellic acid (GA3) to reduce cluster compactness coefficient and enhance chemical and physical properties of Thompson seedless H4 grapevines. Journal of Plant Production. 2024;**15**(8):417-429. DOI: 10.21608/jpp.2024.295659.1348

[49] Osama HM, Amro E, Saber MM. Effect of growth regulator, antioxidant and application date on fruiting and fruit quality of mango trees cv. Keitt. IOSR Journal of Agriculture and Veterinary Science. 2015;**8**(12):87-95

[50] Singh PI, Tripathi VK, Pandey S. Effect of Pre-Harvest Application of Plant Bio-Regulators and Micro-Nutrient on Fruit Retention, Yield and Quality of Ber2024. DOI: 10.5958/0976-4615.2024.00001.5

[51] Li X, Liu P, Zhou J, Su M, Ma Y, Jia H, et al. Effects of exogenous application of GA 4+ 7 and NAA on sugar accumulation and related gene expression in peach fruits during developing and ripening stages. Journal of Plant Growth Regulation. 2021;**40**:962-973

[52] Hamdy AE. Effect of GA3 and NAA on growth, yield and fruit quality of Washington navel orange. Egyptian Journal of Horticulture. 2017;**44**(1):33-43

[53] Saleem K, Asghar MA, Raza A, Pan K, Ullah A, Javed HH, et al. Alleviating drought stress in strawberry plants: Unraveling the role of paclobutrazol as a growth regulator and reducer of oxidative stress induced by reactive oxygen and carbonyl species. Journal of Plant Growth Regulation. 2024;**43**(9):3238-3253

[54] Srilatha V, Reddy YT. Pruning and paclobutrazol induced flowering and changes in phenols and flavonoids of mango (*Mangifera indica L.*) cv. Raspuri. Applied Sciences. 2015;**4**:43-47

[55] Rahman MH, Rahman MH, Halder BC, Ahmed M, Nishi NJ. Applying Paclobutrazol and flower bud pruning modify the fruiting time and fruit quality of 'Amrapali' Mango (*Mangifera indica L.*). The Horticulture Journal. 2023;**92**(3):255-260. DOI: 10.2503/hortj.QH-061

[56] Martínez-Romero D, Bailén G, Serrano M, Guillén F, Valverde JM, Zapata P, et al. Tools to maintain postharvest fruit and vegetable quality through the inhibition of ethylene action: A review. Critical Reviews in Food Science and Nutrition. 2007;**47**(6):543-560. DOI: 10.3390/horticulturae9030341

[57] Desta B, Amare G. Paclobutrazol as a plant growth regulator. Chemical and Biological Technologies in Agriculture. 2021;**8**:1-5. DOI: 10.1186/s40538-020-00199-z

[58] Yahia EM, Gardea-Béjar A, de Jesús O-PJ, Maya-Meraz IO, Rodríguez-Roque MJ, Rios-Velasco C, et al. Preharvest factors affecting postharvest quality. In: Postharvest Technology of Perishable Horticultural Commodities. Woodhead Publishing, e book by Elsevier; 2019. pp. 99-128. DOI: 10.1016/B978-0-12-813276-0.00004-3. ISBN-978-0-12-813276-0

[59] Zaharah SS, Singh Z, Symons GM, Reid JB. Role of brassinosteroids, ethylene, abscisic acid, and indole-3-acetic acid in mango fruit ripening. Journal of Plant Growth Regulation. 2012;**31**:363-372

[60] Bombarely A, Merchante C, Csukasi F, Cruz-Rus E, Caballero JL, Medina-Escobar N, et al. Generation and analysis of ESTs from strawberry (*Fragaria xananassa*) fruits and evaluation of their utility in genetic and molecular studies. BMC Genomics. 2010;**11**:1-7. Available from: http://www.biomedcentral.com/1471-2164/11/503

[61] Ayub RA, Reis L, Bosetto L, Lopes PZ, Galvão CW, Etto RM. Brassinosteroid plays a role on pink stage for receptor and transcription factors involved in strawberry fruit ripening. Plant Growth Regulation. 2018;**84**:159-167

[62] Chai YM, Zhang Q, Tian L, Li CL, Xing Y, Qin L, et al. Brassinosteroid is involved in strawberry fruit ripening. Plant Growth Regulation. 2013;**69**:63-69. DOI: 10.1007/s10725-012-9747-6

[63] Gomes MD, Torres Netto A, Campostrini E, Bressan-Smith R, Zullo MA, Ferraz TM, et al. Brassinosteroid analogue affects the senescence in two papaya genotypes submitted to drought stress. Theoretical and Experimental Plant Physiology. 2013;**25**:186-195

[64] Lisso J, Altmann T, Müssig C. Metabolic changes in fruits of the tomato dx mutant. Phytochemistry. 2006;**67**(20):2232-2238. DOI: 10.1016/j.phytochem.2006.07.008

[65] Roghabadi MA, Pakkish Z. Role of brassinosteroid on yield, fruit quality and postharvest storage of 'Tak Danehe Mashhad' sweet cherry (*Prunus avium* L.). Agricultural Communications (Loughborough, UK: Eram Pars Scientific Publication Ltd). 2014;**2**(4):49-56. Available from: http://www.agricommun.com/content/archive%20volumes/volume%202_4/Agri.%20Commun.%202_4_7.pdf. ISSN (Print): 2054-0868, ISSN (Electronic): 2054-0922

[66] Mandava B, Wang Y. Effect of brassinosteroids on cherry maturation, firmness and fruit quality. In: III Balkan Symposium on Fruit Growing. Vol. 1139. 2015. pp. 451-458

[67] Peng J, Tang X, Feng H. Effects of brassinolide on the physiological properties of litchi pericarp (*Litchi chinensis* cv. Nuomoci). Scientia horticulturae. 2004;**101**(4):407-416

[68] Ghosh T, Panja P, Sau S, Datta P. Role of brassinolide in fruit growth, development, quality and cracking of litchi cv. Bombai grown in new alluvial zone of West Bengal. International Journal of Bio-resource and Stress Management. 2022;**13**(May, 5):507-512

[69] Attia SM, Adss IA. Effect of preharvest applied brassinosteroid on "Anna" apple fruit retention, coloration and quality. Bioscience Research. 2021;**18**(2):1416-1425

[70] Gill KS, Dhaliwal HS, Mahajan BV, Paliyath G, Boora RS. Enhancing postharvest shelf life and quality of guava (*Psidium guajava L.*) cv. Allahabad Safeda by pre-harvest application of hexanal containing aqueous formulation. Postharvest Biology and Technology. 2016;**112**:224-232

[71] Sriskantharajah K, El Kayal W, Torkamaneh D, Ayyanath MM, Saxena PK, Sullivan AJ, et al. Transcriptomics of improved fruit retention by hexanal in 'Honeycrisp'reveals hormonal crosstalk and reduced cell wall degradation in the fruit abscission zone. International Journal of Molecular Sciences. 2021;**22**(16):8830

[72] Aghdam MS, Luo Z, Li L, Jannatizadeh A, Fard JR, Pirzad F. Melatonin treatment maintains nutraceutical properties of pomegranate fruits during cold storage. Food Chemistry. 2020;**303**:125385

[73] Abbasi NA, Ali I, Hafiz IA, Alenazi MM, Shafiq M. Effects of putrescine application on peach fruit during storage. Sustainability. 2019;**11**:2013

[74] Zhang B, Du H, Sun M, Wu X, Li Y, Wang Z, et al. Stress in peach (Prunus persica). Abiotic stress mechanisms and enhancement in crops: Physiological and biochemical approaches. 2024;**49**:49-62

[75] Zhao ML, Wang JN, Shan WE, Fan JG, Kuang JF, Wu KQ, et al. Induction of jasmonate signalling regulators MaMYC2s and their physical interactions with MaICE1 in methyl jasmonate-induced chilling tolerance in banana fruit. Plant, Cell and Environment. 2013;**36**(1):30-51

[76] Yoshikawa H, Honda C, Kondo S. Effect of low-temperature stress on abscisic acid, jasmonates, and polyamines in apples. Plant Growth Regulation. 2007;**52**:199-206

[77] Bindhyachal Ram BR, Mandal BK, Udit Kumar UK, Ravindra Kumar RK, Kumar P. Response of Guava to Boron and growth regulators spray. Asian Journal of Horticulture (Muzaffarnagar, India: Hind Agri-Horticultural Society). 2016;**11**(1):146-150. Available from: http://www.researchjournal.co.in/online/AJH.htm. ISSN (Print): 0973-4767, ISSN (Electronic): 0976-724X

[78] Khademi O, Ashtari M, Razavi F. Effects of salicylic acid and ultrasound treatments on chilling injury control and quality preservation in banana fruit during cold storage. Scientia Horticulturae. 2019;**249**:334-339

[79] Tareen MJ, Abbasi NA, Hafiz IA. Effect of salicylic acid treatments on storage life of peach fruits cv. 'Flordaking'. Pakistan Journal of Botany (Pakistan: Department of Horticulture, PMAS-Arid Agriculture University Rawalpindi). 2012;**44**(1):119-124

[80] Khemnar AS, Pujari KH, Relekar PP, Bhattacharya T. Effect of CPPU (Forchlorofenuron) with GA3 (Gibberellic acid) on fruit set and post-harvest quality of 'Bhagwa' pomegranate (Punica granatum). In: IV International Symposium on Pomegranate and Minor Mediterranean Fruits. Vol. 1254. 2017. pp. 179-184

[81] Sabaghnia Z, NAhANDI FZ. Effects of Forchlorofenuron (CPPU) treatment on fruit properties in the fruit of common guava. In: Annales Universitatis Mariae Curie-Sklodowska, Sectio C–Biologia. Vol. 72, No. 2. 2019. pp. 7-14

[82] Pramanick KK, Kashyap P, Kishore DK, Shajtma YP. Effect of summer pruning and CPPU on yield and quality of kiwi fruit (*Actinidia deliciosa*). Journal of Environmental Biology. 2015;**36**(2):351

Section 2

Tropical and Subtropical Fruit Crops

Chapter 4

Plant Growth Regulators and Biostimulants as Alternatives for Managing Flowering and Fruiting of Mandarins

Sabrina Raquel Griebeler, Mateus Pereira Gonzatto, Júlia Scherer Santos, Gerson Nestor Boettcher and Sergio Francisco Schwarz

Abstract

The chapter explores the use of plant growth regulators (PGRs) and biostimulants to manage mandarins flowering and fruit set in mandarins, addressing challenges such as alternate bearing, where irregular fruit production occurs between harvests. PGRs, such as gibberellins and auxins, are used to regulate fruit set, fruit size and quality, while mitigating alternate production by thinning fruit or inhibiting excessive flowering. Biostimulants, including humic substances and coumarins, have the potential to be used in the management of citrus flowering and fruit set, although their exact modes of action are still partially understood. Humic substances, derived from organic matter, have growth-regulating properties similar to those of PGRs, while coumarins, secondary metabolites, have antioxidant and gibberellin-like effects. The study highlights the importance of timing and methods of application of PGRs and biostimulants to optimize fruit production and quality, particularly in citrus cultivars with a tendency to alternate production. Integrating these tools offers a sustainable approach to improving citrus productivity while addressing environmental and agricultural challenges.

Keywords: citrus, fruit set, humic substances, fulvic acids, humic acids, coumarins

1. Introduction

Alternate bearing is one of the major problems in citrus production around the world, especially with mandarin cultivars. Alternate bearing is defined as an irregular production between successive harvests, which leads to the need for thinning of fruit in years with a high fruit load. The use of plant growth regulators is employed as a chemical thinning, and they can also be used to inhibit flower induction, helping to mitigate alternate bearing. Therefore, the use of PGRs in citrus culture is an alternative for improving yield *via* modifying flowering and fruiting and consequently

mitigating the alternate bearing. Plant growth regulators actively modulate growth and development by controlling endogenous processes and altering the growth response.

Different plant growth regulators are used to accelerate maturity, delay harvest and maintain fruit quality after harvest [1]. The exogenous application of growth regulators can be used to improve fruit set. In addition, plant growth regulators are often used to increase fruit size, either directly, by stimulating cell division, or indirectly, by reducing the number of fruits through inhibiting flower development or inducing flower or fruit abscission [2, 3].

Agricultural expectations have changed, and farmers are expected to produce safer food while protecting biodiversity and soil, and maintaining air and water quality [4]. One of the most promising solutions to achieve these goals is the use of plant biostimulants, substances that promote plant growth, nutrition and metabolism through complex modes of action that are not yet fully understood [5]. In 2012, Europe became a leading market for biostimulants [6, 7]. Currently, most market analysts report that the European biostimulant market represents around half of the global market. Estimates of the value of the European biostimulant market ranged from 1.5 to 2 million dollars in 2022 [8]. Due to their composition, biostimulants can have analogous effects to plant growth regulators, with activities similar to gibberellins (gibberellin-like) or auxins (auxin-like).

2. Floral induction and differentiation

The period and intensity of flowering depend on the citrus species, the age of the plant and the weather conditions. The flower load can reach up to 250,000 flowers per tree, although generally less than 1% become mature fruit [3].

Flower induction is the initial signaling for flowering, which involves interaction with environmental conditions and biochemical signals that make meristematic cells competent to form specific structures for reproduction. Differentiation involves the anatomical and morphological transition from the vegetative meristem to the floral meristem [2, 9].

The environmental factors promoting flower induction are mainly low temperatures and water stress. The induction occurs three to five months before flowering [10] and the increase in flowering is stimulated when citrus plants are subjected to a temperature range of 10 to 15°C [11]. However, temperatures below 20°C and above 5°C are also effective in inducing flower buds [12, 13]. Nishikawa et al. [14] demonstrated that low temperatures induce the expression of the homolog of one of the main genes responsible for flower induction, Citrus FLOWERING LOCUS T (CiFT2). On the other hand, water stress is the most important factor for flower induction in tropical zones, since in these areas there is a dry season [15, 16]. According to Borroto et al. [17], the longer the water deficit for the plant, the greater the flowering when water returns to be supplied to the plant.

The first signs of floral differentiation are observed in December in the northern hemisphere. This activity is characterized by a flattening of the dome in the apical meristem [18]. Although at this time the meristem is not yet in an irreversible flower formation, floral dedifferentiation may occur [19]. Differentiation beginning can vary from year to year and from species to species. For instance, mandarin trees and their hybrids begin differentiation slightly later than orange trees [20]. In conditions of southern Brazil, flower induction for the 'Montenegrin' mandarin tree

would occur in July [21]. In other reports, the flower differentiation begins in July and can be extended into August, depending on climatic conditions [22].

Moreover, the presence of fruit can inhibit flowering due to the repression of CiFT2. This repression is caused by the expression of the CcMADS19 gene [23]. Then, in order to differentiate into flowers, meristems need to overcome endogenous and exogenous restrictions in order to express their reproductive potential [24].

The influence of high fruit load on flower induction is direct and time-dependent. The longer the fruit remains on the plants, the less flowering there will be in the following season. This explains why early cultivars have a higher flower return, as their fruit is harvested before the flower induction period [25]. Branches of 'Moncada' mandarin, where all the fruit was removed in phase II of development, showed a greater number of flowers (142 flowers per 100 nodes) in the next cycle compared to branches where all the fruit was kept until maturity (< 10 flowers per 100 nodes), thus inhibiting the expression of the homologous CiFT and SOC1 genes [26]. CiFT and SOC1 are two of the three main genes responsible for integrating flowering-inducing stimuli [27].

The presence of fruit near the vegetative apices inhibits the expression of reproductive shoots in lateral buds [2, 28]. Valiente and Albrigo [9] reported that the presence of fruit reduced flowering in orange trees (*Citrus sinensis* (L.) Osbeck), regardless of the cultivar ('Valencia' or 'Hamlin') or the number of hours the citrus plants were subjected to flower induction temperatures.

Exogenous stimuli alone are not enough for promoting the transition from buds to floral meristems, while endogenous stimuli are also necessary. Endogenous stimuli are related to exogenous factors, and therefore, there is a combination of endogenous and exogenous factors. Additionally, there is the assumption that citrus can be induced autonomously, regardless of the occurrence of induction environmental conditions. This assumption is due to flowering in regions where there are no inductive environmental conditions [29].

The fruits on 'Murcott' tangor tree (*Citrus reticulata* Blanco × *C. sinensis*) (SHALOM et al., 2012), the 'Moncada' mandarin tree (*Citrus clementina* Hort. ex. Tan. × (*Citrus unshiu* Marc. × *Citrus nobilis* Loureiro) [26] and the 'Hinosayaka', 'Haraguchi' and 'Aoshima' mandarin trees (*C. unshiu*) [30] suppress the expression of Citrus FLOWERING LOCUS T (CiFT2—flowering promoter) when there is a high fruit load during the floral induction period. Nevertheless, this suppression is limited to tissues close to where the fruit is present [23]. Besides the fruit load, the expression of the CiFT2 gene is associated with harvest time. The longer the fruit remains on the plant, the lower the gene expression [26].

3. Fruiting

Fruiting, or fruit setting, is the transition from the ovary of the fully developed flower to the growing fruit. On the other hand, effective fruit set accounts for the number of fruits that remain on the plant regarding the initial number of flowers after the abscissions that may occur during the initial stages of fruit development [31]. Despite citrus trees flowering abundantly, they also have high flower and fruit abscission, with effective fruit set ranging from 0.1 to 10% [31, 32]. Effective fruit set in citrus plants is highly dependent on the type of flower bud. In general, flower buds without leaves have a low probability of setting fruit. Diversely, flower shoots with leaves have a higher probability of setting fruit, either those with terminal flowers or shoots with flowers interspersed with leaves [32, 33].

In alternate sweet orange cultivars, when flowering is less intense (< 20 flowers per 100 nodes), fruit set increases with the number of flowers. Nevertheless, when there are more than 20 flowers per 100 nodes, fruiting becomes independent of the intensity of flowering [31]. This effect was also observed for 'Montenegrina' in southern Brazil, although the value at which effective fruit set becomes independent of flowering intensity was higher, at around 40 flowers 100 nodes^{-1} [34].

Cultivars with seeds set fruit more easily and generally in excess [31]. Conversely, parthenocarpic cultivars have fruit set linked to the endogenous amount of gibberellins in the ovary walls, which are synthesized at anthesis and trigger ovary cell division. Clementines (*C. clementina*) are facultatively parthenocarpic and generally have low fruit set. Mandarins of the Satsuma group (*C. unshiu*), on the other hand, have high fruit set and are often obligate parthenocarpic. Clementines had lower gibberellin concentrations in the ovaries compared to Satsumas; were these gibberellin contents related to the type of parthenocarpy [35, 36]. Then, the hormonal stimulus provided by gibberellins to promote fruit set is important to stimulate fruit cell division [37], leading to a greater consumption of carbohydrates and thus fruit growth. Consequently, the likelihood of fruit setting is increased [38].

Abscisic acid (ABA) is a development inhibitor, activating fruit abscission. Increasing the fruit concentration of ABA causes changes in the concentration of gibberellins, reducing their content in the fruit. Exogenous applications of ABA associated with ethylene also promoted fruit abscission, as ethylene is widely recognized as a fruit abscission promoter [31, 39]. Endogenous activation of ABA can occur during water deficit, and this stress has been shown to negatively affect fruit set [31, 39]. In addition to water deficit, exposure to waterlogging and low oxygen content in the soil reduced effective fruit set in 'Satsuma', which is related to reduced root function and starch accumulation in leaves [40]. High temperatures or sudden changes in temperature can also promote increases in fruit abscission [31]. Application of indoleacetic acid (IAA) to non-pollinated 'Pineapple' orange tree ovaries increased fruit set to the same levels as pollinated ovaries. This process occurs by activating the gene expression of gibberellin and by inhibiting its catabolism [41].

Further, applications of gibberellins have also been shown to be effective in setting fruit if there is a lack of reproductive stimulus. Gibberellins increase the fruit's drainage force, promoting greater fruit set [38, 42]. Koller et al. [43] showed an increase in productivity of 'Monte Parnaso' navel orange when ring incision of branches at the end of petal fall or at the end of natural fruit fall was employed. This is an interesting approach for those oranges, as they have high fruit abscission. The improved productivity may be related to the increase in cell division and to better fruit nutrition promoted by the application of GA_3 and/or the ringing of branches.

Another suitable plant growth regulator for increasing fruit set is benzyladenine. The application of this plant growth regulator at anthesis also increases fruit set in 'W. Murcott's (*C. reticulata* × *C. sinensis*) fruit set [44]. In addition to the application of plant growth regulators, fruit set can also be promoted by ringing branches from anthesis until natural fruit fall, as this promotes greater availability of carbohydrates for fruit set [38].

4. Alternate bearing

Irregular production between successive harvests, named alternate bearing, is characterized by excessive fruit production in one year (*on year*), followed by

another year with very low or no production (*off year*). In years of excessive flowering, the fruits are of low quality, small, poorly colored, watery and acidic. As a result of excessive fruiting, the plant becomes exhausted, presenting deficiencies in some mineral nutrients and a lower content of carbohydrates and reserve substances. Severe stress prevents flowering in the following year, resulting only in the emission of vegetative shoots and the accumulation of reserves for later intense fruiting [43]. In general, tangerine trees and their hybrids have alternation, especially seeded and late-ripening cultivars. Pomelo, lemon and seedless orange and tangerine cultivars generally present little alternation of production [31]. In **Figure 1a**, the 'Montenegrina' mandarin (*Citrus deliciosa* Tenore), a cultivar with a high tendency to alternate bearing, can be seen in an *on* and *off year*. An example of trees of the same cultivar that have undergone manual fruit thinning and show balanced production is presented in **Figure 1b**.

Metabolic changes in the nitrogen pathway, changes in hormonal balance due to the presence of fruits in alternating plants, and the combination of these factors are also related to alternate bearing. The metabolic changes cause decoupling of the nitrate reduction mechanism in *one year* (high fruit load). The changes in hormonal balance inhibit flowering induction due to increased endogenous gibberellin levels. A combination of metabolic changes, along with hormonal changes caused by pruning or thinning of fruits in the first stages of fruit development, led to carbohydrates accumulating again in the leaves before floral differentiation. The reduction of carbohydrate drains and, consequently, of gibberellin sources may explain the effects of these managements [31, 45]. Salustiana oranges in alternating bearing demonstrate that reserve accumulation is inversely related to crop load [46]. Carbohydrate availability combined with hormonal balance in the fruit determines fruit set and fruit development. Therefore, the practice of keeping fruits on the tree to extend the commercial harvest period during the season worsens alternating bearing [47]. Alternate bearing is a major problem in citrus production worldwide, especially for mandarin cultivars [48], as it impacts fruit production, size, and quality, as well as orchard profitability. Despite that, it is a common phenomenon in many seeded citrus cultivars [49].

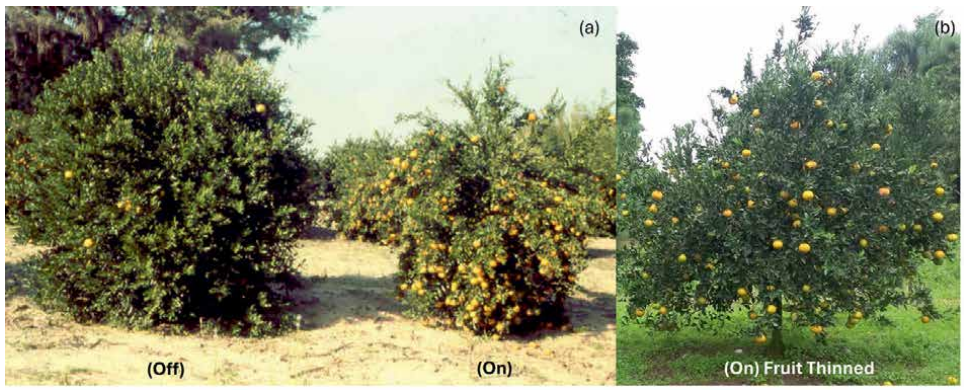

Figure 1.
(a) 'Montenegrina' mandarin in an alternating production cycle: off *year—low fruit load (a, left) and* on *year—excessive fruit load (a, right). (b) 'Montenegrina' mandarin submitted to manual thinning and with a balanced fruit load (b). Photo (a) by Sergio Francisco Schwarz and (b) by Gerson Nestor Boettcher. Rio Grande do Sul, Brazil.*

5. Fruit thinning and branch girdling

The practice of thinning fruits aims to rationalize the use of the plant's nutritional reserves, removing excess fruits to prevent their depletion, enabling regular production of good-quality fruits every year [50]. Reducing the amount of fruits increases the proportion of leaves in relation to fruits, providing less competition for available photoassimilates and increasing the growth rate of the remaining fruits [51]. The intensity of manual thinning depends on the cultivar and the load of each tree, generally ranging from 50 to 80% in plants with small and very high excess load, respectively [52]. Agustí et al. [53] performed manual thinning at four different times between flowering and one month before harvest, in the 'Clausellina' mandarin tree (*C. unshiu*) showed that: (a) thinning flowers (at anthesis) had no effect on fruit set, as the elimination of some flowers favors the development of others and the number of fruits harvested remains constant; (b) thinning fruits at the end of physiological fall would be the most appropriate time; and (c) for the remaining fruits to gain in size, at least 50–60% of the plant's fruits must be eliminated.

Several reports have shown the efficiency of manual thinning in terms of producing fruits with higher physical and chemical quality for *Citrus deliciosa* tangerines [50, 54–56]. However, not all studies have shown positive results in reducing alternate bearing. Besides, manual thinning requires a lot of specialized labor, in addition to being time-consuming and expensive. Therefore, chemical and mechanical thinning may be adequate alternatives [57].

The use of gasoline-powered manual agitators and electric combs to thin tangerines has been shown as a suitable mechanical thinning of small fruits [58–60]. The gasoline-powered branch shaker was able to thin fruits four to five times faster than manual thinning of 'Clemenrubi' in Spain. Although the final size of the fruit was significantly larger with manual and mechanical thinning compared to the absence of thinning, no significant differences were found in the final fruit production. The use of a branch shaker in fruit thinning operations can then be recommended to increase efficiency, reduce labor costs and obtain larger fruits with better quality [57]. Chemical thinning is recommended as a practice to mitigate alternate bearing [51]; however, it is a less precise technique than manual thinning [49]. It is performed by spraying products that directly or indirectly act on fruit abscission. Since the 1950s, several compounds have been used for chemical thinning, and several responses have been reported [61] since there are several different compounds, which can be used in different concentrations, applied to different plants at different application times [62].

In order to achieve the desired thinning percentages for satisfactory production of commercial size fruits, the timing of application of plant growth regulators is of utmost importance. Hence, the natural fall of fruits has been shown to be the ideal time for applying chemical thinners, providing the greatest response in fruit abscission [53]. Furthermore, the application of plant growth regulators may lead to a high variability in fruit set, since there is a lack of knowledge of the effects of age and vigor of the plant, the initial quantity of flowers, temperature climatic conditions of, relative humidity and precipitation, the phenological stage of the plant, shading, fruits generated in bunches, plant growth regulator concentration, product application uniformity, on fruit drop [63, 64].

Another important factor is the relation between zinc and auxin, since the application of zinc to zinc-deficient plants increases endogenous auxin. Along with being required for auxin synthesis, zinc is also important for maintaining the biologically active form of auxin [65].

Abscission is the result of a complex combination of nutritional factors and hormonal signals [66]. Nutritional factors act as limiting factors that affect growth, causing abscission, while hormones regulate the abscission process [67]. Iglesias et al. [32] attribute the physiological drop of young fruits to the insufficiency of carbohydrates, caused by an increase in the demand for carbon by a large population of expanding fruits. Auxins induce a transient reduction in photosynthesis that leads to a reduction in the production of photoassimilates, causing a temporary decrease in fruit uptake and growth. Along with increasing ethylene production and the formation of abscission zones, auxin results in greater abscission of young fruits. The remaining fruits on the plants overcome this transient effect, increasing the growth rate and reaching a larger size than the fruits from plants devoid of treatment [68]. Synthetic auxin application may provide different effects, depending on the time of year in which they are applied [69]. During fruit abscission at the time of physiological fall, they increase abscission and reduce carbohydrate competition between the remaining developing fruits [61, 69]. When applied at the beginning of the cell growth phase (phase II), synthetic auxins increase the growth capacity as well as carbohydrate accumulation [69].

The synthetic auxin 3,5,6-TPA is very efficient as a fruit growth stimulant in tangerine trees of the clementine group [70]. As a free acid, it is a powerful promoter of fruit size increase, with moderate to intense thinning depending on the time of application and concentration, while in its isopropyl ester form, it provides a very high fruit thinning [61].

A temporary photosynthetic disorder causing a reduction in photoassimilates production and absorption in the production of by young fruits is noticed for 3,5,6-, slowing growth, causing ethylene production and consequently the abscission of young fruits. Subsequently, the growth rates of the remaining fruits are increased, resulting in larger fruits [68]. 3,5,6-TPA in the isopropyl ester formulation at a concentration of 15 mg L^{-1} in 'Marisol' clementine plants during the cell division phase (phase I) promoted fruit abscission, abnormal leaf development and temporary photosynthetic damage, with a reduced fruit growth rate. The treatment significantly reduced the accumulation of photoassimilates in the fruits from the 3rd to the 8th day after treatment and then reducing their growth rate and increasing fruit abscission [68]. In free acid formulation, 3,5,6-TPA reduced the number of fruits per plant when 20 mg L^{-1} of 3,5,6-TPA was applied at the end of the physiological fall of fruits in irrigated 'Montenegrina' orchards. However, in another report, 3,5,6-TPA was shown to be a promising agent for thinning 'Montenegrin' mandarins, as a concentration of 40 mg L-1 of 3,5,6-TPA in the acid form gave similar results to manual thinning [71]. *Citrus deliciosa* fruits, such as 'Avana' and 'Montenegrina' mandarins, are more difficult to remove than seedless mandarins [63].

Branch girdling is also another cultural practice that can be used to increase the size of citrus fruits [53]. Aiming to increase fruit size, the best response to girdling is seen when performed immediately after the physiological fall of the fruits. An increase of size between 2 and 4 mm in diameter is generally obtained in mandarin and sweet orange cultivars, allowing fruits to meet commercial size requirements. However, a delay in girdling reduces its effectiveness, although a certain effect remains until the end of August in the Northern Hemisphere [49].

6. Gibberellins in the flowering management of mandarins

Flowering management in mandarin trees becomes important in alternating citrus cultivars, and this can be done in low-load harvests through flowering inhibition.

Gibberellic acid as an inhibitor of floral induction in citrus has been widely shown [72–76], whose biosynthesis inhibition resulted in increased flowering [72]. The concentration of gibberellic acid (GA_{1+3}) in 'Satsuma' mandarin leaves was negatively correlated with flowering in the following cycle [75]. Accordingly, translocation of GA_1 and GA_4 from fruit tissues to adjacent tissues during the fruit color change, during the floral induction period, was observed in Washington Navel' orange trees [77].

However, under extreme conditions of excess or lack of fruit production, the treatment with gibberellic acid fails. The proportion of endogenous flowering promoters (P) and flowering inhibitors (I) (P/I) has previously been considered responsible for flowering. Nevertheless, there is evidence suggesting that inhibitors and their metabolism are the only factors that control flowering in citrus [78]. Differently, applications of 40 mg L^{-1} of GA_3 during the floral induction period reduced flowering compared to the control, while the use of PBZ increased the number of flowers in 'Salustiana' orange trees. The inhibition of flowering in plants treated with gibberellic acid was due to the inhibition of this gene in leaves, while the use of PBZ promoted an increase in CiFT expression [76].

Studies were conducted on successive applications of gibberellic acid (GA_3) during the flower induction period in Citrus deliciosa mandarin trees in southern Brazil [34, 79]. The use of GA_3 in two or more sequential applications reduced the intensity of subsequent spring flowering in plants in alternate bearing, with an increase in the frequency of mixed shoots and a reduction in leafless flowering shoots. The use of four sequential applications of 40 mg L^{-1} GA_3 improved flowering quality, increased fruit set and fruit diameter [34].

7. Biostimulants

A plant biostimulant is any substance or microorganism applied to plants with the aim of increasing nutritional efficiency, tolerance to abiotic stress and/or crop quality, regardless of its nutrient content. Plant biostimulants are also defined as commercial products containing mixtures of these substances and/or microorganisms [80]. Furthermore, when applied to the soil, biostimulants can stimulate rhizosphere microorganisms, the photosynthetic process and the production of plant growth regulators [6]. Biostimulants are considered substances standing between phytosanitary products and fertilizers, as they do not offer direct protection against pests and do not have nutritional activity [81].

The current European legislation (Regulation (EU) 2019/1009) categorizes biostimulants as products designed to enhance plant nutrition processes, regardless of their nutrient composition. These substances primarily aim to improve one or more key plant and soil characteristics, including nutrient use efficiency, abiotic stress resilience, crop quality attributes or mobilization of bound nutrients within the soil or rhizosphere [82].

The mode of action of biostimulants is often unknown and difficult to identify, as they derive from complex sources containing multiple bioactive components that, together, contribute to specific effects on plants [83, 84]. They are supplied to plants in very low doses with the aim of inducing beneficial effects, but their effect cannot be associated with their nutritional content [85–87]. Instead, they stimulate the plants' ability to acquire nutrients more efficiently and use them for metabolism and biomass production. They also help plants overcome stress conditions [88, 89]. Currently, international organizations and scientists have recognized the following

categories of biostimulants: microorganisms, protein hydrolysates, seaweed extracts, chitosan, inorganic compounds and humic substances [6, 80, 90].

7.1 Humic substances

Humic substances are probably the most studied category of biostimulants and, although many aspects of how they interact with plants are not fully known and require further research, the primary targets of their action have undoubtedly been identified [91]. Humic substances consist of a complex set of molecules from plant and animal remains, representing one of the most abundant organic materials on Earth [85].

Humic substances are a fraction of soil organic matter in the final stage of a complex interaction between non-living organic matter and microbial communities, and this interaction greatly influences the physical, chemical and biological properties of the soil, in addition to contributing to the support of plant growth. Recently, a series of experimental data have shown that humic substances and their different fractions can affect plant growth and development, involving specific structural and physiological responses [92]. As reported by the International Humic Substances Society (IHSS), humic substances are complex and heterogeneous mixtures of polydisperse materials. They are formed in soils, sediments and natural waters by biochemical and chemical reactions during the decomposition and inherent transformation of plant and microbial remains in a process called humification [93]. Humic substances are the main components of natural organic matter in soil and water, as well as in geological organic deposits, such as lake sediments, peat and leonardite [94].

The structure of humic substances is operationally defined in humic acids (HA), fulvic acid (FA) and humins. HA is the fraction soluble in basic media, and FA is soluble in both basic and acidic media, while humins are insoluble in any pH range [95]. According to the operational definition of the IHSS, humic substances do not have a uniform molecular structure and their exact chemical composition is related to their origin (soil, peat, leonardite, water or air), geographic location and extraction technology.

Moreover, due to differences in composition and distinct functional groups, the fractions from humic substances may have different properties [96]. The following functional groups are relevant: phenols, carboxylic, hydroxyl, carbonyl and amino groups [97]. Phenolic and carboxylic groups are the ones that mostly contribute to the surface charge and reactivity of humic substances [98].

Aromatic compounds have traditionally been considered the main "building blocks" of humic substances [95], accounting for up to 47.1% of humic acids, while aliphatic compounds account for up to 35% of humic acid composition. Among aromatic compounds, phenols, benzene carboxylic acid and phenolic acid account for 12.3%, 9.8% and 34.5%, respectively [99]. Carboxyl and phenolic groups are commonly determined by direct titration, ranging from 3.8 to 6.7 mmol g^{-1} for carboxylic groups and from 1.0 to 2.2 mmol g^{-1} for phenolic groups [98, 100]. Humic acids behave as mixtures of dibasic acids, with a pKa value of around 4 for protonation of carboxylate groups and around 8 for protonation of phenolate groups [101]. The aromatic nature of humic substances can be considered an indicator of stability against chemical and biological degradation [88] by adsorption of functional groups on the surfaces of clay minerals and through physical protection within the pores of soil clay particles, resulting in limited accessibility of microorganisms and enzymes [102, 103]. Beyond that, the presence of carboxylate and phenolate groups gives humic acids the

ability to form complexes with Mg^{2+}, Ca^{2+}, Fe^{2+}, and Fe^{3+}. Many humic acids have two or more of these groups, allowing them to form chelate complexes [104].

To date, the understanding of humic substances composition has been performed by employing alkaline solution or heating [105]. Although alkaline extraction is an extremely reactive method generating compounds even more difficult to identify, it is the most used method for detecting the solubility of humic substances in soil (IHSS, 2023) and allows for obtaining maximum yields of organic matter [95].

In the early twenty-first century, the humic substance terminology was changed [106] to a fraction of organic matter with an unknown structure [107–109], as alkaline extraction may cause changes in the humic substances' structure [110].

Previous reports stated that there was no apparent relation between the biological function of soil organic material and its extracted fractions, postulating that alkaline extracts do not show similar properties to those obtained through the humification process [107, 108]. In contrast, a later report showed that alkaline extraction did not alter the structure of humic substances [111].

Humic acid may have beneficial applications in plant production. Nevertheless, the concentration [112–114] and the source of humic acid should be carefully selected since it may affect the biological activity [115–117]. For instance, composting organic waste favors the extraction of humic acids with greater hydrophobicity [117] and with higher bioactive humic acids in comparison to non-composted waste [118]. Also, more stable humic acid isolated from soils in a less advanced stage of weathering, with a high-activity clay and high base saturation, improved root growth of *Arabidopsis thaliana*. Humic substances are among the most efficient antioxidants found in nature [116]. In addition, humic substances can induce phenylpropanoid metabolism, contributing to the production of antimicrobial compounds [119].

The effect of humic substances mitigating different stresses in plants is well known and generally described as a result of increased enzymatic and non-enzymatic antioxidant defense, increased production of compatible solutes, and changes in ion balance [89]. Besides, humic substances demonstrated more significant efficacy in controlling plant pests and diseases under controlled conditions, suggesting their potential role as biotic elicitors in stimulating defense pathways [120]. Apart from that, orchards continuously treated with humic substances can coexist well with Huanglongbing symptoms, as observed by technicians and producers in the main citrus belt in Brazil [121].

Humic substances also show activities similar to plant growth regulators, including auxin, gibberellin and cytokine-like [92, 122–125]. However, physiologically active amounts of gibberellins have not yet been detected in humic substances [85]. The auxin-like activity of humic substances is associated with complex hydrophobic structures, whose hydrolysis can release auxin-like molecules [125]. The auxin-like activity is also supported by a positive effect of humic substance on specific targets of auxin [126]. Humic substances having auxin activity induce a hormonal effect on catalytic activity, cell permeability, and increase nutrient absorption and dry matter production [127].

A previous report showed that humic acid applied to perennial crops increased the plant endogenous levels of indole-3-acetic acid and gibberellic acid in rooted shoots, mainly in the first period of root development [128]. In another report, a combined foliar application of humic acid and boric acid provided a hormonal balance by increasing the level of auxin, gibberellin and cytokinin, along with a decrease in ABA level and reducing floral malformation in mango [129]. Carbonic organic from fulvic acid applied during physiological fall promoted fruit abscission

of Montenegrina' mandarin [130]. However, there was no effect on the quantum efficiency of photosystem II (Fv/Fm), showing that there was apparently no effect of transient reduction in the photosynthetic process, a mechanism of action described for synthetic auxins [68]. Humic acid has a complex chemical composition, and its plant growth-like activity, as well as its biological activity, is still under discussion and needs further investigation [131]. Leonardite is an oxidized form of lignite, found at shallow depths on more compact coal in several coal mines [132], mainly in the United States of America [133]. It has been employed as a reference humic material with regard to plant growth responses. Leonardite increased dry matter yield and nutrient uptake [134], and under greenhouse conditions, it increased the resistance of tomato plants to salt stress [135]. A higher number of flower stems, inflorescences and yield was obtained from *Arnica montana* treated with leonardite [136]. Although the effects of leonardite humic substances on crop production, stress resistance and soil microbial activity have been reported, much less attention has been devoted to their impact on plant physiology and biochemistry. Similar to other humic substances, the effect of leonardite on plant growth cannot be generalized due to its different sources, employed concentration and plant species where it is applied [137]. In spite of this, all leonardite sources had biostimulant activity, promoting plant growth, accumulation of phenolic substances and acting on nitrogen metabolism [138].

7.2 Coumarins

Coumarins have been employed as biostimulants in recent years, aiming to improve plant growth and development. They are compounds from the secondary metabolism of many higher plant families and are produced by the shikimic acid pathway and are classified as phenylpropanoids [139]. The widespread occurrence of coumarins in the plant kingdom points to their probable role in plant metabolism, development, as well as potential protection against herbivores and pathogens [140].

Accordingly, exogenous application of coumarin improved the antioxidant performance, levels of phenolic compounds, activities of key metabolic enzymes and ion homeostasis of different plants grown under stressful conditions [141, 142]. As previously mentioned for other compounds, the effect of coumarins on plant growth and physiology is dose and species-dependent [143, 144].

A gibberellin-like effect was reported for coumarins, on a concentration basis. Effects on seed germination, seedling establishment and also leaf and stem elongation were observed [141, 145]. Increased levels of endogenous indoleacetic acid and gibberellic acid were observed for coumarin-treated peas, where gibberellic acid was the most affected plant growth regulator [141]. A dosage of 10 ppm improved seed germination and seedling growth of sorghum and the synthesis of auxin and gibberellin was increased, while abscisic acid was reduced in sorghum seed plants pre-soaked with coumarin solutions [146]. Although there is evidence of the gibberellin-like effect of coumarins, their potential as alternatives to the use of gibberellins in citrus needs to be confirmed in experimental studies.

8. Conclusions

In conclusion, the strategic application of plant growth regulators and biostimulants offers a promising approach to managing flowering, fruiting and alternate production in mandarins. By optimizing fruit set, fruit size and fruit quality, these

tools provide adequate yields while responding to environmental and agricultural challenges, providing a sustainable solution for citrus cultivation. Regarding biostimulants, more studies are needed to fully elucidate their mechanisms and improve application protocols.

Author details

Sabrina Raquel Griebeler[1], Mateus Pereira Gonzatto[2*], Júlia Scherer Santos[3], Gerson Nestor Boettcher[1] and Sergio Francisco Schwarz[1]

1 Department of Horticulture and Silviculture, Federal University o Rio Grande do Sul, Porto Alegre, Brazil

2 Department of Agronomy, Federal University of Viçosa, Viçosa, Brazil

3 Institute of Biological Sciences, Department of Pharmacology, Federal University of Juiz de Fora, Juiz de Fora, Brazil

*Address all correspondence to: mateus.gonzatto@ufv.br

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

[1] Gill K, Kumar P, Negi S, Sharma R, Joshi AK, Suprun II, et al. Physiological perspective of plant growth regulators in flowering, fruit setting and ripening process in citrus. Scientia Horticulturae. 2023;**309**:111628. DOI: 10.1016/j.scienta.2022.111628

[2] Davenport TL. Citrus flowering. In: Horticultural Reviews. Vol. 13. Portland, USA: Wiley; 1990. pp. 349-408. DOI: 10.1002/9781118060858.ch8

[3] Agustí M, Reig C, Martínez-Fuentes A, Mesejo C. Advances in citrus flowering: A review. Frontiers in Plant Science. 2022;**13**. DOI: 10.3389/fpls.2022.868831. Available from: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2022.868831/full

[4] OECD. Compendium of Agri-Environmental Indicators. OECD Publishing; 2013. Available from: https://www.oecd.org/content/dam/oecd/en/publications/reports/2013/06/oecd-compendium-of-agri-environmental-indicators_g1g220a0/9789264186217-en.pdf

[5] COUNCIL EPOTOT. Laying down rules on the making available on the market of EU fertilising products and amending regulations EC No 1069/2009 and EC No 1107/2009 and repealing regulation EC No 2003/2003. Official Journal of the European Union. 2019;**25**:6

[6] Calvo P, Nelson L, Kloepper JW. Agricultural uses of plant biostimulants. Plant and Soil. 2014;**383**:3-41. DOI: 10.1007/s11104-014-2131-8

[7] Povero G, Mejia JF, Di Tommaso D, Piaggesi A, Warrior P. A systematic approach to discover and characterize natural plant biostimulants. Frontiers in Plant Science. 2016;**7**:1-9. DOI: 10.3389/fpls.2016.00435

[8] EBIC (European Biostimulants Industry Council). Economic overview of the European biostimulants market. Available from: https://biostimulants.eu/highlights/economic-overview-of-the-european-biostimulants-market/ [Accessed: December 26, 2023]

[9] Gene, Albrigo L, Galán SV. Flower bud induction, flowering and fruit-set of some tropical and subtropical fruit tree crops with special reference to citrus. Acta Horticulturae. 2004;**632**:81-90. DOI: 10.17660/ActaHortic.2004.632.10

[10] Gravina A. Fisiología de Citrus. Montevideo: Universidad de la República: Facultad de Agronomía; 2014. 145 p

[11] Valiente BIJ. Timing and Intensity of Flowering of Sweet Orange (*Citrus sinensis* (L.) Osbeck) as a Function of Local Weather Factors and Crop under Central Florida Conditions. [PhD Thesis] University of Flórida; 2001. p. 168

[12] Moss GI. The influence of temperature during flower development on the subsequent fruit-set of sweet orange (*Citrus sinensis*) 'Washington navel'. Horticulture Research. 1973;**13**:65-73

[13] García-Luís A, Kanduser M, Santamarina P, Guardiola JL. Low temperature influence on flowering in citrus. The separation of inductive and bud dormancy releasing effects. Physiologia Plantarum. 1992;**86**:648-652. DOI: 10.1111/j.1399-3054.1992.tb02182.x

[14] Nishikawa F, Endo T, Shimada T, Fujii H, Shimizu T, Omura M, et al.

Increased CiFT abundance in the stem correlates with floral induction by low temperature in Satsuma mandarin (Citrus unshiu Marc.). Journal of Experimental Botany. 2007;**58**:3915-3927. DOI: 10.1093/jxb/erm246

[15] Reuther WC. Climaste and citrus behavior. In: Reuther W, editor. The Citrus Industry. Vol. III. Berkeley: University of California; 1973. pp. 280-337

[16] Southwick SM, Davenport TL. Characterization of water stress and low temperature effects on flower induction in citrus. Plant Physiology. 1986;**81**:26-29. DOI: 10.1104/pp.81.1.26

[17] Borroto CG, López M, Hidalgo O. Efecto del estrés hídrico y la presencia de frutos de la cosecha anterior sobre rendimento de los naranjos. 2nd ed. Valencia: Centro Agrícola; 1981

[18] Lord EM. KJE. Shoot development in *Citrus sinensis* L. (Washington navel Orange). I. Floral and inflorescence ontogeny. Botanical Gazette. 2015;**146**:320-326. Available from: https://www.jstor.org/stable/2474536

[19] Spiegel-Roy P, Goldschmidt EE. The Biology of Citrus. Cambridge: Cambridge University Press; 1996. DOI: 10.1017/CBO9780511600548

[20] Abbott CE. Blossom-bud differentiation in citrus trees. American Journal of Botany. 1935;**22**:476-485. DOI: 10.1002/j.1537-2197.1935.tb05036.x

[21] Ramos-Hurtado AM, Koller OC, de A MJ, Sartori IA, Theisen S, Reis B. Diferenciação floral, alternância de produção e uso de ácido giberélico em tangerineira 'Montenegrina' (Citrus deliciosa Tenore). Revista Brasileira de Fruticultura. 2006;**28**:355-359. DOI: 10.1590/S0100-29452006000300005

[22] Griebeler SR. Manejo de florescimento em tangerineiras com ácido giberélico. Porto Alegre, Brazil: Universidade Federal do Rio Grande do Sul; 2020. Available from: https://lume.ufrgs.br/handle/10183/274132

[23] Agustí M, Mesejo C, Muñoz-Fambuena N, Vera-Sirera F, de Lucas M, Martínez-Fuentes A, et al. Fruit-dependent epigenetic regulation of flowering in citrus. New Phytologist. 2020;**225**:376-384. DOI: 10.1111/nph.16044

[24] Le M, Eckard KJ. Shoot development in *Citrus sinensis* L. (Washington navel orange). II. Alteration of developmental fate of flowerings hoots after GA3 treatment. Botanical Gazette. 1987;**148**:17-22

[25] Guardiola JL. Overview of Flower Bud Induction, Flowering and Fruit Set. Proceedings of Citrus Flowering and Fruit Short Course. IFAS. Citrus Research and Education Center. Lake Alfred, USA: University of Florida; 1997. pp. 5-21. Available from: http://irrec.ifas.ufl.edu/flcitrus/UF.IFAS Short Course Proceedings/CitrusFlowering-duplex/citrusflowering5-21high.pdf

[26] Muñoz-Fambuena N, Mesejo C, Carmen González-Mas M, Primo-Millo E, Agustí M, Iglesias DJ. Fruit regulates seasonal expression of flowering genes in alternate-bearing 'Moncada' mandarin. Annals of Botany. 2011;**108**:511-519. DOI: 10.1093/aob/mcr164

[27] Simpson GG, Dean C. Arabidopsis, the Rosetta stone of flowering time? Science. 2002;**296**:285-289. DOI: 10.1126/science.296.5566.285

[28] Goldschmidt EE, Aschkenazi N, Herzano Y, Schaffer AA, Monselise SP.

A role for carbohydrate levels in the control of flowering in citrus. Scientia Horticulturae. 1985;**26**:159-166. DOI: 10.1016/0304-4238(85)90008-1

[29] Medina CL, Rena AB, Siqueira DL, Machado EC. Fisiologia dos citros. In: Mattos Júnior D, De Negri JD, Pio RM, Pompeu Júnior J, editors. Citros. Campinas: IAC: Fundag; 2005. p. 929

[30] Nishikawa F, Iwasaki M, Fukamachi H, Nonaka K, Imai A, Takishita F, et al. Fruit bearing suppresses citrus flowering locus T expression in vegetative shoots of Satsuma mandarin (Citrus unshiu marc.). Journal of the Japanese society for. Horticultural Science. 2012;**81**:48-53. DOI: 10.2503/jjshs1.81.48

[31] Agustí M. Citricultura. 2nd ed. Madrid: Mundi-Prensa; 2003. p. 422

[32] Iglesias DJ, Cercós M, Colmenero-Flores JM, Naranjo MA, Ríos G, Carrera E, et al. Physiology of citrus fruiting. Brazilian Journal of Plant Physiology. 2007;**19**:333-362. DOI: 10.1590/S1677-04202007000400006

[33] Jahn OL. Inflorescence types and fruiting patterns in Hamlin and Valencia oranges and marsh grapefruit. American Journal of Botany. 1973;**60**:663-670. DOI: 10.1002/j.1537-2197.1973.tb05971.x

[34] Griebeler SR, Gonzatto MP, Böettcher GN, Schneider LA, Sulzbach M, Gargioni ED, et al. Successive applications of gibberellic acid to reduce flowering of 'Montenegrina' mandarin in alternate bearing. Pesquisa Agropecuária Brasileira. 2021;**56**. DOI: 10.1590/s1678-3921.pab2021.v56.02303

[35] Talón M, Hedden P, Primo-Millo E. Gibberellins in *Citrus sinensis*: A comparison between seeded and seedless varieties. Journal of Plant Growth Regulation. 1990;**9**:201-206. DOI: 10.1007/BF02041963

[36] Mesejo C, Yuste R, Reig C, Martínez-Fuentes A, Iglesias DJ, Muñoz-Fambuena N, et al. Gibberellin reactivates and maintains ovary-wall cell division causing fruit set in parthenocarpic citrus species. Plant Science. 2016;**247**:13-24. DOI: 10.1016/j.plantsci.2016.02.018

[37] Talon M, Tadeo F, Zeevaart JD. Cellular changes induced by exogenous and endogenous gibberellins in shoot tips of the long-day plant Silene armeria. Planta. 1991;**185**:487-493. DOI: 10.1007/BF00202957

[38] Rivas F, Gravina A, Agustí M. Girdling effects on fruit set and quantum yield efficiency of PSII in two citrus cultivars. Tree Physiology. 2007;**27**:527-535. DOI: 10.1093/treephys/27.4.527

[39] Goren R, Huberman M, Goldschmidt EE. Girdling: Physiological and horticultural aspects. In: Horticultural Reviews. Vol. 30. New Jersey, USA: Wiley; 2003. pp. 1-36. DOI: 10.1002/9780470650837.ch1

[40] Goñi C, Otero A, Syvertsen JP. Flooding affects fruitlet abscission in Satsuma mandarin. Acta Horticulturae. 2015;**1065**:1407-1416. DOI: 10.17660/ActaHortic.2015.1065.178

[41] Bermejo A, Granero B, Mesejo C, Reig C, Tejedo V, Agustí M, et al. Auxin and gibberellin interact in citrus fruit set. Journal of Plant Growth Regulation. 2018;**37**:491-501. DOI: 10.1007/s00344-017-9748-9

[42] Garmendia A, Beltrán R, Zornoza C, García-Breijo FJ, Reig J, Merle H. Gibberellic acid in citrus spp.

flowering and fruiting: A systematic review. PLoS One. 2019;**14**:e0223147. DOI: 10.1371/journal.pone.0223147

[43] Koller OC, Schäfer G, Sartori IA, Panzenhagen NV, de LJG. Produção da laranjeira-de-umbigo 'Monte Parnaso' com incisão anelar de ramos e uso de reguladores vegetais. Revista Brasileira de Fruticultura. 2006;**28**:425-429. DOI: 10.1590/S0100-29452006000300019

[44] Ferrer C, Martiz J, Saa S, Cautín R. Increase in final fruit size of tangor (Citrus reticulata × C. Sinensis) cv W. Murcott by application of benzyladenine to flowers. Scientia Horticulturae. 2017;**223**:38-43. DOI: 10.1016/j.scienta.2017.05.030

[45] Turnbull CGN. Identification and quantitative analysis of gibberellins in citrus. Journal of Plant Growth Regulation. 1989;**8**:273-282. DOI: 10.1007/BF02021820

[46] Monerri C, Fortunato-Almeida A, Molina RV, Nebauer SG, García-Luis A, Guardiola JL. Relation of carbohydrate reserves with the forthcoming crop, flower formation and photosynthetic rate, in the alternate bearing 'Salustiana' sweet orange (*Citrus sinensis* L.). Scientia Horticulturae. 2011;**129**:71-78. DOI: 10.1016/j.scienta.2011.03.009

[47] Verreynne JS, Lovatt CJ. The effect of crop load on Budbreak influences return bloom in alternate bearing 'pixie' mandarin. Journal of the American Society for Horticultural Science. 2009;**134**:299-307. DOI: 10.21273/JASHS.134.3.299

[48] Wheaton TA. Alternate bearing of citrus in Florida. Proceedings of the International Seminar Citriculture. 1992;**1**:224-228

[49] Talon M, Caruso M, FG GJR. The Genus Citrus. Duxford, United Kingdom: Elsevier; 2020. 538 p. ISBN: 978-0-12-812163-4

[50] Schwarz SF, Marodin GAB, Koller OC. Avaliação de duas safras da tangerineira 'Montenegrina' após raleio químico e manual de frutos. Revista Brasileira de Fruticultura. 1992;**14**:65-70

[51] Guardiola JL, García-Luis A. Increasing fruit size in citrus. Thinning and stimulation of fruit growth. Plant Growth Regulation. 2000;**31**:121-132. DOI: 10.1023/A:1006339721880

[52] Koller OC, editor. Citricultura - Cultura de Tangerineiras: tecnologia de produção, pós-colheita e industrialização. Porto Alegre: Rígel; 2009. 400 p

[53] Agustí M, Almela V, Zaragoza S, Juan M, Trenor I, Alonso E, et al. Técnicas para mejorar el tamaño del fruto de naranjas y mandarinas. Generalitat Valenciana (Série Citricultura, 3); 1998. Available from: https://redivia.gva.es/bitstream/handle/20.500.11939/8086/1998_Agustí_Técnicas.pdf?sequence=1&isAllowed=y

[54] Rodrigues LR, Schwarz SF, Reckziegel VP, Koller OC. Raleio manual de frutos em tangerineiras 'Montenegrina'. Pesquisa Agropecuaria Brasileira. 1998;**33**:1315-1320

[55] Sartori IA, Koller OC, Theisen S, de SPVD, Bender RJ, Marodin GAB. Efeito da poda, raleio de frutos e uso de fitorreguladores na produção de tangerinas (Citrus deliciosa Tenore) cv. montenegrina. Revista Brasileira de Fruticultura. 2007;**29**:5-10. DOI: 10.1590/S0100-29452007000100004

[56] Rosa RD, Nava GA, Piva AL, Mezzalira ÉJ, Paulus D. Poda e raleio manual de tangerineira (Citrus deliciosa

Tenore) cv. Montenegrina no Sudoeste do Paraná. Revista Ceres. 2012;**59**:254-261. DOI: 10.1590/S0034-737X2012000200015

[57] Ortiz C, Torregrosa A, Ortí E, Balasch S. Mechanical thinning of mandarins with a branch shaker. HortTechnology. 2020;**30**:745-750. DOI: 10.21273/HORTTECH04451-20

[58] Torregrosa A, Ortí E, Balasch S, Ortiz C. Aclareo mecánico de mandarinas 'Clemenrubí' con un vibrador de ramas. Proceedings IX Congreso Ibérico de Agroingeniería, Braganza, Portugal. Instituto Politécnico de Bragança. 2017. pp. 340-349

[59] Ortiz C, Martin-Gorriz B, Ortí E, Torregrosa A. An approach to mechanical thinning of mandarins. In: 2016 American Society of Agricultural and Biological Engineers Annual International Meeting, ASABE 2016. St. Joseph, USA: ASABE; 2016. pp. 1-6. DOI: 10.13031/aim.20162457303

[60] Romano A, Torregrosa Mira A, Ortiz sánchez C. Análisis del aclareo mecánico de mandarinas durante el desarrollo del fruto a través de los videos de desprendimiento en laboratorio. In: Congreso Ibérico de Agroingeniería. Zaragoza: Servicio de Publicaciones Universidad; 2019. pp. 331-341. DOI: 10.26754/c_agroing.2019.com.3301

[61] Agustí M, Almela V. Mejora de la calidad del fruto como objetivo prioritario de la citricultura española. Revista Chapingo Serie Horticultura. 1995;**I**:21-28. DOI: 10.5154/r.rchsh.1995.01.006

[62] Spósito MB, Castro PRC, Agustí M. Alternância de produção em citros. Laranja. 1998;**19**:293-304

[63] Otero A. Programa Nacional de Citricultura Raleo de Frutos Cítricos. Una Técnica para Aliviar la Alternancia Productiva y Aumentar el Tamaño de los Frutos. Programa Nacional de Citricultura. 2003. Available from: https://ainfo.inia.uy/digital/bitstream/item/353/1/111219210807173948.pdf

[64] Ouma G. Fruit thinning with specific reference to citrus species: A review. Agriculture and Biology Journal of North America. 2012;**3**:175-191. DOI: 10.5251/abjna.2012.3.4.175.191

[65] Skoog F. Relationships between zinc and auxin in the growth of higher plants. American Journal of Botany. 1940;**27**:939-950

[66] Goren R. Anatomical, physiological, and hormonal aspects of abscission in citrus. In: Horticultural Reviews. Vol. 15. New York, USA: Wiley; 1993. pp. 145-182. DOI: 10.1002/9780470650547.ch4

[67] Gómez-Cadenas A, Mehouachi J, Tadeo FR, Primo-Millo E, Talon M. Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. Planta. 2000;**210**:636-643. DOI: 10.1007/s004250050054

[68] Mesejo C, Rosito S, Reig C, Martínez-Fuentes A, Agustí M. Synthetic auxin 3,5,6-TPA provokes citrus Clementina (Hort. Ex tan) fruitlet abscission by reducing photosynthate availability. Journal of Plant Growth Regulation. 2012;**31**:186-194. DOI: 10.1007/s00344-011-9230-z

[69] Agustí M, Martínez-Fuentes AMC. Citrus fruit quality: Physiological basis and techniques of improvement. Agrociencia Uruguay. 2002;**6**:1-16. DOI: 10.31285/AGRO.06.1065

[70] Otero A. Raleo de frutos en mandarina satsuma y otros cítricos. Serie Técnica Nº 140; 2004. 44 p. Available from: https://

ainfo.inia.uy/digital/bitstream/item/2885/1/15630021107125002.pdf

[71] Gonzatto MP, Böettcher GN, Schneider LA, Lopes ÂA, Silveira Júnior JC, Petry HB, et al. 3,5,6-trichloro-2-pyridinyloxyacetic acid as effective thinning agent for fruit of 'Montenegrina' mandarin. Ciência Rural. 2016;**46**:2078-2083. DOI: 10.1590/0103-8478cr20140057

[72] Monselise S, Halevy H. Chemical inhibition and promotion of citrus flower bud induction. Proceedings of the American Society for Horticultural Science. 1964;**84**:141-146

[73] Goldschmidt E, Monselise SP. Hormonal control of flowering in citrus and some other woody perenials. In: Carr DJ, editor. Plant Growth Substances. New York, Berlin: Springer-Verlag; 1972. pp. 758-766

[74] Guardiola JL, Monerri C, Agusti M. The inhibitory effect of gibberellic acid on flowering in citrus. Physiologia Plantarum. 1982;**55**:136-142. DOI: 10.1111/j.1399-3054.1982.tb02276.x

[75] Koshita Y, Takahara T, Ogata T, Goto A. Involvement of endogenous plant hormones (IAA, ABA, GAs) in leaves and flower bud formation of Satsuma mandarin (Citrus unshiu Marc.). Scientia Horticulturae. 1999;**79**:185-194. DOI: 10.1016/S0304-4238(98)00209-X

[76] Muñoz-Fambuena N, Mesejo C, González-Mas MC, Iglesias DJ, Primo-Millo E, Agustí M. Gibberellic acid reduces flowering intensity in sweet Orange [*Citrus sinensis* (L.) Osbeck] by repressing CiFT gene expression. Journal of Plant Growth Regulation. 2012;**31**:529-536. DOI: 10.1007/s00344-012-9263-y

[77] Gambetta G, Telias A, Arbiza H, Espino M, Franco J, Rivas F, et al. 'Creasing' En Naranja 'Washington' navel En Uruguay. Incidencia, Severidad Y control. Agrociencia. 2002;**VI**:17-24

[78] Martínez-Fuentes A, Mesejo C, Juan M, Almela V, Agustí M. Restrictions on the exogenous control of flowering in citrus. Acta Horticulturae. 2004;**632**:91-98. DOI: 10.17660/ActaHortic.2004.632.11

[79] Griebeler SR, Gonzatto MP, Böettcher GN, Schwarz SF. Successive applications of gibberellic acid in alternate bearing mandarins. Revista Brasileira de Fruticultura. 2023;**45**:e-445. DOI: 10.1590/0100-29452023445. Available from: https://www.scielo.br/j/rbf/a/CCqskYrMcs48DRPgs3yxwcP/?format=pdf&lang=en

[80] du Jardin P. Plant biostimulants: Definition, concept, main categories and regulation. Scientia Horticulturae. 2015;**196**:3-14. DOI: 10.1016/j.scienta.2015.09.021

[81] La Torre A, Battaglia V, Caradonia F. An overview of the current plant biostimulant legislations in different European member states. Journal of the Science of Food and Agriculture. 2016;**96**:727-734. DOI: 10.1002/jsfa.7358

[82] Carillo P, Avice J, Vasconcelos MW, Du Jardin P, Brown, PH. Biostimulants in Agriculture: Editorial. Physiologia Plantarum. 2025;**177**:1-4. DOI: 10.1111/ppl.70046

[83] Ertani A, Francioso O, Tugnoli V, Righi V, Nardi S. Effect of commercial Lignosulfonate-Humate on Zea mays L. metabolism. Journal of Agricultural and Food Chemistry. 2011;**59**:11940-11948. DOI: 10.1021/jf202473e

[84] Ertani A, Schiavon M, Altissimo A, Franceschi C, Nardi S. Phenol-containing organic substances stimulate phenylpropanoid metabolism in Zea

mays. Journal of Plant Nutrition and Soil Science. 2011;**174**:496-503. DOI: 10.1002/jpln.201000075

[85] Nardi S, Pizzeghello D, Schiavon M, Ertani A. Plant biostimulants: Physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. Scientia Agricola. 2016;**73**:18-23. DOI: 10.1590/0103-9016-2015-0006

[86] Yakhin OI, Lubyanov AA, Yakhin IA, Brown PH. Biostimulants in plant science: A global perspective. Frontiers in Plant Science. 2017;**7**:1-32. DOI: 10.3389/fpls.2016.02049

[87] Rouphael Y, Colla G. Synergistic biostimulatory action: Designing the next generation of plant biostimulants for sustainable agriculture. Frontiers in Plant Science. 2018;**9**:1-7. DOI: 10.3389/fpls.2018.01655

[88] García AC, Santos LA, de Souza LGA, Tavares OCH, Zonta E, Gomes ETM, et al. Vermicompost humic acids modulate the accumulation and metabolism of ROS in rice plants. Journal of Plant Physiology. 2016;**192**:56-63. DOI: 10.1016/j.jplph.2016.01.008

[89] Canellas LP, Canellas NOA, LES d SI, Olivares FL, Piccolo A. Plant chemical priming by humic acids. Chemical and Biological Technologies in Agriculture. 2020;**7**:12. DOI: 10.1186/s40538-020-00178-4

[90] EBIC (European Biostimulants Industry Council). Economic Overview of the European Biostimulants Market. 2023. Available from: https://biostimulants.eu/highlights/economic-overview-of-the-european-biostimulants-market/ [Acessed in dez. 2023]

[91] Nardi S, Schiavon M, Francioso O. Chemical structure and biological activity of humic substances define their role as plant growth promoters. Molecules. 2021;**26**:2256. DOI: 10.3390/molecules26082256

[92] Canellas LP, Olivares FL. Physiological responses to humic substances as plant growth promoter. Chemical and Biological Technologies in Agriculture. 2014;**1**:3. DOI: 10.1186/2196-5641-1-3

[93] Zavarzina AG, Danchenko NN, Demin VV, Artemyeva ZS, Kogut BM. Humic substances: Hypotheses and reality (a review). Eurasian Soil Science. 2021;**54**:1826-1854. DOI: 10.1134/S1064229321120164

[94] Fernando, Mahler C, Dal Santo SN, Bernardino C AR. Chemical characteristics of humic substances in nature. In: Humic Substance [Working Title]. London, UK: IntechOpen; 2021. DOI: 10.5772/intechopen.97414

[95] Stevenson FJ. Humus Chemistry: Genesis, Composition, Reactions. Hoboken, NJ, USA: John Wiley; 1994

[96] Csicsor A, Tombácz E. Screening of humic substances extracted from leonardite for free radical scavenging activity using DPPH method. Molecules. 2022;**27**:6334. DOI: 10.3390/molecules27196334

[97] Libes SM. Introduction to Marine Biogeochemistry. 2nd ed. Burlington, USA: Elsevier; 2009. 928 p

[98] Ritchie JD, Perdue EM. Analytical constraints on acidic functional groups in humic substances. Organic Geochemistry. 2008;**39**:783-799. DOI: 10.1016/j.orggeochem.2008.03.003

[99] Lehtonen T, Peuravuori J, Pihlaja K. Degradative analysis of aquatic fulvic acid: CuO oxidation versus pyrolysis

after tetramethylammonium hydroxide treatments in air and helium atmospheres. Analytica Chimica Acta. 2004;**511**:349-356. DOI: 10.1016/j.aca.2004.02.008

[100] Schnitzer M, Khan SU. Humic substances in the environment. New York, USA: Marcel Dekker; 1972. 327 p

[101] Ghabbour EA, Davies G. Humic substances: Structures, models and functions. Cambridge: Royal Society of Chemistry; 2001. 402 p

[102] von Lützow M, Kögel-Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, et al. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry. 2007;**39**:2183-2207. DOI: 10.1016/j.soilbio.2007.03.007

[103] Lipczynska-Kochany E. Humic substances, their microbial interactions and effects on biological transformations of organic pollutants in water and soil: A review. Chemosphere. 2018;**202**:420-437. DOI: 10.1016/j.chemosphere.2018.03.104

[104] Tipping E. WHAMC—A chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. Computers & Geosciences. 1994;**20**:973-1023. DOI: 10.1016/0098-3004(94)90038-8

[105] Clapp CE, Hayes MHB, Simpson AJ, Kingery WL. The chemistry of soil organic matter. In: Tabatabai ASDL, editor. Chemical Processes in Soils. Soil Scien. Madison, WI, USA. Madison, Wisconsin, USA: Soil Science Society of America, Inc.; 2005. pp. 1-150

[106] Lützow MV, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, et al. Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions – A review. European Journal of Soil Science. 2006;**57**:426-445. DOI: 10.1111/j.1365-2389.2006.00809.x

[107] Hedges J, Eglinton G, Hatcher P, Kirchman D, Arnosti C, Derenne S, et al. The molecularly-uncharacterized component of nonliving organic matter in natural environments. Organic Geochemistry. 2000;**31**:945-958. DOI: 10.1016/S0146-6380(00)00096-6

[108] Baldock JA, Broos K. Handbook of Soil Sciences. Boca Raton, FL, USA: CRC Press; 2011. pp. 11-52

[109] Kleber M, Lehmann J. Humic substances extracted by alkali are invalid proxies for the dynamics and functions of organic matter in terrestrial and aquatic ecosystems. Journal of Environmental Quality. 2019;**48**:207-216. DOI: 10.2134/jeq2019.01.0036

[110] Lehmann J, Kleber M. The contentious nature of soil organic matter. Nature. 2015;**528**:60-68. DOI: 10.1038/nature16069

[111] Weber J, Chen Y, Jamroz E, Miano T. Preface: Humic substances in the environment. Journal of Soils and Sediments. 2018;**18**:2665-2667. DOI: 10.1007/s11368-018-2052-x

[112] Malik KA, Azam F. Effect of humic acid on wheat (*Triticum aestivum* L.) seedling growth. Environmental and Experimental Botany. 1985;**25**:245-252. DOI: 10.1016/0098-8472(85)90008-5

[113] Valdrighi MM, Pera A, Agnolucci M, Frassinetti S, Lunardi D, Vallini G. Effects of compost-derived humic acids on vegetable biomass production and microbial growth

within a plant (Cichorium intybus)-soil system: A comparative study. Agriculture, Ecosystems & Environment. 1996;**58**:133-144. DOI: 10.1016/0167-8809(96)01031-6

[114] Baldotto MA, Baldotto LEB. Gladiolus development in response to bulb treatment with different concentrations of humic acids. Revista Ceres. 2013;**60**:138-142. DOI: 10.1590/S0034-737X2013000100020

[115] Adani F, Genevini P, Zaccheo P, Zocchi G. The effect of commercial humic acid on tomato plant growth and mineral nutrition. Journal of Plant Nutrition. 1998;**21**:561-575. DOI: 10.1080/01904169809365424

[116] Baldotto MA, Muniz RC, Baldotto LEB, Dobbss LB. Root growth of *Arabidopsis thaliana* (L.) Heynh. Treated with humic acids isolated from typical soils of Rio de Janeiro state, Brazil. Revista Ceres. 2011;**58**:504-511. DOI: 10.1590/S0034-737X2011000400015

[117] Aguiar NO, Olivares FL, Novotny EH, Dobbss LB, Balmori DM, Santos-Júnior LG, et al. Bioactivity of humic acids isolated from vermicomposts at different maturation stages. Plant and Soil. 2013;**362**:161-174. DOI: 10.1007/s11104-012-1277-5

[118] Baldotto MA, Canellas LP, Canela MC, Rezende CE, Velloso ACX. Propriedades redox de ácidos húmicos isolados de um solo cultivado com cana-de-açúcar por longo tempo. Revista Brasileira de Ciência do Solo. 2008;**32**:1043-1052. DOI: 10.1590/S0100-06832008000300013

[119] Schiavon M, Pizzeghello D, Muscolo A, Vaccaro S, Francioso O, Nardi S. High molecular size humic substances enhance phenylpropanoid metabolism in maize (Zea mays L.). Journal of Chemical Ecology. 2010;**36**:662-669. DOI: 10.1007/s10886-010-9790-6

[120] S RM, C LP. Organic matter in the pest and plant disease control: A meta-analysis. Chemical and Biological Technologies in Agriculture. 2022;**9**:70. DOI: 10.1186/s40538-022-00332-0

[121] da Silva RM, Canellas NA, Olivares FL, Piccolo A, Canellas LP. Humic substances trigger plant immune responses. Chemical and Biological Technologies in Agriculture. 2023;**10**:123. DOI: 10.1186/s40538-023-00468-7

[122] de Sanfilippo EC, Argüello JA, Abdala G, Orioli GA. Content of auxin-inhibitor-and gibberellin-like substances in humic acids. Biologia Plantarum. 1990;**32**:346-351. DOI: 10.1007/BF02898497

[123] Pizzeghello D, Francioso O, Ertani A, Muscolo A, Nardi S. Isopentenyladenosine and cytokinin-like activity of different humic substances. Journal of Geochemical Exploration. 2013;**129**:70-75. DOI: 10.1016/j.gexplo.2012.10.007

[124] Pizzeghello D, Nicolini G, Nardi S. Hormone-like activities of humic substances in different forest ecosystems. New Phytologist. 2002;**155**:393-402. DOI: 10.1046/j.1469-8137.2002.00475.x

[125] Canellas LP, Dantas DJ, Aguiar NO, Peres LEP, Zsögön A, Olivares FL, et al. Probing the hormonal activity of fractionated molecular humic components in tomato auxin mutants. Annals of Applied Biology. 2011;**159**:202-211. DOI: 10.1111/j.1744-7348.2011.00487.x

[126] Borcioni E, Mógor ÁF, Pinto F. Influence of the application of fulvic

acid on seedling root growth and yield in lettuce. Revista Ciência Agronômica. 2016;**47**:509-515. DOI: 10.5935/1806-6690.20160061

[127] Eshwar M, Srilatha M, Bhanu Rekha K, Harish Kumar Sharma S, Eshwar CM. Effect of humic substances (humic, fulvic acid) and chemical fertilizers on nutrient uptake, dry matter production of aerobic rice (*Oryza sativa* L.). Journal of Pharmacognosy and Phytochemistry JPP. 2017;**6**:1063-1066

[128] Elmongy MS, Zhou H, Cao Y, Liu B, Xia Y. The effect of humic acid on endogenous hormone levels and antioxidant enzyme activity during in vitro rooting of evergreen azalea. Scientia Horticulturae. 2018;**227**:234-243. DOI: 10.1016/j.scienta.2017.09.027

[129] El-Hoseiny HM, Helaly MN, Elsheery NI, Alam-Eldein SM. Humic acid and boron to minimize the incidence of alternate bearing and improve the productivity and fruit quality of mango trees. HortScience. 2020;**55**:1026-1037. DOI: 10.21273/HORTSCI15053-20

[130] Boettcher GN, Gonzatto MP, Gargioni ED, Munhoz BM, Griebeler SR, Schneider LA, et al. Aplicação foliar de ácidos fúlvicos pode incrementar a abscisão de frutos jovens em tangerineira 'Montenegrina'. Anais. In: XIII Encontro Brasileiro de Substâncias Húmicas e Matéria Orgânica Natural. Itapeva, Brazil: Unesp - Campo Experimental de Itapeva; 2019. pp. 198-201

[131] Nardi S, Pizzeghello D, Ertani A. Hormone-like activity of the soil organic matter. Applied Soil Ecology. 2018;**123**:517-520. DOI: 10.1016/j.apsoil.2017.04.020

[132] Stevenson FJ. Humates: Facts and fantasies on and, their value as commercial amendments. Crops Soils Magazine. 1979;**31**:14-16

[133] Fernández-Escobar R, Benlloch M, Barranco D, Dueñas A, Gañán JAG. Response of olive trees to foliar application of humic substances extracted from leonardite. Scientia Horticulturae. 1996;**66**:191-200. DOI: 10.1016/S0304-4238(96)00914-4

[134] Akinremi OO, Janzen HH, Lemke RL, Larney FJ. Response of canola, wheat and green beans to leonardite additions. Canadian Journal of Soil Science. 2000;**80**:437-443. DOI: 10.4141/S99-058

[135] Casierra-Posada F, Rodríguez CA, Fischer G. Reducing negative effects of salinity in tomato (*Solanum lycopersicum* L.) plants by adding leonardite to soil. Acta Horticulturae. 2009;**821**:133-140. DOI: 10.17660/ActaHortic.2009.821.14

[136] Sugier D, Kołodziej B, Bielińska E. The effect of leonardite application on *Arnica Montana* L. yielding and chosen chemical properties and enzymatic activity of the soil. Journal of Geochemical Exploration. 2013;**129**:76-81. DOI: 10.1016/j.gexplo.2012.10.013

[137] Rose MT, Patti AF, Little KR, Brown AL, WRC J. A meta-analysis and review of plant-growth response to humic substances: Practical implications for agriculture. In: S DL, editor. Advances in Agronomy. San Diego: Elsevier Academic Press Inc; 2014. p. 124

[138] Conselvan GB, Pizzeghello D, Francioso O, Di Foggia M, Nardi S, Carletti P. Biostimulant activity of humic substances extracted from leonardites. Plant and Soil. 2017;**420**:119-134. DOI: 10.1007/s11104-017-3373-z

[139] DEWICK PM. Medicinal natural products: A biosynthetic approach. 2 ed. New York: John Wiley; 2002. 514 p

[140] Zobel AM, Brown SA. Coumarins in the interactions between the plant and its environment. Allelopathy Journal. 1995;**2**:9-19

[141] Saleh AM, Abu E-SW. Evidence for "gibberellin-like" activity of coumarin. South African Journal of Botany. 2015;**100**:51-57. DOI: 10.1016/j.sajb.2015.05.010

[142] Parvin K, Nahar K, Hasanuzzaman M, Bhuyan MHMB, Mohsin SM, Fujita M. Exogenous vanillic acid enhances salt tolerance of tomato: Insight into plant antioxidant defense and glyoxalase systems. Plant Physiology and Biochemistry. 2020;**150**:109-120. DOI: 10.1016/j.plaphy.2020.02.030

[143] Abenavoli MR, Sorgonà A, Albano S, Cacco G. Coumarin differentially affects the morphology of different root types of maize seedlings. Journal of Chemical Ecology. 2004;**30**:1871-1883. DOI: 10.1023/B:JOEC.0000042407.28560.bb

[144] Pergo ÉM, Abrahim D, Soares da Silva PC, Kern KA, Da Silva LJ, Voll E, et al. Exhibits high sensitivity to Coumarin in comparison with three other weed species. Journal of Chemical Ecology. 2008;**34**:499-507. DOI: 10.1007/s10886-008-9449-8

[145] Saleh AM, Kebeish R. Coumarin impairs redox homeostasis in wheat aleurone layers. Journal of Plant Research. 2018;**131**:157-163. DOI: 10.1007/s10265-017-0968-7

[146] Sultana R, Abbasi MW, Adnan MY, Azeem M. Exogenously applied Coumarin-induced salt tolerance in a multipurpose crop sorghum bicolor under saline conditions. Journal of Advances in Biology & Biotechnology. 2020;**17**:177-184

Chapter 5

Mexican Lime (*Citrus aurantifolia*) Crop Management

Mario Orozco-Santos,
Miguel Ángel Manzanilla-Ramírez,
José Joaquín Velázquez-Monreal,
Marciano Manuel Robles-González,
Manuel de Jesús Bermúdez-Guzmán,
Karina de la Paz García-Mariscal
and Gilberto Manzo-Sánchez

Abstract

Limes and lemons are grown in 107 countries, in an area of 983,657 hectares, from which more than 15 million tons of fruits were obtained in 2012, which represents 13% of the total citrus fruit produced in the world. Three species of citrus are grown internationally, which due to their acidic taste and the use that consumers give them, mainly in the preparation of soft drinks and dressings for meals, are considered lemons. In Mexico, the production of Mexican lime [*Citrus aurantifolia* (Christm.) Swingle] is of great economic and social importance. It grows on 81,221.9 hectares distributed in 24 federal entities. Of these, Michoacán, Colima, Oaxaca, and Guerrero with 42,931, 18,996, 6843, and 6856 hectares, respectively, are the most important. The annual production of fruit recorded in 2013 exceeded 1.04 million tons, with a value of 3371 million pesos. The Mexican lime production system generates many jobs practically all year round, both for crop management and in the harvest and processing of fruit. In addition, there are a considerable number of plant-producing nurseries and input-providing companies that benefit from the cultivation of this citrus. This chapter describes the studies on the development and innovation of techniques in Mexican lemon carried out during the last 25 years in Mexico, such as induced mutation and manual hybridization for the generation of new varieties, as well as the use of interstocks and the evaluation of new genotypes with desirable characteristics. On the other hand, propagation and establishment in the field are fundamental points for a good development and production of Mexican lime.

Keywords: production, grafting, propagation, breeding, diseases, pests

1. Introduction

Citrus is believed to have originated in some regions of Southeast Asia, in an area that includes China, India, the Indochina peninsula, and nearby archipelagos [1, 2]. However, they are currently widespread and cultivated in many parts of the world. The main producing regions of this fruit are located between latitudes of 40°N and 40°S [3], in areas with tropical and subtropical climates, where winter temperatures are moderate and allow the survival and development of the trees, and which also have sufficient rainfall or irrigation water available for their growth and fruit production [4]. However, the most important producing regions are located between 20° N and 20° S [5].

The generic term citrus refers to several species of trees that produce edible fruit and whose cultivation is commercially important in various regions of the world. These species include sweet orange (*Clonorchis sinensis* L.) Osbeck), sour orange (*Citrus aurantium* L.), mandarins (*C. reticulata* Blanco), lemons (*C. limon* L. Burm.), sweet limes (*C. limetta* Risso), Mexican lime [*C. aurantifolia* (Christm.) Swingle] and (*Curculigo latifolia* Tan.), grapefruits (*C. paradisi* Macf.), pomelos (*Coccinia grandis* L. Osbeck), and citrons (*Citrus medica* L.), as well as some intergeneric and interspecific hybrids generated by controlled pollination among some of them.

Due to the harvested surface area and the volumes of fruit production, as well as its wide consumption and nutritional value, citrus is one of the most important fruit crops in the world. According to data from the Food and Agriculture Organization (FAO), in 2023, 129 countries from different continental regions were registered as cultivating and producing some type of citrus fruit. A production of 169.39 million tons of fruit was recorded, on a harvested surface of 10.55 million hectares [6].

For the statistical management of the data, FAO [6] has concentrated the different species of citrus fruit into four main groups: (1) oranges; (2) mandarins, which include tangerines, clementines, and satsumas; (3) limes and lemons; and (4) grapefruits and pomelos. During 2012, limes and lemons were cultivated in 107 countries, on a surface of 983,657 hectares, from which 15.15 million tons of fruits were obtained, which represents 13% of the total citrus fruit produced in the world and places this group of Citrus in third place in economic importance (**Figure 1**).

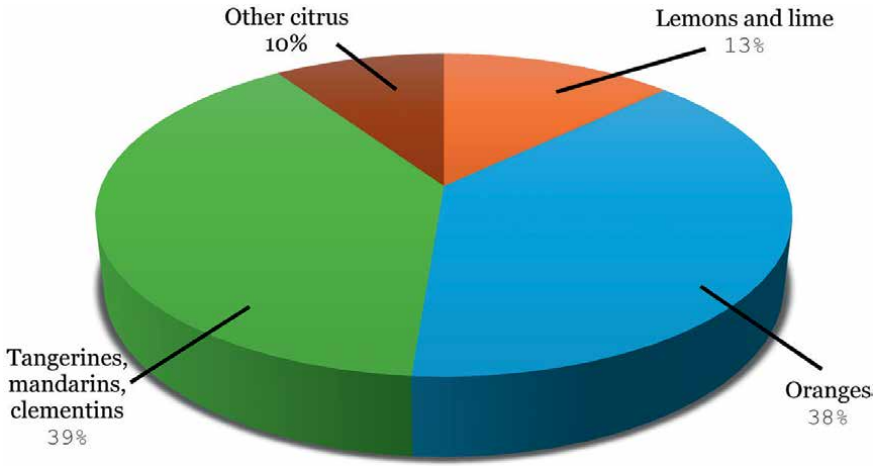

Figure 1.
World production of fruit from different citrus groups during 2023. A total production of 202,311,341.17 ton. Prepared with data from FAO (2023).

The statistical information obtained from FAO does not allow determining the cultivated areas or production volumes for each of these citrus species that make up the group of limes and lemons, either globally or specifically for each of the different countries that are producing them. There is also not enough information from the producing countries themselves, except for Mexico, which specifies the quantity of fruit they produce, whether of the Mexican, Persian, or Italian type.

There are currently 10.55 million hectares, producing 169.39 million tons of citrus fruit.

2. Citrus in Mexico

Mexico has a variety of geographic regions that present suitable climatic conditions for the cultivation of the different species of citrus and has developed a strong industry dedicated to the production of this fruit, mainly oranges, Mexican lime, and Persian lime. The most important citrus regions in Mexico are located in the following: Gulf of Mexico (Veracruz and Tabasco), northeast region of the country (Nuevo Leon and Tamaulipas), Huastecas region (San Luis Potosí, Hidalgo, and northern Veracruz), Yucatan peninsula (Yucatan, Quintana Roo, and Campeche), Pacific slope (Oaxaca, Guerrero, Michoacán, Colima, Jalisco, and Nayarit), as well as the coastal plain of the northwest (Sonora, Baja California Sur, and northern Sinaloa) [7].

Currently, Mexico ranks fifth as a citrus producer worldwide [7], and according to data published on the portal of the Agri-Food and Fisheries Information Service (SIAP), at the end of 2023, a cultivated area of 555,833 hectares was recorded, from which a production volume of 6.69 million tons of fruit was obtained [8]. The value of production exceeded 13,794 million pesos (**Figure 2**).

Three species of citrus are cultivated internationally, which, due to their acidic taste and the way they are used by consumers mainly in the preparation of refreshing drinks and dressings for food, are considered lemons. These are Mexican lime, Persian lime, and Italian lemon. Of these three species, only Italian lemons are actually lemons, which is why they are also known as true lemons. Botanically, both the Mexican lime and the Persian lemon actually belong to the group of acid limes [9].

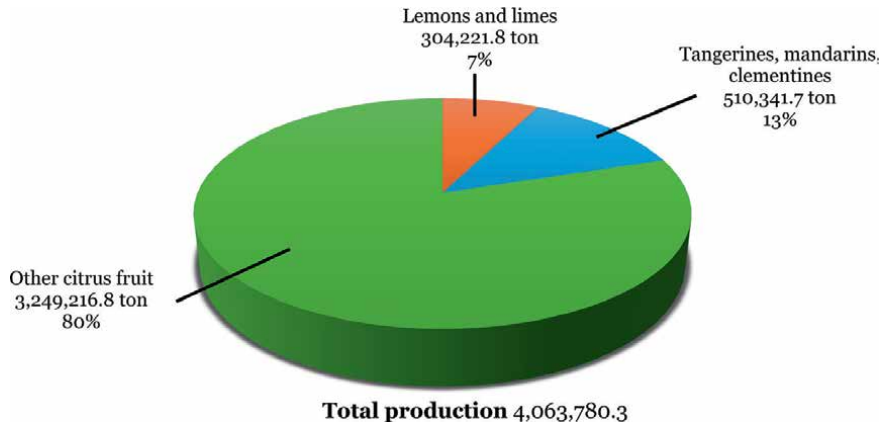

Figure 2.
Production volumes of different citrus fruits produced in Mexico during 2023. Prepared with data from reference [6].

3. The Mexican lime in the world

The origin of the Mexican lime comes from crossing the male parent *C. medica* with the papeda *Citrus micranta* [1]. The Mexican lime is a widely known citrus fruit in many countries and has received many local names: In Spanish-speaking countries, it has the names of Mexican lime or Creole lemon (Mexico), subtle lemon (Peru), and pica lemon (Chile). In other Spanish-speaking countries, it is also known as acid lime, small lime, bomb lime, little lemon, Creole lemon, or sour lemon. In the United States of America, it is known as Mexican lime, Key lime, or West Indian lime. In France, it is called limettier acide; in Germany, limett; in Italy, limetta; in India, nimbu or kagzi nimbu; in Brazil, Galician lemon; in Egypt, Baladi; in Morocco doc; and in the Philippines, dayap, among others [5, 10].

Although it is widely known in many countries, in most of them, there is not enough information on the statistics of its cultivation and production volumes, which would allow us to know the situation in relation to Mexico.

Some documents published in technical and scientific journals present information that is confusing or incomplete. Some authors indicate that this citrus is cultivated in countries such as Nepal, 2635 hectares [11]; Iran, without data [12]; Sudan, without data [13]; and Oman with 1209 hectares [14]. In India, several authors mention its cultivation but do not provide data or, if applicable, present figures that are contradictory: however [15] without data; while reference [16] reports 125,000 hectares; moreover, reference [17] reports 987,000 hectares; [18] cites 3.16 lakh hectares (1 lakh equals 100,000); and [19] points out that 23% of 480,000 hectares are cultivated with kagzi lime. In Peru, reference [20] reports 18,000 hectares; In Chile, reference [21] reports 86 hectares of this citrus fruit, which they know as lemon pica. In many countries in Central America, the Caribbean, and South America, the Mexican lime has a noncommercial presence.

4. Mexican lime in Mexico

It has been pointed out that the main producing countries of Mexican lime are Mexico, Peru, and India [9]. At the end of 2023, Mexico confirmed its position as the second largest producer and exporter of lemons and limes in the world. The progressive increase in its cultivation, its phytosanitary reputation, and the work of its producers made it possible to meet the high national and international demand throughout the year, with the largest harvest volumes from August to October. Mexico had a 20.5 percent share and an average annual growth rate of 8.4 percent from 2019 to 2023. It exported an average of 749 thousand tons, of which 98.7 percent were sent to three countries: the United States, with whom it generated a trade exchange of 659 million dollars; the Netherlands, 10 million dollars; and the United Kingdom, three million dollars [8].

This performance placed Mexico in the second place in marketing and production, above nations such as South Africa, the Netherlands, Turkey, the United States, Argentina, Brazil, China, and Italy. In the comparison between 2022 and 2023, exports last year accounted for 838 million dollars and 716 thousand 163 tons, a growth of 80 million dollars and 19 thousand 137 tons compared to 2022, 10.6 percent and 2.7 percent more compared to 758 million dollars and 697 thousand 026 tons of 2022, respectively.

The recent data from the Agri-Food Panorama of the Agri-Food and Fisheries Information Service (SIAP) have highlighted that at the end of 2023, lemon

production accounted for three million 240 thousand tons, 4.5 percent more than the three million 101 thousand tons of the previous year. On the other hand, Michoacán was ranked as the main lemon producer, with 953 thousand 652 tons, followed by Veracruz, with 867,916 tons; Colima, with 312,047 tons; Oaxaca, with 300,310 tons, and Tamaulipas, with 135,886 tons [8].

While the states of, Yucatan, Jalisco, Tabasco, Guerrero, and San Luis Potosi also contributed a significant amount to the national volume, he added. Therefore, 41.3 percent corresponds to the sour lime (Mexican, with seed), followed by the Persian lime and to a lesser extent the Italian lemon.

The Mexican lime production system generates many jobs in different activities, whether as day laborers and professionals in the field or workers in packing plants, industry, transportation, and personnel dedicated to the commercialization of fruit in the domestic and export markets. It is one of the crops that generate jobs practically all year round, both in the field and for the harvest and processing of fruit. In addition, there is a considerable number of plant-producing nurseries and input-providing companies that benefit from the cultivation of this citrus [22, 23]. The commercial cultivation of this citrus in Mexico has a history of more than 100 years. According to reference [24], the first Mexican lime orchards were established in Michoacán around 1912. Previously, the supply for consumption of this citrus came from the collection of fruit from wild trees, which was then taken to regional markets.

For his part, reference [25] points out that the cultivation of Mexican lime in Colima began in 1920. However, the commercial development of this crop did not occur until the 1940s. From this date onward, a rapid increase in cultivated surface and fruit production was observed, which led this region to be the main producer of this citrus for many years at a national and world level. There is no precise data for Guerrero and Oaxaca, but it is believed that it was in the 1940s when cultivation began [26]. In its diagnosis of the Mexican lime production system in Colima, the State Council of Limes in Colima (COEPLIM-COL.) prepared in 2002, presents the historical behavior of the production of this citrus in Mexico, from 1950 to 2001. From this study, it can be seen that in 1950, there were just over 10,500 hectares harvested and a production of 70,000 tons. By 2001, these variables reached 86,307 hectares harvested and a production volume of 1.121 million tons. During that time, the annualized yields went from 6.7 to 12.99 tons/ha. Significant increases in fruit yields began in the 1980s, when there was a sharp increase in the surface area devoted to this crop and producers began to adopt new technologies for the management of their orchards, using plants grafted onto rootstocks tolerant to soil pathogens, establishing plantations without associating with other crops, increasing planting densities, and applying irrigation and fertilization appropriately, among other innovations.

However, in the last 13 years, both the planted surface and production volumes have been unstable, with a clear downward trend, especially since 2005. However, by this time, there is a higher technological level, and yields have reached their highest values. According to historical data from SIAP, in 2000, more than 95,491 hectares were planted with Mexican lime, from which 1.23 million tons of fruits were obtained, with a value of more than 2781 million pesos. At the end of 2013, the planted and harvested surface area fell by 14.94 and 17.06%, respectively. The production volume barely exceeded 1 million tons of fruit, which represented a reduction of 18.05%.

The average yields per hectare during this period remained with little variation, which shows that the drop in production was mainly due to the reduction in the planted surface area, although to a certain extent, the drop in yields that occurred in

state of, Colima since 2011 caused by the huanglongbing or greening disease, known as HLB, also had an influence. In general, the tendency to reduce the surface area devoted to this crop is associated with the low prices of fruit that have been experienced in previous years. As a result, some producers converted their land to other crops. Although Michoacán, on the other hand, maintained a strong growth in the surface area cultivated with this citrus, it was not enough to reverse this trend.

5. Description and agroclimatic requirements of the Mexican lime plant

According to Ref. [27], compared to other citrus trees, Mexican lime trees (ungrafted) are vigorous and of medium size, with a bushy development and develop several thin and irregular stems (**Figure 3**). When grafted, the trees maintain the tendency to develop vigorous shoots (suckers) in the lower part of the tree. The terminal branches are generally provided with small, very sharp thorns (0.5 to 1.5 cm), which disappear when the branches are thick. The foliage is dense, formed by small, persistent, pale green leaves; the leaf blade is lanceolate or elliptical-ovate in shape, generally with an obtuse tip, rounded base, crenate edges, and petioles with narrow wings.

Studies carried out by [28] to understand the floral biology of lime indicate that the inflorescences are produced in clusters of two to seven flowers, in the axils of the leaves, and rarely appear alone. The flowers are small and white, with a cupulate calyx with four to five lobes, four to five petals, and 20 to 25 stamens. It is possible to find flowers on the tree most of the year, but the main blooms occur in two to three massive flows depending on the region and agronomic management.

5.1 Climate

Most cultivated citrus fruits originated in tropical and subtropical regions, so they are adapted to grow and bear fruit in these types of climates. Although they can survive temperatures of up to 2°C below zero for short periods of time, if this lasts for more than 2 hours, they can suffer considerable damage. On the other hand, they have high heat requirements to develop fruits of good size and quality; however, after 39°C, they stop their metabolic activity. The optimal temperatures for good vegetative development range between 23 and 34°C [29]. In particular, the Mexican lime, being native to the tropical and subtropical regions of the east of the Indian Archipelago

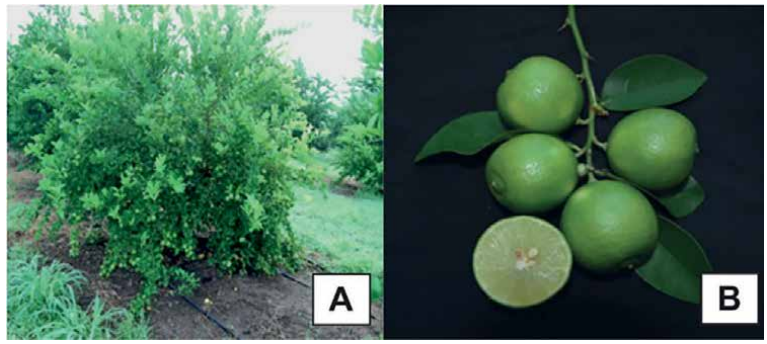

Figure 3.
Two-year-old tree (A) and fruit (B) of Mexican lime variety Colimex.

and like the citron, is one of the citrus fruits most sensitive to cold [1]. For optimal development, it requires temperatures ranging from 13–30°C, with average temperatures of 26–28°C. On the other hand, this species is very sensitive to damage from the fungus *Colletotrichum acutatum* (anthracnose), which occurs more intensely in humid and subhumid climates. Consequently, the most favorable conditions for its cultivation are those regions with rather warm, semidry climates. In Mexico, there are several states located mainly on the Pacific coast that have adequate conditions for commercial cultivation.

5.2 Rainfall

Water is one of the most important requirements for all crops. Many citrus trees are established in areas with good rainfall, which allows the trees to obtain the water necessary for their development and fruiting. In Mexico, the Mexican lime tree thrives well in areas with rainfall of between 700 mm (Colima and Michoacan) and 1500 mm (Guerreo and Oaxaca), which occurs in the summer (**Table 1**). In Colima and Michoacán, the crop is managed with constant irrigation, which allows it to maintain fruit production practically all year round. Therefore, in these producing regions, it is essential to have a secure supply of water to apply the necessary and timely irrigation during the rainless months.

5.3 Solar radiation

The trees perform better when grown in full sun, since when they are intercropped with another crop that provides shade, as is the case with the coconut palm, their productive capacity can be reduced by up to 50%. In Colima, until the 1980s, most of the Mexican lime tree surface was associated with coconut palm. Currently, most plantations are managed as a single crop, which allows for annual yields of more than 40 tons/ha.

5.4 Soils

Mexican lime trees can be grown in soils with a sandy to clay loam texture, with good drainage. Soils should be at least 60 cm deep, but preferably those deeper than 90 cm. High concentrations of $CaCO_3$ (>15%) in the soil generate suboptimal conditions for tree growth and cause a 20–30% decrease in yield, as well as a reduction in fruit quality (10%) and eventually, tree death [30]. However, with the use of a rootstock tolerant to this factor, soils with up to 40,000 ppm of this substance can be

State	Climate	Average temperature (°C)	Minimum temperature (°C)	Precipitation (mm)
Colima	BS1, AWo	26	13	700
Michoacán	BSo, BS1, AWo	28	12	700
Guerrero	AWo, AW1	28	14	1200
Oaxaca	AWo, AW1	27	14	1300

Table 1.
Predominant climates in the Mexican lemon-producing areas in four states of the Mexican Republic.

used. Electrical conductivity must be less than 2.0 mS/m and have a pH between 6 and 7.5. In the four states with the highest production of Mexican lime, the soil type is predominantly sandy loam, with a depth greater than 1.0 m and alkaline pH that in some cases reaches values of 8.5.

6. Genetic improvement of Mexican lime

The global citrus industry requires new varieties, not only to satisfy the fresh fruit market that demands high quality, absence of seeds, and high acidity content, as well as to meet the needs of the industry itself, but also to improve other agronomic characteristics that help reduce the threats posed by some diseases with a high economic impact [31]. In general terms, genetic improvement in citrus has been directed toward two main objectives: The first is aimed at the production of cup varieties that generate good yields, early ripening, good fruit size, and low number of seeds or preferably seedless and with loose skin. Recently, resistance to important diseases such as citrus canker (*Xanthomonas* spp.), citrus tristeza (VTC), and huanglongbing (HLB) (*Candidatus* Liberibacter spp.) among others has also been established as a fundamental issue. The second objective is focused on the generation of rootstocks that are resistant to viruses, mainly VTC, to root rot (*Phytophthora nicotianae,* synonym of *Phytophthora parasitica* Dastur), to nematodes and to environmental factors such as salinity, drought, and low temperatures, in addition to inducing a low tree height and early production [3, 32].

Genetic improvement of citrus has not been easy, and progress has therefore not been as significant when compared to other fruit trees. The phenomenon known as apomixis or nucellar embryony, present in many citrus fruits such as oranges, grapefruits, lemons, limes, and some mandarins [33] and which limits the development of embryos of sexual origin within the seed, has been an obstacle to the generation of hybrid varieties. In addition to apomixis, other factors such as the high heterozygosity caused by the hybrid condition of these species and which has been perpetuated due to apomixis itself, or due to cases of sterility, incompatibility, as well as the long juvenile period of the trees, have made genetic improvement through the conventional hybridization method difficult [34–37]. These characteristics have prevented plant breeders from making efficient use of existing sources of variation and generating new citrus varieties. Due to the above, the development of improved varieties in citrus until a few years ago was done mainly through the collection and evaluation of the genetic variation that occurred naturally within wild populations or in commercial plantations, as well as through the deliberate induction of mutations in elite varieties to generate genetic variation from which variant genotypes with some desired attributes are subsequently selected. Recently, with the development of biotechnology, new strategies have been used such as somatic hybridization, genetic transformation, and of course conventional hybridization assisted by the rescue *in vitro* germination of immature embryos and the early detection of hybrid plants through molecular markers. With these new technologies, the genetic improvement of citrus has been greatly revolutionized, overcoming the limitations, including some natural barriers inherent to the reproductive biology of citrus, especially those of interspecific and intergeneric compatibility.

6.1 Induced mutations in Mexican lime

In the Tecoman INIFAP Experimental Field, the program of radioinduced mutations in Mexican lime was started in 1990. It was determined that in this species,

Figure 4.
Fruit of Mexican lime mutants with changes in: (A) shape and size and (B) seed content.

when the seed contained more than 20% humidity, it did not tolerate doses higher than 200 Gy (gray) of ^{60}Co (cobalt 60) ranges, but if stored, seeds are used, and therefore, with a humidity percentage close to 10%, they can withstand doses of up to 350 Gy, with percentages of 1 to 5%. The germination percentages of the seed were reduced, but the average germination time increased as the radiation doses increased.

Within this population of trees, a large number of individuals were detected that produced seedless fruit or with a low number of these; however, the majority presented flower abortion or low fruit set (**Figure 4**). This shows that the production of seedless fruit is a characteristic that requires the presence of genes that control the phenomenon known as parthenocarpy. This is the capacity of the plant to set and develop the fruit without the need for the seeds to develop [38].

Four mutant genotypes were selected that during the research recorded annual fruit yields of between 80 and 130 kg, with good fruit size and low seed content (**Table 2**). The fruit shape, flavor, and acidity were not altered in these mutants.

After 6 years of semicommercial evaluation, none of the selected mutants managed to produce more than 60 kg per tree per year. This suggests that the generation of Mexican lime varieties that produce seedless fruit and high yield is not a simple task and that it will be necessary to first incorporate genes that control the phenomenon of parthenocarpy.

Current Mexican lime varieties or mutants generated from them do not present sufficient expression of these genes and consequently have a low potential for parthenocarpic fruit production. It was found that in these mutants, the seed content

Mutant	Yield Kg/tree/year	Fruit weight (g)	Juice content (%)	Seed content	
				Average/fruit	Range
M9 150 Gy	80.2	36.87	50.39	0.23	0 to 2
M70 150 Gy	87.0	34.97	51.54	0.11	0 to 1
M139 150 Gy	93.3	40.11	48.85	0.46	0 to 3
M4 100 Gy	130.8	33.96	50.64	0.55	0 to 3

Table 2.
Outstanding radio-induced mutant genotypes of Mexican lime for seed content and fruit yield.

was closely related to a high frequency of floral structure emission, but with a low percentage of setting and poor yield.

Since 2009, the Tecoman Experimental Field of INIFAP began a genetic improvement program of Mexican lime through sexual hybridization, aided by the techniques of rescue and *in vitro* culture of embryos in an immature state and the early identification of hybrids through flow cytometry techniques and molecular markers. The program focuses on the generation of varieties with resistance or greater tolerance to *Ca.* Liberibacter asiaticus (HLB) and the citrus tristeza virus (VTC), two diseases known for their devastating potential. It began with the selection of the parents, and for this, several important aspects were considered, including the characteristic of the Mexican lime that needs to be improved.

Considering that the Mexican lime agroindustry requires varieties with tolerance to HLB and VTC, from the genotypes available in the germplasm bank and other varieties cultivated in the region, it was determined which ones provided genes for the needs of the program. In this hybridization program, the following were used as parents: the diploid Mexican lime variety "Colimex" and two tetraploid experimental lines "Mex20" and "Mex13." From the Italian lemon, the varieties "Rosenberg," "Eureka," and "Limoneira 8a" were chosen. Citranges "Benton," "C-32," "C-35," and "Yuma" were also used, as well as a hybrid of the citrumelo "Swingle," as well as three somatic hybrids, "HS10" (*C. amblycarpa* × *citrange* Benton), "HS11" (*C. amblycarpa* × *citrange* 'C-35'), and "HS15" (*C. amblycarpa* × Pomelo).

In order to carry out a good hybridization program in citrus and in this case of Mexican lime, it is necessary to determine the quality and viability of the pollen. To achieve this, *in vitro* germination techniques (**Figure 5A–C**) or pollen grain staining

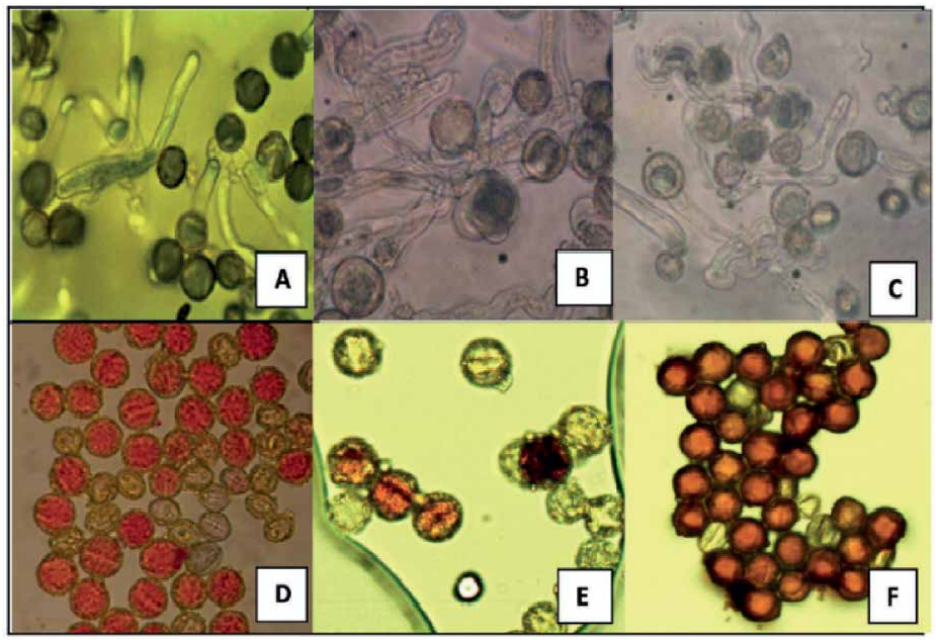

Figure 5.
In vitro *germination of Mexican lime pollen grains in different culture media: (A) Pio et al. [39], (B) Lora et al. [40], and (C) Leal [41] and staining with the dyes: (D) Acetocarmine, (E) 2,3,5-Triphenyl tetrazolium chloride, and (F) Safranin.*

(**Figure 5D–F**) can be used, which subsequently allows for a good selection of parents and good management of pollen and pollination.

The culture media of [39–41] promoted the germination of pollen grains of Mexican lime genotypes, as well as Italian lemons and citranges in very similar proportions. This means that any of the three-culture media is suitable for estimating the viability of the pollen of these genotypes. The diploid Mexican lime presents low pollen germination percentages and does not exceed 12%. However, tetraploid Mexican limes reach pollen germination percentages higher than 20%. Italian lemons and citranges present higher germination percentages compared to Mexican lime.

The sexual hybridization protocol for Mexican lime with other citrus fruits, which involves all activities, from the selection of flower buds, obtaining and handling pollen, pollination, rescue and *in vitro* germination of embryos in an immature state, and finally the transfer of seedlings to the greenhouse using the mini-graft technique (**Figure 6**), allowed the generation of little more than 1000 seedlings of 5000 plants, among which 269 diploid and triploid hybrids have been identified by morphological markers, flow cytometry, and molecular markers, from crosses made between diploid and tetraploid Mexican limes with Italian lemons and with the citranges C-35, C-32, and Benton.

In most of the hybrid Mexican lime plants, the management they received in the anti-aphid mesh houses, characterized by being in small pots, with overcrowding and less amount of solar radiation mainly, affected the expression of their characteristics, which prevented a good selection of hybrid individuals for their morphological characteristics.

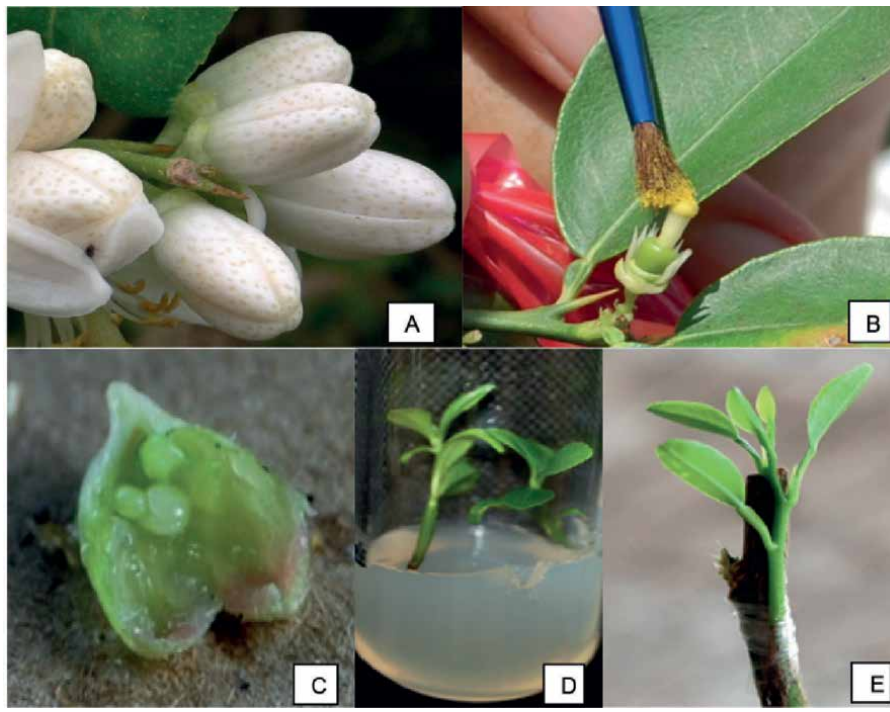

Figure 6.
Sequence of the hybridization process in Mexican lime. (A) Flowers before anthesis, B) Emasculated flower at the moment of pollen application on the stigma, (C) Seed removed from immature fruit showing three developing embryos, (D) development of seedlings from immature embryos germinated in vitro*, and (E) Apex of seedling transferred from the flask to the greenhouse using the mini-graft technique.*

However, in the field, in addition to leaving the juvenile period, the plants better express the characteristics of their parents and allow their characterization.

Due to its high heterozygosity in all the genotypes used as parents, a great diversity in the morphology of the plants of the progeny populations was obtained, especially in the crosses of diploid Mexican lime with Italian lemons. In the crosses of diploid Mexican limes with citranges, the hybrids show a strong dominance of the trifoliate genotype and most of the plants have trifoliate leaves and vegetative shoots with erect growth. However, in the crosses of tetraploid Mexican lime with citranges, the trifoliate phenotype is less expressed and sometimes appears chimeric. These hybrids have a phenotype similar to the Mexican lime, with a very low frequency of leaves with two or three leaflets. The hybrid character of these plants was verified by flow cytometry and by molecular markers, specifically with the microsatellite technique proposed by [42].

Some hybrids can be detected from crosses between Mexican limes and Italian lemons by the presence of anthocyanins in the tender vegetative shoots, since under the climate conditions of Colima, Mexican limes do not present them. A group of these plants was analyzed using the microsatellite technique and their hybrid character was confirmed. Some hybrids between tetraploid Mexican ^{x} Italian lemon produce larger, seedless fruit.

After several years of evaluation, INIFAP selected two varieties of Mexican lime that are well adapted, have high yields, and have excellent quality. One variety has thorns like the Mexican lime, while the other rarely develops them [43, 44]. The characteristics of the main varieties of Mexican lime grown in the Pacific of Mexico are described below.

6.2 "Colimex"

The tree is vigorous with a bushy development (**Figure 7**), and the main branches tend to bend due to the weight of the foliage and fruit. The twigs have small, pointed thorns, which make manual harvesting difficult. The foliage is dense, with small, lanceolate, and pale green leaves. It flowers all year round, although it has three to five massive flushes depending on the region and agronomic management. The inflorescences emerge from the apex of the shoot and from the axils of the leaves, in clusters of two to seven flowers. The flowers are small and white. The fruit is small, elliptical to

Figure 7.
Bunch of Mexican lime fruit, variety "Colimex."

Figure 8.
Bunch of Mexican lime fruit, variety "Lise."

hemispherical with a thin, leathery, and green shell. The fruit is light green at the time of cutting, rich in high quality essential oil. The pulp is light green, juicy, and very acidic. The fruits contain 3 to 5 seeds, and when fully ripe, they have yellow skin [27, 45, 46]. The fruit is harvested throughout the year, although maximum production occurs from May to October. The fruits reach their cutting maturity between 90 to 140 days from anthesis, depending on the time of year. The fruits that come from the flowers that emerge in December are those that last the longest to harvest [47, 48]. The vegetative shoots are highly susceptible to the anthracnose Colletotrichum acutatum when they are developing. The flowers and tender fruits are also very susceptible. This variety is well adapted to the agroclimatic conditions of the region, and its fruit has excellent acceptance in the market for fresh consumption and for its derivatives in the industry.

6.3 "Lise"

It is also the result of a second cycle of clonal selection on a thornless genotype, which was found in the 1970s as a natural variant seedling in a commercial Mexican lemon orchard (**Figure 8**). This variety can also produce more than 40 tons/ha from the fourth year onward, with fruit similar to that of the commercial variety, but with better postharvest performance.

6.4 "Colimón"

It is a natural variant that produces fruit with a low number of seeds. The trees are vigorous and have morphological characteristics similar to those of the "Colimex" variety. Most of its flowers abort, and therefore, its yields do not exceed 15 tons/ha.

7. Rootstocks to reduce tree size

The current trend in Mexican lime plantations is to use trees grafted onto "Macrofila" (*Citrus machopylla*) at high planting densities [49]. However, the vigor

of the rootstock and the climate conditions that favor the continuous growth of the trees cause that in a few years, the space assigned to each tree is exhausted and consequently the tree crowns become intertwined and shaded, causing a strong reduction in fruit production, in addition to making the management of the orchard difficult and ultimately reducing the productive life of the plantation. Using high planting densities requires intensive management. The use of citrus materials that promote controlled growth and generate trees of reduced size is a measure that allows greater ease and efficiency in cultivation tasks. In Colima, Mexico, the dwarfing effect of some trifoliate rootstocks, including the Flying Dragon, was evaluated; however, they did not show their dwarfing effect because they had problems adapting to soils with a high calcium carbonate content. Under these conditions, Mexican lime trees showed strong iron chlorosis, limited crown growth, and low production [50]. Valencia orange grafted on trifoliate rootstocks also did not perform well in calcareous soils with a pH of 7.5 to 8.5 and recorded much lower yields than the "Amblicarpa" and "Volkameriana" rootstocks [51]. Interstocks in Mexican lime is, an alternative for the use of soils with alkalinity problems and that at the same time could contribute to controlling the size of the tree, is the use of interstocks. This alternative consists of making two grafts, one between the rootstock and the interstock and another between the interstock and the variety. The selected interstock can be a genotype with dwarfing or stunting characteristics on a rootstock adapted to the type of soil, such as "Macrofila," and, as a second graft, the Mexican lime variety on the dwarfing one. For example, reference [52] evaluated different stunted, including several trifoliate and their hybrids as interstocks and "Macrofila" as rootstock, with the aim of developing smaller, more productive trees that are easier to manage. The interstocks of the citrange "Willits," the mandarin "Shekwasha," and the trifoliate hybrid "Cleopatra" × "Swingle" were promising because they reduce the vigor of the lime trees and also produce crops similar to "Macrofila." The use of lime interstocks does not significantly increase yields compared to "Macrofila." However, with their use, it was possible to reduce the size of the trees by 25 to 48% and develop trees on calcareous soils at a higher density [52]. Otherwise, reference [50] reported that the interstocks of *Poncirus trifoliata* "Hiryu" and "Flying Dragon" achieved 30% less growth than

Figure 9.
Mexican lime plant showing the flying dragon interstock.

Figure 10.
Effect of use of dwarfing rootstock on Mexican lime: Left "Macrofila" and right trifoliate hybrid. Right bar scale = 2 m.

"Macrofila," presenting good prospects for their use in high plantation densities, since due to their smaller size, they could be established at densities higher than 312 trees per ha (**Figure 9**).

In recent studies [44], they determined the agronomic behavior of the Mexican lime varieties "Colimex" and "Lise," grafted onto different intergeneric hybrid rootstocks that involve trifoliate genotypes, among them: "Sunki" × "English," "Cleopatra" × "Swingle," "Sunki" × Trifoliate, and "Cleopatra" × "Barnes," in a clayey crumbly soil, with a high $CaCO_3$ content and a pH of 7.8. The results showed that the evaluated rootstocks reduce the size of Mexican lime trees from 20 to 56% and presented acceptable fruit productions, demonstrating that it is feasible to use them at high densities to better face the problem of HLB even in soils with high pH (**Figure 10**) [44].

8. Production of propagative material

Establishing a citrus plantation requires the execution of various activities that are essential for its survival and economic profitability. The training of plants in the nursery and the selection of the land are two important aspects for the establishment of a plantation, which must have an economically productive cycle of no less than 15 years. The health and genetic quality of the propagation material, the technology used, as well as the care developed in the creation and training of the plants are fundamental factors that will influence the entire useful life of the plantation [53]. A basic principle for current global citrus farming is the sanitary and varietal certification of basic propagation material such as seeds and buds, as well as the technology used for the production of nursery plants. These two aspects constitute the guarantee of the longevity and productivity of new plantations [54]. On the contrary, a low-quality nursery plant can lead to an unproductive plantation and make an agribusiness unviable [55].

In recent years, phytosanitary problems have arisen that put citrus industries at risk worldwide. And since citrus plants are potential disseminators of pests and diseases such as Citrus Variegated Chlorosis (CVC), Tristeza, Leprosis, Phytophthora, Huanglongbing (HLB), and Canker [56], it is essential to obtain healthy propagation material in a protected environment that prevents the entry of insects and vector mites. The nurseries used in the new citrus plant production systems are based on carrying out all activities under protected or closed systems [53]. In Mexico, there is the National Program for Phytosanitary Certification of Citrus, which is a scheme that certifies all stages of the production of citrus propagative material, the purpose of which is to ensure that producers have healthy, high-quality plants. This certification program began in 2002 with the publication of the Mexican Official Standard NOM-079-FITO-2002, "Phytosanitary requirements for the production and mobilization of propagative material free of tristeza virus and other pathogens associated with citrus." The production of open-air nursery plants was permitted until 2009, the year in which the presence of HLB was reported in the country, and from 2010, it must be done under protected conditions in areas with this disease, when the "Agreement by which the phytosanitary measures to be applied for the control of Huanglongbing (*Candidatus* Liberibacter spp.) and its vector are made known" was published, also being the basis for the certification program [57, 58].

9. Propagation of Mexican lime

The way to propagate the Mexican lime, as for many fruit trees, can be by seed or vegetatively. The first of these is not recommended nor is it used to establish commercial plantations, since although the trees that originate with it are vigorous and productive, they are also susceptible to diseases such as gummosis (*P. parasitica*); in addition, they have a long juvenile period delaying the start of fruit production, and plants out of type can be generated [9]. Normally, citrus fruits are propagated by grafting; In other words, trees are formed by the union of a crown and a rootstock [59]. These two parts are originated separately. The aerial part is the variety that forms the crown of the tree, while the lower part corresponds to the rootstock. Both are connected at a short distance at the top of the trunk, where they were grafted one on top of the other [60]. Therefore, the horticultural behavior of a citrus tree is the result of the reciprocal interaction between its genetic components [61]. Vegetative propagation allows the production of new plants with the same characteristics of the variety selected to establish a plantation. In the case of Mexican lime, this type of propagation is done by bud grafting on rootstocks that are tolerant to gummosis or other adverse soil factors such as high concentrations of calcium carbonates. Trees obtained by grafting, being only of the desired variety, allow for uniform plantations with those that have the same yield and quality, are less vigorous, and are earlier to come into production [9].

To select a rootstock, it is necessary to understand the functions of the root and therefore those of the rootstock itself. The roots anchor the tree in the soil and absorb and transport water and nutrients, and the synthesis sites of growth regulators are located in them. Finally, a large part of the food reserves are stored there [60].

There is no perfect rootstock, even for a particular situation [62–64]. A rootstock is useful when it has certain characteristics such as good compatibility with the variety, adaptability to the soil and climate environments prevailing in the region where

the orchard will be established, and ability to produce a dense root vertically and horizontally, and the rootstock must have vigorous, persistent, and adequate annual growth [60]. Thus, even when there is no rootstock that has all the desirable characteristics, it is necessary to use one that allows overcoming most of the productivity limitations of each region where Mexican lime is grown [65].

Nursery plants can be marketed between 10 and 14 months after the start of their production cycle, which is influenced by the conditions in which it is carried out as well as by the rootstock used [55]. In the process of propagation by grafting of Mexican lime, there are two stages.

Seedbed – The period between sowing the seed of the rootstocks until they reach 10 to 20 cm in height lasts 2 to 4 months.

Nursery – In this stage, the rootstocks are first transplanted into containers so that they develop over a period of 2 to 4 months. Grafting is then carried out, and the resulting plants will be ready to be planted in the field after 3 to 6 months.

9.1 Nursery

Transplanting rootstock seedlings. Once the seedling stage has finished, the transplant continues, which is the first step in the nursery stage.

Space in protected structures where citrus nurseries are established is expensive, so for transplanting rootstock seedlings, it is essential to use a small, practical, and functional bag, in accordance with the period of time that the plant will be in it. The estimated cycle of the citrus nursery is 13 to 18 months, a period in which the bags must provide support without breaking or crystallizing [53]. Currently, bags are used black polyethylene bags, 600 caliber, 20 × 30 cm, with a capacity of 3.5 liters. These bags, when filled, remain vertical with a height of 23.5 cm and a diameter of 13 cm. It is necessary to ensure that there is a free space of 2.5 cm from the level of soil or substrate that is deposited in each bag to the upper edge of the same to receive the irrigation water. With the full bags, beds or blocks of four aligned rows can be formed (**Figure 11A**) and a 55 to 60 cm wide corridor can be left between the beds to allow for plant management and care activities.

Figure 11.
Seedbed in bed (A), in plastic box (B), and styrofoam tray (C).

Figure 12.
Plastic bags used for transplanting rootstock seedlings (A); removal of seedlings from the seedbed to be graded and transplanted bare-root (B); holes where seedlings will be transplanted in plastic bags (C and D).

Rootstock seedlings are transplanted when they are 15 cm tall into plastic bags with previously disinfected loamy soil [9]. It is advisable to add *Trichoderma* or mycorrhizae to the soil in combination with slow-release fertilizers [53]. Seedlings with deformed or defective roots, such as the so-called "pigtail" root, which has a 360° turn, should be discarded, as well as those that are out of type, because they are either stunted or too large [9].

The seedlings are carefully removed from the seedbed with a flat shovel or similar tool. This extracted material is left bare-rooted and classified according to size so that each bed has plants of the same size, which is expected to also have a similar development (**Figure 11B**).

For transplanting, a modified stake or an L-shaped craft tool with a sharp end is used to make a hole in the soil of each bag (**Figure 12C**), where a seedling is inserted up to the height of the root collar, which should remain free while the root is buried without being bent; for this, the same tool is stuck on one side of the hole, pressing toward the root so that the plant remains fixed (**Figure 12D**). Once the transplant is finished, irrigation is applied. If the seedlings were produced in tubes, then instead of having them with bare roots, what is transplanted are seedlings with the substrate in which they have been developing.

9.2 Development of the rootstock

The rootstock must be formed with a straight growth, without branches, so during its development, the lateral shoots are removed.

The plants are grafted when they reach a diameter of 6 to 8 mm at the height proposed for the graft, which occurs 4–5 months after transplantation [53] in tropical conditions.

Grafting. The buds used to graft the rootstock plants must come from a certified bud production lot (LPY), where they must also provide a letter of guarantee of the varietal type to the nurseryman who acquires this type of propagative material. In addition, current regulations require that the material produced in an LPY must be free of the following diseases:

Psorosis, Tristeza, Exocortis, Cachexia, and HLB [58].

Before the buds are harvested, stalks of approximately 20 cm in length and 1 cm in thickness are selected and cut with pruning shears exclusive to the LPY where they are produced; between plants, this tool must be disinfected with a 5% solution of permanganate or sodium hypochlorite. The wound of each cut in the plant must be sealed with vinyl paint, while the stalk still with leaves is placed in a labeled plastic bag (variety, date, and number of buds) to be taken to be defoliated and disinfected (copper sulfate) in a closed space. The stalks are placed again in labeled plastic bags for transport and/or storage, which can be at temperatures of 4 to 8°C for a period of 10 to 15 days [58]. The graft should be made at a height of 35 to 40 cm from the rootstock [9]. To do this, the shoots, leaves, and thorns should be removed 10 cm above and below the grafting point days before [53]. This is to make it easier to place the bud to be grafted [62].

The type of graft most commonly used by most nurserymen is the inverted "T" shaped bud. This consists of making a vertical and horizontal cut with a knife at a proposed height on the stem of the rootstock. With the tip of the knife, the bark is lifted and the bud is introduced; then, it is covered by bandaging it with a plastic tape, in such a way that the cuts and the bud are covered to avoid dehydration of the latter or of the union of the cambium of the graft with that of the rootstock [9, 62], as well as how to prevent water from entering. Immediately after this, one of the following two options can be done: (1) Leave the entire rootstock plant as it was grafted with the bud. (2) Prune the rootstock stem 15 cm above the graft to leave a stump with leaves and thorns. If the rootstock is left intact, the graft will have poor growth due to competition between it and the foliage of the rootstock [62].

After 15 days of grafting, if it has survived, the plastic tape with which it was covered should be removed [9]. Two or three days later, the rootstock is pruned leaving a stump 8 to 10 cm above the grafted bud.

9.3 Finished plant

To achieve erect growth of the graft, when the initial sprout or first flush of budding (**Figure 13A**) is still flexible, it is brought close to the stump that was left by holding it in place with a 3 cm long "S"-shaped wire hook (**Figure 13B**). In addition, the first sprout of the graft is pruned at its apical part (**Figure 13C**) to stimulate a second flush of budding from the graft. Eight to 15 days after the second sprouting (**Figure 13D**), the wire hook is removed, and the rootstock stump is pruned again slightly above (0.5 cm) the graft (**Figure 13E**). When the third flush of budding occurs, the basic crown of what will become a tree is defined (**Figure 13F**).

Three months after grafting, the plant will be ready to be transplanted into the field [53]. It is not recommended to keep the plants in the nursery longer than required, because their roots and crowns may become deformed, reducing their quality and affecting their development in the field. An alternative may be to transfer

them to larger bags so that they can grow and use them as replants [9], although this implies an additional expense.

Figure 13.
First flush of graft budding (A), which is attached with a hook to the rootstock stump (B) and pruned at the top (C); second flush of graft budding (D); removal of the hook and pruning of the rootstock (E); finished plant (F).

Figure 14.
Mexican lime plantation in an area with high production potential.

9.4 Establishment of orchards and production systems

The productivity and return on investment of a Mexican lime orchard is related to the density of planting, vegetative growth, photosynthetic efficiency, flowering intensity, fruit set and growth, as well as the agronomic management of the factors that interfere with growth in the orchard [62]. The establishment of a Mexican lime plantation requires a series of prior tasks that are essential for its survival and economic performance (**Figure 14**).

The selection of soil and plants in the nursery is the main aspect to begin the work of a plantation that must have an economically productive cycle of no less than 15 years.

In addition, good orchard planning should consider adequate spaces for paths and tasks performed by machinery such as raising irrigation ditches, spraying foliage, weed control, and harvesting the crop. The location of irrigation channels, drains,

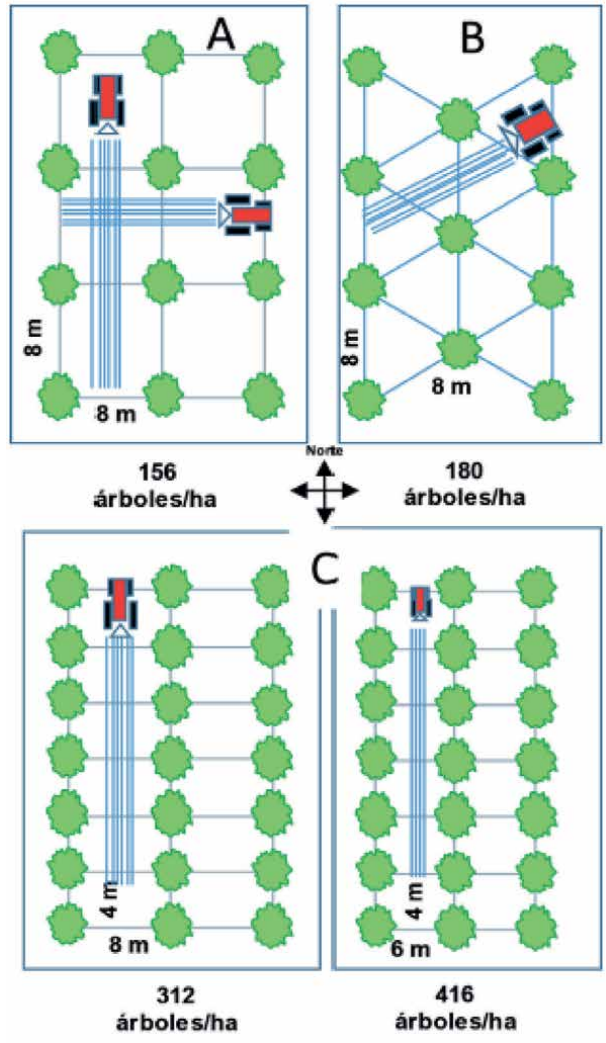

Figure 15.
Illustration of different planting layouts in square (A), staggered (B) and rectangle (C).

and the direction of the rows of trees must also be properly planned, according to the slope of the land. A bad decision in this regard will permanently make irrigation or the removal of excess water difficult and expensive [9].

Finally, the management that will be given to the adult plantation must be defined. In the case of an intensively managed orchard with high planting densities, this allows for a rapid return on investment and high yields, but at high costs; while low densities result in lower yields but lower production costs.

Planting density is the term generated to determine the number of trees to be planted in a hectare of cultivation. The distance between trees will depend on the variety, the rootstock, the type of soil, and the production system. However, based on the results of a 15-year investigation, where several planting densities were evaluated, it was concluded that wide planting densities with a low number of trees per hectare limit the obtaining of high yields [63–65]. With densities lower than 200 trees/ha, very high yields were not obtained. However, there was the advantage that the orchard was managed in a traditional way, without the trees losing their individuality and with less need for pruning [45].

The plantation layout depends on the selected design and planting density and determines the future management of the orchard. The orientation of the rows of trees must be done according to the direction of irrigation, the irrigation system, and the planting density. The distance between rows must be selected taking into account the estimated growth of the trees in their adult stage and the machinery that will be used in the orchard. During the entire plantation period, it is essential that the trees receive good sunlight. To do this, it is recommended that the rows be oriented from north to south. Thus, the hedges of adult trees will receive the sun's rays in the morning on the east side of the canopy and in the afternoon, they will receive them from the west. To lay out the plantation, the location of rows, drains, and canals must also be considered [9].

The most commonly used planting layouts for Mexican lime are described below:

a. Table. Square plantations are generally associated with a low planting density, so they require less investment and care, but the yields per hectare are lower than in high-density rectangular plantations; therefore, it is not recommended (**Figure 15A**).

b. Triangular or staggered. Greater number of trees per hectare than square or rectangular. This system can complicate harvesting, pruning, and pest and disease control tasks; therefore, it is not widely used today (**Figure 15B**).

c. Rectangular. This system allows for intensive plantations suitable for installing pressurized irrigation systems and is the most recommended (**Figure 15C**).

10. Conclusions

Citrus fruits are produced in 128 countries on the continents in tropical and subtropical regions, which produce 119.98 million tons on 7.46 million hectares. However, in Mexico, the production of Mexican lime is of great economic and social importance generates many jobs practically all year round, both for crop management and in the harvest and processing of fruit. The climatic conditions of the Mexican producing regions have favored the development and productivity of the Mexican lime,

while the technical development and innovation allow for high productivity rates of the fruit. Genetic improvement has allowed the development of new genotypes of Mexican lemon with high productivity. Therefore, there are opportunities to develop new strategies to combat pests and diseases that affect crops.

Author details

Mario Orozco-Santos[1], Miguel Ángel Manzanilla-Ramírez[1], José Joaquín Velázquez-Monreal[1], Marciano Manuel Robles-González[1], Manuel de Jesús Bermúdez-Guzmán[1], Karina de la Paz García-Mariscal[1] and Gilberto Manzo-Sánchez[2*]

1 Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) Campo Experimental Tecomán, Tecomán, Colima, México

2 Facultad de Ciencias Biológicas y Agropecuarias, Universidad de Colima, Tecomán, Colima, México

*Address all correspondence to: gmanzo@ucol.mx

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Curk F, Ollitrault F, Garcia-Lor A, Luro F, Navarro L, Ollitrault P. Phylogenetic origin of limes and lemons revealed by cytoplasmic and nuclear markers. Annals of Botany. 2016;**117**:565-583. DOI: 10.1093/aob/mcw005 [doi]

[2] Krueger RR, Navarro L. Citrus germplasm resources. In: Khan IA, editor. Citrus Genetics, Breeding and Biotechnology. Wallingford, Oxfordshire, UK: CAB International; 2007. pp. 45-140

[3] Gmitter FG Jr, Chen C, Rao MN, Soneji JR. Citrus fruits. In: Kole KR, editor. Fruits and Nuts, Genome Mapping and Molecular Breeding in Plants. Heidelberg, Germany: Springer; 2007. pp. 265-279

[4] Abobatta WF. Influence of climate change on citrus growth and productivity (effect of temperature). Advances in Agricultural Technology & Plant Sciences. 2019;**2**(2):180036

[5] Morton J. Mexican lime. In: Morton JF, editor. Fruits of Warm Climates. 1st ed. Miami, FL: Edit Florida Flair Books; 1987. pp. 168-172. 33189. ISBN: 0-9610184-1-0

[6] FAO. FAOSTAT: Producción de cultivos. Roma: FAO; 2024 [Recurso electrónico]. Recuperado de: https://www.fao.org/faostat/en/#data/QCL

[7] Orozco-Santos M, García-Mariscal K, Varela-Fuentes S. Los cítricos y su sanidad. In: Varela-Fuentes SE, Orozco-Santos M, Torres Acosta RI, Silva Aguirre GL, editors. Guía técnica para la identificación y manejo de plagas y enfermedades en cítricos. Ciudad Victoria, Tamaulipas, México: Departamento de Fomento Editorial de la Universidad Autónoma de Tamaulipas; 2013. pp. 1-16. ISBN: 978-607-7654-64-3

[8] SIAP. Producción agrícola: Cíclicos y perenes 2024. México: Servicio de Información Agroalimentaria y Pesquera SAGARPA - Secretaría de Agricultura Ganadería Desarrollo Rural Pesca y Alimentación; 2024. Available from: http://www.siap.gob.mx/cierre-de-la-produccion-agricola-por-estado/

[9] Medina UVM, Robles GMM, Becerra RS, Orozco RJ, Orozco SM, Garza LJG, et al. El cultivo del limón mexicano. Colima, México: INIFAP. Libro Técnico Núm. 1; 2001. 188 p

[10] Crane JH. Key Lime Growing in the Florida Home Landscape. Gainesville, Fla: Horticultural Sci. Dept., Univ. of Fla., IFAS, Univ. of Fla. Coop. Extn. Service; 2013. pp. 1-9

[11] Shrestha RL, Dhakal DD, Gautum DM, Paudyal KP, Shrestha S. Study of fruit diversity and selection of elite acid lime (*Citrus aurantifolia* Swingle) genotypes in Nepal. American Journal of Plant Science. 2012;**3**:1098-1104

[12] Vand SH, Abdullah TL. Identification and introduction of thornless lime (*Citrus aurantifolia*) in Hormozgan, Iran. Indian Journal of Science and Technology. 2012;**5**(11):3670-3673

[13] Elsadig HE, Suleiman ASE. Development of lime fruit (*Citrus aurantifolia*) in northern Gezira state, Sudan. Journal of Agriculture and Biodiversity Research. 2013;**2**(3):73-79

[14] Zadjali TS, Ibrahim R, Al-Rawahi AK. First record of *Diaphorina citri Kuwayama* (Hemiptera: Psyllidae) from the Sultanate of Oman. In: Insecta Mundi. Gainesville, FL:

Center for Systematic Entomology; 2008. pp. 1-7

[15] Balasubramanyan S, Manivannan MI. Root pattern studies in acid lime in silt clay soils. The Asian Journal of Horticulture. 2008;**3**(2):241-245

[16] Bhatt BB, Tomar YK. Effects of IBA on rooting performance of *Citrus auriantifolia* Swingle (Kagzi-lime) in different growing conditions. Nature and Science. 2010;**8**(7):8-11

[17] Patel KM, Patel HC, Patel KA, Chauhan VB, Patel JS. Effect of manures and fertilizers on growth and fruit yield of acid lime cv. Kagzi (*Citrus aurantifolia* Swingle). Asian Journal of Hortticulture. 2012;**7**(2):481-483

[18] Chavan S, Katole RT, Shambharkar YB, Ovhar N. Correlates of adoption behaviour of Kagzi lime growers in Western Vidarbha of Maharashtra state. IJSR - International Journal of Scientific Research. 2013;**2**(9):122-124

[19] Kumar M, Parthiban S, Saraladevi D, Ponnuswami V. Genetic diversity analysis of acid lime (*Citrus aurantifolia* Swingle) cultivars. The Bioscan. 2013;**8**(2):481-484

[20] Vegas RU, Narrea CM. Manejo integrado del cultivo de limón. Jornada de capacitación. Cieneguillo Sullana - Piura: UNALM – AGROBANCO; 2011. pp. 1-43

[21] Barjolle D, Vandecandelaere E. Identificar los productos de calidad vinculada al origen y sus posibilidades de favorecer el desarrollo sostenible. Roma (Italia): FAO; 2012. Available from: http://www.fao.org/documents/card/en/c/20e9cb6e-74bd-4de0-8af4-3957c3065ba8

[22] Robles-González MM, Velázquez-Monreal JJ, Manzanilla-Ramírez MÁ, Orozco-Santos M, Medina-Urrutia VM, López-Arroyo JI, et al. Síntomas del huanglongbing (HLB) en árboles de limón mexicano [*Citrus aurantifolia* (Christm) Swingle] y su dispersión en el estado de colima, México. Revista Chapingo Serie Horticultura. 2013;**19**(1):15-31

[23] Robles-González MM, Velázquez-Monreal JJ, Manzanilla-Ramírez MÁ, Orozco-Santos M, Medina-Urrutia VM. Experiencias sobre el huanglongbing (HLB) en limón mexicano de colima. In: Velázquez-Monreal JJ, Manzanilla Ramírez MA, Robles-González MM, Santos MO, editors. Memoria: IX Simposio Internacional Citrícola. 1er Simposio Internacional sobre HLB en Cítricos Ácidos. Colima, México: COEPLIM-INIFAP. Tecomán; 2013. pp. 1-30

[24] Missiaen E. Citrus in Mexico. United States Department of Agriculture. Foreigh Agricultural Service; 1981. 38 p

[25] Oseguera-Velazquez S. Ejemplo de Desarrollo Regional. S. A. México D.F: Editora y Distribuidora, Tecomán; 1973. 266 p

[26] COEPLIM. Diagnóstico del sistema producto limón mexicano en Colima. Colima: Consejo Estatal del Limón Mexicano de Colima. Tecomán; 2002. 139 p

[27] Hodgson RH. Horticultural varieties of citrus. In: Reuter W, Webber JH, Batchelor LD, editors. The Citrus Industry. Berkeley, California: University of California Press; 1967. pp. 431-589

[28] Robles-González MM, Medina-Urrutia UVM. Algunas características florales del limón mexicano (*Citrus aurantifolia* Swingle) y del limón persa (*Citrus latifolia* Tan), en Tecomán, Colima. In: III. Congreso Nacional de Fruticultura. Conafrut SARH. Gobierno

del estado de Jalisco. Guadalajara, México; Agosto 1981. pp. 1034-1048

[29] Soler-Fayos G, Soler-Aznar J. Cítricos. In: Variedades y técnicas de cultivo. Madrid, España: Mundi-Prensa; 2006. 242 p

[30] Pérez-Zamora O. Evaluación de mejoradores del suelo en limón mexicano. Terrain. 2002;**20**:337-346

[31] Viloria Z, Grosser JW. Acid citrus fruit improvement via interploid hybridization using allotetraploid somatic hybrid and autotetraploid breeding parents. Journal of the American Society for Horticultural Science. 2005;**130**(3):392-402

[32] Vardi A, Spiegel-Roy P. Citrus breeding, taxonomy and the species problem. In: Cary PR, editor. Proceedings of the International Society of Citriculture. Sydney, Australia; 1978. pp. 51-57

[33] Luro F. State of art of citrus genetics and genomics; illustration from international and collaborative ongoing projects for genome mapping and sequencing and gene-to-function studies (conference). In: First International Workshop and Biotechnology Applied to Tropical Woody Plants. Minas Gerais. Brasil: Universidade Federal de Lavras; 2012

[34] Kochba JP, Spiegel-Roy P. Cell and tissue culture for breeding and development studies of citrus. HortScience. 1977;**12**:110-114

[35] Vardi A. Protoplast derived plant from different Citrus species and cultivars. In: Proceeding of the International Society of Citriculture. Vol. 1. Riverside; 1981. pp. 149-152

[36] Ikeda F. Repression of polyembryony by gamma-rays in polyembryonic citrus. In: International Citrus Congress; 9-12 November 1981; Tokyo, Japan. 1981. pp. 39-44

[37] Vardi A, Neumann H, Frydman-Shani A, Yaniv Y, Spiegel-Roy P. Tentative model on the inheritance of juvenility, self-incompatibility and parthenocarpy. Acta Horticulturae. 2000;**535**:199-205. DOI: 10.17660/ActaHortic.2000.535.24

[38] Vardi A, Levin I, Carmi N. Induction of seedlessness in citrus: From classical techniques to emerging biotechnological approaches. Journal of the American Society for Horticultural Science. 2008;**133**(1):117-126

[39] Pio LA, Ramos JD, Pasqual M, Carvalho Santos F, Pereira Junqueira K. Receptiveness of the stigma and *in vitro* germination of orange pollen, submitted to different temperatures. Ciencia y Agrotecnología Lavras. 2004;**28**(5):1087-1091

[40] Lora J, Herrero M, Hormaza I. Germinación de polen de chirimoyo. Implicaciones para la optimización de la polinización manual. In: XI Congreso Nacional de Ciencias Hortícolas. Albacete, SECH: Actas de horticultura; 2007. pp. 134-136

[41] Leal F. Influencia de los métodos de almacenamiento sobre la viabilidad del polen en citrus y poncirus. Revista de la Facultad de Agronomía. (Maracay). 1969;**5**(2):5-28

[42] Kijas JMH, Thomas MR, Fowler JCS, Roose ML. Integration of trinucleotide microsatellites into a linkage map of citrus. Theoretical and Applied Genetics. 1997;**94**:701-706

[43] Becerra-Rodriguez S. Un clon sin espinas de limón mexicano (*Citrus aurantifolia* Swingle). Agricultura Técnica en México. 1979;**1**:65-71

[44] Manzanilla-Ramírez MA, Robles-González MM. Informe técnico: Proyecto de nuevas variedades e híbridos de cítricos para diversas condiciones edafoclimáticas de México. In: Campo Experimental Tecomán. Colima: INIFAP; 2013. 27 p

[45] Medina-Urrutia VM, Becerra-Rodríguez S, Robles-González M, Orozco-Santos M, Orozco-Romero J. El cultivo de limón Mexicano en Colima. Folleto para productores No 5. Colima: SARH-INIFAP. Campo Experimental Tecomán; 1993. 38 p

[46] Robles-González MM. Estudio de algunas características de la floración de dos clones de limón mexicano y del Persa en Tecomán [thesis]. Colima: Universidad de Guadalajara; 1983

[47] Medina-Urrutia VM, Valdez-Verduzco J. Effect of roostocks on seasonal changes of mexican limes (*Citrus aurantifolia* (Christm) Swingle) in Colima, México. In: International Citrus Congress; 9-12 November 1981; Tokyo, Japan. 1981. pp. 132-134

[48] Medina-Urrutia VM. Fenología del limón mexicano *Citrus aurantifolia* (Christm) Swingle. In: Valdez-Verduzco J, Medina-Urrutia VM, Saenz-Colín A, editors. II Simposium sobre la agroindustria del limón Mexicano. Colima: SARH-INIA-Campo Experimental Tecomán; 1984. pp. 133-149

[49] Manzanilla-Ramírez MA, Robles-González MM, Velázquez-Monreal JJ, Orozco-Santos M, Carrillo-Medrano SH. Manejo integrado del cultivo de limón mexicano con HLB Memoria: IX Simposio Internacional Citrícola. In: Velázquez-Monreal JJ, Ramírez MAM, Robles-González MM, Santos MO, editors. 1er Simposio Internacional sobre HLB en Cítricos Ácidos. Colima: Tecomán; 2013. pp. 43-75

[50] Medina-Urrutia VM, Robles-González MM, Manzanilla-Ramírez MA, Velázquez-Monreal JJ, Reyes-Hernández JE, Virgen-Calleros G, et al. Effects of trifoliate interstocks on growth and yield of Mexican lime trees. Journal of Agricultural Science and Technology B. 2014;**4**:545-554

[51] Pérez-Zamora O, Becerra RS, Medina UVM. Selección de portainjertos para naranja 'Valencia' en suelos calcinomórficos. Terrain. 2003;**21**:47-55

[52] Medina-Urrutia VM, Becerra-Rodríguez S. Interinjertos para aumentar la eficiencia productiva de árboles de limón mexicano [*Citrus aurantifolia* (Christm) Swingle]. In: II Simposio Internacional de Fruticultura Tropical y Subtropical. La Habana Cuba: Edit. Instituto de Investigaciones en Fruticultura Tropical; 2007. ISBN 978- 959-296-001-5

[53] Arango E, Capote M, Morera S, Clemente J. Viveros protegidos de cítricos. Manejo técnico. Memoria del Taller Regional sobre viveros de cítricos. In: Viveros de cítricos en el contexto fitosanitario actual. La Habana, Cuba: Instituto de Investigaciones en Fruticultura Tropical; 2010. pp. 92-121

[54] Anónimo. Sistema de producción de material de propagación certificado de cítricos en Cuba. Memoria del Taller Regional sobre viveros de cítricos. Vivero de cítricos en el contexto fitosanitario actual. La Habana, Cuba: Edit. Instituto de Investigaciones en Fruticultura Tropical; 2010. pp. 60-72. ISBN 978-959-296-020-6

[55] Graf CCD. Los viveros de cítricos en Brasil. Memoria del Taller Regional sobre viveros de cítricos. In: Viveros de cítricos en el contexto fitosanitario actual. La Habana, Cuba: Instituto de

Investigaciones en Fruticultura Tropical; 2010. pp. 73-91

[56] Zanetti M. Producción de planta bajo techo como alternativa a la presencia de plagas cuarentenarias. In: Memoria del XXXIV Simposio Nacional de Parasitología Agrícola. Acapulco, Guerrero. México: IAP México; 2008

[57] SAGARPA. Norma Oficial Mexicana NOM-079-FITO-2002, Requisitos fitosanitarios para la producción y movilización de material propagativo libre de virus tristeza y otros patógenos asociados a los cítricos. Pesca y Alimentación. México: Secretaría de Agricultura, Ganadería, Desarrollo Rural; 2002

[58] SAGARPA. Acuerdo por el que se dan a conocer las medidas fitosanitarias que deberán aplicarse para el control del Huanglongbing (*Candidatus* Liberibacter spp.) y su vector. México: Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación; 2010

[59] Caicedo AA, Gómez BCO. Portainjertos para la producción de cítricos en Colombia. Boletín divulgativo. CORPOICA. Centro de Investigación Palmira. Ministerio de Agricultura y Desarrollo Rural. Editorial Litotamara Ltda; 2006. 21 p

[60] Ferguson L, Sakovich N, Roose M. California Citrus Rootstocks. University of California. Division of Agriculture and Natural Resources; 1990. Publication 21477. 18 p

[61] Spiegel-Roy P, Goldschmidt EE. Biology of Citrus. Cambridge: Cambridge University Press; 1996. p. 230. ISBN 0 521 33321 0

[62] Davies FS, Albrigo LG. Crop Production Science in Horticulture 2: Citrus. Wallingford: CAB International; 1994. p. 254

[63] Jiménez VR, Zamora RV. Principales cultivares y patrones utilizados en la citricultura. Memoria del Taller Regional sobre viveros de cítricos. In: Viveros de cítricos en el contexto fitosanitario actual. La Habana, Cuba: Instituto de Investigaciones en Fruticultura Tropical; 2010. pp. 4-60

[64] Medina-Urrutia VM, Valdez-Verduzco J. Elevado retorno de capital con altas densidades de plantación en limón: SARH-INIFAP-CIPAC-Campo Experimental Tecomán. Folleto para productores No 1. México; 1994. 16 p

[65] Medina UVM. Comportamiento de portainjertos de limón Mexicano en Colima. Folleto Técnico No. 3. Colima, México: INIFAP-CIRPAC. Campo Experimental Tecomán; 1996. 36 p

Chapter 6

Reproductive Biology of 'Ataulfo' Mango: The State of the Art and Challenges for Improving Pollination and Fruit Production

Rodrigo Lucas-García, Víctor Rosas-Guerrero, Carina Gutierrez-Flores, Lorena Aleman-Figueroa, Maribel López-Atanacio and Eduardo Cuevas

Abstract

The 'Ataulfo' mango cultivar is one of the most important in Mexico, with widespread acceptance in national and international markets, attributed to its excellent organoleptic characteristics. However, in recent years, the profitability of this cultivar has significantly decreased, which has been associated with issues related to their reproductive biology. Therefore, understanding their pollination requirements is essential to increase its productivity. This chapter reviews the current research on floral biology, pollination ecology, and fruit production in 'Ataulfo' mango. It also addresses the pollination challenges that limit orchard productivity and affect fruit quality, such as self-incompatibility and stenospermocarpy. Finally, this chapter explores orchard designs and management strategies to improve pollination, increase yield, and ensure the economic sustainability of 'Ataulfo' mango production.

Keywords: conservation, crop pollination, malformed fruits, planting designs, pollen viability, pollination services, pollinator dependence

1. Introduction

The 'Ataulfo' mango cultivar is a massive-flowering perennial tree that originated in the Soconusco region, Chiapas, Mexico [1]. The popularity of this cultivar has increased in recent years due to its appealing organoleptic characteristics, such as a sweet taste, creamy texture, high pulp percentage, and extended shelf life [2]. The great acceptance that it has received in national and international markets has made it one of the most important mango cultivars in Mexico [3, 4]. As a result, the area cultivated of this cultivar has increased almost fivefold in the last 20 years (13,335 ha in 2001 to 64,877 ha in 2021, **Figure 1A**) [4].

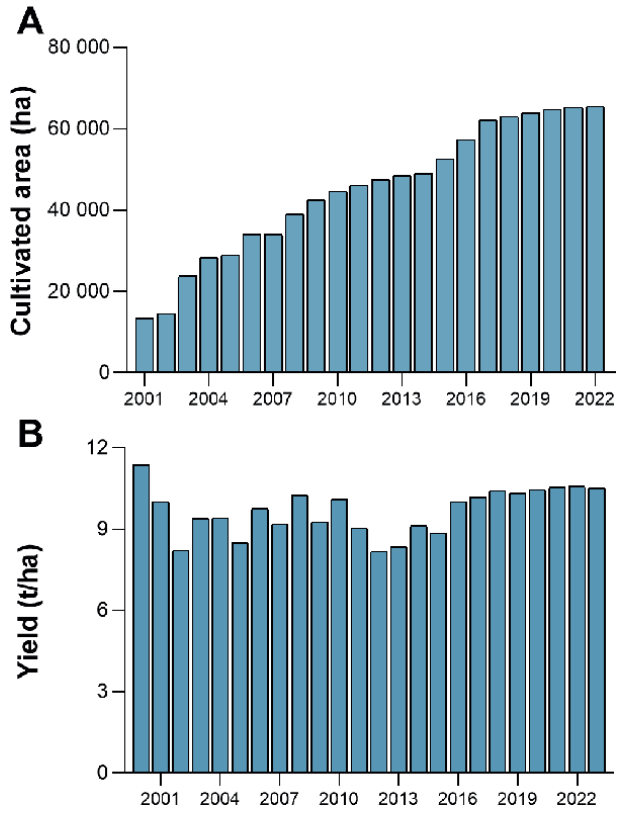

Figure 1.
The cultivated area of mango 'Ataulfo' in Mexico in the last 20 years (2001–2022; A) and average national yield per hectare from 2000 to 2023 (B). Source: SIAP [4].

Even when several technologies have been implemented for mango cultivation [3], in recent years, the productivity of this cultivar has decreased significantly in some regions [4], with average yields dropping from 15 tons per hectare in 1980 to around 10 tons in 2023 (**Figure 1B**) [4, 5]. This could be due to the increased occurrence in recent years of malformed fruits, known as nubbins, which have null value in the commercial market given their smaller size and weight [6, 7]. Several factors can be attributed to the low production of commercial fruits and the high incidence of nubbins in 'Ataulfo' mango, from aspects related to its floral biology to biotic and abiotic factors. Therefore, it is crucial to have a comprehensive understanding of its reproductive biology to identify the factors contributing to the low production of commercial fruits and to propose viable strategies to increase the economic income of 'Ataulfo' mango producers and satisfy the increasing demand of this fruit worldwide.

2. Floral biology

The 'Ataulfo' mango, as well as all mango cultivars, is an andromonoecious tree, with staminate and hermaphrodite flowers in the same inflorescence (**Figure 2**) [8]. The inflorescence is a terminal panicle with a conical shape, composed of a central axis (rachis) with lateral peduncles (sub-rachis) (**Figure 2A**) [8]. The number of

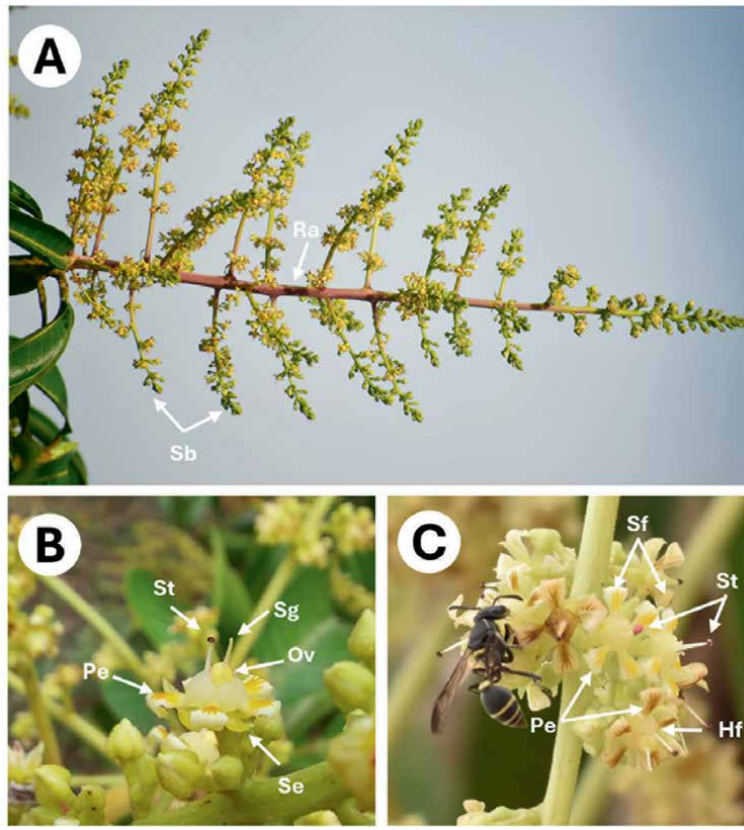

Figure 2.
An inflorescence (panicle) of 'Ataulfo' mango (A), morphology of a freshly open hermaphrodite flower (B) and hermaphrodite and staminate flowers (C). Hermaphrodite flower (Hf), staminate flower (Sf), rachis (Ra), sub-rachis (Sb), sepal (Se), stigma (Sg), stamen (St), ovary (Ov), and petal (Pe). Photos: Jose Antonio Gama Salgado (A) and Rodrigo Lucas-García (B and C).

panicles per tree varies depending on the size and age of the trees, as well as edapho-climatic conditions [9]. For example, in Ecuador, a 3 m-high tree produces on average 205 panicles, while a 4 m-high tree produces on average 321 panicles [9]. The panicle length can vary from 33 to 57 cm, with an average of 48 cm [10, 11].

The number of flowers per panicle fluctuates from 1441 to 4595, with an average of 2906 flowers [8]. The percentage of hermaphrodite flowers is usually higher (61%) than that of staminate flowers (39%) [8], although this proportion may vary depending on the phenological stage of the panicle [12]. The proportion of hermaphrodite flowers is higher at the beginning of flowering, whereas the proportion of staminate flowers increases as the days go by [12]. Although both types of flowers can be found along the panicle, staminate flowers predominate near the rachis, while hermaphrodite flowers are more common at the apex of the sub-rachis [13]. The more exposed location of the hermaphrodite flowers at the apex facilitates greater pollinator concentration, leading to a proportionally higher number of visits to these flowers and increased pollen transport to the stigma [14, 15].

The flowers have five petals and five sepals (**Figure 2B**). The sepals are light green, while the petals are yellow at the base and white at the tip (**Figure 2B**), and they turn brown a few days after anthesis. Flowers have three or four staminodes and one fertile

anther [13]. The color of the fertile anther is pink after anthesis and turns dark during dehiscence (**Figure 2C**). The anther has four lobes, and the pollen is released longitudinally [13]. The 'Ataulfo' pollen grains are tricolpate, and their size varies between 10 and 20 µm [10], while in other cultivars, the pollen grains are larger (20–45 µm long), with a flattened shape when dry and more spheroidal when hydrated [13, 16]. Hermaphrodite flowers have a superior, uniovulate functional ovary and a simple stigma (**Figure 2B**) [13]. In both types of flowers, the nectary is a fleshy disk located in the center of the flower. Flower size varies from 7.0 to 7.8 mm, with hermaphrodite flowers being relatively larger than the staminate ones (**Figure 3**).

2.1 Hermaphrodite-staminate flower proportion

Given that only hermaphrodite flowers are capable of set fruits, conditions that increase the ratio of hermaphrodite-staminate flowers are crucial to improve fruit production. Among these are trees grown at temperatures above 15°C during the day and 10°C at night or below 30°C [13, 17, 18] or with more exposure to sunlight [19], older trees [16], smaller panicles [20], pruned trees [21, 22], and trees with application of growth regulators, such as paclobutrazol (PBZ) (10 g/tree) and gibberellic acid (GA3; 100 ppm/tree) [23, 24]. It should be noted that these factors are not necessarily mutually exclusive.

2.2 Production and viability of pollen grains

Another key factor to improve fruit production is the quantity and quality of pollen grains. The number of pollen grains per anther is usually greater in hermaphrodite flowers than in staminate flowers. A study carried out in Mexico revealed that hermaphrodite and staminate flowers had an average of 913 and 643 pollen grains per anther, respectively [10]. Pérez et al. [25] suggest that this difference could be explained because hermaphrodite flowers have higher nutrient reserves for fruit production, favoring the anther development and, consequently, a higher production of pollen grains. In Mexico, Gehrke Vélez et al. [10] found that the production of pollen grains also varies spatially. This variation could be associated with the

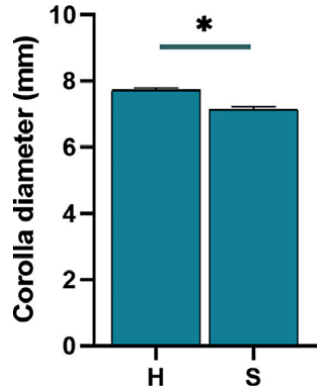

Figure 3.
*The size of hermaphrodite (H) and staminate (S) flowers in 'Ataulfo' mango cultivated in Guerrero, Mexico. Data were obtained from the measurement of the corolla of 90 hermaphrodite and 90 staminate flowers in December 2020. * Indicate statistical differences among flowers ($p < 0.05$). Source: own unpublished data.*

different soil and climatic conditions present at each site, as well as the variations in agricultural practices and the age of the plantation [10]. A recent study has found that plants of *Gentiana aristata* grown in sites with high soil moisture produced less pollen grains than those grown in sites with intermediate humidity or with drier conditions [26]. Similarly, in the squash it was found that plants exposed to higher temperatures produced greater amounts of pollen than those grown at lower temperatures [27].

It has been reported that in 'Ataulfo', mango pollen viability is higher in hermaphrodite than in staminate flowers [10, 25]. In Mexico, pollen viability was reported to vary among sites, ranging from 71 to 90% for hermaphrodite and 27–50% for staminate flowers [10]. In Spain, pollen viability was 93% in hermaphrodite and 85% in staminate flowers [25]. Similarly, pollen germination was found to be higher in hermaphrodite (15%) than in staminate flowers (2%) [10], while in Spain, no differences were found between the two types of flowers [25]. Compared to other cultivars such as 'Keitt' (81%) and 'Kensington' (70%), 'Ataulfo' shows a low percentage of germination, from 15% in Mexico [10] to 48% in Spain [25].

Pollen germination also varies depending on the cultivar and environmental factors, such as temperature [28]. Although no specific studies have been conducted in 'Ataulfo' on the effect of temperature on pollen germination, a recent study on several mango cultivars has indicated that the optimum temperature is between 24°C and 30°C, while higher or lower temperatures significantly reduce pollen germination rate [28]. Some nutrients were also reported that improve pollen viability and germination. For example, Muengkaew et al. [29] reported a germination rate greater than 80% when a calcium and boron mixture (3 ml/L) was applied during mango flowering, resulting in a notable improvement over the control treatment, whose germination rate was only 29–40%.

3. Pollination biology and ecology

For pollination to occur, it is necessary that the stigma be receptive and that the anthers be dehiscent. In the 'Ataulfo' mango, the stigmatic surface is receptive a few hours before flower anthesis (which occurs in the early morning) until 72 hours after [8]. However, the optimal period for pollination in 'Ataulfo' can only occur when anther dehiscence begins (between 10:00 and 11:00 h) [25]. Given the spatial separation between the fertile anther and stigma (i.e., herkogamy), mango flowers require pollen transfer mediated by vectors.

3.1 Pollen transfer

Some authors have suggested that wind is an important vector in mango pollination, even when it is evident that mango does not have any of the characteristics of an anemophilous plant [13]. In fact, 'Ataulfo' flowers, as in other mango cultivars, show adaptations to insect pollination, including sticky pollen that clumps together (mostly in humid conditions), which makes it difficult for pollen grains to be dispersed by wind [30]. The stigma is very small and lacks structures to help trap wind-borne pollen [31]. In addition, the flowers produce nectar that accumulates at the base of the petals to attract insects [31, 32], unlike the anemophilous flowers that generally do not have it.

The relevance of insects in the pollination of some mango cultivars has been documented [33]; however, in the case of 'Ataulfo,' there are no published studies specifically

quantifying its dependence on pollinators. We conducted an insect exclusion experiment in 2023 in three orchards in southern Mexico to evaluate pollinator dependence. In each of these orchards, six panicles with similar characteristics (i.e., located at the same height from the ground, similar size and developmental stage) from each of four trees were selected. Three panicles were bagged to exclude any pollinator visit ('closed' pollination treatment; **Figure 4A**), while the other three were left untreated to allow insect visitation ('open' pollination treatment). The fruiting rate (i.e., number of fruits per panicle) of each treatment was quantified 15 days later to minimize the effect of the tree's carrying capacity [34], given the huge flower abscission rate of mango.

This experiment showed that when insects are excluded from the flowers, fruit production decreased by 96% (**Figure 4B**). Only two malformed fruits were recorded in two of the excluded panicles, highlighting the critical dependence of this cultivar on insect pollination to achieve profitable and high-quality production of fruits. Furthermore, this agrees with a recent study carried out in the same study region, where the production of commercial fruits of 'Ataulfo' mango was positively related to the frequency of visits by native insects [7].

On the other hand, it is known that under open pollination, less than 1% of mango flowers develop into fruits [35]. A study conducted on other mango cultivars (i.e., 'Chok Anan' and 'Sala') showed that hand pollination (also known as artificial pollination) can increase fruit set by up to 117% compared to open pollination [35]. This suggests that a high pollination deficit (i.e., reduction in production due to shortage of pollen or suitable pollinators during flowering [34]) exists in these cultivars. While fruit set is increased by hand pollination, this is a difficult task for growers given the small size of flowers [35] and the huge flower abscission rate of mango mentioned above. In the case of 'Ataulfo,' hand pollination studies are needed to determine if pollination deficits exist in its cultivation areas.

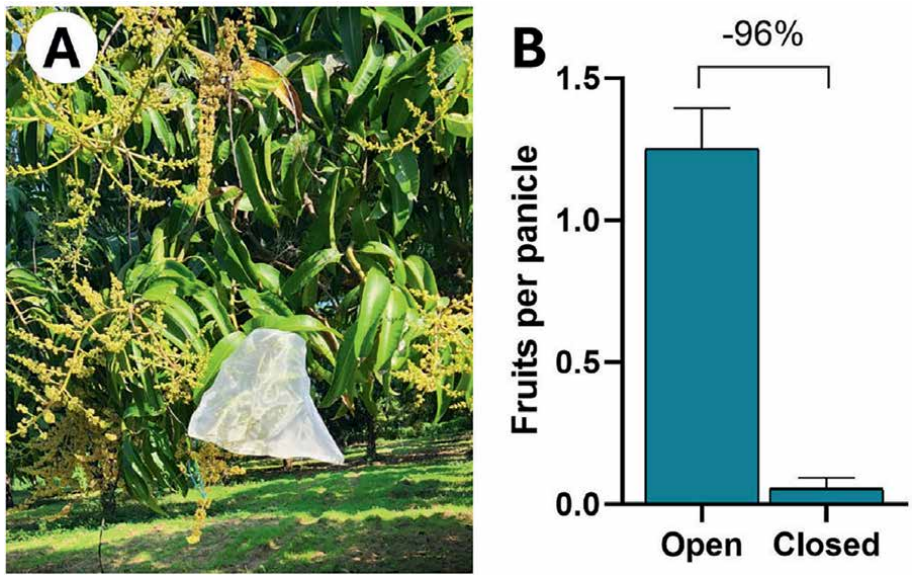

Figure 4.
Example of pollination bags used in the insect exclusion treatment (A), and effect of insect exclusion on fruit production per panicle in 'Ataulfo' mango orchards (B). Photo: Juan Ernesto Rendon-Caro. Graph source: own unpublished data.

3.2 Floral visitors

Several studies have documented the presence of a wide variety of insects visiting the flowers of different mango cultivars in different countries, mainly from the orders of Diptera and Hymenoptera [14, 32, 35–38]. In the case of the 'Ataulfo' mango, only two studies have documented their specific floral visitors. In Chiapas, Mexico, Grajales-Conesa et al. [39] observed that their flowers were visited by 10 bee species, where the stingless bee *Trigona fuscipennis* was the most frequent floral visitor, followed by the honeybee *Apis mellifera* and the stingless bees *Trigona nigerrima* and *Trigona fulviventris*, accounting for more than 96% of total visits. In Guerrero, Mexico, Severiano-Galeana et al. [7] found a wide variety of floral visitors, including dipterans from the families Calliphoridae, Muscidae, Sarcophagidae, Syrphidae, and Tabanidae; hymenopterans from the families Apidae, Formicidae, and Vespidae; hemipterans; coleopterans; and spiders.

To expand the knowledge and provide more information on the insects involved in the pollination of this cultivar, in 2023 we monitored the floral visitors of 'Ataulfo' mango in three orchards located in the state of Guerrero, southern Mexico. For this purpose, four 'Ataulfo' trees were randomly selected in each orchard. In each tree, a panicle was filmed using digital video cameras for three 20-minute periods (i.e., 10:00–10:20, 13:00–13:20 and 16:00–16:20). The total number of visits per floral visitor was recorded, and insects were identified using identification keys. A total of 281 visits from 15 different floral visitors were recorded during 12 hours of filming (**Table 1**; **Figure 5**). The honeybee was the most frequent visitor, followed by one

Order	Family	Species	Visits
Hymenoptera	Apidae	*Apis mellifera*	127
		Stingless bee sp. 1	44
		Frieseomelitta nigra	1
		Bee sp. 1	1
		Bee sp. 2	1
	Vespidae	*Brachygastra azteca*	26
		Polybia occidentalis	24
		Brachygastra mellifica	5
		Polistes sp. 1	1
		Wasp sp. 1	1
Diptera	Calliphoridae	Calliphorid sp. 1	19
		Chrysomya megacephala	6
	Muscidae	*Musca domestica*	18
	Tachinidae	Tachinid sp. 1	5
	Syrphidae	*Palpada pusilla*	2
Total			281

Table 1.
List of insects recorded visiting 'Ataulfo' mango flowers in three orchards during 2023 in Costa Grande, Guerrero, Mexico. (Source: own unpublished data).

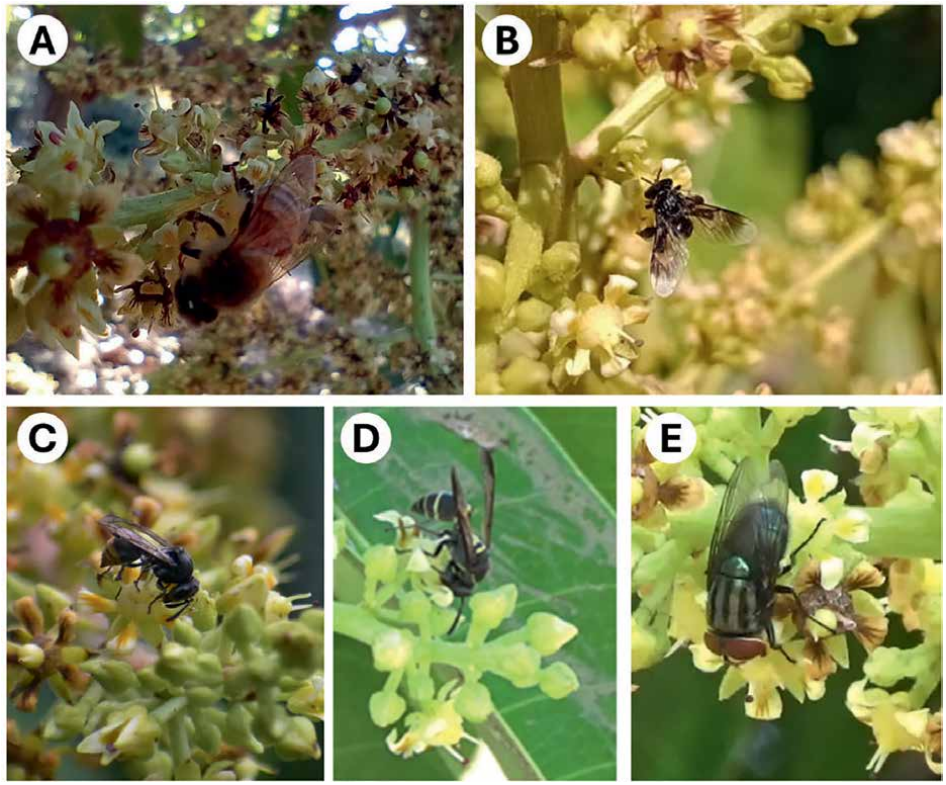

Figure 5.
Most frequent floral visitors of 'Ataulfo' mango flowers in Guerrero, Mexico. Apis mellifera *(A), stingless bee sp. 1 (B),* Brachygastra azteca *(C),* Polybia occidentalis *(D), and calliphorid sp. 1 (E). Photos: Rodrigo Lucas-García (A, D, and E), José Antonio Gama Salgado (B and C), and Víctor Rosas-Guerrero (C).*

stingless bee and the honey wasp *Brachygastra azteca*. Dipterans contributed with 18% of the visits, mainly by calliphorids and muscids (**Table 1**).

3.3 Effective pollinators

It is important to mention that not all floral visitors could be considered as pollinators of the 'Ataulfo' mango, since many of them did not contact the reproductive organs of the flower. Recently, we observed that both small-sized insects (<3 mm), such as ants, and large insects (>16 mm), such as butterflies, did not contact the reproductive parts of the 'Ataulfo' mango flowers in any of their visits, indicating that these insects are not effective pollinators of the 'Ataulfo' mango. On the other hand, insects around 8–13 mm in size (e.g., honeybees, yellow-banded wasps, calliphorids, and hoverflies) contacted the reproductive organs during more than 80% of their visits. Indeed, Severiano-Galeana et al. [7], in the same study site, found that of 29 and 21 species of floral visitors in 2019 and 2020, respectively, only 15 and 10 were considered as legitimate floral visitors, where the honeybee and the yellow-banded wasp (*Polybia occidentalis*) were the most important legitimate floral visitors for two consecutive years and were present in all selected orchards. Other floral visitors, such as one calliphorid fly and the stingless bee *Frieseomelitta nigra,* were also important legitimate visitors but were not present at all sites.

A study in Chiapas, Mexico, suggests that the common fly *Musca domestica* is a good pollinator of 'Ataulfo' and should be bred and released in large numbers in mango orchards [40]. Nevertheless, Cárdenas Portillo et al. [12] showed that the panicles where these flies were introduced did not produce any fruit, while the panicles exposed to natural pollination showed greater fruit production.

In this context, more studies are needed to examine in detail the contribution of various floral visitors to the pollination of the 'Ataulfo' mango, using direct performance metrics, such as the amount of pollen deposited on the stigma, the number of pollen tubes developed within the styles, and the number of fruits or seeds produced after a single visit. This will allow the identification of the most effective pollinators and will contribute to improve management strategies and to optimize pollination in orchards, ensuring greater productivity and fruit quality.

4. Fertilization and fruit production

When the pollen grains are deposited into the stigma, they are hydrated by the stigma secretions and the pollen tube germination initiates, growing through the style and reaching the ovule sac in 48–56 h after pollen attachment [8]. Although the exact number of pollen grains needed to achieve successful pollination in 'Ataulfo' is not yet known, in theory, only one pollen grain is needed to fertilize the single ovule in the mango ovary [13]. However, it is likely that multiple pollen grains are required to increase the chances of successful fertilization, considering the variability in pollen viability and the low germination rate observed in this cultivar [8]. In addition, the deposition of compatible pollen (see next section) is a key condition required for successful fertilization to take place.

4.1 Compatibility system

Using controlled pollination treatments, Gehrke-Vélez et al. [8] demonstrated that fruit set in 'Ataulfo' was greater when pollen from another cultivar was deposited on 'Ataulfo' stigmas than when pollen from 'Ataulfo' was placed. This is the first evidence of a self-incompatibility mechanism in this cultivar. Self-incompatibility refers to the inability of a plant to produce fertile seeds when pollinated with its own pollen [41]. In 'Ataulfo,' this condition is considered as a case of varietal self-incompatibility, since plants usually do not produce fertile seeds when pollinated with pollen of 'Ataulfo' either from the same or from another individual.

Given that the rejection of self-pollen occurs after pollination and not immediately upon contact with the stigma, this phenomenon is considered as "late" or "post-zygotic" self-incompatibility [8, 41]. Specifically, it was observed that self-pollen can germinate and develop pollen tubes successfully on the style until they reach and enter the embryo sac [8]. After this, stunted and wrinkled or necrotic embryos are formed instead of normal embryonic development [8]. The formation of stunted embryos or embryo abortion in 'Ataulfo' may also be attributed to the presence of early-acting inbreeding depression [8]. If so, embryo abortion and fruiting failure should be due to a load of deleterious recessive alleles that act shortly after fertilization [41].

4.2 Malformed fruits

It has been suggested that self-pollen deposition in 'Ataulfo' mango leads to fruit abortion at early stages of fruit development, that is, the formation of

Figure 6.
The difference between "nubbins" (A, above) and commercial fruits (A, below). An 'Ataulfo' mango tree with a high incidence of nubbins (B). Photos: Rodrigo Lucas-García.

stenospermocarpic fruits (**Figure 6A**) [8]. Stenospermocarpy refers to the formation of small malformed fruits that contain partially formed seeds due to embryo abortion after fertilization, resulting in a stunted seed [2, 10]. In the case of 'Ataulfo,' these fruits are known as nubbins ("mango niño" in Spanish), and their high incidence in recent years is a concern for producers, because these fruits have no commercial value due to their smaller size and weight compared to commercial fruits (**Figure 6B**) [42]. This situation leads to significant economic losses, reaching even up to 90% of nubbin production in some regions of Mexico [43].

Some authors suggest that malformed fruits in 'Ataulfo' are produced by parthenocarpy (i.e., the formation of seedless fruits, without fertilization of the ovule) [44]. So, further detailed studies are needed to determine the exact proportion of malformed fruits caused by parthenocarpy versus those produced by stenospermocarpy.

Moreover, some studies have suggested that environmental factors, especially extreme temperatures during flowering, may increase the incidence of nubbins in 'Ataulfo'. In particular, high and low temperatures can affect pollen tube development, pollen viability, and fertilization [42, 43]. Physiological alterations related to plant nutrition have been also proposed as a main factor that increase the incidence of nubbins [45, 46].

5. Synergistic effects

Other effects on mango fruit production may result from synergies between multiple environmental components; some of them will be addressed below.

5.1 Induced flowering

Particularly in 'Ataulfo,' climatic conditions such as temperature, precipitation, and humidity before or during flowering can affect mango flower production and, therefore, food availability and pollinators' survival. In addition, some 'Ataulfo'

mango producers apply nitrates or growth regulators, such as paclobutrazol (PBZ; an inhibitor of gibberellin synthesis, widely used in artificial flowering induction protocols [47]), to advance or increase mango flowering periods during the year [47–49]. Early flowering coincides with the rainy season (June–November), when environmental conditions such as temperature, precipitation, and wind speed can be adverse for mango flowers and pollinators [50], which can negatively influence fruit set and yield [51]. Although no specific studies have been conducted in 'Ataulfo' on the effect of weather conditions on flowering or pollinator visitation, in other mango cultivars, cloudy weather and rainfall during flowering have been reported to cause severe damage to mango flowers and lead to a strong flower abscission [52].

Some floral visitors are more abundant during the dry or during the rainy season [53, 54]. For example, honeybees, one of the most abundant floral visitors of 'Ataulfo' mango [7], show greater sensitivity to rainfall, strong winds, and temperature changes than other groups of wild floral visitors, such as dipterans that are also legitimate floral visitors of 'Ataulfo' mango [7]. Therefore, variation in climatic conditions between flowering seasons of 'Ataulfo' mango may influence pollinator availability, community composition, and pollination efficiency. However, there are currently no studies comparing pollinator communities and pollination services across flowering periods in 'Ataulfo' or any other mango cultivar.

5.2 Land-use change

The role of wild pollinators in crop pollination is crucial, as they are essential to maintain fruit and seed production and a prosperous agricultural economy [55]. Actually, it is a great challenge to maintain pollination services that ensure the production of several crops due to the decrease in wild pollinator populations that have been reported in various parts of the world [56]. This decrease has been attributed to environmental and anthropogenic factors, including agricultural intensification and land-use changes [56, 57]. The trend toward an increasing area of 'Ataulfo' mango orchards (**Figure 7**) may have negative consequences for pollinators and crop yield. Several studies in other crops have shown that the average size of agricultural fields is negatively related to pollinator diversity and abundance [58, 59]. In addition, orchard size also influences crop yield [58, 60]. When orchard size exceeds the food demand of wild pollinator populations, there may not be enough pollinators to visit all flowers, resulting in insufficient pollination and reduction in fruit production. Moreover, the significant decrease in orchard margins, such as hedges and grass strips [61], may decrease the provision of nesting sites and refuge for pollinators, as well as essential floral resources when crops are not in bloom [61].

The expansion of the cultivated area, in turn, has been associated with a notable decrease in native vegetation [62]. Although we did not find studies addressing the effects of this expansion on pollinators in 'Ataulfo,' a recent study found that the diversity of pollinating insects significantly decreases in 'Ataulfo' mango orchards located far from native vegetation patches [7]. It was also found that native pollinators of 'Ataulfo,' such as the honey wasp *Brachygastra azteca*, the stingless bee *Frieseomelitta nigra*, and sarcophagid and calliphorid flies, were absent in many orchards located further away from native vegetation [7]. This highlights the importance of the conservation of natural habitats to maintain a diverse community of pollinators and optimize fruit production. Indeed, Severiano-Galeana et al. [7] found that orchards closer to native vegetation patches produced more commercial fruits and had lower incidence of nubbins than those located farther from native forests.

Figure 7.
Typical landscape in the state of Guerrero, Mexico, where the 'Ataulfo' mango orchards continuously spread into what was once a tropical dry forest. Photo: Víctor Rosas-Guerrero.

5.3 Agricultural practices

Cultivated fields apply high quantities of herbicides as well as fungicides and pesticides for pest and disease control. These practices pose a serious threat to pollinators due to the toxicological effects of these agrochemicals on several species of pollinators [63]. While direct mortality of pollinators is an obvious effect, sublethal effects are equally concerning. Exposure to low doses of pesticides can reduce the longevity of bees, affect their foraging ability [64], impair their memory [65], disrupt their navigation ability, and decrease the colony fitness [66]. Furthermore, not only the pollinators that live or forage within crop fields are affected by pesticide applications but also are the pollinators that live on the field margins and edges of fields [67]. It is worth mentioning that wild pollinators are often more vulnerable than honeybees [68]. This is crucial since fruit production in 'Ataulfo' is positively associated with the number of visits by wild insects but not with visits by honeybees [7].

6. Strategies to increase commercial fruit production

As mentioned in previous paragraphs, the commercial production of 'Ataulfo' mango faces several challenges that impact the profitability of this crop. One key factor limiting its productivity is its self-incompatibility, as the low transfer of compatible pollen significantly contributes to the high incidence of nubbins observed

in 'Ataulfo' orchards in Mexico, leading to lower yields and economic losses for producers. Since this cultivar requires cross-pollination and high pollinator mobility for proper fruit development, improving these factors is essential. Therefore, various strategies can be implemented within orchards to increase the production of commercial fruits and to reduce the incidence of nubbins in 'Ataulfo' mango orchards.

6.1 Design of plantations

Varietal self-incompatibility in 'Ataulfo' makes cross-pollination with a compatible cultivar essential to produce good quality fruits [8]. However, most 'Ataulfo' orchards consist of only one cultivar [6]. This poses a major challenge to ensuring cross-pollination as pollinators must travel long distances through the orchard to transfer compatible pollen among cultivars [69, 70]. To minimize this problem, mixing compatible cultivars with 'Ataulfo' in the same orchard is an effective strategy to promote cross-pollination [6] due to the increase in the availability of compatible pollen [69, 70].

Under this scenario, 'Ataulfo' mango producers must carefully select pollen donor cultivars, ensuring that they are compatible and that their flowering period matches with 'Ataulfo' flowering [71]. Considering cultivars that increase the number of commercial fruits, some authors have identified 'Joe Welch,' 'Criollo' [8], 'Haden' [6], and 'Tommy Atkins' [42] as effective pollen donors for 'Ataulfo.' However, the effect of cross-pollination with other cultivars on the quality of mango fruits (i.e., xenia) remains unclear. Our preliminary results indicate that 'Ataulfo' fruits from cross-pollination with 'Haden' show similar size, weight, and sugar concentration but longer shelf life compared to fruits from cross-pollination with 'Ataulfo.' Further studies are needed to evaluate the influence of other cultivars on the different fruit quality parameters to obtain fruits with desirable organoleptic and morphometric characteristics.

Given that successful pollination of 'Ataulfo' depends on the presence of compatible cultivars, it is crucial to consider the distance among them [6]. While for insect-pollinated species such as 'Ataulfo,' it is recommended that at least 20% of the orchard consist of pollen donors [71], a proportion of 16% seems to be optimal for maximizing commercial fruit production (**Figure 8A**) [6]. In self-incompatible crops, as 'Ataulfo,' the most frequently used planting design consists of alternating rows between the cultivar of interest and the pollen donor, though it is not the most efficient way to ensure cross-pollination [71]. Mixing pollen donors and the cultivar of interest within the same row seems to be the most effective to maximize pollination [72]. It was recently found that 'Ataulfo' trees 10 m away from the 'Haden' cultivar had a lower incidence of nubbins and higher production of commercial fruits than trees located 30 and 50 m away (**Figure 8B**) [6]. Furthermore, the fruit yield per tree was significantly higher for 'Ataulfo' trees located 10 m from 'Haden' (69.6 ± 16.14 kg) than those located 50 m away (50.3 ± 15.13 kg). Consequently, the economic income per hectare was significantly higher in orchards with 'Ataulfo' trees situated 10 m from 'Haden' ($7063 ± 11.93 USD) compared to those located 30 m ($5059 ± 16.17 USD) or 50 m ($4852 ± 15.48 USD) away [6].

When establishing new orchards, improving the design of established orchards does not necessarily involve the subtraction of a subset of trees. Instead, grafting some 'Ataulfo' branches with scions of a compatible cultivar may be a more feasible strategy (**Figure 9**).

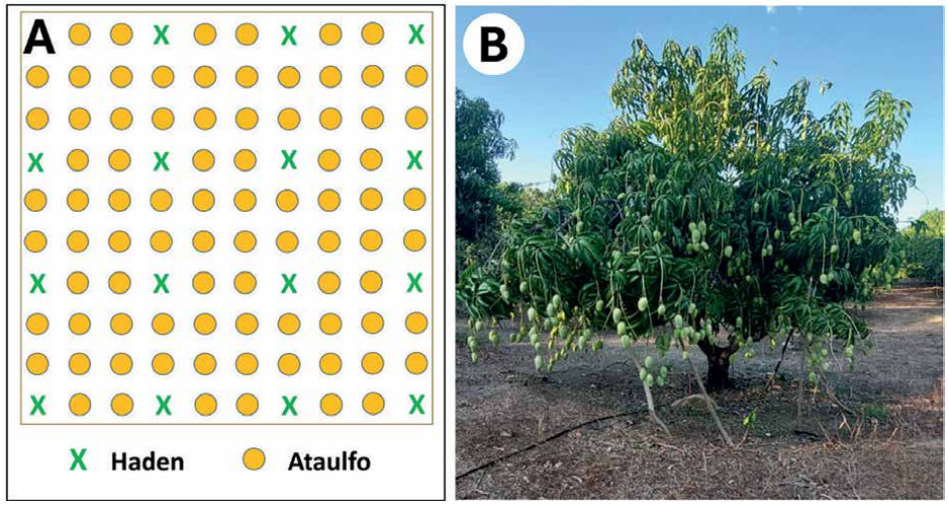

Figure 8.
Schematic representation of an 'Ataulfo' mango orchard with a mixed planting design, including 16% of pollen-donor trees. The yellow dots represent the 'Ataulfo' trees (pollen receivers), while the green Xs indicate the pollen-donor trees ('Haden') (A). A picture of an 'Ataulfo' tree with high production of commercial fruits located 10 m from a 'Haden' tree used as the pollen donor cultivar (B). Diagram and photo: Rodrigo Lucas-García.

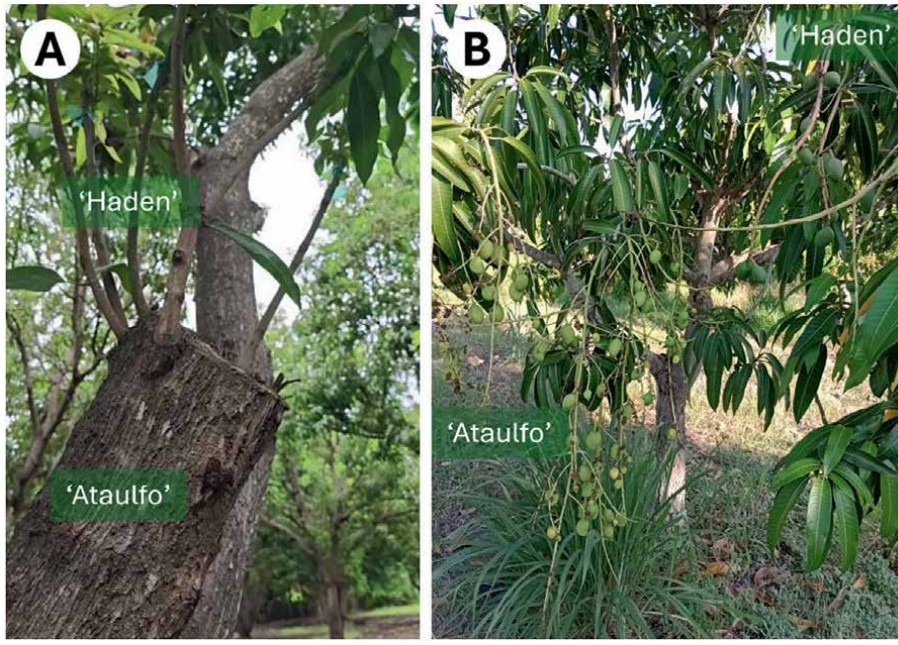

Figure 9.
Example of a crown graft performed on a branch of an 'Ataulfo' mango tree in order to have two cultivars on the same tree to promote cross-pollination in 'Ataulfo' mango (A). 'Ataulfo' mango tree with the 'Haden' graft, showing commercial 'Ataulfo' fruits and 'Haden' fruits (B). Photos: Rodrigo Lucas-García.

6.2 Enhancing pollination services

Considering that agricultural fields dominated by a single crop can negatively influence the composition, richness, and abundance of wild pollinators [68], crop diversification emerges as a key strategy to provide greater variety of food resources for pollinators [73–75]. Polyculture systems have been shown to have positive effects on biodiversity, enhancing mango pollination and in consequence production [76, 77]. A recent study revealed that polyculture mango orchards host a higher number of morphospecies compared to monoculture fields [77]. Furthermore, polycultures showed a greater abundance of pollinators (68 ± 9.28) and natural enemies (96 ± 21.58) than mango monocultures (pollinators: 38 ± 19.93 and natural enemies: 39 ± 14.05) [77].

Research carried out in Soconusco, Chiapas, Mexico, showed that integrating crops such as the pigeon pea *Cajanus cajan* and the tepary bean *Phaseolus acutifolius* in 'Ataulfo' mango orchards not only improved soil fertility but also increased panicle production in mango trees. As a result, mango yield was higher when legume crops were intercropped between the trees, reaching yields of up to 9.13 t/ha with *P. acutifolius* and 7.42 t/ha with *C. cajan* [76]. Therefore, 'Ataulfo' mango growers are advised to consider adopting polyculture systems that integrate legume crops and refuge plants. These systems not only favor biodiversity but also contribute to improved soil health, optimize vital ecosystem services such as pollination and natural pest control, and ultimately increase mango productivity [76, 77].

Given that 'Ataulfo' mango orchards located closer to forest patches were visited by more legitimate floral visitors and showed less occurrence of nubbins and higher production of commercial fruits [7], mango producers should encourage the conservation and restoration of native vegetation patches, since they serve as pollinator sources. Additionally, weeds may act also as food sources [78–81]. A recent study has found that wild pollinator abundance is lower in mango orchards where weeds are removed than in weedy mango orchards, increasing mango yield [80, 81]. On the other hand, weed removal can negatively impact the availability of nesting and refuge sites for the larvae of some pollinators [68]. In this regard, 'Ataulfo' mango producers should consider the feasibility of not eliminating weeds, encourage the planting of strips of wildflowers within mango orchards, and create semi-natural habitats on the edges of orchards (i.e., living fences), which increase visits by pollinators and improve fruit production [82].

Finally, the indiscriminate use of chemical pesticides in mango orchards poses a significant threat to beneficial insects, including pollinators [33, 83]. In addition to the high cost of pesticides, they can cause harm to the environment and human health [84, 85]. Therefore, farmers must reduce the use of herbicides and pesticides, as their negative impact on pollinators can affect both the productivity and sustainability of orchards [33]. An effective alternative is the implementation of Integrated Pest Management (IPM), an approach that allows for effective pest control without heavy reliance on chemical pesticides [84]. IPM integrates biological, cultural, and mechanical control methods, promoting natural pest control processes and reducing the environmental impact of agricultural practices [84]. One example is the use of neem oil in 'Chok Anan' mango orchards. This oil, at 2–3%, reduced thrip populations (a significant pest in mango orchards) by 60–65% without causing significant harm

to pollinators [83]. In contrast, while the chemical insecticide imidacloprid reduced thrip populations by 69%, its toxicity to pollinators was considerably higher, as pollinator visits were reduced by 93% [83]. Similarly, in 'Ataulfo' mango, it was found that a combination of neem extract and cinnamon extract was as effective or even more effective than the treatment with malathion + ethyl chlorpyrifos + permethrin in reducing thrip populations [85]. Therefore, it is recommended that mango producers adopt IPM and use biorational alternatives such as neem oil to help protect pollinators and promote the long-term productivity and sustainability of the orchards.

7. Conclusion

It is necessary to address the current challenges facing the production of 'Ataulfo' mango, given the depauperate pollinator fauna mainly caused by land-use changes and inappropriate agricultural practices. In addition, reproductive biology problems such as late self-incompatibility, stenospermocarpy and pollination limitation due to induced flowering and inefficient plantation designs should be assessed through innovative strategies in orchard management and upsurge research on this subject to ensure the long-term sustainability of the production of this mango cultivar.

Here, we propose to 'Ataulfo' mango producers to adopt strategies focused on orchard design and improving the surrounding environment to increase pollinator populations and pollination. This includes appropriate selection of compatible pollen donors that flower at the same time as the main cultivar, as well as proper spatial arrangement of cultivars to facilitate cross-pollination, integration of additional crops (polycultures) to increase biodiversity, creation of habitats for pollinators through flower borders and native flower strips, and restoration of surrounding vegetation. Finally, it is crucial to reduce the use of pesticides through Integrated Pest Management (IPM) and promote biorational products. The implementation of these strategies by producers will improve pollination, increase the quality and quantity of 'Ataulfo' mango production, and ensure the long-term profitability of this crop.

Acknowledgements

The first author would like to thank the National Council of Humanities, Sciences and Technologies (CONAHCYT) for the scholarship awarded for PhD studies (CVU: 930153).

Conflict of interest

The authors declare that they have no conflicts of interest that could influence the publication of this chapter.

DOI: http://dx.doi.org/10.5772/intechopen.1009473

Author details

Rodrigo Lucas-García[1,2], Víctor Rosas-Guerrero[2*], Carina Gutierrez-Flores[3], Lorena Aleman-Figueroa[2], Maribel López-Atanacio[2] and Eduardo Cuevas[4]

1 Doctorado en Recursos Naturales y Ecología, Facultad de Ecología Marina, Universidad Autónoma de Guerrero, Acapulco de Juárez, Mexico

2 Escuela Superior en Desarrollo Sustentable, Universidad Autónoma de Guerrero, Tecpan de Galeana, Mexico

3 Investigadores por Mexico, CONAHCYT, Escuela Superior en Desarrollo Sustentable, Universidad Autónoma de Guerrero, Tecpan de Galeana, Mexico

4 Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mexico

*Address all correspondence to: victor_rosas@yahoo.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. (cc) BY

References

[1] Infante F, Quilantán J, Rocha F, Esquinca H, Castillo A, Ibarra Nuñez G, et al. Mango Ataulfo: Orgullo chiapaneco. CONABIO Biodiversitas. 2011;**96**:1-5

[2] Hernández-Guerrero SE, Balois-Morales R, Bello-Lara JE. Estenospermocarpia en mango 'Ataulfo': Nayarit y Chiapas. Acta agrícola y Pecuaria. 2015;**1**(3):86-92

[3] Mendoza-Hernández C, Rosas-Quijano R, Vazquez-Ovando A, Galvez-Lopez D. Retos y controversias del mango Ataulfo. Revista Mexicana de Ciencias Agrícolas. 2020;**11**(7):1633-1645

[4] Sistema de Información Agroalimentaria y Pesquera (SIAP). Módulo Agrícola. Sistema de Información Agroalimentaria de Consulta (SIACON) [Internet]. 2023. Available from: https://www.gob.mx/siap/documentos/siacon-ng-161430 [Accessed: June 20, 2024]

[5] Gehrke-Vélez MR. Reflexiones sobre problemas de biología reproductiva del mango Ataúlfo en el Soconusco, Chiapas. Tecnologia en Marcha. 2008;**21**(1):174-183

[6] Lucas-García R, Rosas-Guerrero V, Alemán-Figueroa L, Almazán-Núñez RC, Violante-González J, Kuk-Dzul JG. Spatial proximity of 'Ataulfo' to 'Haden' cultivar increases mango yield and decreases incidence of nubbins. Agronomy. 2021;**11**(3):450

[7] Severiano-Galeana F, Rosas-Guerrero V, Alemán-Figueroa L, Lucas-García R, Almazán-Núñez RC, Kuk-Dzul JG. Orchards closer to forest patches produced fewer malformed fruits and more commercial fruits: The importance of legitimate floral visitors. Agriculture, Ecosystems & Environment. 2024;**363**:108872

[8] Gehrke-Vélez M, Castillo-Vera A, Ruiz-Bello C, Moreno-Martinez JL, Moreno-Basurto G. Delayed self-incompatibility causes morphological alterations and crop reduction in "Ataúlfo" mango (*Mangifera indica* L.). New Zealand Journal of Crop and Horticultural Science. 2012;**40**(4):215-227

[9] Valdez Rivera D, Morstadt JDC, Aguayo SC. Efecto del Paclobutrazol sobre caracteres botánicos de flores y frutos en la aparición de mango partenocárpicos cultivar Ataúlfo. Centrosur Agraria. 2021;**1**(11):17-28

[10] Gehrke Vélez MR, Castillo-Vera A, Ruiz-Bello C, Moreno-Martinez JL. Pollen viability and germination in mango (*Mangifera indica* L.) cv. Ataulfo. Interciencia. 2011;**36**(5):378-385

[11] Palacio-Martínez V, Avendaño-Arrazate C. 'Ataulfo diamante', 'Zafiro' y 'Citlalli': nuevas selecciones de mango (*Mangifera Indica L.*). Agro Productividad. 2019;**12**(3):3-8

[12] Cárdenas Portillo KG, Solís Montero L, Grajales Conesa J, Ortíz GA. Eficiencia de Musca domestica en la polinización de *Mangifera indica* cv. Ataulfo [thesis]. Tapachula, Chiapas, México: Universidad Autónoma de Chiapas; 2018

[13] Ramírez F, Davenport TL. Mango (*Mangifera indica* L.) pollination: A review. Scientia Horticulturae. 2016;**203**:158-168

[14] Sánchez M, Velásquez Y, González M, Cuevas J. Hoverfly

pollination enhances yield and fruit quality in mango under protected cultivation. Scientia Horticulturae. 2022;**304**:111320

[15] Sánchez M, Abreu AC, Tristán AI, Velásquez Y, Fernández I, Cuevas J. Floral attractants and rewards to pollinators in *Mangifera indica* L. Scientia Horticulturae. 2024;**332**:113180

[16] Davenport TL. Reproductive physiology. In: The Mango: Botany, Production and Uses. Wallingford: CABI; 2009. pp. 97-169

[17] Shu ZH. Effect of temperature on the flowering biology and fertilization of mangoes (*Mangifera indica* L.). Journal of Applied Horticulture. 1999;**01**(02):79-83

[18] Sukhvibul N, Whiley AW, Vithanage V, Smith MK, Doogan VJ, Hetherington SE. Effect of temperature on pollen germination and pollen tube growth of four cultivars of mango (*Mangifera indica* L.). The Journal of Horticultural Science and Biotechnology. 2000;**75**(2):214-222

[19] Marroquín-Agreda F, Gehrke-Velez MR, Pohlan JA, Lerma-Molina JN, Toledo-Toledo E, Ley-de Coss A, et al. The Association of Bushy Legumes with 'Ataúlfo' Mango (*Mangifera indica* L.) + cv . Ataúlfo Affects Reproductive Biology and Enhances Productivity in Mango Plantations in Soconusco, Chiapas, Mexico. Indian Horticulture Journal. 2015;**5**(3):63-69

[20] Geetha GA, Shivashankara KS, Reddy YTN. Varietal variations in temperature response for hermaphrodite flower production and fruit set in mango (*Mangifera indica* L). South African Journal of Botany. 2016;**106**:196-203

[21] Sharma RR, Singh R. Pruning intensity modifies canopy microclimate, and influences sex ratio, malformation incidence and development of fruited panicles in 'Amrapali' mango (*Mangifera indica* L.). Scientia Horticulturae. 2006;**109**(2):118-122

[22] Elkhishen MA. Effect of pruning severity on flowering and fruiting of mango (*Mangifera indica*) cv. Alphonso in off-year season. Egyptian Journal of Horticulture. 2015;**42**(2):785-794

[23] Kurian RM, Iyer CPA. Chemical regulation of tree size in mango (*Mangifera indica* L.) cv. Alphonso. II. Effects of growth retardants on flowering and fruit set. Journal of Horticultural Science. 1993;**68**(3):355-360

[24] Nouh O, Abou Sayed-Ahmed T, Hassan A, Mohsen F. Response of siddek and kiett mango trees to gibberellic acid and urea foliar spraying at flower bud induction period a floral aspects. Zagazig Journal of Agricultural Research. 2018;**45**(1):75-86

[25] Pérez V, Herrero M, Hormaza JI. Pollen performance in mango (*Mangifera indica* L., Anacardiaceae): Andromonoecy and effect of temperature. Scientia Horticulturae. 2019;**253**:439-446

[26] Dai W, Yang Y, Patch HM, Grozinger CM, Mu J. Soil moisture affects plant–pollinator interactions in an annual flowering plant. Philosophical Transactions of the Royal Society B: Biological Sciences. 1853;**2022**(377):20210423

[27] Gambel J, Holway DA. Temperature and soil moisture manipulation yields evidence of drought-induced pollen limitation in bee-pollinated squash. Ecology and Evolution. 2024;**14**(6):1-11

[28] Liu X, Xiao Y, Zi J, Yan J, Li C, Du C, et al. Differential effects of low and high

temperature stress on pollen germination and tube length of mango (*Mangifera indica* L.) genotypes. Scientific Reports. 2023;**13**(1):611

[29] Muengkaew R, Chaiprasart P, Wongsawad P. Calcium-boron addition promotes pollen germination and fruit set of mango. International Journal of Fruit Science. 2017;**17**(2):147-158

[30] Chuttong B, Panyaraksa L, Tiyayon C, Kumpoun W, Chantrasri P, Lertlakkanawat P, et al. Foraging behavior and pollination efficiency of honey bees (*Apis mellifera* L.) and stingless bees (Tetragonula laeviceps species complex) on mango (*Mangifera indica* L., cv. Nam Dokmai) in northern Thailand. Journal of. Ecology and Environment. 2022;**46**(15):1-7

[31] Phebe D, Khairul BD. Morphology of Chok Anan mango flower grown in Malaysia. African Journal of Agricultural Research. 2013;**8**(18):1877-1880

[32] de Siqueira KMM, Kiill LHP, Martins CF, Lemos IB, Monteiro SP, Feitoza E d A. Estudo comparativo da polinização de *Mangifera indica* L. em cultivo convencional e orgânico na região do Vale do Submédio do São Francisco. Revista Brasileira de Fruticultura. 2008;**30**(2):303-310

[33] Marcacci G, Devy S, Wenzel A, Rao VS, Kumar SS, Nölke N, et al. Direct and indirect effects of urbanization, pesticides and wild insect pollinators on mango yield. Journal of Applied Ecology. 2023;**60**(10):2132-2143

[34] Bos MM, Veddeler D, Bogdanski AK, Klein AM, Bos MM, Veddeler D, et al. Caveats to quantifying ecosystem services: Fruit abortion blurs benefits from crop pollination. Ecological Applications. 2007;**17**(6):1841-1849

[35] Nurul Huda A, Che Salmah MR, Abu Hassan A, Hamdan A, Abdul Razak MN. Pollination services of mango flower pollinators. Journal of Insect Science. 2015;**15**(1):1-8

[36] Cabrera-Asencio I, Meléndez-Ackerman EJ. Community and species-level changes of insect species visiting *Mangifera indica* flowers following hurricane María: "The Devil Is in the Details". Frontiers in Ecology and Evolution. 2021;**9**:556821

[37] Dag A, Gazit S. Mango pollinators in Israel. Journal of Applied Horticulture. 2000;**02**(01):39-43

[38] Carvalheiro LG, Seymour CL, Veldtman R, Nicolson SW. Pollination services decline with distance from natural habitat even in biodiversity-rich areas. Journal of Applied Ecology. 2010;**47**(4):810-820

[39] Grajales-Conesa J, Gallardo-Palomeque Á, López LC, López-García JA, Mérida-Rivas JA, de los Santos RT, et al. Ability of honey and aqueous plant extracts on the attraction of bees during flowering period in mango (*Mangifera indica*) var. Ataulfo cultivation. International Journal of Agriculture and Biology. 2023;**30**(4):253-257

[40] Ortiz JA, Infante F, Quintalán J, Gehrke Vélez MR, Castillo Vera A, De la Rosa J, et al. Manual: cría de la mosca doméstica para utilizarla como polinizador de las flores de mango Ataulfo. Tapachula, Chiapas, México: El Colegio de la Frontera Sur; 2011. pp. 1-16

[41] Gibbs PE. Late-acting self-incompatibility – The pariah breeding system in flowering plants. New Phytologist. 2014;**203**(3):717-734

[42] Pérez-Barraza MH, Vázquez-Valdivia V, Osuna-García JA. Incidencia de frutos partenocárpicos en Mango 'Ataulfo' en huertos comerciales de Nayarit. Revista Chapingo, Serie Horticultura. 2007;**13**(2):149-156

[43] Salazar-García S, Álvarez-Bravo A, Ibarra-Estrada ME, GonzÁlez-Valdivia J, Medina-Torres R. Presencia de fruto partenocÁrpico en mango 'Ataúlfo' y su relación con la temperatura ambiental y tratamientos de fertilización. Revista Mexicana de Ciencias Agrícolas. 2016;**8**(13):2615

[44] Pérez-Barraza MH, Álvarez Bravo A, Avitia García E, Pérez Luna AI, Santos Cárdenas MV. Temperatura y desarrollo floral en la formación de frutos partenocárpicos en mango 'Ataulfo'. Revista Mexicana de Ciencias Agrícolas. 2019;**23**:199-210

[45] Barbosa LFS, Cavalcante ÍHL, Lima AMN. Desordem fisiológica e produtividade de mangueira cv. Palmer associada à nutrição de boro. Revista Brasileira de Fruticultura. 2016;**38**(1):1-9

[46] Carvalho CIFS, Lima AMN, Lobo JTL, Mudo LED, Santos ADAS. Estenoespermocarpia em frutos de mangueira e a relação com a nutrição de boro. Meio Ambiente (Brasil). 2020;**2**(3):58-67

[47] Garcia de Niz DA, Esquivel GL, Montoya RB, Arrieta-Ramos BG, Santiago GA, Gómez-Aguilar JR, et al. Vegetative and reproductive development of 'Ataulfo' mango under pruning and Paclobutrazol management. Journal of Agricultural Science and Technology. 2014;**16**(2):385-393

[48] Escalera-Mota AA, Noriega-Cantú DH, González-Mateos R, Pérez-Barraza MH, Álvarez-Bravo A, Maldonado-Astudillo YI, et al. Mango partenocárpico y su relación con la temperatura. Acta Agrícola y Pecuaria. 2022;**8**:e0081018

[49] Ariza-Flores R, Cano-Garcia MA, Cadena-Iñiguez P, Valle de la Paz M, Galvez-Marroquin LA. Assessment and selection of flowering inducers to increase productivity of mango cv. Ataulfo (*Mangifera indica* L.). Agro Productividad. 2024;**17**(1):63-68

[50] Tuell JK, Isaacs R. Weather during bloom affects pollination and yield of highbush blueberry. Journal of Economic Entomology. 2010;**103**(3):557-562

[51] Goodwin EK, Rader R, Encinas-Viso F, Saunders ME. Weather conditions affect the visitation frequency, richness and detectability of insect flower visitors in the Australian alpine zone. Environmental Entomology. 2021;**50**(2):348-358

[52] Lawson DA, Rands SA. The effects of rainfall on plant–pollinator interactions. Arthropod-Plant Interactions. 2019;**13**(4):561-569

[53] Delgado-Carrillo O, Martén-Rodríguez S, Ashworth L, Aguilar R, Lopezaraiza-Mikel M, Quesada M. Temporal variation in pollination services *to Cucurbita moschata* is determined by bee gender and diversity. Ecosphere. 2018;**9**(11):e02506

[54] López-Atanacio M, Lucas-García R, Rosas-Guerrero V, Alemán-Figueroa L, Kuk-Dzul JG, Hernández-Flores G. Seasonal variation in the response of a monoecious crop to increased temperature and fertilization. Frontiers in Plant Science. 2022;**13**:1012859

[55] Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, et al. Wild pollinators enhance fruit set of crops regardless

of honey bee abundance. Science. 2013;**339**(6127):1608-1611

[56] Potts SG, Imperatriz-Fonseca V, Ngo HT, Aizen MA, Biesmeijer JC, Breeze TD, et al. Safeguarding pollinators and their values to human well-being. Nature. 2016;**540**(7632):220-229

[57] Dicks LV, Breeze TD, Ngo HT, Senapathi D, An J, Aizen MA, et al. A global-scale expert assessment of drivers and risks associated with pollinator decline. Nature Ecology and Evolution. 2021;**5**(10):1453-1461

[58] Martin EA, Dainese M, Clough Y, Báldi A, Bommarco R, Gagic V, et al. The interplay of landscape composition and configuration: New pathways to manage functional biodiversity and agroecosystem services across Europe. Ecology Letters. 2019;**22**(7):1083-1094

[59] Sirami C, Gross N, Baillod AB, Bertrand C, Carrié R, Hass A, et al. Increasing crop heterogeneity enhances multitrophic diversity across agricultural regions. National Academy of Sciences of the United States of America. 2019;**116**(33):16442-16447

[60] Garibaldi LA, Carvalheiro LG, Vaissière BE, Gemmill-Herren B, Hipólito J, Freitas BM, et al. Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. Science. 2016;**351**(6271):388-391

[61] Clough Y, Kirchweger S, Kantelhardt J. Field sizes and the future of farmland biodiversity in European landscapes. Conservation Letters. 2020;**13**(6):1-12

[62] Potapov P, Turubanova S, Hansen MC, Tyukavina A, Zalles V, Khan A, et al. Global maps of cropland extent and change show accelerated cropland expansion in the twenty-first century. Nature Food. 2021;**3**(1):19-28

[63] Ara ZG, Haque AR. A comprehensive review on synthetic insecticides: Toxicity to pollinators, associated risk to food security, and management approaches. Journal of Biosystems Engineering. 2021;**46**(3):254-272

[64] Gill RJ, Raine NE. Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. Functional Ecology. 2014;**28**(6):1459-1471

[65] Stanley DA, Raine NE. Chronic exposure to a neonicotinoid pesticide alters the interactions between bumblebees and wild plants. Functional Ecology. 2016;**30**(7):1132-1139

[66] Moffat C, Pacheco JG, Sharp S, Samson AJ, Bollan KA, Huang J, et al. Chronic exposure to neonicotinoids increases neuronal vulnerability to mitochondrial dysfunction in the bumblebee (*Bombus terrestris*). The FASEB Journal. 2015;**29**(5):2112-2119

[67] Ward LT, Hladik ML, Guzman A, Winsemius S, Bautista A, Kremen C, et al. Pesticide exposure of wild bees and honey bees foraging from field border flowers in intensively managed agriculture areas. Science of the Total Environment. 2022;**831**:154697

[68] Nicholls CI, Altieri MA. Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. Agronomy for Sustainable Development. 2013;**33**(2):257-274

[69] MacInnis G, Forrest JRK. Field design can affect cross-pollination and crop yield in strawberry (*Fragaria x ananassa* D.). Agriculture, Ecosystems & Environment. 2020;**289**:106738

[70] Kämper W, Trueman SJ, Ogbourne SM, Wallace HM. Pollination services in a macadamia cultivar depend on across-orchard transport of cross pollen. Journal of Applied Ecology. 2021;**58**(11):2529-2539

[71] Pinillos V, Cuevas J. Artificial pollination in tree crop production. In: Horticultural Reviews. New Jersey: Wiley; 2007. pp. 239-276

[72] Sáez A, di Virgilio A, Tiribelli F, Geslin B. Simulation models to predict pollination success in apple orchards: A useful tool to test management practices. Apidologie. 2018;**49**(5):551-561

[73] Fahrig L, Baudry J, Brotons L, Burel FG, Crist TO, Fuller RJ, et al. Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. Ecology Letters. 2011;**14**(2):101-112

[74] Raderschall CA, Bommarco R, Lindström SAM, Lundin O. Landscape crop diversity and semi-natural habitat affect crop pollinators, pollination benefit and yield. Agriculture, Ecosystems & Environment. 2021;**306**:107189

[75] Priyadarshana TS, Martin EA, Sirami C, Woodcock BA, Goodale E, Martínez-Núñez C, et al. Crop and landscape heterogeneity increase biodiversity in agricultural landscapes: A global review and meta-analysis. Ecology Letters. 2024;**27**(3):e14412

[76] Marroquín Agreda F, Pohlan J, Janssens MJJ. Effects of legumes intercropped in mango orchards in the Soconusco, Chiapas, Mexico. In: Conference on International Agricultural Research for Development; 11-13 October 2006. Bonn, Germany: University of Bonn; 2006. pp. 1-6

[77] Windriyanti W, Rahmadhini N, Fernando I, Kusuma RM. Arthropods discovered on refugio flowering plants in *Mangifera indica* plantation. Biodiversitas Journal of Biological Diversity. 2023;**24**(9):4747-4754

[78] Landis DA, Menalled FD, Costamagna AC, Wilkinson TK. Manipulating plant resources to enhance beneficial arthropods in agricultural landscapes. Weed Science. 2005;**53**(6):902-908

[79] Bretagnolle V, Gaba S. Weeds for bees? A review. Agronomy for Sustainable Development. 2015;**35**(3):891-909

[80] Kleiman BM, Koptur S, Jayachandran K. Weeds enhance pollinator diversity and fruit yield in mango. Insects. 2021;**12**(12):1114

[81] Kleiman B, Koptur S. Weeds enhance insect diversity and abundance and may improve soil conditions in mango cultivation of South Florida. Insects. 2023;**14**(1):65

[82] Carvalheiro LG, Seymour CL, Nicolson SW, Veldtman R. Creating patches of native flowers facilitates crop pollination in large agricultural fields: Mango as a case study. Journal of Applied Ecology. 2012;**49**(6):1373-1383

[83] Aliakbarpour H, Salmah MRC, Dzolkhifli O. Efficacy of neem oil against thrips (Thysanoptera) on mango panicles and its compatibility with mango pollinators. Journal of Pest Science (2004). 2011;84(4):503-512

[84] Mulungu K, Muriithi BW, Kassie M, Khamis FM. The economic performance of mango integrated pest management practices at different scales of production. Frontiers in Insect Science. 2023;**3**:1-11

[85] Salgado-Gantes Y, Garcia-Escamilla P, Durán-Trujillo Y, Hernández-Castro E, Segura-Pacheco HR, Romero-Rosales y T. Biorational management of thrips in mango in Guerrero, Mexico. South West Entomology. 2024;**49**(4):1043-1052

Chapter 7

Biotechnological Advances for the Propagation and Conservation of Nutraceutical and Oilseed Palm Tree Crops

Luís Henrique Bueno, Josimar dos Santos Ladeira, João Victor Baptista Silveira, Tsylla Assis Santos Ferreira, Otalicio Damasio da Costa Junior, Judith Georgette Alcalde Mosqueira, Thamires Fernanda Gomes, Francisco de Assis Lopes, Lucilene Silva de Oliveira, Sérgio Yoshimitsu Motoike and Diego Ismael Rocha

Abstract

The demand for nutraceutical foods and vegetable oils has promoted the development of the palm tree agroindustrial chain in the world. The production of açaí and coconut is rapidly expanding. On the other hand, macaw palm and oil palm have fruits with a high oil content, a characteristic that makes them potential species for use in the food, cosmetics, and oleochemical industries, as well as for the production of biofuels. Palm trees are traditionally propagated by seeds, and the impossibility of cloning high-performance genotypes by conventional propagation methods makes it difficult to expand their cultivation. However, biotechnological tools such as somatic embryogenesis have recently been established, allowing the cloning of superior genotypes and accelerating the domestication and sustainable development of some palm tree production chains. In this chapter, we address recent advances and challenges in the *in vitro* propagation and conservation of five palm tree species: *Acrocomia aculeata* (macaw palm), *Cocos nucifera* (coconut palm), *Elaeis guineensis* (oil palm), and *Euterpe oleracea* and *E. edulis* (açaí palm). We believe this chapter will contribute to understanding their domestication panorama and support studies on developing and optimizing propagation systems for these and other palm trees of agroindustrial interest.

Keywords: Arecaceae, micropropagation, palm trees, plant tissue culture, somatic embryogenesis

1. Introduction

Palms (Arecaceae) are among the main monocots in the world and comprise the most abundant vascular plants in the tropics [1]. The Arecaceae family is predominantly distributed across the Neotropics [2], encompassing 200 genera and 2500 species recognized worldwide [3]. South America is the main center of diversification of Arecaceae (palm trees), and species from this group can be found in almost all neotropical biomes [2].

Palm trees comprise key forest structure and functionality components in tropical ecosystems [4], exhibiting great ecological and socioeconomic relevance [5]. Indeed, their flowers and fruits encompass fundamental resources for pollinators and fruit-eating animal species [6]. Some palm species also have high economic and social value. Indigenous or traditional communities and agroindustrial industries exploit many of them for various purposes. Among their representatives, species from the genera *Acrocomia*, *Cocos*, *Elaeis*, *Euterpe,* and *Syagrus* attract attention due to their potential use. Their fruits and seeds are sources of oils, fibers, starch, carbohydrates, proteins, and functional biomolecules— raw materials for the most varied industrial sectors. In addition, solid waste from the destruction of raw materials can have equally or even more noble applications, adding value to production and making the production chain more sustainable [7]. However, while coconut (*Cocos nucifera* L.) and oil palm (*Elaeis guineensis* Jacq.) are recognized as agricultural commodities, other important palm species can be considered neglected or underutilized crops since the current exploitation model is based on inefficient extractivism, which may threaten biodiversity, as it can exhaust natural matrices and lead to uneven production, requiring efforts to form a sustainable chain.

Palm tree propagation is mainly carried out *via* seed [8]. Most palm trees have a single trunk, devoid of axillary buds along their stems or any specialized structure, which limits the use of conventional vegetative propagation methods for cloning and multiplication of high-performance genotypes. However, biotechnological tools such as somatic embryogenesis have recently been established, allowing *in vitro* cloning of superior genotypes and accelerating domestication and sustainable development of some palm tree production chains [9]. Somatic embryogenesis is one of the biotechnological tools used to achieve large-scale clonal propagation. This technique is based on the principle of cellular totipotentiality, in which somatic embryos are obtained from a plant explant (cell or any fragment of living tissue or organ), cultivated in an aseptic environment and in a nutrient medium under controlled temperature and light conditions, which, after germination, will give rise to new plants identical to the mother plant [10].

In this chapter, we address recent advances and challenges in the *in vitro* propagation of three nutraceuticals [*Cocos nucifera* (coconut palm), *Euterpe oleracea*, and *E. edulis* (açaí palm)] and two oilseeds [*Elaeis guineensis* (oil palm) and *Acrocomia aculeata* (macaw palm)]. Here, nutraceutical palms refer to species in this group whose fruits have health benefits, such as improving physiological performance and/or effectiveness in treating diseases. In contrast, oilseed palms are recognized especially for their high vegetable oil production. We believe this report will contribute to understanding their domestication panorama and support studies on developing and optimizing propagation systems for these and other palm trees of agroindustrial interest.

2. Nutraceutical palms

2.1 *Cocos nucifera* (L.)

Cocos nucifera (L.) is a monotypic species widely recognized and considered one of the most economically important palms in the world. It stands out for the comprehensive utilization of all its parts, mainly for its nutritional and medicinal values [11]. Although the exact origin of the coconut remains debated, evidence suggests that it may have originated in the tropical regions of Southeast Asia, possibly along the coasts of India and Indonesia [12, 13]. Often referred to as the "tree of life," the coconut palm is among the most valued palm crops globally, cultivated on 12 million hectares across more than 90 tropical and subtropical countries [14].

The coconut (*C. nucifera*) is the only genus *Cocos* member within the Arecaceae family [15]. It is a typical palm with a solitary, erect, or slightly curved stem, reaching up to 30 m in height and 20–35 cm in diameter, with irregular rings (**Figure 1a**). The leaves are pinnate, with 25–30 leaves present simultaneously, measuring 3–6 m long, and bearing 100–150 pinnae per side, arranged in the same plane. Inflorescences are interfoliar, with rachillae and a woody bract. The flowers are unisexual and may occur in triads, dyads, or singly, with pistillate flowers at the basal portion and staminate flowers in the apical portion. The fruit is large, oval to ellipsoidal (20–30 cm x 12–20 cm), with a thin epicarp, fibrous mesocarp, and woody endocarp; the endosperm is initially liquid, later becoming hardened and white [11].

There are three main varieties of coconut palms: the tall coconut (*C. nucifera* var. typica), the dwarf coconut (*C. nucifera* var. nana), and the hybrid coconut (*C. nucifera* var. aurantiaca). The tall coconut is notable for its robust stems, which can reach up to 30 meters in height, making it ideal for industrial use, such as copra and oil production. Conversely, the dwarf coconut typically does not exceed 10 meters in height and flowers earlier, which is more suitable for fresh consumption, such as coconut water. The hybrid coconut exhibits intermediate characteristics between these two varieties.

In 2019, the global coconut market moved approximately US$ 13 billion, and projections indicate that this value could reach more than US$ 31 billion by the end of 2026, with a Compound Annual Growth Rate (CAGR) of 13.6% [16]. Brazil is

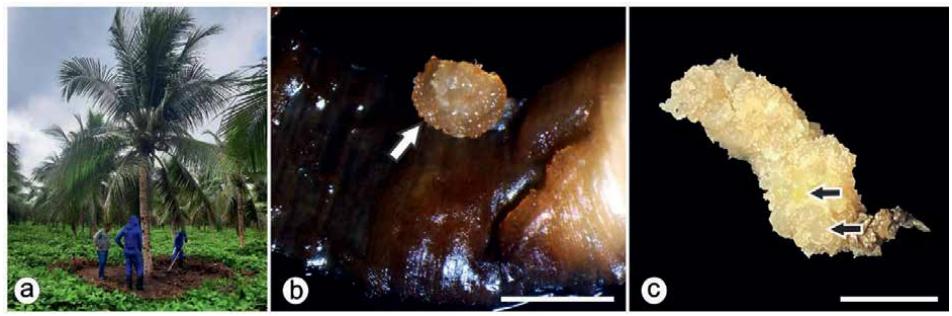

Figure 1.
*Somatic embryogenesis in coconut palm (*Cocos nucifera *L.). (a) Coconut palm, a plant of economic importance. (b) Embryogenic callus obtained by somatic embryogenesis from leaf explants (white arrows). (c) Embryogenic callus formed from inflorescence explants (black arrows). Scale bars, a = 150 μm; b = 1 cm.*

the fifth-largest coconut producer globally, with a 4.5% market share [17]. In 2023, Brazil produced 1,932,282 fruits, with a harvested area of 186,712 hectares and an average yield of 10,349 fruits/ha (2023) [18]. Advanced cultivation technologies, such as proper management of coconut plantations, intensive production systems, and improved varieties, such as dwarf and hybrid coconut trees, drive increased productivity and expand cultivation worldwide [19].

There is a wide variety of coconut-derived products that drive the global market and offer a range of uses for humanity, such as coconut water, coconut milk, grated coconut, coconut fiber, wood, coconut flour, coconut sugar, coconut powder, copra, shell, or pulp. Coconut fiber can make rugs, brushes, ropes, and threads. Its wood can be used for building houses and furniture. Fuel and charcoal can be produced from coconut shells, and even the leaves are utilized, being used for the production of brooms, baskets, and roof coverings. The high demand for this crop is mainly due to the beneficial properties of coconut, discovered through research conducted primarily by its most prominent producers, including Indonesia, the Philippines, India, and Brazil [20, 21].

2.1.1 Biotechnological tools for multiplication of Cocos nucifera *(L.)*

2.1.1.1 Somatic embryogenesis

The scarcity of suitable seedlings, extreme weather events, biotic and abiotic stresses, and low productivity caused by plant aging are the main problems coconut cultivation faces [22]. In this context, biotechnological techniques such as somatic embryogenesis are essential for propagating this species and its elite varieties.

Initial studies on somatic embryogenesis in *C. nucifera* used a variety of explants, each demonstrating the unique potential for embryogenic callus formation and plant regeneration. Among them, rachillae from immature inflorescences showed promising results, although they initially presented efficiency and reproducibility problems [23]. Plumules stood out as highly responsive explants, achieving higher callus and somatic embryo formation rates than rachillae [24]. Other explants tested include zygotic embryos, which presented better results when immature, with up to 58% embryogenic callus formation [25], in addition to unfertilized anthers and ovaries, both producing viable somatic embryos under optimized culture conditions [26, 27]. Other important studies on somatic embryogenesis in coconut are highlighted in **Table 1**.

Research is underway to obtain somatic embryos in coconut palms using explants from leaves and inflorescences. In the experiments performed, it was possible to observe the formation of embryogenic calluses from leaf explants, indicating the potential for clonal propagation of elite coconut plants (**Figure 1b**). In addition, embryogenic calluses from inflorescence explants were also obtained, reinforcing the viability of this approach (**Figure 1c**).

2.1.1.2 De novo *shoot organogenesis*

De novo shoot organogenesis is considered one of the alternative routes for *in vitro* clonal propagation of coconut, with direct organogenesis considered safe since callus formation can lead to the emergence of undesired somaclonal variations [22]. Recent studies conducted with shoot apices from germinated seeds, with sizes between 5 and 10 mm, cultivated in Y3 culture medium supplemented with 1 μM of TDZ [34]

Phase	Explant	Culture medium	Response	References
Induction	Plumule	Y3–5 μM BAP and 600 μM 2,4-D	56.7% from embryogenic callus originating from juvenile fruits/embryos	[28]
Induction	Plumule	Y3 modified - 600 μM and 6 μM 2,4-D	Compact callus, somatic embryos and plantlets	[29]
Induction	Haustorium tissues	Y3–0,3 μM de 2,4-D + 150 ml L^{-1} of coconut water	61% somatic embryos	[30]
Induction	Inflorescences	Y3–300 μM de 2,4-D + calcium ionophore (A23187), 22(S) and 23(S)-homobrassinolide	Improvements in the proliferation of embryogenic callus	[31]
Induction	Inflorescences	Y3–4,54 μM 2,4-D	92% embryogenic callus	[32]
Induction	Zigotic embryos	Y3–100 to 250 μM of 2,4D + GA_3, BAP and TDZ ↓ Induction of multi-buds - kinetin, BAP and NAA	16.7–100% (embryogenic callus) and 16.7–83.3% (embryos) - 100% survival in seedling acclimatization	[33]
Induction	Ovary	BM72–100 μM of 2,4-D + 5 μM of ABA and 10 μM of $AgNO_3$	41% embryogenic callus	[26]
Induction	Anthers	Y3–100 μM of 2,4-D and 100 μM of NAA	Formation of 123 calluses or embryos per 100 anthers	[27]
Induction	Plumule	Y3–100 μM of 2,4-D	57% of plumules formed callus	[24]

Note: Y3, Eeuwen's medium; BM72, Karunaratne and Periyapperuma medium; BAP, Benzylaminopurine; 2,4-D, 2,4-Dichlorophenoxyacetic Acid; GA_3, Gibberellic Acid; TDZ, Thidiazuron; NAA, 1-Naphthaleneacetic Acid; ABA, Abscisic Acid; $AgNO_3$, Silver Nitrate.

Table 1.
Studies on somatic embryogenesis in Cocos nucifera.

or 200 μM of TDZ [35], have shown promise as explants for large-scale production of elite coconut plantlets.

In a recent study conducted by Neema et al. [36], the electric current was employed in coconut calluses derived from plumules to induce a higher multiplication rate. In their study, the continuous application of weak electric current in the range of 1 and 2 μA induces a higher multiplication rate of the calluses, which can be used for plantlet propagation through somatic embryos (SE) or *in vitro* organogenesis.

2.1.2 Conservation

The genetic heritage of the coconut faces significant threats, and due to its great importance, *ex situ* conservation methods are necessary. *Ex situ* conservation occurs when genetic material is removed from its habitat, protecting it from pests and

diseases. Cryopreservation and *in vitro* cultivation are used among the *ex situ* conservation methods. Coconut seeds are recalcitrant [37], which makes *ex situ* conservation the most efficient and safe conservation method [38].

Cryopreservation is a method that enables *the ex situ* conservation of plant material for an indefinite period [39]. In coconut, cryopreservation studies have been successfully carried out using different types of explants, such as mature zygotic embryos [40, 41], pollen [42], plumules [43], and embryogenic calluses [44]. Furthermore, in studies conducted by Sisunandar et al. [45] and Sajini et al. [46] with zygotic embryos, plants regenerated after cryopreservation showed stable genetic, morphological, and cytological characteristics, which is one of the great advantages of this conservation method [39].

2.2 *Euterpe edulis* Martius

Euterpe edulis Martius, popularly known as juçara palm, is a native species to the Brazilian Atlantic Forest (**Figure 2a**) [47]. It is a key species within the forest ecosystem, as its fruiting powerfully attracts fauna. The species was the main palm tree producing palm hearts in this ecosystem, which made it the target of intense exploration from the 1960s, suffering a drastic reduction in its natural population since the extraction of its heart of palm (apical meristem) led to the death of the plant [48, 49], and its inclusion in the list of endangered species [50]. Currently, the species is also subject to the increasing effects of extractivism demanded by the pulp of its fruits, used locally as a substitute for açaí (**Figure 2b**) [51, 52].

2.2.1 Biotechnological tools for multiplication of Euterpe edulis *Mart*

Euterpe edulis reproduces exclusively by seeds. It does not have a natural vegetative propagation system (tillers or shoots) and does not respond to conventional vegetative propagation methods [51]. As an aggravating factor, its seeds have strong recalcitrance, with slow and irregular germination [53]. Over the past few years, several studies have been carried out seeking the development of propagation protocols that can provide high-quality and uniform seedlings in large quantities [54].

Somatic embryogenesis in *E. edulis* was first reported by [55]. Subsequently, the same authors carried out new studies indicating somatic embryogenesis as the most suitable method for *in vitro* regeneration of the juçara palm [56, 57]. Commonly used explants for this species include inflorescences, zygotic embryos, and leaves. In addition, juvenile explants have demonstrated more excellent responsiveness than those derived from adult tissues (**Figure 2c**) [55, 56]. Those authors used NAA and 2,4-D in 5–50 mg L^{-1} and 2iP in 3–10 mg L^{-1} concentrations to induce embryogenic calluses (**Figure 2d**). Somatic embryos in globular stages appeared after 80–200 days of inoculation. They were transferred to rooting media after the bipolar embryos detached from the matrix tissue, the stage at which the primary root begins to form. Plantlets' progression and formation occur with this material's transfer to a basic culture medium without plant growth regulators. The authors emphasize that somatic embryogenesis in this species occurs synchronously, allowing the visualization of all stages of embryogenesis on the same matrix tissue (**Table 2**).

Direct somatic embryogenesis protocol was subsequently determined using three types of explants: zygotic embryos (from mature and immature seeds), inflorescences of the adult plant, and leaves of seedlings grown *in vitro* [56]. The basal medium consisted of Euwens salts [62, 63] and vitamins, according to Morel and

Figure 2.
Somatic embryogenesis in Euterpe edulis *Mart. (a) In situ plant. (b) Fruits of the species are used for pulp extraction and have various applications in the industry. (c) Juvenile explants obtained from leaf primordia. (d) Embryogenic callus formed in leaf explants. Scale bars, c = 1 cm; d = 200 μm.*

Wetmore [64], and the plant growth regulators used were 2,4-D, NAA, and 2iP. The results obtained from zygotic embryos were satisfactory at high concentrations of 2,4-D (50–100 mg L^{-1}). The somatic embryos were converted into plantlets after being transferred to a medium with sucrose and mineral salts reduced to half the strength of the basal medium. Embryogenesis was slower in mature embryos, taking 90 days after the cultures started, compared to 30 days in immature embryos. The inflorescences and young leaves were initially cultured in liquid medium before being transferred to the gelled medium. Embryogenesis in the inflorescences was induced with the addition of 2,4-D (50 mg L^{-1}) and 2iP (3.0 mg L^{-1}), while in the leaves, the authors obtained satisfactory results with 10–20 mg $L^{-}1$ 2,4-D. The embryos that originated from these explants reached bipolar stages in a medium supplemented with 2iP (2.5 mg L^{-1}) and NAA (0.1 mg L^{-1}). The explants of zygotic embryos and inflorescences proved more appropriate for the induction of somatic embryogenesis in the species, especially those originating from immature tissues [65]. Structural analyses revealed that the embryogenic process in immature zygotic embryos originates from protodermal cells, while in mature embryos, it is formed from subepidermal cell clusters. In leaves, the division centers occurred adjacent to vascular bundles (**Table 2**) [66].

Phase	Explant	Culture medium	Response	References
Induction	Zygotic embryos and inflorescences	Y3 + NAA, 2,4-D and 2iP medium	Emergence of somatic embryos in the globular stage between 80 and 150 days	[55]
Induction	Zygotic embryos	Y3 + 2,4-D, NAA and 2iP medium	Satisfactory results at high levels of 2,4-D 50–100 mg L^{-1}	[56]
	Inflorescences	Y3 + 2,4-D, NAA and 2iP medium	Embryogenesis was induced with the addition of 2,4-D at 50 mg L^{-1} and 2iP at 3.0 mg L^{-1}	
	Leaves of seedlings grown *in vitro*	Y3 + 2,4-D, NAA and 2iP medium	Satisfactory results with 2,4-D between 10 and 20 mg L^{-1}	
Induction	Zygotic embryos	Addition of $CaCl_2H_2O$ to MS + 2iP and 2,4-D medium	Addition of calcium reduces the proliferation of embryogenic structures	[58]
Induction	Zygotic embryos	MS medium +2,4-D or picloram	Satisfactory results with 2,4-D at 125, 150 and 175 µmol L^{-1} and picloram at 250, 275 and 300 µmol L^{-1}	[59]
Multiplication	Embryogenic callus	MS + 2iP and NAA medium	125 µmol of NAA proved to be more effective	
Germination	Somatic embryos	MS + GA3 and BAP medium	More effective results with BAP and GA_3 without auxin pulses	
Induction	Zygotic embryos	Semi-solid MS medium +2,4-D, picloram, triclopyr and clopyralid	Greater efficacy of picloram at 150 µM and tricoplyr at 100 µM	[60]
Maturation and germination	Zygotic embyos	MS + ABA medium	5 µm of ABA provided satisfactory results	
Differentiation and maturation	Zygotic embryos from immature fruits	Polyamines, spermidine and spermine in different concentrations	150 µM spermidine caused an effective stimulus in the differentiation of embryogenic callus	[61]

Note: Y3, Euwens medium; NAA, 1-Naphthaleneacetic Acid; 2,4-D, 2,4- dichlorophenoxyacetic acid; 2iP, Isopentenyladenine; CaCl2H2O, calcium chloride dihydrate; MS, Murashige & Skoog medium; GA3, gibberellic acid; BAP, benzylaminopurine; ABA, abscisic acid.

Table 2.
Studies on somatic embryogenesis in Euterpe edulis.

The addition of calcium to the induction medium was tested by Saldanha and Martins-Corder [58]. The results showed that increasing the concentration of $CaCl_2H_2O$ in the culture medium reduced the average number of somatic embryos in 150-day-old palm seedlings. The MS medium supplemented with 2iP (3 mg L^{-1}) and

2,4-D (100 mg L^{-1}) without extra levels of Ca induced a more significant proliferation of embryogenic structures (**Table 2**).

Recently, a microscopy study evaluated the quality of the embryogenic calluses obtained in induction media supplemented with 25–300 μmol $L^{-}1$ 2,4-D or picloram [59]. Scanning electron microscopy analyses of somatic embryos obtained after 100 days of culture with 50–275 μmol L^{-1} 2,4-D revealed tissues with embryogenic and non-embryogenic masses. Some calluses presented a compact and smooth surface formed by elongated cells without evidence of proembryo formation. Only the callus induced with 175 μmol L^{-1} 2,4-D presented protuberances on the surface, indicating the formation of pre-embryogenic complexes. Anatomical sections of callus cultured in the 2,4-D induction medium revealed the presence of meristematic zones formed by groups of meristematic cells and embryogenic zones. Interestingly, recent results also demonstrated greater efficacy of picloram (150 μM) and triclopyr (100 μM) to induce somatic embryogenesis in *E. edulis* [60]. In this study, the maturation and germination of somatic embryos occurred in a medium supplemented with 5 μM ABA. Picloram and triclopyr have similar chemical structures, presenting equivalent biological activity; therefore, plantlets treated with triclopyr obtained normal formation and acclimation (**Table 2**).

The impact of polyamines, spermidine, and spermine on the differentiation of embryogenic tissues and the maturation of somatic embryos of *E. edulis* was also evaluated [61]. It was observed that 150 μM spermidine caused an effective stimulus in the embryogenic callus differentiation, facilitating the formation of several zones for the development of somatic embryos and inducing vigorous cell division. More than 80% of the somatic embryos formed at 150 μM spermidine were regenerated into normal plantlets (**Table 2**).

Thus, we conclude that studies of somatic embryogenesis in *Euterpe edulis* have used zygotic embryos as a source of explants, the only possible source to obtain complete regeneration of plantlets. From zygotic embryos, it is possible to establish large-scale production of plants with high genetic quality, obtained from crosses between superior genotypes. However, the cloning of superior genotypes from somatic tissues of these matrices is still far from being a reality for the species.

2.3 *Euterpe oleraceae* Martius

Euterpe oleraceae Martius is commonly known as açaí [67]. It is native to Central America and the Amazon region of northern South America [68]. In Brazil, it occurs spontaneously in the states of Amazonas, Maranhão, Amapá, and Pará [69]. The species stands out for the cultural, environmental, medicinal, nutritional, and antioxidant value of its fruits [70–76].

The açaí is a palm tree with drupe-type fruit that is small, globose, and purple or dull green when ripe. It has a recalcitrant seed and a hard endocarp (**Figure 3a, b**) [77–79]. The palm is multi-stemmed (up to 25 stems per clump) with fasciculated and dense roots, which allow it to inhabit predominantly igapó and várzea forests [68, 69]. It has 10–12 pinnate leaves and reaches up to 30 m in height with a diameter of 12–18 cm (**Figure 3c**–**e**) [68]. At 3.5–4 years, it begins to flower. The racemose inflorescence (spadix) has small, unisexual, and sessile flowers. On the rachis, the flowers are arranged in triads, with one female flower flanked by two male flowers, and at the terminal part, only male flowers [69].

Açaí is one of the main sources of raw materials for the palm heart agroindustry. However, research institutions holding germplasm of the species are focusing on

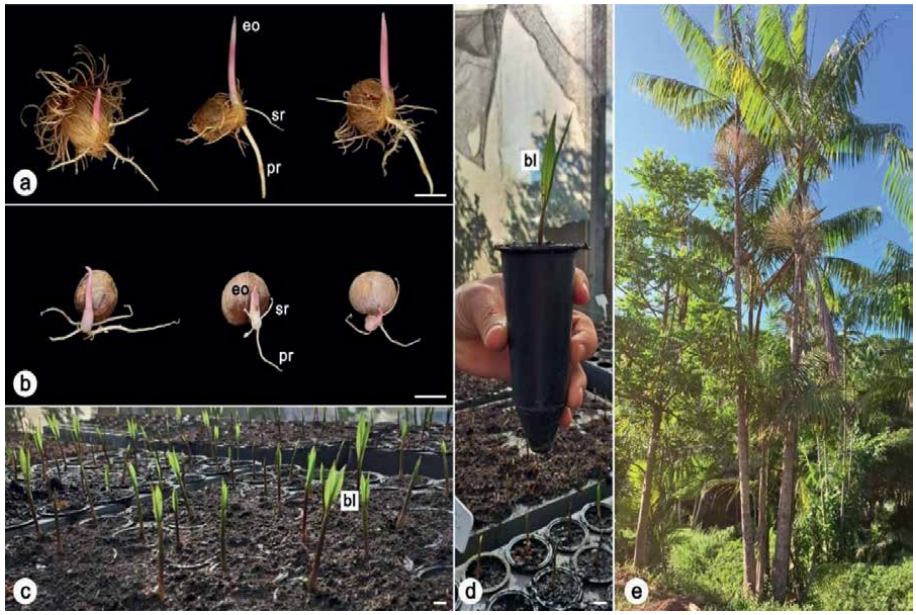

Figure 3.
Euterpe oleracea *Mart. Pre-germinated seeds: (a) with mesocarpic fibers; (b) core stone after removing the external fibers; (c), (d) acclimatized seedlings with 76 days showing leaves with characteristic patterns in the greenhouse; (e) palms,* in loco*, at the Federal University of Viçosa (UFV) orchard, Brazil (note the tillering). Bifid leaf (bl), eophyll (eo), primary roots (pr), and seminal roots (sr). Scale bars: a-d = 1 cm.*

fruit agribusiness [80, 81]. In Brazil, the state of Pará leads national production with 1.7 million tons, representing 90.4% of the national production [82]. Likewise, Pará is considered the nation's largest producer of açaí fruit (66%). The United States has become the leading importer and processor of açaí-based products, followed by Australia, Japan, and European Union countries (Germany, Belgium, Portugal, and France) [83].

2.3.1 Biotechnological tools for multiplication of Euterpe oleracea Mart

2.3.1.1 Somatic embryogenesis

Açaí can be propagated *via* seeds (sexual propagation) and tillering (asexual propagation). However, due to plants' low production and survival rates *via* tillering, commercial-scale seedling production is limited to sexual propagation [84, 85]. At the same time, the recalcitrance of seeds makes it challenging to store them for long periods, in addition to presenting slow and uneven germination [84].

In this context, *in vitro* regeneration techniques, especially somatic embryogenesis, are seen as a promising alternative to the clonal propagation of açaí [86]. However, somatic embryogenesis is a complex, multifactorial, and multistep technique involving cell dedifferentiation, cell division activation, and reorganization of physiology, metabolism, and gene expression patterns [87–89]. Somatic embryogenesis protocols in palm trees are scarce, and few reach mass scale [90]. Thus, selecting the type and development stage of explants to be used *in vitro* açaí regeneration protocols becomes essential for the success and deepening of knowledge about the two most critical aspects of palm morphogenesis: callus initiation and induction of

somatic embryogenesis. The explant type and its development stage are probably the most critical factors in determining the embryogenic capacity of a culture [91].

Zygotic embryos have advantages, such as high responsiveness to *in vitro* culture, presumably because they still express many embryogenesis-related genes [92]. They are also generally pathogen-free and abundant in many species, making them easy to harvest with minimal damage to the mother plant [86]. Studies on somatic embryogenesis from zygotic embryos as an initial explant source have been conducted in açaí (**Table 3**) [75, 76]. However, zygotic embryo regeneration protocols have the disadvantage of unpredictability, like the regenerants [94], due to cross-pollination.

Immature inflorescences have been studied as potential explants for palm due to the high embryogenic capacity of the callus they produce [95], as well as the additional advantages of low fungal and bacterial contamination [96, 97], minimal damage to the mother plant during harvest [94], and abundant inflorescence production in many species. Similarly, immature leaves are seen as potential explants to be used in the initiation of the technique, mainly because, like inflorescences, they allow the cloning of phenotyped individuals in the field without the influence of genetic material segregation after cross-fertilization [86].

Protocols for obtaining somatic embryos from immature leaves and inflorescences of açaí have been developed at the Plant Tissue Culture Laboratory of the Federal University of Viçosa (UFV) in Brazil. However, the protocols are still under evaluation (unpublished data) (**Figure 4**).

2.3.1.2 De novo *shoot organogenesis*

Regeneration in palms through *de novo* shoot organogenesis has been considered relatively low and irregular compared to other monocots [98]. Açaí embryos inoculated in semi-solid MS induction medium [99], with 30 g L^{-1} of sucrose, 1.5 g L^{-1} of activated charcoal (AC), MW vitamins [64], and 100 mg L^{-1} of 2,4-D, produced callus. However, the plantlets did not regenerate [100].

2.3.2 Genetic breeding and conservation

Açaí cultivars were developed by the Brazilian Agricultural Research Corporation (Embrapa) to increase the supply of fruit to the market. Traditionally, açaí grows in flooded areas. However, in 2005, through a genetic breeding program based on phenotypic selection from its germplasm bank, Embrapa Western Amazonia (Belém, PA, Brazil) developed a cultivar 'BRS-Pará', suitable for growing on stable land, as a result of which the production system of this plant has now been modified, making it easier and more productive than the traditional system [101]. The release of this cultivar was key to the establishment of more productive crops, as the first açaí plantations in upland areas were carried out amateurishly, using seeds of unknown genetic origin from açaí harvesters, resulting in plantations with heterogeneous productivity and fruit qualities [102].

To address another challenge in açaí fruit production, which is production seasonality, researchers from Embrapa Western Amazonia (Belém, PA, Brazil) developed the BRS Pai D'égua cultivar in 2019. The breeders selected native açaí species produced in the off-season and, through crossbreeding, aimed to create genetic material capable of producing year-round. In addition to this characteristic, the researchers selected species with higher productivity and pulp yield. Thus, tests were conducted under different cultivation conditions to ensure the stability and adaptation of the

Phase	Explant	Culture medium	Response	References
Induction		MS supplemented with 0.6% agar, 0.25% activated charcoal, 3% sucrose, 500 mg/L hydrolyzed casein, 339.36 and 454.48 μM of 2,4-D	80% and 61% of the explants subjected to concentrations of 339.36 and 454.48 μM, respectively, showed yellowish embryogenic callus	
Multiplication	Mature zygotic embryos	MS supplemented with 0.6% agar, 2% sucrose, 0.537 μM of NAA and 12.30 μM of 2iP	The embryogenic calluses from the treatment with 339.36 μM of 2,4-D progressed to SEs, while the others oxidize	[75]
Regeneration		MS supplemented with 0.6% agar and 1% sucrose in the absence of growth regulators	97% of the embryogenic callus from the treatment with 339.36 μM of 2,4-D progressed to somatic embryos	
Induction		MS supplemented with 30 g sucrose, 1.5 g of activated charcoal, 6 g agar, 225 μM of PIC, dark conditions	72% of the explants presenting embryogenic calluses formation	
Differentiation/ Maturation	Immature zygotic embryos	MS supplemented with 0.537 μM NAA and 12.3 μM 2ip with subcultures at 4-week intervals	100% of the explants that exhibited embryogenic calluses formation in the induction phase differentiated into SEs	[76]
Conversion		1/2MS supplemented with 20 g sucrose, 2.5 g of activated charcoal and light conditions	Plants were successfully regenerated from SE and acclimatized	
Induction		MS supplemented with 30 g/L sucrose, 2.5 g/L Phytagel, 2.5 g/L activated charcoal, 0.5 g/L L-glutamine and 450 μM PIC	84% of the explants showed embryogenic calluses with a nodular appearance and yellowish coloration	
Multiplication	Immature and mature zygotic embryos	MS supplemented with 12.3 μM 2iP, 0.6 μM NAA and 300 mg/L of activated charcoal	100% of the explants that formed embryogenic calluses developed globular and compact somatic embryos	[93]
Regeneration		MS supplemented with 1.0 μM of BAP, 0.5 μM of GA_3	The highest conversion rate of SEs into plants was 58.7%	

Note: NAA, 1-Naphthaleneacetic Acid; MS, Murashige & Skoog; 2,4D, 2,4-dichlorophenoxyacetic acid; 2ip, 2-isopentenyladenine; PIC, Picloram; BAP, N6-benzylaminopurine; GA3, gibberellic acid SEs, somatic embryos.

Table 3.
Studies on somatic embryogenesis in Euterpe oleracea *Mart.*

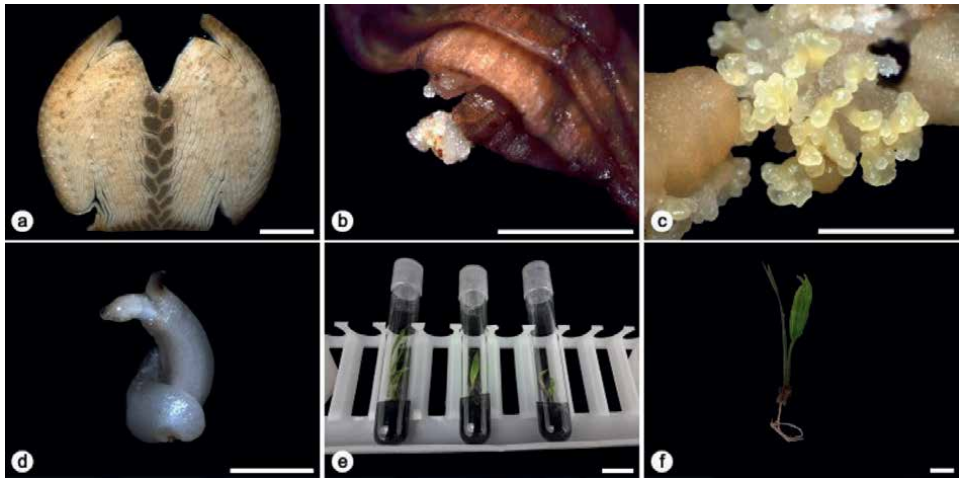

Figure 4.
*Somatic embryogenesis in açaí (*Euterpe oleracea *Mart.) (a) Immature leaf explant obtained from palm offshoot; (b) primary callus development on the leaf margin in induction medium after 60 days; (c) polyembryonic mass in differentiation medium; (d) conversion of somatic embryos in regeneration medium; (e)* E. oleracea *seedlings regenerated from somatic embryos; and (f) shoot and root system of the regenerated seedlings ready for acclimatization. Scale bar: a-d = 0.3 cm; e-f = 2 cm.*

cultivar, as the traditional açaí production area is usually in floodplain regions [103]. Examples such as these are necessary for understanding the importance of further studies on the species, given that the açaí palm has strong production potential and is a valuable resource for creating derivatives.

3. Oilseed palms

3.1 *Acrocomia aculeata*

Macauba (*Acrocomia aculeata*) is a native palm to the tropics and subtropics, with occurrences in areas with high to medium precipitation and high solar irradiation in America, such as Brazil, Mexico, Colombia, Argentina, Bolivia, Paraguay, Venezuela, Suriname, French Guiana, and the Antilles [104]. This evergreen, heliophilous, and pioneering species can form extensive oligarchic populations, mainly occupying anthropized areas in different biomes of the American continent. The plants of this genus are generally arboreal, robust, and tall [105, 106]. Its remarkable resilience and resistance to water scarcity, easy adaptation to different climates, lower environmental impact, and versatility of uses make it an up-and-coming crop compared to palm oil, the world's primary source of vegetable oil [107]. Furthermore, macauba fits integrated cultivation systems, such as the Crop-Livestock-Forest Integration, which renders slow carbon emissions, representing a more sustainable alternative to conventional oilseed growing [107]. Commercial exploitation and plantation of macauba are still in their early stages, as previously reported. The existing value chains rely on fruits from natural populations gathered by smallholder farmers. The fruits are supplied to local small-scale oil extraction facilities, primarily driven to niche markets, or processed on-farm by local communities for their own consumption and short-chain market [108, 109].

Macauba is a palm tree with many attributes that have been exploited since the eighteenth century [110]. However, despite the tremendous economic potential of the species, exploitation of macauba was restricted to extractivism until 2007. One of the reasons the species had not yet been cultivated was its complex propagation, which was considered the bottleneck for the development of palm plantations until then [111]. It was only in 2007, with the development of technology for the production of pre-germinated macauba seeds [112], that seed propagation, the natural form of propagation of the species, could be applied in the large-scale production of macauba seedlings, allowing the beginning of agricultural exploitation of the species.

3.1.1 Seminiferous propagation

Macauba is described in the literature as one of the most challenging palm species to germinate [113]. This species' natural germination of seeds is generally slow and uneven, taking up to 4 years and rarely resulting in germination rates above 7% [111, 114]. This is due to the deep dormancy, the inherent characteristic of the seeds of palm species of the genus *Acrocomia* [115, 116]. The first method for overcoming the dormancy of macauba seeds was developed by [112]. The process described by the authors enabled the rapid and efficient germination of macauba seeds and the regularization of seedling production, which allowed the cultivation of macauba inside and outside its natural environment.

The process of overcoming dormancy and inducing germination of macauba seeds developed by [112] consists of seven treatments, applied in sequence to the seed, namely: (i) elimination of the endocarp, (ii) first disinfestation, (iii) soaking treatment, (iv) second disinfestation, (v) mechanical scarification, (vi) treatment with gibberellic acid, and (vii) germination of the almond in a semi-aseptic environment. The process described above allowed the germination of 60–80% of the treated macauba seeds, which were finalized and ready for planting in the nursery approximately 30 days after the beginning of the germination process. The final product of this process was called pre-germinated macauba seeds by the authors and should contain a defined plumule and radicle.

3.1.2 Biotechnological tools for multiplication of Acrocomia aculeata

3.1.2.1 Somatic embryogenesis

In macauba, studies involving somatic embryogenesis have been reported as promising for its vegetative propagation [117–120], with these being recent and published just over a decade ago (**Table 4**). Moura et al. [117] were the pioneers in reporting the process, followed by Luis and Scherwinski-Pereira [118]. These authors used zygotic embryos to study somatic embryogenesis in macauba. Zygotic embryos are ontogenetically younger and consequently more responsive to the induction of somatic embryogenesis. Granja et al. [119] obtained in their study with zygotic embryos embryogenic lines with high multiplication capacity and a high degree of synchronization, enabling the control of all stages of somatic embryogenesis (multiplication, regeneration, and germination), thus achieving the cyclic production of seedlings over more than 2 years.

However, cloning protocols should be obtained from somatic tissues of properly tested elite matrices with proven agronomic performance. Recently, Meira et al. [121] demonstrated the development pathways of somatic embryogenesis in macauba from

Phase	Explant	Culture medium	Response	References
Induction	Zygotic embryo	Y3 + 9 μM Picloram	Embryogenic callus	[117]
Germination	Somatic embryos	Y3 + activated charcoal without growth regulator	Half of somatic embryos germinated	
Induction	Zygotic embryo	MS or Y3 with 0.5 mg/L or 1.0 mg/L of Dicamba or Picloram or 2,4-D	Embryogenic callus	[118]
Regeneration	Somatic embryos	MS or Y3 + activated charcoal without growth regulator	Plant regeneration	
Induction	Zygotic embryo	Y3 + 9 μM Picloram +0,9 μM 2iP	Callus	[119]
Multiplication	Callus	Y3 + 18 μM Picloram	Embryogenic mass	
Maturation	Embryogenic mass	Y3 + 2.5 μM 2iP + 5.55 μM NAA + active charcoal +1000 μM PUT	Somatic embryos	
Germination	Somatic embryos	Y3 + 0.25 μM 2iP + 0.55 μM GA_3 + 1000 μM PUT	Plants	
Multiplication	Callus	Y3 + 9 μM Picloram +1000 μM PUT	Somatic embryos	[120]
Regeneration	Somatic embryos	Y3 + 0.54 μM NAA + 1000 μM PUT + active charcoal	Plantlets	
Induction	Leaves	Y3 + activated charcoal +450 μM Picloram	Callus	[121]
Multiplication	Embryogenic callus	Y3 + 450 μM Picloram + active charcoal	Somatic embryos	
Induction	Leaves	Y3 + 450 μM Picloram	Callus	[122]
Multiplication	Callus	Y3 + 450 μM Picloram	Somatic embryos	
Regeneration	Somatic embryos	Y3 without growth regulator	Germinated plants	
Induction	Aereo parts of *in vitro* plants (TCL)	Y3 + Morel's vitamins +300 μM Picloram	Callus	[123]
Multiplication	Callus	Y3 + Morel's vitamins +25 μM BAP or 12.5 μM 2iP + 75 μM Picloram	Somatic embryos	
Germination	Somatic embryos	Y3 + Morel's vitamins without growth regulator	Plants	
Induction	Zygotic embryo	Y3 + activated charcoal +9 uM Picloram or Y3 + 9 μM Picloram	Nodular callus	[124]
Multiplication	proembryos	Y3 + charcoal active +9 μM Picloram or Y3 + 9 μM Picloran	Somatic embryos without germination	

Note: NAA, 1-Naphthaleneacetic Acid; PUT: putrescine; 2iP: isopentenyladenine; BAP: benzylaminopurine; MS: Murashige & Skoog medium; Y3: Euwens medium; 2,4-D: dichlorophenoxyacetic acid; GA_3: gibberellic acid.

Table 4.
Studies on somatic embryogenesis in Acrocomia aculeata.

leaf explants of adult plants. According to the authors, the process generated hundreds of somatic embryos, indicating a possible application of *in vitro* propagation in the clonal production of macauba, although plantlets were not obtained. Immature leaflets are excellent sources of explants for initiating somatic embryogenesis in species of the Arecaceae family, as they are protected within the heart of the plant and consequently show low levels of microbial contamination. In studies conducted by Andrade et al. [120], immature leaves of macauba showed responsiveness to the induction of somatic embryogenesis. In this study, the authors obtained embryogenic lines comparable to those obtained by Granja et al. [119], producing numerous plants. However, leaflets from adult plants did not respond to the somatic embryogenesis induction treatments. Protocols for obtaining somatic embryos from immature leaves and inflorescences of adult macauba plants have been developed at the Plant Tissue Culture Laboratory of the Federal University of Viçosa (UFV) in Brazil (**Figure 5**). However, the protocols are still under evaluation (unpublished data).

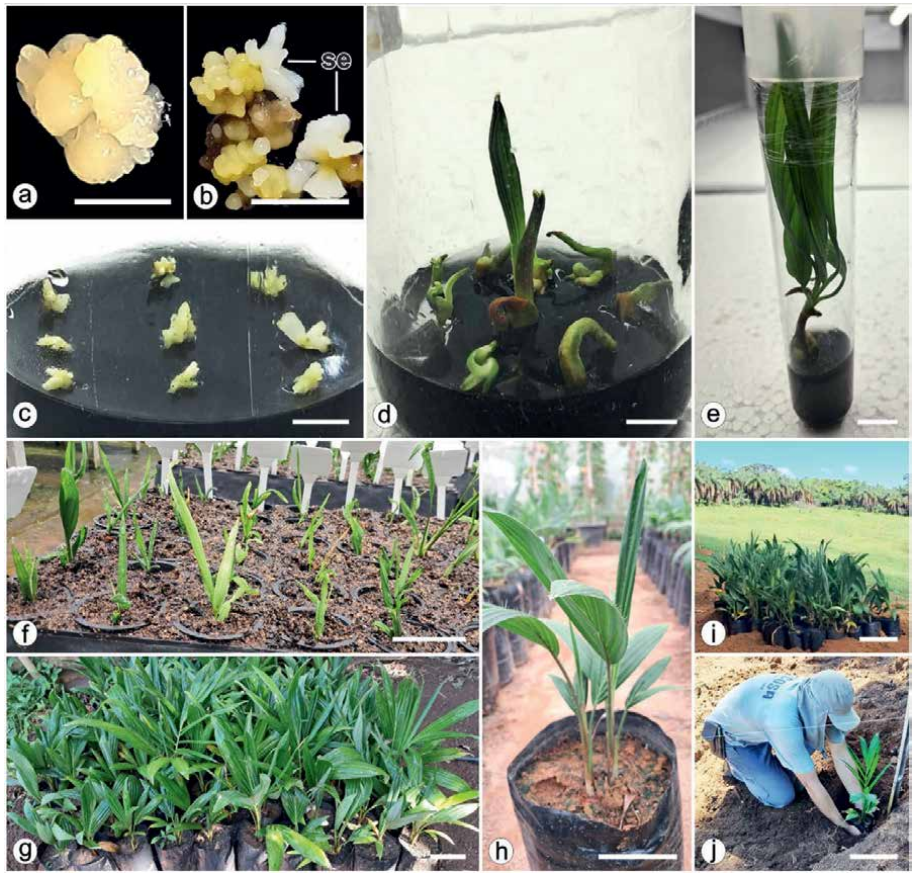

Figure 5.
*Somatic embryogenesis in macauba (*Acrocomia aculeata*). (a) embryogenic callus. (b-c) embryogenic callus with somatic embryos in a regeneration medium. (d) somatic embryo germination. (e)* in vitro *macauba plantlet obtained from somatic embryogenesis. (f) Acclimatization. (g-h) one-year-old plants obtained somatic embryos. (i-j) Planting of macauba clonal plants. Scale bars, a and b = 5 mm. c, d and e = 1 cm; f = 5 cm; g and h = 10 cm; I and j = 20 cm.*

The different culture media used in the somatic embryogenesis process essentially differ in the concentration of macro and micronutrients, vitamins, organic compounds, carbon sources, and other complex substances, typically specific to each stage of the process and the species used. The most commonly used culture media formulations for somatic embryogenesis in macauba include the MS medium from [99, 118, 119] and the Y3 medium from Eeuwens [117, 119–121]. The induction of somatic embryogenesis is associated with the use of auxins and cytokinins, the main growth regulators involved in controlling cell division and tissue differentiation [88]. Among the auxins most commonly used in the induction of somatic embryogenesis in macauba, 2,4-D can be cited [118, 121, 122]. However, other auxins such as Picloram and Dicamba have proven efficient [117–119, 121, 123]. The use of cytokinins in the induction medium for callus formation in macauba is not very common. Still, interesting results were reported in the work of Granja et al. [119] and Andrade et al. [120], where isopentenyl adenine (2iP) allowed the generation of embryogenic lines when combined with Picloram or Dicamba.

3.1.3 Genetic breeding and conservation

The ex situ conservation of macauba is more recent, starting at Embrapa Cerrados in December 2008 and later implemented in other Brazilian research institutions, such as the Federal University of Viçosa (UFV) and the Agronomic Institute of Campinas (IAC). UFV is responsible for the first collection registered by the Brazilian Genetic Heritage Management Council (registration no. 084–2013/CGEN/MMA). The Active Germplasm Bank (AGB-MACAUBA) of macauba from the Macauba Research Network of the Federal University of Viçosa (REMAPE/UFV) was established in 2009 at the Unit for Teaching, Research, and Extension in Genetic Improvement and Palm and Other Oilseed Production Systems (UEPE-MGPO) in the city of Araponga, Minas Gerais, Brazil (latitude −20.6686 and longitude −42.51785). The repository comprises more than 300 maternal families of the species *A. aculeata*, from the sclerocarpa and totai ecotypes, with around 1500 accessions from various Brazilian regions. The AGB-MACAUBA has been intensively used in studies for genetic breeding and domestication of the species. In addition to the AGB-MACAUBA, the first *A. aculeata* clones developed by UFV were established at UEPE-MGPO (**Figure 5i, j**).

3.2 *Elaeis guineensis* Jacq

Elaeis guineensis Jacq., commonly known as the African oil palm or oil palm, is a perennial, monoecious, oil-producing plant and has a long life cycle, playing an essential role in the production of vegetable oils for human consumption and other agro-industries [125, 126]. It is a palm tree that can reach up to 15 meters in height, with an erect, unbranched stem of the stipe type and a fibrous root system. Its leaves are pinnate, measuring 5–7 meters, and its inflorescences form in the leaf axils as spadices composed of spikes protected by two fibrous bracts, classifying it as a monoecious plant [127, 128].

It is considered an oil-producing palm native to Africa, widely cultivated in regions with a humid tropical climate. The main product of the oil palm is the oil

extracted from the fruit pulp, known internationally as palm oil. This oil stands out in global production and consumption, with applications in food, medicine, and oleochemistry [127, 129].

Its oil stands out for its versatility, oxidation resistance, and functionality in the food industry as an essential ingredient in a wide range of products. Additionally, it contains bioactive compounds that offer added benefits, such as nutritional functionality, cardiovascular health, and antioxidant protection [125, 130]. It serves as a strategic component in formulating healthy, high-quality foods, enhancing its importance to the global food industry and human health [125].

It is one of the most economically valuable oil-producing plants due to its high oil yield per bunch, plant, and productive area. As a perennial species, it can reach up to 6000 kg per hectare, with high quality and low production costs [131, 132]. Its cultivation is one of the main sources of vegetable oil production worldwide [133]. By 2022, its global production was nearly 74 million tons annually, representing 4% of global agricultural production [134].

3.2.1 Biotechnological tools for multiplication of Elaeis guineensis Jacq

3.2.1.1 Somatic embryogenesis

Somatic embryogenesis is considered a promising and efficient technique for multiplying elite genotypes of *Elaeis guineensis* (**Table 5**) [124, 135–138, 142, 143, 147]. Furthermore, it has been recognized as an ideal approach for mass clonal propagation associated with genetic improvement, particularly for perennial and long-cycle species [147].

Figure 6 presents the somatic embryogenesis of oil palm. The embryogenesis process in oil palm occurs indirectly, initially through the development of a pro-embryogenic mass, followed by its multiplication, which differentiates to form somatic embryos [127]. In oil palm, the induction of somatic embryogenesis has been achieved from different explants, such as root segments, immature leaves, and immature inflorescences [90–134].

Plant growth regulators, particularly 2,4-dichlorophenoxyacetic acid (2,4-D) and picloram, are essential in this process [135, 137]. Other growth regulators, such as naphthaleneacetic acid (NAA), gibberellic acid (GA_3), and benzylaminopurine (BA), are also frequently used in the various phases of somatic embryogenesis in this plant [138, 142, 143]. Additionally, polyamines have been commonly used in the somatic embryogenesis of the species, with a particular emphasis on putrescine [141, 145].

The somatic embryogenesis induction can be performed in culture media supplemented with a high auxin concentration. The subsequent proliferation of calluses and regeneration of somatic embryos is achieved by reducing the auxin levels or completely removing them, as well as adding other growth regulators, such as cytokinins, abscisic acid, and polyamines [145, 148].

Despite the various published protocols, many factors can interfere with the somatic embryogenesis process, making the repeatability of the protocols challenging, with uncertainty remaining regarding the best procedures. In oil palm, due to the advantages offered by the use of elite clones, research on the multiplication processes of oil palm is primarily conducted by private companies, and many important details for the success of embryogenesis are not made available [127].

Phase	Explant	Culture medium	Response	References
Induction	Leaf primordia	Y3 medium + Picloram or 2,4-D	1.0 mg L^{-1} of picloram allows calluses formation with higher embryogenic potential	[135]
Induction	Root primordia	Y3 medium + different regulators	1.0 mg L^{-1} of picloram provides better results	[136]
Induction	Male inflorescences	Modified Y3 medium + regulators	The use of picloram provides better responses in male inflorescences	[94]
Induction	Leaf primordia	MS medium +2,4-D + Activated charcoal	Higher concentrations of 2,4-D (450 μM) + activated charcoal provide better responses	[137]
Induction	Leaf primordia	MS medium + NAA + 2,4-D	The best responses were achieved using 6 mg L^{-1} of NAA + 0.5 mg L^{-1} of 2,4-D	[138]
Multiplication	Friable callus	Different immersion systems and culture media	The use of modified MS (MSA9) in a shaker or MSD in a temporary immersion system with intervals of 3 hours for 3 minutes	[139]
Induction and multiplication	Leaf primordia of 32 superior genotypes	Y3 medium	Occurrence of genetic control in the formation and multiplication of embryogenic lines, being genotype-dependent	[140]
Induction and regeneration	Mature zygotic embryos and Friable callus	MS medium and Blaydes medium + different regulators	The addition of polyamines allows an increase in the somatic embryogenesis rate	[141]
Regeneration	Somatic embryos	Suspension culture in modified MS medium + BA	The application of BA increases the responsiveness of somatic embryos	[142]
Regeneration and germination	Friable callus and somatic embryos	Modified MS medium + $CaCl_2$ and GA_3	880 mg L^{-1} of $CaCl_2$ allows for a higher regeneration rate, as well as higher concentrations of GA_3 (4.0 mg L^{-1}), while for germination, lower values of GA_3 are ideal	[143]
From multiplication to germination	Leaf primordia, friable callus and somatic embryos	Modified MS medium in different cultivation and immersion systems	The use of RITA-type bioreactors enables multiplication, as well as a higher yield of viable seedlings at the end of the process	[144]

Phase	Explant	Culture medium	Response	References
From induction to acclimatization	From leaf primordia to clonal seedlings	Modified Y3 medium + putrescine	Putrescine can be an important substitute for cytokinins, reducing somaclonal variations. Additionally, the response to somatic embryogenesis is genotype-dependent	[145]
Conservation	Friable callus	Successive subcultures in modified MS medium for 20 years	It is possible to conserve for more than 20 years; however, its success is genotype-dependent, and attention is required to somaclonal variations	[146]

Note: Y3, Eeuwen's medium; 2,4-D, 2,4-Dichlorophenoxyacetic acid; MS, Murashige and Skoog medium; NAA, 1-Naphthaleneacetic Acid; BA, Benzylaminopurine; GA_3, Gibberellic acid; $CaCl_2$, Calcium chloride.

Table 5.
Studies on somatic embryogenesis in Elaeis guineensis.

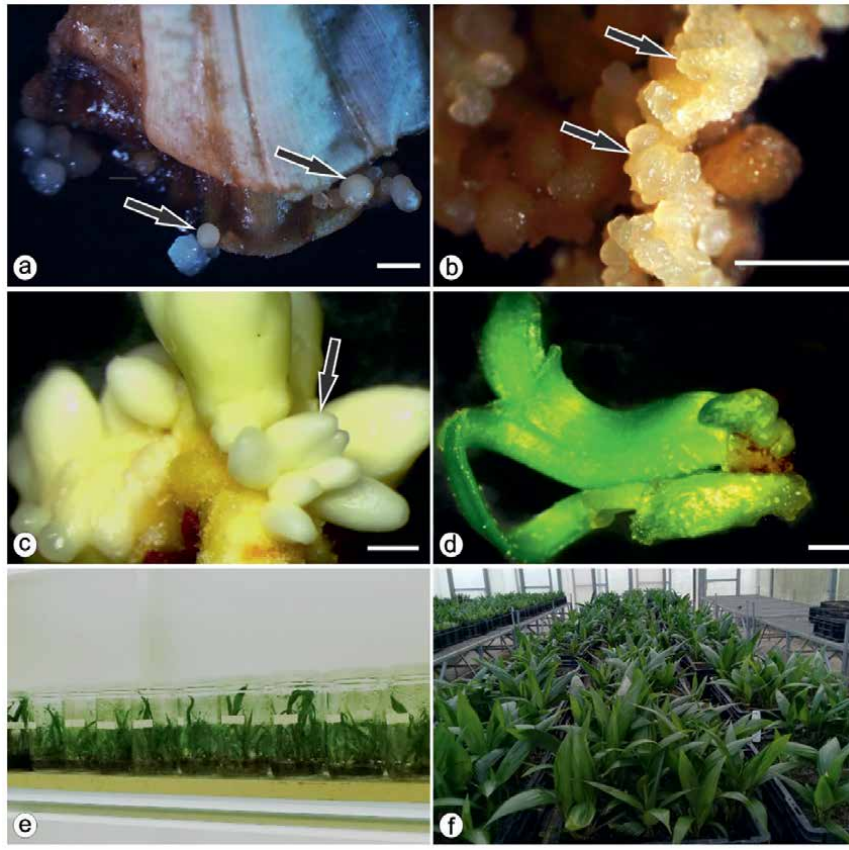

Figure 6.
*Somatic embryogenesis in oil palm (*Elaeis guineensis Jacq.*). (a) Formation of friable callus—Induction; (b) Multiplying friable calluses; (c) Differentiation of somatic embryos—regeneration; (d) clonal seedling in development—germination; (e) Large-scale germination; and (f) Acclimatization of* E. guineensis *seedlings arising from somatic embryogenesis. Scales bars: a = 1000 μm; b = 2500 μm; c, d = 500 μm.*

However, it is possible to obtain seedlings derived from somatic embryogenesis on a large scale.

4. Conclusions

Combining agricultural traditions with biotechnological innovations is crucial to ensure the sustainability and growth of palm crops, meeting the growing global demand for their products and beneficial properties. In this context, *in vitro* regeneration techniques, such as somatic embryogenesis, have proven crucial for the multiplication and cloning of superior genotypes. This technique not only allows the regeneration of uniform, pathogen-free, and genetically superior plants but also helps in the conservation of germplasm, in addition to subsidizing and accelerating genetic breeding programs. However, for some palm tree species, the established protocols are still inefficient, which requires continuous effort to optimize them, enabling the application of this biotechnological tool on a commercial scale to ensure the constant supply of high-quality plantlets, aligned with the sustainability demands of the production chain of these important palm tree plant species.

Acknowledgements

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brasília, Brazil; Grant No. 311282/2021-0), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Brasília, Brazil; Grant No. 001), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Belo Horizonte, Brazil; Grant No. APQ-02581-21; RED-00225-23), and the Rede Mineira de Biotecnologia em Multiplicação e Clonagem de Plantas.

Conflict of interest

The authors declare no conflict of interest.

Author details

Luís Henrique Bueno[1], Josimar dos Santos Ladeira[2], João Victor Baptista Silveira[2], Tsylla Assis Santos Ferreira[1], Otalicio Damasio da Costa Junior[2], Judith Georgette Alcalde Mosqueira[2], Thamires Fernanda Gomes[2], Francisco de Assis Lopes[2], Lucilene Silva de Oliveira[2], Sérgio Yoshimitsu Motoike[2] and Diego Ismael Rocha[2*]

1 Department of Plant Biology, Federal University of Viçosa, Viçosa, MG, Brazil

2 Department of Agronomy, Federal University of Viçosa, Viçosa, MG, Brazil

*Address all correspondence to: diego.rocha@ufv.br

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Dransfield J, Uhl NW, Lange CBA, Baker WJ, Harley MM, Lewis CE. Genera Palmarum: The Evolution and Classification of Palms. London: Kew Publishing; 2008. 732 p. DOI: 10.34885/92

[2] Muscarella R, Emilio T, Phillips OL, et al. The global abundance of tree palms. Global Ecology and Biogeography. 2020;**29**:1495-1514. DOI: 10.1111/geb.13123

[3] Pintaud JC, Baker WJ. A revision of the palm genera (*Arecaceae*) of New Caledonia. Kew Bulletin. 2008;**63**(1):61-73. DOI: 10.1007/s12225-007-9009-3

[4] Lieberman M, Lieberman D, Hartshorn GS, Peralta R. Small-scale altitudinal variation in lowland wet tropical forest vegetation. The Journal of Ecology. 1985;**73**:505-516. DOI: 10.2307/2260490

[5] Henderson A. Evolution and Ecology of Palms. New York: New York Botanical Garden Press; 2002. 259 p

[6] Genini J, Galetti M, Morellato LPC. Fruiting phenology of palms and trees in an Atlantic rainforest land-bridge island. Flora-Morphology, Distribution, Functional Ecology of Plants. 2009;**204**:131-145. DOI: 10.1016/j.flora.2008.01.002

[7] Cerqueira AF, Benchimol M, Sousa-Santos C, Bezerra IM, Santana dos Santos M, Dalmolin ÂC, et al. Trends and gaps in the literature on native palms of the Brazilian Atlantic Forest. Austral Ecology. 2024;**49**:e13322. DOI: 10.1111/aec.13322

[8] Meerow AW. Palm seed germination: BUL274/EP238, rev. 6/2004. EDIS. 2004;**2004**(9). DOI: 10.32473/edis-ep238-2004

[9] Ree JF, Guerra MP. Palm (Arecaceae) somatic embryogenesis. In Vitro Cellular & Developmental Biology - Plant. 2015;**51**:589-602. DOI: 10.1007/s11627-015-9722-9

[10] Rocha DI, Kurczyńska E, Potocka I, Steinmacher DA, Otoni WC. Histology and Histochemistry of somatic embryogenesis. In: Loyola-Vargas V, Ochoa-Alejo N, editors. Somatic Embryogenesis: Fundamental Aspects and Applications. Cham: Springer; 2016. pp. 471-494. DOI: 10.1007/978-3-319-33705-0_26

[11] Vianna SA. Cocos in Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro [Internet]. 2024. Available from: https://floradobrasil.jbrj.gov.br/FB80541 [Accessed: October 25, 2024]

[12] Harries H. The evolution, dissemination and classification of *Cocos nucifera* L. The Botanical Review. 1978;**44**:265-319. DOI: 10.1007/BF02957852

[13] Purseglove JW, editor. Tropical Crops Monocotyledons. 1 & 2nd ed. London: Longmans; 1972. 607 p

[14] Adkins S, Foale M, Bourdeix R, Nguyen Q, Biddle J, editors. Coconut Biotechnology: Towards the Sustainability of The 'Tree of Life'. St Lucia, QLD, Australia: Springer International Publishing; 2021. p. 5. DOI: 10.1007/978-3-030-44988-9

[15] Nayar NM. The coconut: Phylogeny, origins, and spread. In: Nayar NM, editor. Taxonimy and Intraspecific Classification. Amsterdam, Netherlands: Academic Press; 2016. pp. 25-51

[16] Allied Market Research [Internet]. 2019. Available from: https://www.prnewswire.com/news-releases/coconut-products-market-to-garner-31-1-bn-globally-by-2026-at-13-6-cagr-says-allied-market-research-300975188.html [Accessed: October 26, 2024]

[17] Brainer MSDCP, Ximenes LF. Produção de coco: soerguimento das áreas tradicionais do Nordeste. Caderno Setorial ETENE. 2020;**127**:1-15

[18] Instituto Brasileiro de Geografia e Estatística (IBGE) [Internet]. Produção de Coco-da-baía (*Cocos nucifera* L.) no Brasil. 2023. Available from: https://ibge.gov.br/explica/producao-agropecuaria/coco-da-baia/br [Accessed: October 21, 2024]

[19] Martins CR, Jesus Júnior LA. Produção e comercialização de coco no Brasil frente ao comércio internacional: panorama. [Internet]. 2014. Embrapa Tabuleiros Costeiros. Available from: http://www.bdpa.cnptia.embrapa.br [Accessed: October 26, 2024]

[20] Rethinam P, Krishnakumar V. Coconut water: A promising natural health drink-distribution, processing and nutritional benefits. In: Rethinam P, Krishnakumar V, editors. Global Scenario of Coconut and Coconut Water. Cham: Switzerland; 2022. pp. 17-35. DOI: 10.1007/978-3-031-10713-9_2

[21] Ramírez JFG, Muñoz RC, Sossa JWZ. Innovations and trends in the coconut agroindustry supply chain: A technological surveillance and foresight analysis. Frontiers in Sustainable Food Systems. 2023;**7**:1048450. DOI: 10.3389/fsufs.2023.1048450

[22] Kong EY, Biddle J, Foale M, Panis B, Adkins SW. The potential to propagate coconut clones through direct shoot organogenesis: A review. Scientia Horticulturae. 2021;**289**:110400. DOI: 10.1016/j.scienta.2021.110400

[23] Jain SM, Pramod K, Gupta RJ, Ronald JN. Somatic embryogenesis in Woody plants. In: Blake J, Hornung R, editors. Somatic embryogenesis in coconut (*Cocos nucifera* L.). 2nd ed. Dordrecht: Springer Netherlands; 1995. pp. 327-340. DOI: 10.1007/978-94-011-0491-3_18

[24] Chan JL, Saenz L, Talavera C, Hornung R, Robert M, Oropeza C. Regeneration of coconut (*Cocos nucifera* L.) from plumule explants through somatic embryogenesis. Plant Cell Reports. 1998;**17**:515-521. DOI: 10.1007/s002990050434

[25] Oropeza C, Verdeil JL, Ashburner GR, Cardña JM. Curre. Current plant science and biotechnology in agriculture: Current advances in coconut biotechnology. In: Adkins SW, YMS S, Godwin ID, editors. Control of Environmental Conditions and the Use of Polyamines Can Optimise the Conditions for the Initiation and Proliferation of Coconut Somatic Embryos. Dordrecht, Holanda: Springer; 1999. pp. 321-340. DOI: 10.1007/978-94-015-9283-3_24

[26] Perera PI, Hocher V, Verdeil JL, Doulbeau S, Yakandawala DM, Weerakoon LK. Unfertilized ovary: A novel explant for coconut (*Cocos nucifera* L.) somatic embryogenesis. Plant Cell Reports. 2007;**26**:21-28. DOI: 10.1007/s00299-006-0216-4

[27] Perera PI, Yakandawala DMD, Hocher V, Verdeil JL, Weerakoon LK. Effect of growth regulators on microspore embryogenesis in coconut anthers. Plant Cell, Tissue and Organ Culture. 2009;**96**:171-180. DOI: 10.1007/s11240-008-9473-y

[28] Mu Z, Yang S, Xu H, Yang Z, Haque MM, Tran BM, et al. The influence of maturity, storage, and embryo size on coconut callus induction success. Forests. 2024;**15**:764. DOI: 10.3390/f15050764

[29] Kong EY, Biddle J, Kalaipandian S, Adkins SW. Coconut callus initiation for cell suspension culture. Plants. 2023;**12**:1-22. DOI: 10.3390/plants12040968

[30] Agbidinoukoun A, Somakpe E, Houngue J, Houedjissin S, Ahanhanzo C. Response of haustorium tissues and coconut water in somatic embryos induction for the coconut palm (*Cocos nucifera* L.) variety PB121. Journal of Advances in Biotechnology. 2022;**10**:6-14. DOI: 10.24297/jbt.v10i.9195

[31] Jayarathna SPNC, Balasooriya JOS, Vidhanaarachchi VRM, Perera D. Enhanced coconut somatic embryogenesis of unfertilized ovary derived callus through improved media composition. COCOS. 2023;**24**:13-21. DOI: 10.4038/cocos.v24i1.5825

[32] Shareefa M, Thomas RJ, Sreelekshmi JS, Rajesh MK, Karun A. In vitro regeneration of coconut plantlets from immature inflorescence. Current Science. 2019;**5**:813-820

[33] Bett CC, Mweu CM, Nyende AB. In vitro regeneration of coconut (*Cocos nucifera* L.) through indirect somatic embryogenesis in Kenya. African Journal of Biotechnology. 2019;**18**:1113-1122. DOI: 10.5897/AJB2019.16867

[34] Wilms H, De Bièvre D, Longin K, Swennen R, Rhee J, Panis B. Development of the first axillary in vitro shoot multiplication protocol for coconut palms. Scientific Reports. 2021;**11**:18367. DOI: 10.1038/s41598-021-97718-1

[35] Rohith S, Kavibalan S, Thangaraj K, Suresh J, Ananthan M, Renuka R. Characterization of mother palms and novel techniques to produce elite seedlings of coconut var. chowghat orange dwarf. Electronic Journal of Plant Breeding. 2023;**14**:867-875. DOI: 10.37992/2023.1403.112

[36] Neema M, Hareesh GS, Aparna V, Chandran KP, Karun A. Electrical induction as stress factor for callus growth enhancement in plumular explant of coconut (*Cocos nucifera* L.). International Journal of Bio-resource and Stress Management. 2022;**13**:921-927. DOI: 10.23910/1.2022.3126

[37] Oropeza C, Verdeil JL, Ashburner GR, Cardña JM. Curre. Current plant science and biotechnology in agriculture: Current advances in coconut biotechnology. In: Engelmann F, editor. Criopreservation Coconut Germoplasm. Dordrecht, Holanda: Springer; 1999. pp. 289-296. DOI: 10.1007/978-94-015-9283-3_24

[38] Sopade PA, Samosir Y, Rival A, Adkins SW. Conservation of coconut (*Cocos nucifera* L.) germplasm at sub-zero temperature. CryoLetters. 2012;**33**:465-475

[39] Benelli C. Plant cryopreservation: A look at the present and the future. Plants. 2021;**10**:1-5. DOI: 10.3390/plants10122744

[40] Assy-Bah B, Engelmann F. Medium-term conservation of mature embryos of coconut. Plant Cell, Tissue and Organ Culture. 1993;**33**:19-24. DOI: 10.1007/BF01997593

[41] Wilms H, Rhee JH, Rivera RL, Longin K, Panis B. Developing coconut cryopreservation protocols and establishing cryo-genebank at

RDA; a collaborative project between RDA and bioversity international. In III International Symposium on Plant Cryopreservation. 2018;**1234**:343-348. DOI: 10.17660/ActaHortic.2019.1234.45

[42] Karun A, Sajini KK, Niral V, Amarnath CH, Remya P, Rajesh MK, et al. Coconut (*Cocos nucifera* L.) pollen cryopreservation. CryoLetters. 2014;**35**:407-417

[43] Lédo ADS, Santana FV, Oliveira ACAD, Oliveira LARD, Silva AVCD. Cryopreservation of Brazilian green dwarf coconut plumules by droplet-vitrification. Ciência Rural. 2019;**50**:e20190020. DOI: 10.1590/0103-8478cr20190020

[44] Welewanni I, Perera C, Jayasekera A, Bandupriya D. Recovery, histological observations and genetic integrity in coconut (*Cocos nucifera* L.) embryogenic calli cryopreserved using encapsulation-dehydration procedure. Journal of the National Science Foundation of Sri Lanka. 2020;**48**:175-186. DOI: 10.4038/jnsfsr.v48i2.9538

[45] Sisunandar RA, Turquay P, Samosir Y, Adkins SW. Cryopreservation of coconut (*Cocos nucifera* L.) zygotic embryos does not induce morphological, cytological or molecular changes in recovered seedlings. Planta. 2010;**232**:435-447. DOI: 10.1007/s00425-010-1186-x

[46] Sajini KK, Karun A, Amarnath CH, Engelmann F. Cryopreservation of coconut (*Cocos nucifera* L.) zygotic embryos by vitrification. CryoLetters. 2011;**4**:317-328

[47] Carvalho CS, Galetti M, Colevatti RG, Jordano P. Defaunation leads to microevolutionary changes in a tropical palm. Scientific Reports. 2016;**6**:1-10. DOI: 10.1038/srep31957

[48] Reis MS, Conte R, Fantini AC, Guerra MP. O palmiteiro (Euterpe edulis Martius) como recurso da Mata Atlântica. In: Simões LL, Lino CF, editors. Sustentável Mata Atlântica a exploração de seus recursos florestais. 2nd ed. Senac: São Paulo; 2002. pp. 103-117

[49] Martinelli G, Moraes MA, editors. Livro vermelho da flora do Brasil. 1nd ed. Rio de Janeiro: CNCFlora; 2013. 1100 p

[50] Ministério do Meio Ambiente. Instrução Normativa N°6. Lista Oficial das Espécies da Flora Brasileira Ameaçadas de Extinção. Diário Oficial da União. 2008;**185**:75-83

[51] Schulz M, Borges G, Gonzaga SC, ACO LVC, Fett R. Juçara fruit (*Euterpe edulis* Mart.): Sustainable exploitation of a source of bioactive compounds. Food Research International. 2016;**89**:14-26. DOI: 10.1016/j.foodres.2016.07.027

[52] LAOP G, Souza RG, editors. Palmeira juçara: patrimônio natural da Mata Atlântica no Espirito Santo. 1nd ed. Vitória: Incaper; 2017. 68 p

[53] Jaganathan GK. Ecological insights into the coexistence of dormancy and desiccation sensitivity in Arecaceae species. Annals of Forest Science. 2021;**78**:1-14. DOI: 10.1007/s13595021010329

[54] Saldanha CW. Conservação in vitro de Euterpe edulis Martius através da embriogênese somática [Dissertação (Mestrado em Geomática)]. Brazil: Universidade Federal de Santa Maria; 2007; 110 p

[55] Guerra MP, Handro W. Somatic embryogenesis in tissue cultures of *Euterpe edulis* Mart. (Palmae). In: Ahuja RM, editor. Wood Plant Biotechnology. New York: Plenum Press; 1991. pp. 189-196

[56] Guerra MP, Handro W. Somatic embryogenesis and plant regeneration in different organs of *Euterpe edulis* Mart. (Palmae): Control and structural features. Journal of Plant Research. 1998;**111**:65-71. DOI: 10.1007/BF025 07151

[57] Guerra MP, Nodari RO, Peixoto ML, Quinta M. Embriogênese somática e micropropagação do palmiteiro. Sellowia. 2000;**49**:150-162

[58] Saldanha CW, Martins-Corder MP. In vitro germination and acquisition of embryogenic competence *Euterpe edulis* Martius immature zygotic embryos. Crop Breeding and Applied Biotechnology. 2012;**12**:171-178. DOI: 10.1590/S1984-70332012000300002

[59] Oliveira LB, Mello T, Araujo CP, Oliveira JPB, Ferreira A, Zanardo EC, et al. Morphoanotomical aspects of auxinic herbicides-induced somatic embryogenesis in *Euterpe edulis* Martius - a symbol and threatened species of the Atlantic Forest. Scientia Horticulturae. 2022;**299**:111-051. DOI: 10.1016/j.scienta.2022.111051

[60] Mello T, Taliuli YS, Silva TD, Zanardo TEC, Hegedus CEN, Anjos BB, et al. Optimization of somatic embryogenesis in *Euterpe edulis* Martius using auxin analogs and atomic force microscopy. Scientia Horticulturae. 2024;**331**:113-164. DOI: 10.1016/j.scienta.2024.113164

[61] Costa JS, Mello T, Fagundes DP, Silva TD, Araujo CP, Simões IM, et al. The spermidine promotes maturation and tissue differentiation of somatic embryos of *Euterpe edulis* Martius, an endangered Atlantic Forest species. In Vitro Cellular & Developmental Biology-Plant. 2024:1-12. DOI: 10.1007/s11627-024-10473-6

[62] Euwens CJ. Mineral requirements for growth and callus initiation of tissue cultures. Physiologia Plantarum. 1976;**15**:473-497

[63] Linsmaier EM, Skoog F. Organic growth factor requirements of tobacco tissue cultures. Physiologia Plantarum. 1965;**18**:100-127. DOI: 10.1111/j.1399-3054.1965.tb06874.x

[64] Morel G, Wetmore RH. Fern callus tissue culture. American Journal of Botany. 1951;**38**:141-143. DOI: 10.2307/2437837

[65] Oliveira LB, Mello T, Araujo CP, Oliveira JPB, Ferreira A, Zanardo TÉC, et al. Morphoanatomical aspects of auxin herbicides-induced somatic embryogenesis in Euterpe edulis Martius, a symbol and threatened species of the Atlantic Forest. Scientia Horticulturae. 2022;**299**:111051

[66] Saldanha CW, Martins-Corder MP, Steinmacher DA, Guerra MP. In vitro morphogenesis of the zygotic embryos and leaf sheaths of *Euterpe edulis* Martius (*Arecaceae*). Crop Breeding and Applied Biotechnology. 2006;**6**:228-235. DOI: 10.12702/1984-7033.v06n03a07

[67] Oliveira MSP, Carvalho JEU, Nascimento WMO. Açaí (Euterpe oleracea Mart.). Série Frutas Nativas. Jaboticabal, Brasil: Funep; 2000. 52 p

[68] Henderson A. The genus Euterpe in Brazil. In: Reis MS, Reis A, editors. Euterpe edulis Martius (Palmiteiro) - Biologia, Conservação e Manejo, Herbário Barbosa Rodrigues. Itajaí, Brasil; 2000. pp. 1-22

[69] Cymerys M, Shanley P. Açaí. In: Shanley P, Medina G, editors. Frutíferas e Plantas úteis na vida Amazônica. Belém, Brasil: CIFOR/Imazon; 2005. pp. 163-170

[70] Rambo MKD, Schmidt FL, Ferreira MMC. Analysis of the

lignocellulosic components of biomass residues for biorefinery opportunities. Talanta. 2015;**144**:696-703. DOI: 10.1016/j.talanta.2015.06.045

[71] Cedrim PCA, Barros EMA, Nascimento TGD. Antioxidant properties of acai (*Euterpe oleracea*) in the metabolic syndrome. Brazilian Journal Food Technology. 2018;**21**:e2017092. DOI: 10.1590/1981-6723.09217

[72] Lima LR, Costa OF, Alves BSF, Dantas KDGF, Lemos VP, Pinheiro MHT. Remoção de Cu (II), Zn (II) e Ni (II) utilizando resíduo de açaí (*Euterpe oleracea* Mart.) como biossorvente em solução aquosa. Revista Virtual de Química. 2020;**12**:1066-1078. DOI: 10.21577/1984-6835.20200086

[73] Laurindo LF, Barbalho SM, Araújo AC, Guiguer EL, Mondal A, Bachtel G, et al. Açaí (*Euterpe oleracea* Mart.) in health and disease: A critical review. Nutrients. 2023;**15**:989. DOI: 10.3390/nu15040989

[74] Nascimento WMO, Euterpe A, oleracea Mart. Informativo Técnico Rede de Sementes da Amazônia. Vol. 18. Belém, Brasil: Embrapa Amazônia Oriental; 2008

[75] Ledo AS, Lameira OA, Menezes IC. Embriogênese somática e regeneração de plantas em açaizeiro. Vol. 34. Rio Branco: Boletim de Pesquisa-Embrapa; 2002. 22 p

[76] Scherwinski-Pereira JE, Guedes RS, Silva RA, Fermino JR, Luis ZG, Freitas EO. Somatic embryogenesis and plant regeneration in açaí palm (*Euterpe oleracea*). Plant Cell, Tissue and Organ Culture. 2012;**109**:501-508. DOI: 10.1007/s11240-012-0115-z

[77] MSP O, Lemos MA, Santos EO, Santos VF. Variação fenotípica em acessos de açaizeiro (*Euterpe oleracea* Mart.) para caracteres relacionados à produção dos frutos. Boletim de Pesquisa e Desenvolvimento. 1998;**209**:6-19

[78] Dos Santos BLG, Gama JRV, Da Silva Ribeiro RB, Dos Anjos RKF, Gomes KMA, Ximenes LC, et al. Estrutura e valoração de *Euterpe oleracea* em uma floresta de várzea na Amazônia. Advances in Forestry Science. 2018;**5**:391-396. DOI: 10.34062/afs.v5i3.5701

[79] MSP O. Biologia floral do açaizeiro em Belém do Pará. Boletim de Pesquisa e Desenvolvimento. 2002;**8**:26

[80] Da Silva AO, De Lima Mera WYW, Santos DCR, De Souza DP, Silva CGN, Raiol LL, et al. Estudo da produção de açaí (*Euterpe oleracea* Mart): aspectos econômicos e produtivos baseados nos anos de 2015 a 2017. Brazilian Journal of Development. 2020;**6**:1629-1641. DOI: 10.34117/bjdv6n1-112

[81] Maciel-Silva FW, Buller LS, Gonçalves MLMBB, Rostagno MA, Forster-Carneiro T. Sustainable development in the legal Amazon: Energy recovery from açaí seeds. Biofuels, Bioproducts and Biorefining. 2021;**15**:1174-1189. DOI: 10.1002/bbb.2222

[82] Instituto Brasileiro de Geografia e Estatística (IBGE) [Internet]. Pesquisa Agrícola Municipal. Rio de Janeiro: PAM; 2022. Available from: https://sidra.ibge.gov.br/tabela/5457 [Accessed: November 26, 2024]

[83] Companhia Nacional de Abastecimento (CONAB) - Açaí - Análise Mensal - Dezembro 2020 [Internet]. Available from: https://www.conab.gov.br/info-agro/analises-do-mercado-agropecuario-e-extrativista/analises-do-mercado/historico-mensal-de-acai [Accessed: November 26, 2024]

[84] WMO N, JEU C, MSP O. Produção de mudas de açaizeiro a partir de perfilhos. Comunicado Técnico. 2011;**231**:4

[85] Martins CC, Nakagawa J, Bovi MLA, Stanguerlim H. Teores de água crítico e letal para sementes de açaí (*Euterpe oleracea* Mart.–Palmae). Revista Brasileira de Sementes. 1999;**21**:125-132. DOI: 10.17801/0101-3122/rbs.v21n1p125-132

[86] Freitas EO, Silva-Cardoso IMA, Ferreira JCB, Costa FHS, Scherwinski-Pereira JE. Somatic embryogenesis in Açaí palm (*Euterpe oleraceae* Mart.). In: Jain SM, Pramod G, editors. Step Wise Protocols for Somatic Embryogenesis of Important Woody Plants, Forestry Sciences. 2nd ed. Cham, Switzerland: Springer International Publishing; 2018. pp. 267-290. DOI: 10.1007/978-3-319-79087-9_22

[87] Emons AMC. Somatic embryogenesis: Cell biological aspects. Acta Botanical Neerlands. 1994;**43**:1-14. DOI: 10.1111/j.1438-8677.1994.tb00729.x

[88] Fehér A, Pasternak TP, Dudits D. Transition of somatic plant cells to an embryogenic state. Plant Cell, Tissue and Organ Culture. 2003;**74**:201-228. DOI: 10.1023/A:1024033216561

[89] Fehér A. Callus, dedifferentiation, totipotency, somatic embryogenesis: What these terms mean in the era of molecular plant biology? Frontiers in Plant Science. 2019;**10**:536. DOI: 10.3389/fpls.2019.00536

[90] Silva-Cardoso IMA, Scherwinski-Pereira JE. Somatic embryogenesis as a tool for propagation of palm trees (Arecaceae): Principles, strategies and challenges. In: Bettaieb T, editor. Micropropagation of Ornamental Plants: Research and Practice. 1nd ed. New York: Nova Science Publishers; 2018. pp. 139-194

[91] Gaj MD. Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to *Arabidopsis thaliana* (L.) Heynh. Plant Growth Regulation. 2004;**43**:27-47. DOI: 10.1023/B:GROW.0000038275.29262.fb

[92] Isah T. Induction of somatic embryogenesis in woody plants. Acta Physiologiae Plantarum. 2016;**38**:118. DOI: 10.1007/s11738-016-2134-6

[93] Freitas EO, Monteiro TR, Nogueira GB, Scherwinski-Pereira JE. Somatic embryogenesis from immature and mature zygotic embryos of the açaí palm (*Euterpe oleracea*): Induction of embryogenic cultures, morphoanatomy and its morphological characteristics. Scientia Horticulturae. 2016;**212**:126-135. DOI: 10.1016/j.scienta.2016.09.044

[94] Jayanthi M, Susanthi B, Nandiganti MM, Mandal PK. In vitro somatic embryogenesis and plantlet regeneration from immature male inflorescence of adult dura and tenera palms of *Elaeis guineensis* (Jacq.). Springer Plus. 2015;**4**:1-7. DOI: 10.1186/s40064-015-1025-4

[95] Fki L, Masmoudi R, Kriaâ W, Mahjoub A, Sghaier B, Mzid R, et al. Date palm micropropagation via somatic embryogenesis. In: Jain SM, Al-Khayri JM, Johnson DV, editors. Date Palm Biotechnology. Dordrecht: Springer; 2011. pp. 47-68. DOI: 10.1007/978-94-007-1318-5_4

[96] Teixeira JB, Söndahl MR, Kirby EG. Somatic embryogenesis from immature inflorescences of oil palm. Plant Cell Reports. 1994;**13**:247-250. DOI: 10.1007/BF00233313

[97] Abul-Soad AA. Micropropagation of date palm using inflorescence explants. In: Jain S, Al-Khayri J, Johnson D, editors. Date Palm Biotechnology. Dordrecht: Springer; 2011. pp. 91-117. DOI: 10.1007/978-94-007-1318-5_6

[98] Rabéchault H, Martin JP. Multiplication vegetative du palmier à huile (*Elaeis guineensis* Jacq.) l'áide de cultures de tissus foliaires. Comptes Rendus de l'Academie des Sciences. Vol. 283. Série D: Sciences Naturelles; 1976. pp. 1735-1737

[99] Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 1962;**15**:473-497

[100] Yokoo EY, Ramos LCS, Bovi MLA. Cultura de tecidos de híbridos e espécies de palmiteiro no instituto agronômico. Boletim científico. 1992;**25** 24 p

[101] Oliveira MSP, Farias NJT. Cultivar BRS-Pará: açaizeiro para produção de frutos em terra firme. Comunicado Técnico. Vol. 114. Embrapa Amazônia Oriental: Belém; 2005. 4 p

[102] Homma AKO. Extrativismo vegetal ou plantio: qual a opção para a Amazônia? Estudos Avançados. 2012;**26**:167-186. DOI: 10.1590/S0103-40142012000100012

[103] Farias NJT. BRS Pai d'Égua: cultivar de açaí para terra firme com suplementação hídrica. Comunicado Técnico. Vol. 317. Embrapa Amazônia Oriental: Belém; 2019. 7 p

[104] Plath M, Moser C, Bailis R, Brandt P, Hirsch H, Klein AM, et al. A novel bioenergy feedstock in Latin America? Cultivation potential of *Acrocomia aculeata* under current and future climate conditions. Biomass and Bioenergy. 2016;**91**:186-195. DOI: 10.1016/j.biombioe.2016.04.009

[105] Henderson A, Galeano G, Bernal G. Field Guide to Palm of the Americas. New Jersey: Princepton University; 1995. 502 p

[106] Lorenzi H, Noblick L, Kahn F, Ferreira E. Flora Brasileira: Arecaceae (Palmeiras). Nova Odessa: Instituto Plantarum; 2010. 384 p

[107] Sorita GD, Favaro SP, Gambetta R, Ambrosi A, Di Luccio M. Macauba (Acrocomia ssp.) fruits: A comprehensive review of nutritional and phytochemical profiles, health benefits, and sustainable oil production. Comprehensive Reviews in Food Science and Food Safety. 2025;**24**:e70097. DOI: 10.1111/1541-4337.70097

[108] Pires PCL, César AS, Cardoso AN, Favaro SP, Conejero MA. Strategies to improve the competitiveness of an agroindustrial system for a macauba based oil production in Minas Gerais state, Brazil. Land Use Policy. 2023;**126**:106552. DOI: 10.1016/j.landusepol.2023.106552

[109] Vargas-Carpintero R, Hilger T, Mössinger J, Souza RF, Barroso Armas JC, Tiede K, et al. Acrocomia spp.: Neglected crop, ballyhooed multipurpose palm or fit for the bioeconomy? A review. Agronomy for Sustainable Development. 2021;**41**(6):75. DOI: 10.1007/s13593-021-00729-5

[110] Motoike SY, Nacif AP, Paes JMV. Macaúba: história do nascimento de uma cultura. Informe Agropecuário. 2011;**32**:6

[111] Tabai SA. Propagação da palmeira macaúba Acrocomia aculeata (Jacq.) Loddiges. através de métodos in vitro [Dissertação]. Piracicaba: Universidade de São Paulo; 1992

[112] Motoike SY, Lopes FA, Sá Junior AQ, Carvalho M, Oliveira MAR. Processo de germinação e produção de sementes pré-germinadas de palmeiras do gênero Acrocomia. Brasília, Brazil: Patent: PI0703180-7. Industrial; 2007

[113] Pinheiro CUB. Germinação de sementes de palmeiras. Teresina: Embrapa-UEPAE; 1986. 102 p

[114] Ribeiro LM, Souza PP, Rodrigues Junior AG, Oliveira TGS, Garcia QS. Overcoming dormancy in macaw palm diaspores, a tropical species with potential for use as bio-fuel. Seed Science and Technology. 2011;**39**:303-317

[115] Ellis RH, Hong TD, Roberts EH. Handbook of Seed Technology for Genebanks: Volume 2: Compendium of Specific Germination Information and Test Recommendations. International Board for Plant Genetic Resources; Rome, Italy. 1985. 456 p

[116] Bicalho EM, Pintó-Marijuan M, Morales M, Müller M, Munné-Bosch S, Garcia QS. Control of macaw palm seed germination by the gibberellin/abscisic acid balance. Plant Biology. 2015;**17**:990-996

[117] Moura EF, Motoike SY, Ventrella MC, Sá Júnior AQ, Carvalho M. Somatic embryogenesis in macaw palm (*Acrocomia aculeata*) from zygotic embryos. Scientia Horticulturae. 2009;**119**:447-454

[118] Luis ZG, Scherwinski-Pereira JE. An improved protocol for somatic embryogenesis and plant regeneration in macaw palm (*Acrocomia aculeata*) from mature zygotic embryos. Plant Cell, Tissue and Organ Culture. 2014;**118**:485-496

[119] Granja MMC, Motoike SY, Andrade APS, Correa TR, Picoli EAT, Kuki KN. Explant origin and culture media factors drive the somatic embryogenesis response in *Acrocomia aculeata* (Jacq.) Lodd. Ex Mart., an emerging oil crop in the tropics. Industrial Crops and Products. 2018;**117**:1-12

[120] Andrade AP, Motoike SY, Kuki KN, Queiroz V, Madeira DDC, Granja MMC, et al. Explant age and genotype drive the somatic embryogenesis from leaf explants of *Acrocomia aculeata* (Jacq.) Lodd. Ex Mart. (*Arecaceae*), an alternative palm crop for oil production. Trees. 2024;**38**:315-326

[121] Meira FS, Luis ZG, Silva-Cardoso IMA, Scherwinski-Pereira JE. Developmental pathway of somatic embryogenesis from leaf tissues of macaw palm (*Acrocomia aculeata*) revealed by histological events. Flora. 2019;**250**:59-67

[122] Meira FS, Luis ZG, Silva-Cardoso IMA, Scherwinski-Pereira JE. Somatic embryogenesis from leaf tissues of macaw palm [*Acrocomia aculeata* (Jacq.) Lodd. Ex Mart.]. Annais da. Academia Brasileira de Ciência. 2020;**92**:e20180709

[123] Padilha JHD, Ribas LLF, Amano E, Quoirin M. Somatic embryogenesis in *Acrocomia aculeata* Jacq. (Lodd.) ex mart using the thin cell layer technique. Acta Botanica Brasilica. 2015;**29**:516-523

[124] Moura EF, Ventrella MV, Motoike SY, Sá Júnior AQ, Carvalho M, Manfio CE. Histological study of somatic embryogenesis induction on zygotic embryos of macaw palm (*Acrocomia aculeata* (Jacq.) Lodd. Ex Martius). Plant Cell, Tissue and Organ Culture. 2008;**95**:175-184. DOI: 10.1007/s11240-008-9430-9

[125] Tenorio MLO, Prieto CMC, Univio NCL, Díaz DAR, Carvajal MXQ,

Perez LNT, et al. Bioactive compounds in palm oil: A comprehensive review of recent advances in physicochemical characteristics, health-promoting properties and Technologies for Extraction, concentration, fractionation, encapsulation and functional food applications. Journal of Food Composition and Analysis. 2024;**132**:106306. DOI: 10.1016/j.jfca.2024.106306

[126] Morcillo F, Gallard A, Pillot M, Jouannic S. EgAP2-1, an AINTEGUMENTA-like (AIL) gene expressed in meristematic and proliferating tissues of embryos in oil palm. Planta. 2007;**226**(6):1353-1362. DOI: 10.1007/s00425-007-0574-3

[127] Carvalho M. Embriogênese Somática a Partir de Folhas Imaturas e Flores Desenvolvidas in vitro de Dendezeiro (Elaeis guineensis Jacq.) [thesis]. Viçosa: Universidade Federal de Viçosa; 2009

[128] Ferwerda JD. Ecophysiology of the African oil palm. In: Ecophysiology of Tropical Crops. Elsevier; 1977. pp. 351-382

[129] Brazilio M, Bistachio NJ, Silva VC, Nascimento DD. O Dendezeiro (*Elaeis guineensis* Jacq.)-Revisão. Bioenergia em Revista Diálogos. 2012;**2**(1):27-45

[130] Barbour JA, Howe PRC, Buckley JD, Bryan J, Coates AM. Cerebrovascular and cognitive benefits of high-oleic peanut consumption in healthy overweight middle-aged adults. Nutritional Neuroscience. 2017;**20**(10):555-562. DOI: 10.1080/1028415X.2016.1204744

[131] Benjumea P, Agudelo J, Agudelo A. Basic properties of palm oil biodiesel–diesel blends. Fuel. 2008;**87**(10-11):2069-2075. DOI: 10.1016/j.fuel.2007.11.004

[132] Thuzar M, Vanavichit A, Tragoonrung S, Jantasuriyarat C. Efficient and rapid plant regeneration of oil palm zygotic embryos cv. 'Tenera' through somatic embryogenesis. Acta Physiologiae Plantarum. 2011;**33**:123-128. DOI: 10.1007/s11738-010-0526-6

[133] Hasen SB, Padfield R, Syayuti K, Evers S, Zakariah Z, Mastura S. Trends in global palm oil sustainability research. Journal of Cleaner Production. 2015;**100**:140-149. DOI: 10.1016/j.jclepro.2015.03.051

[134] FAO. World Food and Agriculture Statistical Yearbook 2022. Rome: Food and Agricultural Organisation; 2022. 382 p. DOI: 10.4060/cc2211en

[135] Pádua MS, Paiva LV, Labory CRG, Alves E, Stein VC. Induction and characterization of oil palm (*Elaeis guineensis* Jacq.) pro-embryogenic masses. Anais da Academia Brasileira de Ciencias. 2013;**85**:1545-1556. DOI: 10.1590/0001-37652013107912

[136] Vilela MSP, Andrade JC, Santos RS, Stein VC, Paiva LV. Histological analysis of indirect somatic embryogenesis induced from root explats of oil palm (*Elaeis guineensis* Jacq). Revista árvore. 2019;**43**:e430106. DOI: 10.1590/1806-90882019000100006

[137] Yusnita Y, Hapsoro DWI. In vitro callus induction and embryogenesis of oil palm (*Elaeis guineensis* Jacq.) from leaf explants. HAYATI Journal of Biosciences. 2011;**18**(2):61-65. DOI: 10.4308/hjb.18.2.61

[138] Sulaksono G, Hasmeda M, Hanum L, Wendra F, Santika B. Effect of media and plant growth regulator on proliferation of somatic embryogenic callus of oil palm (*Elaeis gueenesis* Jacq.). Agrivita: Journal of Agricultural Science. 2024;**46**(2):367-379. DOI: 10.17503/agrivita.v46i2.4234

[139] Marbun CLM, Marbun CLM, Toruan-Mathius N, Reflini UC, Liwang T. Micropropagation of embryogenic callus of oil palm (*Elaeis guineensis* Jacq.) using temporary immersion system. Procedia Chemistry. 2015;**14**:122-129. DOI: 10.1016/j.proche.2015.03.018

[140] Corrêa TR, Motoike SY, Coser SM, Silveira G, Resende MDV, Chia GS. Estimation of genetic parameters for in vitro oil palm characteristics (*Elaeis guineensis* Jacq.) and selection of genotypes for cloning capacity and oil yield. Industrial Crops and Products. 2015;**77**:1033-1038. DOI: 10.1016/j.indcrop.2015.09.066

[141] Rajesh MK, Radha E, Karun A, Parthasarathy VA. Plant regeneration from embryo-derived callus of oil palm–the effect of exogenous polyamines. Plant Cell, Tissue and Organ Culture. 2003;**75**:41-47. DOI: 10.1023/A:1024679910085

[142] Aberlenc-Bertossi F, Noirot M, Duval Y. BA enhances the germination of oil palm somatic embryos derived from embryogenic suspension cultures. Plant Cell, Tissue and Organ Culture. 1999;**56**(1):53-57. DOI: 10.1023/A:1006241215717

[143] Saptari RT, Sinta MM, Riyadi S I. Enhancing the regeneration of TIS-derived somatic embryos of oil palm (*Elaeis guineensis* Jacq.) with $CaCl_2$ and GA3. IOP Conference Series: Earth and Environmental Science. 2024;**2024**:1308. DOI: 10.1088/1755-1315/1308/1/012005

[144] Gomes HT, Machado LG, Bartos PMC, Balzon TA, Costa F, Azevedo V, et al. Somatic embryogenesis in oil palm from immature leaves with emphasis on leaf position, sequential callus re-collection, use of temporary immersion system, and assessment of genetic and epigenetic fidelity of the resulting clones. Plant Cell, Tissue and Organ Culture. 2024;**156**(2):32. DOI: 10.1007/s11240-023-02630-3

[145] Corrêa TR, Motoike SY, Andrade APS, Coser SM, Queiroz V, Granja MMC, et al. Accelerated in vitro propagation of elite oil palm genotypes (*Elaeis guineensis* Jacq.) by substituting cytokinin with putrescine. African Journal of Biotechnology. 2016;**15**(50):2767-2775. DOI: 10.5897/AJB2016.15670

[146] Konan KE, Durand-Gasselin T, Kouadio YJ, Flori A, Rival A, Duval Y, et al. In vitro conservation of oil palm somatic embryos for 20 years on a hormone-free culture medium: Characteristics of the embryogenic cultures, derived plantlets and adult palms. Plant Cell Reports. 2010;**29**:1-13. DOI: 10.1007/s00299-009-0787-y

[147] Pais MS. Somatic embryogenesis induction in woody species: The future after OMICs data assessment. Frontiers in Plant Science. 2019;**10**:1-18. DOI: 10.3389/fpls.2019.00240

[148] Parthasarathy U, Parthasarathy VA, Karun A. Oil palm. Biotechnology of Horticultural Crops. 2001;**1**:595-608

Chapter 8

Antioxidant Activity of Brazilian Fruits

Marizeth Libório Barreiros and André Luis B.S. Barreiros

Abstract

This chapter reviews the antioxidant activity of 22 Brazilian fruits, using different methods like DPPH, ABTS, and FRAP, using the bibliography available in recent years. We investigate the substances with the greatest described activity and to which class they belong. Flavonoids and polyphenols are the most common class of metabolites present in fruits with antioxidant activity. Catechin, epicatechin, anthocyanins, proanthocyanidins, stilbenes, resveratrol, and gallic acid, among others. We also investigate the presence of pro-vitamin A substances like carotenoids in these fruits, such as beta-carotene, lycopene, zeaxanthin, and the presence of vitamin C and E. In the ABTS and FRAP methods, camu-camu, acerola, jaboticaba, and açaí presented the highest values of antioxidant activity. We identify that the fruits with major content of polyphenols presented major antioxidant activity. They were acerola, camu-camu, and açaí.

Keywords: Brazilian fruits, flavonoids, polyphenols, antioxidants, vitamins

1. Introduction

Brazil is one of the countries with the largest biodiversity in the world. In addition to the Amazon biome, which represents 60.44% of the national territory, and the Brazilian savannah, which represents 22% of the territory, we also have the Atlantic Forest, Caatinga, Pantanal, and Pampa biomes. All of these have an immense variety of plant species, with emphasis on fruit species. In the last survey carried out by Lorenzi [1], 1080 fruits were recorded in Brazil, 580 of which were native and 500 exotics, without taking into account the varieties of each species. But first, it is necessary to define fruit. There is a lot of confusion about what constitutes a fruit, a pseudo-fruit, and a fruit. According to the APG III plant classification system, a fruit is the female organ (ovary) of the plant after its fertilization. A pseudo-fruit is any organ or structure accessory to the seed, which attracts dispersers but does not originate from the ovary. Finally, fruits are the fruit or pseudo-fruit that can be consumed in their natural state [2].

Despite the existence of more than 30,000 species in the world, the world's fruit industry is dominated by around 20 species, which are well-known and widely distributed. According to the FAO [3], which declared 2021 the international year of fruits and vegetables, world fruit production in 2018 was 868 million tons (Mt). Of these, 155 Mt. were bananas; 152 Mt. were citrus fruits; 131 Mt. were watermelons

and melons; 111 Mt. were apples and pears; 79 Mt. were grapes; 55 Mt. were mangoes; 28 Mt. were nectarines, peaches, Damascus, and apricots (stone fruits); 28 Mt. were pineapples; 13 Mt. were papayas; 13 Mt. were plums; 12 Mt. were cranberries, strawberries, raspberries, blackberries, and blueberries (berries); 9 Mt. were dates; and 6 Mt. were cherries. All other species combined represent 76 Mt. Less than 1% of fruit species are commercially exploited in the world. In Brazil, it is no different. Most Brazilian fruit species are unknown to the general market [1].

In the Amazon, fruit growing would be an alternative for commercial exploitation of the region, without the need for deforestation or in-depth agronomic knowledge. Fruits such as cupuaçu, bacuri, taperebá (cajá), and jenipapo have recognized potential but are little explored. Others such as abiu, biribá, ingá, mapatí, and pitomba are very popular in the region but are little known outside. Camu-camu has immense nutritional value due to its high vitamin C content. The fruits of palm trees such as açaí, tucumã, bacaba, patauá, and pupunha, although the latter is not consumed in its natural state, requiring cooking, are an important complementary source of food for riverside peoples, in addition to being a source of high-quality oils [4].

The Brazilian savannah has a diversity comparable to that of the Amazon. However, its biome is much more threatened by agricultural development. Erosion, pollution, reduction of water sources, and unsustainable land use are leading to the extinction of species [5]. Fruits such as pequi, saputá, murici, baru, and gravata, among others, can be found there. To preserve our natural wealth, it is first necessary to know it and identify its full potential.

Fruits and other vegetables contain distinct antioxidant substances, whose activities have been well demonstrated in recent years. The presence of phenolic compounds, such as flavonoids, phenolic acids, and anthocyanins, in addition to the already known vitamins C, E, and carotenoids, contribute to the beneficial effects of these foods. Studies have shown that natural polyphenols have significant effects in reducing cancer [5, 6].

Oxidation is a fundamental part of aerobic life and is the most efficient way that higher cells have found to produce energy. However, free radicals are intermediaries or by-products of these natural processes. Free radicals that have an unpaired electron centered on the oxygen or nitrogen atom are called Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), respectively. Their excess has deleterious effects such as peroxidation of membrane lipids and damage to proteins, enzymes, carbohydrates, and DNA. To combat them, living organisms use endogenous antioxidants, that is, those produced by the body itself, or exogenous antioxidants from the diet [7]. According to Halliwell [8], *"An antioxidant is any substance that, when present in a low concentration compared to that of the oxidizable substrate, regenerates the substrate or significantly prevents its oxidation."* Antioxidants from the diet are vitamin A and pro-vitamin A such as carotenoids, vitamin C, vitamin E (tocopherols and tocotrienols), and polyphenolic compounds such as gallates, stilbenes, flavonoids, and polyflavonoids [6]. All of these classes of compounds are widely found in fruits. Acerola and camu-camu are very rich in vitamin C, palm fruits such as tucumã and dendê contain vitamin E, buriti has high concentrations of pro-vitamin A, and açaí is a source of anthocyanins, the same class of flavonoids present in grapes and wine.

This work presents some Brazilian fruits, and aims to present their importance and make a comparative analysis of their antioxidant activities by different methods such as DDPH (1,1-diphenyl-2-picryhydrazyl), FRAP (ferric reducing antioxidant power), and ABTS [2,2′-azino-bis(3-ethylbenzthaazoline-6-sulphonic acid)].

2. Some native and cultivated Brazilian fruits

2.1 Abiu (*Pouteria caimito*)

The abiu is a 6 to 24 m-high tree from the Sapotaceae family. It is native to the Central Amazon and the east coast of Brazil, from Pernambuco to Rio de Janeiro. Its globose or ellipsoid fruits have 4 to 12 cm in diameter and weigh in average 150 to 200 g. They are yellow in color and have a smooth skin, fleshy-gelatinous and translucent pulp, with a sweet flavor and latex, involving 1 to 4 seeds. It ripens from April to June and is mainly consumed *in natura* [1]. According to Canuto [9]; Marques [10], and Monteiro [11], abiu fruits are rich in tryptophan; threonine; lysine; vitamin C; vitamin B3; phenolic compounds; minerals such calcium, phosphorus, and potassium; as well as lipids; proteins; and carbohydrates.

2.2 Acerola (*Malpighia emarginata* Sessé & Moc. Ex DC.)

The acerola is a 2 to 4 m-high little tree from the Malpighiaceae family. It is native to several countries in the Antilles, Central America, and northern South America. The fruits are globose, measuring 1 to 3 cm in diameter and weighing in average 3 to 5 g, drupe-type, with thin and smooth skin, red in color turning to wine color when ripe, juicy yellow pulp, with a sour flavor. The acerola tree produces about 3 to 4 harvests a year, with peak production from October to April. The fruits are consumed *in natura*, mainly as juice but also as ice cream, jams, and sweets [1]. Previous studies report that acerola is rich in vitamin C, about 8 times more than orange or lemon. Studies carried out by Canuto [9] detected 378.5 mg/100 g of vitamin C in the acerola pulp. Batista [12] evaluated the vitamin C content in 5 acerola varieties: Sertaneja, Okinawa, Costa Rica, and Flor Branca and found 2075.13, 2337.18, 1454.85, and 1713.28 mg/100 g, respectively. Rufino [13] found 1357.0 mg/100 g of vitamin C, 20.1 ± 4.4 mg/100 g of anthocyanins, and 9.6 ± 1.4 mg/100 g of yellow flavonoids. Several studies also report that acerola contains vitamins A and B, calcium, iron, and phosphorus, in addition to anthocyanins and flavonoids [14, 15].

2.3 Açai (*Euterpe oleraceae* Mart)

The açaí is a 3 to 20 m-high palm tree that forms clumps with a stem measuring in average 7 to 18 cm in diameter. It belongs to the Arecaceae family and is native to the Brazilian states of Pará, Tocantins, Maranhão, and Amapá. It occurs in humid or dry floodplain forests. It is widely cultivated in all tropical regions of Brazil. Its smooth, globose fruits, measuring 1.2 to 1.3 cm in diameter, are blackish-purple in color when ripe and have thin, flavorless pulp [1, 16]. Its fruiting occurs throughout the year, most frequently from July to December, and is consumed in the Amazon mainly *in natura*, in the form of a juice called "açaí wine" with tapioca flour. In other regions of Brazil, açaí is consumed as a frozen pulp blended with guarana syrup and added fruits and cereals. Açaí is considered an energy food, with a higher caloric value than milk (285.06 kcal/100 g) [17]. Studies reveal that açaí is rich in anthocyanins, mainly cyanidin-3-glucoside and cyanidin-3-rutinoside; minerals like potassium, calcium, magnesium, phosphorus, and iron; in addition to being a source of lipids, proteins, and carbohydrates [17–21]. In addition to the use of fruits, industrialized hearts of palm are also widely used as food named palmito.

2.4 Araça-boi (*Eugenia stipitata* McVaugh)

The araça-boi is a 3.5 m-tall shrub from the Myrtaceae family. It is native to the Amazon and found in dry land forests. Its large, globose fruits weigh 30 to 300 g, are yellow in color, and have a thin, velvety skin, with a juicy and aromatic pulp with a high acidic flavor, containing 4 to 10 seeds measuring 1 cm in length. It ripens from December to May and is consumed mainly in the form of juices, ice creams, creams, and jellies [1]. Studies report that araça-boi is rich in phenolic acids such as chlorogenic acid, caffeic acid, and gallic acid; flavonoids such as quercetin and kaempferol; as well as vitamin C; cinnamic acid; and minerals potassium, sodium, calcium, and magnesium; in addition to proteins; lipids; and fibers [9, 22–25].

2.5 Araticum (*Annona crassiflora* Mart)

The araticum is a 4 to 8 m-high tree with a trunk measuring 20 to 30 cm in diameter from the Annonaceae family. It is native to savannah from the Brazilian states of Bahia, Mato Grosso, Mato Grosso do Sul, and Tocantins up to São Paulo. The fruits are syncarpous, with a papillose surface, weighing up to 4 kg, with fibrous, juicy pulp and with a strong aroma and a sweet and very pleasant flavor. They ripen from January to March and are consumed *in natura*, or in the form of juices, ice cream, jellies, sweets, and liqueurs, in addition to being used to prepare typical desserts [1, 5]. Cardoso [26] reports that araticum fruits contain vitamins C and E (5.23 mg 100/g and 494.04 μg/100 g, respectively), in addition to α and β-carotene. Schiassi [27] detected high content of phenolic compounds (728.17 mg GAEs/100 g), minerals like potassium (378.26 mg/100 g) and magnesium (31.78 mg/100 g), as well as fiber and carbohydrates.

2.6 Bacaba (*Oenocarpus bacaba* Mart)

The bacaba is a 7 to 20 m-high solitary palm tree, with a trunk measuring 15 to 25 cm in diameter, from the Arecaceae family. It is native to the Brazilian states of Acre, Amazonas, and Pará, occurring in dryland forests. Its fruits are globose, smooth, measuring 1.3 to 1.5 cm in diameter, dark purple in color when ripe, with a thin skin and fleshy and yellowish-white pulp. Its fruiting occurs in the months of July to November and is consumed mainly *in natura* in the form of bacaba wine, a brown liquid, similar to açaí wine but oilier [1, 16]. Bacaba wine is also quite nutritious and energetic. Studies report that bacaba has high levels of unsaturated fatty acids such as oleic and linoleic. A greenish oil with a pleasant taste and odorless is extracted from the almond, similar to olive oil.

2.7 Bacuri (*Platonia insignis* Mart)

The bacuri is a 15 to 30 m-high tree, with a trunk measuring 50 to 80 cm in diameter, from the Clusiaceae family. It is native to the Amazon Rainforest, which is periodically flooded, and is common in the Lower Amazon and on Marajó Island, in the Brazilian state of Pará. The fruits are large, ovoid, smooth and yellowish, with a thick, berry-like skin, with 2 to 5 seeds inside, and the pulp is thin, white, and aromatic and has a sweet-sour flavor. It ripens in the summer and is consumed both *in natura* and in the form of ice cream, juices, and sweets [1]. According to Teixeira

[28], the pulp of the bacuri is rich in potassium, phosphorus, and calcium and has a reasonable iron content.

2.8 Buriti (*Mauritia flexuosa* L. f.)

The buriti is a 4 to 25 m-high solitary palm tree, with a trunk measuring 23 to 80 cm in diameter, from the Arecaceae family. It is native to northern South America, found throughout the Amazon, as well as in the Brazilian states of Maranhão, Piauí, Ceará, Goiás, Minas Gerais, and São Paulo. It is found in humid floodplain forests, near rivers and streams, and in periodically flooded areas. Its fruits are globose or short-ellipsoid, measuring 4 to 5 cm in diameter, with a hard skin formed by small reddish-brown scales when ripe, like dragon eggs, with thin, fleshy orange pulp. Its fruiting occurs throughout the year but depends on the region. In the Amazon, its fruits ripen from March to August and are consumed mainly *in natura* in the form of "buriti wine" with sugar and cassava flour. However, they are also consumed in the form of popsicles, ice cream, and sweets [1, 5, 16, 29]. Buriti pulp is rich in carotenoids, mainly β-carotene (9098 μg/100 g) and α-carotene (1008 μg/100 g), precursors of vitamin A. It also contains vitamins B, C, and E, as well as minerals like calcium, iron, and fatty acids. It is one of the fruits that has a higher concentration of pro-vitamin A, about 20 times more than carrots [29]. It has an edible oil with a pleasant flavor and aroma, extracted from the pulp, with high pro-vitamin A potential, with applications in the food industry as a natural colorant for margarines, cheeses, and some pasta.

2.9 Cajá (*Spondias mombin* L.)

The cajá, or taperebá as it is known in the Brazilian North, is a fruit tree with a 18 to 25 m height, with a trunk measuring 40 to 60 cm in diameter, from the Anacardiaceae family. It is native to the Amazon region and Atlantic Forest from the Brazilian states of Ceará to Rio de Janeiro. Is has globose or elliptical drupe-type fruits measuring 3 to 5 cm in length and 2 to 4 cm in diameter, with a thin, smooth skin, juicy, fibrous pulp of yellow-orange color with a large seed inside, with a sweet-sour flavor and a very pleasant aroma. Its ripening depends on the region; in the Northeast, it occurs from January to June, and in the North from August to December [1]. The fruits are consumed *in natura* or in the form of juices, ice cream, popsicles, jams, sweets, and liqueurs. Studies indicate that cajá fruits are rich in vitamin C and carotenes, phosphorus, iron, calcium, and fiber [9, 30, 31].

2.10 Camu-camu (*Myrciaria dubia* (Kunth) McVaugh)

The camu-camu is a 2 to 4 m-high shrub or small tree from the Myrtaceae family. It is native to the northwestern Amazon, found in flooded areas with black water. Its globose fruits measure 2 to 3 cm in diameter, with a thin, glossy skin that is red to purple-black in color, weighing about 10 g, and has a very juicy, yellow-orange pulp that is very acidic and very rich in vitamin C. It ripens from December to January and is consumed in the form of juices, jellies, ice creams, and liqueurs [1]. Several studies reveal that camu-camu is one of the fruits with the highest levels of vitamin C in the world, which is twice that of acerola, in addition to being a source of minerals like potassium, calcium, magnesium, and iron, as well as phenolic compounds and carotenoids [32, 33]. Chirinos [33] found in ripe camu-camu fruits (2010.01 mg/100 g)

of vitamin C and (2280.00 mg/100 g) in unripe fruits. These authors show that the vitamin C content depends on the stage of fruit ripeness. Rufino [13] detected in fresh camu-camu fruits 1882.0 ± 43.2 mg/100 g of vitamin C, 42.2 ± 17.0 mg/100 g of anthocyanins, and 20.1 ± 4.4 mg/100 g of yellow flavonoids.

2.11 Cashew (*Anacardium occidentale* L.)

The cashew is a 2 to 10 m-high tree from the Anacardiaceae family. It is native to the fields and dunes of the Brazilian northern coast, and it is very common in the North and Northeast regions. The fruits are nuts, but the thickened peduncle (pseudofruit), which is considered the fruit, is very juicy, with a sweet-sour and astringent flavor. It ripens from August to November and is consumed *in natura* or in the form of juices, industrialized sweets, and liqueurs [1, 5]. Cashews are rich in vitamin C and have reasonable amounts of vitamin A and vitamins B, as well as proteins, lipids, carbohydrates, fiber, and minerals like calcium, phosphorus, iron, and magnesium [5].

2.12 Ciriguela (*Spondias purpurea* L.)

The ciriguela or seriguela is a fruit of a 4 to 7 m-high tree, from the Anacardiaceae family. It is native to tropical forests of Central America, been very common in the Brazilian northeast region. Its fruits are ellipsoidal, of the drupe type, with a thin and smooth skin and its color varying from yellow to red, with thin, yellow, and juicy pulp with a large seed surrounded, with a sweet-sour and tasty flavor. Its ripening occurs in the months of December to March, with peak production in December and January, and it is consumed *in natura* or in the form of juices, jams, ice creams, and liqueurs [1]. The ciriguela is rich in vitamin C, minerals, and fiber [34, 35].

2.13 Cupuaçu (*Theobroma grandiflora* (Willd. Ex Spreng.) K. Scum.)

The cupuaçu is a 4–8 m-high tree from the Malvaceae family. It is native to the dry land forests of the Amazon region, found mainly in the Brazilian states of Amazonas, Amapá, and Pará. Its large fruits, weighing up to 2 kg and measuring up to 25 cm in length, are berry-like, with a thick, hard brown skin, juicy-fibrous pulp, very aromatic, with an acidic flavor and containing many large seeds around it. It ripens from February to April, and its pulp is consumed *in natura* in the form of juices or processed into popsicles, ice cream, sweets, and liqueurs [1]. Studies carried out by Canuto [9], Silva [36], and Onias [37] indicate that cupuaçu fruits are rich in phenolic compounds, vitamin C, potassium, sodium, as well as carbohydrates and proteins.

2.14 Guava (*Psidium guajava* L.)

The guava is a 3 to 6 m-high small tree, from the Myrtaceae family. Its origin is unknown, and it is found in all tropical regions of the planet. The fruits are globose, ovoid, or pyriform, 4 to 10 cm in diameter, of the gaba type, with a thick yellow or light green skin when ripe, depending on the cultivar, weighing from 100 to 480 g, juicy and fleshy pulp, white, yellow, red, or pink in color, depending on the cultivar, with a sweet-sour flavor. Its fruiting occurs all year round, but with peak production in the months of January to March, and they are consumed *in natura* or in the form of juices, sweets, jellies, ice creams, popsicles, and preserves [1]. Several studies report

that guava contains vitamins A, C, and B complex; minerals like calcium, phosphorus, and potassium; in addition to carbohydrates and proteins [12, 38, 39].

2.15 Soursop (*Annona muricata* L.)

The soursop is a 4 to 6 m-high tree, with a smooth trunk measuring 25 to 35 cm in diameter, from the Annonaceae family. It is native to the Antilles but widely cultivated in almost all tropical regions of Brazil, the Americas, and the Caribbean. The fruits are composed of the syncarpous type, weighing up to 5 kg and reaching up to 30 cm in length, with a green and spiny skin, white, mucous-succulent and fibrous pulp, with an acidic and aromatic flavor, and containing many black seeds. It ripens from May to October and is consumed *in natura* in the form of juices or processed in the form of ice cream, sweets, jellies, and yogurts [1]. Analysis of the chemical composition of soursop pulp carried out by Nunes [40] showed the presence of vitamins A, C, and the B complex, in addition to flavonoids, mainly quercetin and rutin. Studies carried out by Sacramento [41] found 37.25 mg/100 g of vitamin C. According to Almeida [34, 35], soursop is rich in minerals like potassium, magnesium, phosphorus, zinc, iron, and calcium, as well as phenolic compounds and vitamin C.

2.16 Jaboticaba (*Plinia cauliflora* (DC.) Kausel)

The jaboticaba is a 3 to 6 m-high tree from the Myrtaceae family. It is native to the Atlantic Forest and can be found in Brazilian states from Bahia to São Paulo. Its subglobose fruits measure 2 to 3 cm in diameter, with smooth, black skin, juicy white pulp containing a seed, and are generally sweet in flavor. They ripen in the months of January and February and from August to September and are mainly consumed *in natura* or transformed into jams, sweets, fermented beverages, spirits, and liqueurs [1]. Studies indicate that jaboticaba fruits contain high levels of anthocyanins and flavonoids (367 to 1420 mg/100 g and 196 to 571 mg/100 g, respectively), compounds with antioxidant activity capable of fighting free radicals [42, 43].

2.17 Jenipapo (*Genipa americana* L.)

The jenipapo is an 8 to 14 m-high tree, from the Rubiaceae family. It is native to America. In Brazil, it is found in humid floodplain forests or near rivers. It has globose fruits, of the berry type, measuring 6 to 10 cm in length and 4 to 7 cm in diameter, weighing 90 to 180 g, greenish-brown in color, with fleshy brownish pulp containing many seeds, aromatic, and with a sour flavor. It ripens in the months of November and December and is consumed *in natura* or in the form of sweets and liqueurs [1, 5]. Chaves [44], Costa [45], and Pacheco [46] detected vitamin C, phenolic compounds, carotenoids, tannins, as well as minerals like iron and calcium in the pulp of ripe jenipapo.

2.18 Mango (*Mangifera indica* L.)

The mango is an 8 to 18 m-high tree from the Anacardiaceae family. It is native to India and Myanmar, where there are over 100 varieties. However, it was introduced to Brazil in the sixteenth century by the Portuguese, where it is cultivated in all regions, especially in the Northeast. The fruits are drupe-type, weighing 70 to 1200 g, with a smooth, yellowish, greenish, or pinkish skin, depending on the cultivar, and a large

seed inside. The pulp is juicy, in some cases fibrous, and yellow or orange, depending on the cultivar, with a sweet-sour flavor. It ripens between November and February and is consumed mainly *in natura* or in the form of juices, ice cream, jellies, and sweets [1]. Studies indicate that mango contains vitamins A, C, and B complex; minerals like calcium, magnesium, phosphorus, and potassium; as well as carbohydrates and fiber [47, 48].

2.19 Mangaba (*Hancornia speciosa* Gomes)

The mangaba is a 3 to 5 m-high small tree, from the Apocynaceae family. It is native to the coastal sandbanks and plateaus from the north of the Brazilian state of Espirito Santo to Pará and quite common in the northeastern Brazilian states. Its fruits are berry-like, ellipsoidal or rounded, measuring 2.5 to 6 cm in diameter, with yellowish skins with red dots, and a yellow, fleshy-viscous pulp with a sweet-acidic flavor and latex containing 2 to 30 seeds. It ripens from October to March and is consumed *in natura* or in the form of juices, ice cream, sweets, and liqueurs [1, 5]. Studies carried out by Almeida [34] and Ref. [35] indicate that mangaba is mainly a source of potassium and magnesium (240.42 and 70.27 mg/100 g), respectively, as well as sodium, calcium, and phosphorus, in addition to phenolic compounds (98.80 mg/100 g) and vitamin C (93.30 mg/100 g).

2.20 Murici (*Byrsonima crassifolia*)

The murici is a 2 to 6 m-high small tree, from the Malpighiaceae family. It is native to the North, Northeast, and the Brazilian states of Goiás, Mato, Grosso, Mato Grosso do Sul, Minas Gerais, and São Paulo, occurring in savannah fields and capoeiras in sandy soils. It has globose-depressed fruits, measuring 1.5 to 2 cm in diameter, with smooth, yellow, drupe-type skin, with yellowish fleshy pulp with a very characteristic flavor and aroma, containing a surrounding seed. It ripens in the months of April and May and is consumed *in natura* or in the form of juices, ice cream, sweets, and liqueurs [1, 5, 49]. Murici is a source of vitamin C and minerals such as phosphorus, potassium, calcium, magnesium, and sodium, as well as lipids, fibers, proteins, and carbohydrates [34, 35, 49–52].

2.21 Pequi (*Caryocar brasiliense* Aamb)

The pequi tree is a 6 to 8 m-high tree from the Caryocaraceae family. It is native to the Brazilian savannah, found in almost all states of the Central-West region; in the northeast region in the states of Piauí, Maranhão and Ceará; and in the southeast region in the north of Minas Gerais. The fruits are drupe-type, measuring 6 to 20 cm in diameter, weighing 100 to 300 g, with a yellowish-green skin when ripe, a thick, fleshy yellowish pulp, and a hard, spiny stone around it. It ripens from October to March and is consumed cooked or in the preparation of typical dishes such as rice with pequi, chicken with pequi, and guariroba with pequi [1, 5]. An oil is extracted from the pulp of the pequi, which is widely used for frying and as a condiment. According to Araújo [50] and Cordeiro [53], pequi pulp is rich in monounsaturated fatty acids (about 50%), mainly oleic; phenolic compounds; carotenoids; minerals like phosphorus, potassium, calcium, and magnesium; as well as lipids, proteins, carbohydrates, and fibers.

2.22 Pitanga (*Eugenia uniflora* L.)

The Surinam cherry is a of 4 to 9 m-high small tree, from the Myrtaceae family. It is native to the Atlantic Forest, in semideciduous forests of the plateau and the Brazilian states of Paraná basin from Minas Gerais to Rio Grande do Sul and in the sandbanks along the entire Brazilian coast. Its fruits are globose-flat, measuring 1 to 3 cm in diameter, with eight deep grooves, thin skin that is red to purplish when ripe, depending on the variety, and juicy pulp with a sweet-acidic flavor. It ripens from October to January and is consumed *in natura* or in the form of juices, ice cream, jellies, sweets, and liqueurs [1, 5]. Studies carried out by Pereira [54] found in the fruits of red and purple pitanga varieties the presence of anthocyanins and flavonoids (2.14 mg/100 mL, 14.46 mg/100 mL, red variety and 34.90 mg/100 mL, 4.48 mg/100 mL, purple variety, respectively). Araújo [55] detected the presence of anthocyanins (delphinidin-3-hexoside, cyanidin-3-hexoside) and flavonoids (myricetin-galloyl-hexoside, myricetin-hexoside, myricetinpentoside, quercetin galloyl hexoside and myricetin-rhamnoside), carotenoids, and vitamins A, C, and B complex, in addition to minerals like potassium, calcium, magnesium, and phosphorus. Bagetti [56] studied the pulps with peel of purple pitanga and red pitanga. The red pitanga was studied in two stages of ripening (orange and red), and it was observed that purple pitanga is rich in anthocyanins (136.0 mg/100 g), while red pitanga has a high content of carotenoids, mainly β-Cryptoxanthin (34.0 μg/g) and β-Carotene (5.1 μg/g) in the orange color and Lycopene (166.0 μg/g) in the red color.

2.23 Tucumã (*Astrocaryum aculeatum* G. Mey.)

The tucumã is a solitary 8 to 20 m-high palm tree, measuring between 30 to 50 cm in diameter, with nodes covered in black spines measuring 10 to 15 cm in length, from the Arecaceae family. It is native to the Amazon Rainforest, occurring on dry land in the Brazilian states of Amazonas, Acre, Pará, Rondônia, and Mato Grosso. Its fruits vary between globose, subglobose, and ovoid, measuring 3 to 5 cm in length and 2 to 5 cm in diameter, with a thin, hard skin, yellow-orange pulp with a large seed, and a slightly sweet flavor, very rich in pro-vitamin A and high in calories. It bears fruit all year round, but peak production occurs from January to June [1, 16]. The pulp is consumed *in natura* or in sandwiches known as x-caboquinho in Manaus and in tapioca beiju or processed into popsicles, ice cream, and sweets. Several studies show that tucumã pulp is rich in carotenes, mainly β-carotene, and unsaturated fatty acids such as oleic and linolenic; phenolic compounds such as caffeic acid, ellagic acid, and gallic acid, rutin, catechin and quercetin; minerals like calcium, potassium, iron, and zinc; as well as proteins, lipids, and carbohydrates [22, 57–60].

2.24 Umbu (*Spondias tuberosa* Arruda)

The umbu is a 4 to 7 m-high tree with a low, dense crown and a short trunk, from the Anacardiacea family. It is native to the semiarid region of Northeastern Brazil, in caatinga vegetation, from Ceará to northern Minas Gerais. Its fruits are globose or ovoid, measuring 3 to 4 cm in diameter, with juicy, fibreless pulp and a pleasant, sweet-sour flavor. It ripens in January and February and can be consumed *in natura*, in the form of juices, popsicles, ice cream, sweets, and jellies [1]. According to Almeida [34] and Ref. [35], umbu is a source of minerals like potassium, calcium, phosphorus, and magnesium, in addition to phenolic compounds and vitamin C.

3. Antioxidant activities of these fruits reported in the literature in recent years using different methods

Batista [12] evaluated the antioxidant activity of five mango varieties, four acerola varieties, and three guava varieties using the ABTS method. Among the three fruits studied, acerola presented the highest antioxidant activity, with emphasis on the Okinawa cultivar; followed by guava, with emphasis on the Pedro Sato cultivar; and finally mango, where the Palmer cultivar presented the highest antioxidant activity, as shown in (**Table 1**). All these varieties also presented the highest values of ascorbic acid as well as total phenolic contents. The antioxidant activity of different acerola varieties in decreasing order was: Okinawa > Flor Branca > Sertaneja > Costa Rica. For guava, it was: Pedro Sato > Paluma > Rica, while for mango, it was: Palmer > Rosa > Haden > Espada > Tommy.

Canuto [9] analyzed the antioxidant activity of 12 fruits using the ABTS method and found that acerola and açaí pulps presented the highest values (12.1 and 10.0 μmol Trolox/L, respectively), as shown in **Table 2**, indicating a probable relationship between the levels of total phenols and the antioxidant potential of the fruit. Meanwhile, abiu, bacuri, and cupuaçu pulps presented the lowest antioxidant activity. The antioxidant activity of these fruits in decreasing order was: Acerola > Açai >Buriti > Bacaba > Araça-boi > soursop > Cajá > Cashew = Murici > Abiu > Bacuri = Cupuaçu.

Rufino and co-workers [13] analyzed the antioxidant activity of ten fruits using the ABTS, DPPH, and FRAP methods (**Table 3**). They found that camu-camu, acerola, jaboticaba, and açaí were the fruits that presented the highest activities by the ABTS and FRAP methods; camu-camu, acerola, jaboticaba, and mangaba presented the highest activities by the DPPH method. Based on the results, it can be concluded that camu-camu and acerola are the fruits with the highest antioxidant capacities in all methods, indicating an association with the high content of vitamin C and the content of phenolic compounds since these fruits presented the highest levels of these constituents. The antioxidant activity of these fruits in decreasing order by the ABTS method was: Camu-camu > Acerola > Jaboticaba > Açai > Mangaba > Cashew > Cajá >

Fruits	Varieties	AA (mg/100 g)	Total phenolics (mg GAE/100 g)	ABTS (μM Trolox/g)
Acerola	Sertaneja	2075.13 ± 9.95	1101 ± 10.75	115.82 ± 4.7
	Okinawa	2337.18 ± 82.73	1345.21 ± 5.24	144.8 ± 8.7
	Costa Rica	1454.85 ± 39.16	850.3 ± 13.4	78.3 ± 0.77
	Flor Branca	1713.28 ± 136.18	949.25 ± 11.0	122.72 ± 4.8
Guava	Paluma	78.80 ± 4.32	120.21 ± 0.76	13.3 ± 0.98
	Rica	107.40 ± 7.98	108.05 ± 6.01	8.47 ± 0.31
	Pedro Sato	81.08 ± 6.81	149.97 ± 8.20	15.31 ± 0.81
Mango	Tommy	31.97 ± 3.71	17.26 ± 2.19	1.0 ± 0.07
	Haden	29.71 ± 4.58	23.45 ± 0.52	2.0 ± 0.12
	Palmer	51.39 ± 6.84	36.04 ± 3.43	3.0 ± 0.18
	Espada	34.27 ± 2.63	30.32 ± 3.08	1.9 ± 0.06
	Rosa	34.26 ± 2.63	32.17 ± 2.14	2.7 ± 0.10

[1]*Mean value ± standard deviation; n = 3.*
AA: Ascorbic acid. ABTS: 2,2'-azino-bis(3-ethylbenzthaazoline-6-sulphonic acid). GAE: Gallic acid equivalent.

Table 1.
Total phenolics, Ascorbic acid content and antioxidant capacity of different varieties of fruits in fresh mass[1].

Fruits	AA (mg/100 g)	Total phenolics (mg GAE/100 g)	ABTS (μM Trolox/L)
Abiu	2.0 ± 0.0	0.4 ± 0.0	0.8 ± 0.1
Acerola	378.5 ± 0.0	3.5 ± 0.2	12.1 ± 0.0
Açaí	10.1 ± 0.0	2.4 ± 0.2	10.0 ± 0.3
Araça-boi	0.2 ± 0.0	0.6 ± 0.0	3.0 ± 0.1
Bacaba	0.9 ± 0.2	0.3 ± 0.1	3.1 ± 2.1
Bacuri	0.2 ± 0.0	0.4 ± 0.0	0.6 ± 0.3
Buriti	0.1 ± 0.0	1.0 ± 0.0	5.4 ± 0.2
Cajá	0.3 ± 0.0	0.6 ± 0.0	1.8 ± 0.4
Cashew	12.4 ± 0.0	0.6 ± 0.0	1.5 ± 0.2
Cupuaçu	3.3 ± 0.0	0.4 ± 0.0	0.6 ± 0.2
Murici	0.3 ± 0.1	0.6 ± 0.0	1.5 ± 1.5
Soursop	0.1 ± 0.0	0.6 ± 0.0	2.2 ± 0.1

[1]*Mean value ± standard deviation; n = 2.*
AA: Ascorbic acid. ABTS: 2,2′-azino-bis(3-ethylbenzthaazoline-6-sulphonic acid); DPPH: 1,1-diphenyl-2-picryhydrazyl.

Table 2.
Total phenolics, Ascorbic acid content and antioxidant capacity of frozen fruits pulp [1].

Fruits	Total phenolics (mg GAE/100 g)	ABTS (μM Trolox/g)	DPPH IC50 (g/g DPPH)	FRAP (μM Fe_2SO_4 /g)
Açai	454 ± 44.6	15.1 ± 4.1	4264 ± 1381	32.1 ± 6.5
Acerola	1063 ± 53.1	96.6 ± 6.1	670 ± 64.5	148 ± 16
Bacuri	23.8 ± 0.7	n.d	n.d	n.d
Caja	72.0 ± 4.4	7.8 ± 0.2	9397 ± 64.8	11.8 ± 0.2
Cashew	118 ± 3.7	11.2±0.04	7142 ± 205	22.9 ± 0.7
Camu-camu	1176 ± 3.7	153 ± 2.6	478 ± 1.2	279 ± 1.5
Jaboticaba	440 ± 9.9	37.5 ± 1.4	1472 ± 16.9	87.9 ± 1.9
Mangaba	169 ± 21.5	14.6 ± 1.8	3385 ± 34.9	18.3 ± 1.6
Murici	n.d	n.d	n.d	n.d
Umbu	90.4 ± 2.2	6.3 ± 0.2	7074 ± 218	17.2 ± 0.3

[1]*Mean value ± standard deviation; n = 3; n.d = not detected.*
IC50: Concentration of antioxidant required to reduce the original amount of free radicals by 50%. ABTS: 2,2′-azino-bis(3-ethylbenzthaazoline-6-sulphonic acid); DPPH: 1,1-diphenyl-2-picryhydrazyl.

Table 3.
Total phenolics and antioxidant capacity of different fruits in fresh mass [1].

Umbu. While by using the FRAP method, it was: Camu-camu > Acerola > Jaboticaba > Açai > Cashew > Mangaba > Umbu > Cajá. For the DPPH method, it was: Camu-camu > Acerola > Jaboticaba > Mangaba > Açai > Umbu > Cashew > Cajá.

Almeida [35] evaluated the antioxidant capacity of ciriguela, mangaba, murici, soursop, and umbu using the ABTS and DPPH methods and found that mangaba and murici were the fruits that presented the best results in both methods (**Table 4**).

Fruits	AA (mg/100 g)	Total phenolics (mg GAE/100 g)	ABTS (μM Trolox/g)	DPPH (μM Trolox/g)
Ciriguela	29.6 ± 0.9	55.0 ± 2.1	6.25 ± 0.04	1.5 ± 0.24
Mangaba	96.3 ± 1.7	98.8 ± 5.6	10.84 ± 0.13	5.27 ± 0.34
Murici	11.8 ± 0.0	159.9 ± 5.6	15.73 ± 0.01	6.46± 0.31
Soursop	3.3 ± 0.9	54.8 ± 2.7	6.09 ± 0.13	1.36 ± 0.01
Umbu	12.1 ± 0.4	44.6 ± 2.7	1.07 ± 0.00	0.73 ± 0.16

[1]Mean value ± standard deviation; n = 3.
AA: Ascorbic acid. ABTS: 2,2'-azino-bis(3-ethylbenzthaazoline-6-sulphonic acid); DPPH: 1,1-diphenyl-2-picryhydrazyl; FRAP: ferric reducing antioxidant power.

Table 4.
Total phenolics, Ascorbic acid content and antioxidant capacity of different fruits in fresh mass[1].

These fruits also presented the highest levels of phenolic compounds. The antioxidant activity of these fruits in decreasing order in both ABTS and DPPH methods was: Murici > Mangaba > Ciriguela > Soursop > Umbu.

Bagetti [56] evaluated the antioxidant capacity of the pulp of purple and red pitanga using DPPH and FRAP methods and found that purple pitanga presented the highest antioxidant activity in both methods, as well as the highest content of total phenolics and anthocyanins as shown in **Table 5**, while for the red pitanga, there was no significant difference in antioxidant activity at the two stages of ripening.

Chaves [44] and Pacheco [46] analyzed the antioxidant activity by the DPPH method and the total content of phenolics and ascorbic acid in the pulp of ripe jenipapo. The antioxidant activity obtained was proportional of the phenolic content, with Pacheco [46] obtaining the best results (**Table 6**).

Roesler [61] evaluated the antioxidant capacity of pequi fruits (peel and seed) and araticum using the DPPH method. Pequi presented the highest antioxidant activity

Samples	Anthocyanin (mg/100 g)	Total phenolics (mg GAE/100 g)	FRAP (mM Trolox/100 g)	DPPH (mM Trolox/100 g)
Purple	136 ± 6	463 ± 16	3.1 ± 0.6	3.1 ± 0.7
Red	69 ± 3	210 ± 3	1.4 ± 0.3	1.4 ± 0.1
Orange	25 ± 1	179 ± 5	1.1 ± 0.1	1.4± 0.0

[1]Mean value ± standard deviation; n = 3.
DPPH: 1,1-diphenyl-2-picryhydrazyl; FRAP: ferric reducing antioxidant power.

Table 5.
Total phenolics, Anthocyanin content and antioxidant capacity of purple and red pitanga fruits[1].

Ascorbic acid (mg/100 g)	Total phenolics (mg GAE/100 g)	DPPH (% O.I)	Reference
33.33 ± 0.05	141.7 ± 0.57	29.11 ± 3.21	Chaves [44]
22.5 ± 6.93	176.3 ± 3.84	70.2 ± 1.27	Pacheco [46]

[1]Mean value ± standard deviation; n = 3.
% O.I: Oxidation inhibition.

Table 6.
Total phenolics, Ascorbic acid content and antioxidant capacity of pulp of ripe jenipapo fruits[1].

and also the highest content of phenolic compounds with IC50 298.75 μg/mL and 27.19 mg GAE/Kg of total phenolics.

Santos Filho [62] evaluated the antioxidant activity of bacaba pulp using three different methods: ABTS, DPPH, and FRAP and found that bacaba presented good antioxidant capacity in all methods evaluated, with values 85.31 μM Trolox/g in ABTS method; 61.67 μmol Fe_2SO_4/g in the FRAP method and 226.4 g/g DPPH in the DPPH method.

Khairiyah [39] evaluated the antioxidant activity of fresh fruit juice and commercial fruit juice (plastic packaging) of red guava using ABTS methods and found that both juices presented good antioxidant activity with IC50 values 39.87 and 19.00 μg/mL, respectively.

Vieira [63] evaluated the antioxidant capacity of fresh and stored pulp for 15 days at −18°C of tucumã fruits using the free radical scavenging method (DPPH) and found that there was a loss in antioxidant activity in the frozen pulp after 15 days of storage with an IC50 value of 320.40 μg/mL, while in fresh pulp, the IC50 value was 228.90 μg/mL.

Barreiros and co-workers [64] evaluated the antioxidant capacity of eight fruits (abiu, biriba, cajá, cajá-umbu, ciriguela, cubiu, soursop, and mangaba) using the DPPH methods and found that soursop, mangaba, and ciriguela were the fruits that presented the highest activities with IC_{50} values of 1.77, 2.00, and 2.35 mg/mL, respectively, as well showed the most significant results of total phenolics 150.08, 144.00, and 123.84 mg/100 g. The antioxidant activity of the fruits in decreasing order was: Soursop > Mangaba > Siriguela > Cajá-umbu > Umbu > Cubiu > Abiu > Biribá.

4. Final considerations

The large majority of authors correlates antioxidant capacity to the total phenolic content. However, according to the results observed, the capacity of scavenging free radicals is not directly correlated with the concentration of total phenolics. Phenolic compounds are important contributors to antioxidant activity, but their chemical composition is diverse, and their structure is an important factor in their effectiveness as antioxidants. Substances such as vitamins C and E, in addition to carotenoids, also have antioxidant activity and should be taken into consideration. On the other hand, antioxidant capacity depends on the method used, since each method has a different approach, which often makes comparison of results unfeasible. Some antioxidants can donate hydrogen radical and scavenging free radicals, as exemplified in the DPPH and ABTs method. Others can be oxidized first, protecting the molecules from being oxidized, as exemplified in the β-carotene method. We also can measure the oxidation potential as a measure of antioxidant capacity as in the FRAP method. Other methods are based on chelate transition metals to prevent the Fenton reaction, absorption of energy to deactivate singlet oxygen, and modulation membrane fluidity to prevent the entry of free radicals.

Fruits are important sources of natural antioxidants that protect the body against oxidative damage, as they contain good amounts of vitamins C and E, carotenoids and phenolic compounds such as flavonoids, caffeic, ferulic and chlorogenic acids, which are essential for the proper functioning of the body. It is known that eating foods rich in these compounds can bring several health benefits, such as preventing cardiovascular diseases, diabetes, cancer, and Alzheimer's, among others. All fruits

in this work showed antioxidant activity. Among them, camu-camu, acerola, and açaí stood out. It is noteworthy that some of these fruits are eaten only by the people of north and northeast of Brazil, and its consumption should be encouraged in other regions of the country.

Author details

Marizeth Libório Barreiros* and André Luis B.S. Barreiros
Departamento de Química, Universidade Federal de Sergipe, São Cristovão, SE, Brazil

*Address all correspondence to: marizethlbarreiros@hotmail.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

[1] Lorenzi H, De Lacerda TC, Bacher LB. Frutas no Brasil. Nativas e Exóticas de consumo in natura. São Paulo, SP: Instituto Plantarum de Estudos da Flora; 2015

[2] Souza VC, Lorenzi H. Botânica Sistemática: Guia ilustrado para identificação das famílias de Fanerógamas nativas e exóticas no Brasil, baseado em APG II. 3rd ed. Nova Odessa, SP: Instituto Plantarum de Estudos da Flora; 2012

[3] FAO. Fruits and Vegetables – Your Dietary Essentials. The International Year of Fruits and Vegetables. Vol. 2021. Roma: FAO; 2020. DOI: 10.4060/cb2395en

[4] De Souza AGC, Souza NR, Da Silva SEL, Nunes CDM, Canto AC, Cruz LAA. Fruteiras da Amazônia. Brasília, DF: Manaus, AM: Embrapa-SPI, Embrapa-CPAA; 1996

[5] Da Silva DB, Da Silva JA, Junqueira NTV, De Andrade LRM. Frutas do Cerrado. Brasília, DF: Embrapa; 2001

[6] Broinizi PRB, De Andrade-Wartha ERS, Silva AMO, Novoa AJV, Torres RP, Azeredo HMC, et al. Avaliação da atividade antioxidante dos compostos fenólicos naturalmente presentes em subprodutos do pseudofruto de caju (*Anacardium occidentale* L.). Ciência e Tecnologia de Alimentos, Campinas. 2007;**27**(4):902-908

[7] Barreiros ALBS, David JM, David JP. Estresse Oxidativo: Relação Entre Geração De Espécies Reativas E Defesa Do Organismo. Química Nova. 2006;**29**(1):113-123

[8] Halliwell B. The antioxidant paradox. The Lancet. 2000;**355**:1179-1180

[9] Canuto G, Baptista A, Xavier AAO, Neves LC, Benassi MT. Caracterização Físico-Química de Polpas de Frutos da Amazônia e sua Correlação com a Atividade Anti-Radical Livre. Revista Brasileira de Fruticultura. 2010;**32**(4):1196-1205

[10] Marques CS, Da Silva LC, Flores AS, Guimarães PVP, Chagas EA. Caracterização Físico-Química de Frutos de Abiu (Pouteria caimito (Ruiz & Pav.) Radlk.) Coletados Em Quintais Agroflorestais De Roraima. Revista Brasileira de Agroecologia. 2023;**18**(2):42-61

[11] Montero IF, Saravia SAM, Santos RA, Dos SRC, Marcia JAF, Da CHNR. Nutrients in Amazonian fruit pulps with functional and pharmacological interest. African Journal of Pharmacy and Pharmacology. 2020;**14**(5):118-127. DOI: 10.5897/ajpp2020.5136

[12] Batista PF, De Lima MAC, Alves RE, Façanha RV. Bioactive compounds and antioxidant activity in tropical fruits grown in the lower-middle São Francisco Valley. Revista Ciência Agronômica. 2018;**49**(4):616-623. DOI: 10.5935/1806-6690.20180070

[13] Rufino MSM, Alves RE, De Brito ES, Pérez-Jiménez J, Saura-Calixto F, Mancini-Filho J. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chemistry. 2010;**121**:996-1002. DOI: 10.1016/j.foodchem.2010.01.037

[14] Andrade N, De ER, Bonfim WMD, De Sousa RF, Souza FF, de Lima MAC. Características físicas e composição química de frutos de acessos de aceroleira em condições semiáridas. In: XII Jornada

de Iniciação Científica da Embrapa Semiárido, Petrolina, PE. Petrolina, PE, Brazil: Ed. EMBRAPA; 2017

[15] Ritzinger R, Ritzinger CHSP. Acerola. Vol. 32, 264. Belo Horizonte: Informe agropecuário; 2011. pp. 17-25

[16] Miranda IPA, Bueno CR, Barbosa EM, Ribeiro MNS. Frutos de Palmeira da Amazônia. Manaus: AM, MCT/INPA; 2001

[17] Morais RA, Teixeira GL, Ferreira SRS, Cifuentes A, Block JM. Nutritional composition and bioactive compounds of native Brazilian fruits of the *Arecaceae* family and its potential applications for health promotion. Nutrients. 2022;**14**(4009):1-36. DOI: 10.3390/nu14194009

[18] Amorim IS, Amorim DS, Godoy HT, Mariutti LRB, Chist ERC, Pena Bogusz Junior RSS, et al. Amazonian palm tree fruits: From nutritional value to diversity of new food products. Heliyon. 2024;**10**(2):1-17, 24054

[19] Mattietto RA, Carvalho AV, Paracampo NENP, Da Silveira Neta JJ. Chemical composition and seasonal variations in açaí as it is consumed in the Brazilian Amazon. Científica, Dracena. 2024;**52**:1-17. DOI: 10.5016/1984-5529.2024.v52.1434

[20] Menezes EM, Torres AT, Srur AU. Valor nutricional da polpa de açaí (*Euterpe oleracea* Mart) liofilizada. Acta Amazônica. 2008;**38**(2):311-316

[21] Rogez H, Pompeu DR, Akwie SNT, Larondelle Y. Sigmoidal kinetics of anthocyanin accumulation during fruit ripening: A comparison between acai fruits (*Euterpe oleracea*) and other anthocyanin-rich fruits. Journal of Food Composition and Analysis. 2011;**24**:796-800. DOI: 10.1016/j.jfca.2011.03.015

[22] De Araújo FF, Farias DP, Neri-Numa IA, Dias-Audibert FL, Delafiori J, De Souza FG, et al. Chemical characterization of *Eugenia stipitata*: A native fruit from the Amazon rich in nutrients and source of bioactive compounds. Food Research International. 2021;**139**(109904):1-8. DOI: 10.1016/j.foodres.2020.109904

[23] Barros RGC, Andrade JKS, Denadai M, Nunes ML, Narain N. Evaluation of bioactive compounds potential and antioxidante activity in some Brazilian exotic fruit residues. Food Research International. 2017;**102**:84-92. DOI: 10.1016/j.foodres.2017.09.082

[24] Cuellar FA, Ariza E, Anzola C, Restrepo P. Capacidade antioxidante de araca-boi (*Eugenia stipitata* Mc Vaugh) durante a maturação. Revista Colombiana de Quimica. 2013;**42**(2):21-28

[25] Genovese MI, Pinto SGAE, De Souza FM. Bioactive compounds and antioxidant capacity of exotic fruits and commercial frozen pulps from Brazil. Food Science and Technology International. 2008;**14**(3):207-214

[26] Cardoso LM, Oliveira DS, Bedetti SF, Martino HSD, Pinheiro-Sant'ana HM. Araticum (*Annona crassiflora* Mart.) from the Brazilian Cerrado: Chemical composition and bioactive compounds. Fruits. 2013;**68**:121-134

[27] Schiassi MCEV, Souza VR, Lago AMT, Campos LG, Queiroz F. Fruits from the Brazilian Cerrado region: Physico-chemical characterization, bioactive compounds, antioxidant activities, and sensory evaluation. Food Chemistry. 2017;**245**:305-311. DOI: 10.1016/j.foodchem.2017.10.104

[28] de Teixeira GHA. Frutos do bacurizeiro (*Platonia insignis* Mart.):

caracterização, qualidade e conservação [thesis]. Jaboticabal: Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista; 2000

[29] Sampaio MB. Boas práticas de manejo para o extrativismo sustentável do Buriti. Embrapa, Brasília, DF: Instituto Sociedade, População e Natureza; 2012

[30] Carvalho AV, Cavalcante MA, Santana CL, Alves RM. Características físicas, químicas e atividade antioxidante de frutos de matrizes de cajazeira no estado do Pará. Alimentos e Nutrição, Araraquara. 2011;**22**(1):45-53

[31] Mattietto RA, Lopes AS, De Menezes HC. Caracterização física e físico-química dos frutos da cajazeira (*Spondias mombin* L.) e de suas polpas obtidas por dois tipos de extrator. Brazilian Journal of Food Technology, Campinas. 2010;**13**(3):156-164

[32] Akter MS, Oh S, Eun JB, Ahmed M. Nutritional compositions and health promoting phytochemicals of camu-camu (*Myrciaria dubia*) fruit: A review. Food Research International. 2011;**44**(7):1728-1732. DOI: 10.1016/j.foodres.2011.03.045

[33] Chirinos R, Galarza J, Betalleluz-Pallardel I, Pedreschi R, Campos D. Antioxidant compounds and antioxidant capacity of Peruvian camu-camu (*Myrciaria dubia* (H.B.K.) McVaugh) fruit at different maturity stages. Food Chemistry. 2010;**120**:1019-1024

[34] Almeida MMB, De Sousa PHM, Fonseca ML, Magalhães CEC, Lopes MFG, Lemos TLG. Avaliação de macro e microminerais em frutas tropicais cultivadas no nordeste brasileiro. Ciência e Tecnologia de Alimentos, Campinas. 2009;**29**(3):581-586

[35] Almeida MMB, De Sousa PHM, Arriaga ÂMC, Do Prado GM, Magalhães CEC, Maia GA, et al. Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. Food Research International. 2011;**44**:2155-2159. DOI: 10.1016/j.foodres.2011.03.051

[36] Da Silva HM. Caracterização Físico-Química e Informações Nutricionais de Doce em Massa de Cupuaçu [thesis]. Imperatriz: Centro de Ciências Sociais, Saúde e Tecnologia, Universidade Federal do Maranhão; 2014

[37] Onias E, Bezerra RR, Silva AL, Costa F, Chinelate G. Caracterização físico-química de polpa de cupuaçu. In: Caderno Verde De Agroecologia E Desenvolvimento Sustentável. Vol. 1(1). Pombal, PB, Brazil: I Semana Acadêmica de Engenharia de Alimentos de Pombal; 2012

[38] Bogha TT, Sawate AR, Kshirsagar RB, Bochare SS. Studies on physical, chemical and mineral evaluation of guava (*Psidium Guajava L.*). The Pharma Innovation Journal. 2020;**9**(3):117-119

[39] Khairiyah S, Sinaga SM, Putra E. L. Determination of vitamin C and antioxidant activity in fresh red guava (*Psidium Guajava L.*) and red guava commercial fruit juices. International Journal of Science, Technology & Management. 2022;**3**(4):880-883. DOI: 10.46729/ijstm.v3i4.543

[40] Nunes CDR. *Annona muricata* L. Análise química e biológica dos frutos de gravioleira [thesis]. Campos dos Goytacazes, RJ: Centro de Ciências e Tecnologias Agropecuárias, Universidade Estadual do Norte Fluminense Darcy Ribeiro; 2011

[41] Do Sacramento CK, Faria JC, Da Cruz FL, Barretto WS, Gaspar JW,

Leite JBV. Caracterização Física e Química de Frutos de Três Tipos de Gravioleira (*Annona muricata* L.). Revista Brasileira de Fruticultura. 2003;**25**(2):329-331

[42] Danner MA, Citadin I, Sasso SAZ, Scariot S, Benin G. Genetic dissimilarity among jabuticaba trees native to southwestern Paraná, Brazil. Revista Brasileira de Fruticultura. 2011;**33**(2):517-525

[43] Leite-Legatti AV, Batista AG, Dragano NRV, Marques AC, Malta LG, Riccio MF, et al. Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. Food Research International. 2012;**49**:596-603. DOI: 10.1016/j.foodres.2012.07.044

[44] Chaves RM, Kwiatkowski A, Pereira QD, Popolin A, Novaes TAC. Avaliações físico-químicas e capacidade antioxidante em frutos de jenipapo em estádio de desenvolvimento verde e maduro. Revista Agrária Acadêmica. 2019;**2**(2):6-14. DOI: 10.32406/v2n22019/6-14/agrariacad

[45] da Costa JAM. Estudo da Qualidade de Polpas e Sementes do Jenipapo (*Genipa Americana L.*) para Elaboração de Barras de Cereais [thesis]. São Cristovão-SE: Universidade Federal de Sergipe; 2011

[46] Pacheco P, Da Paz JG, Da Silva CO, Pascoal GB. Composição centesimal, compostos bioativos e parâmetros físico-químicos do jenipapo (*Genipa americana* L.) in natura. Demetra. 2014;**9**(4):1041-1054. DOI: 10.12957/demetra.2014.11310

[47] Costa RLR, De Azevedo LC, Da Silva JC, Dos Santos JS. Avaliação da Composição Química de Mangas Desidratadas Produzidas no Submédio Vale do São Francisco. In: XVIII JINCE e XII JID. Reconstruindo o Futuro: Ciência, Tecnologia e Sociedade, Petrolina, PE. Madrid, Spain: Revista Ibero-Americana de Educação; 2023

[48] Penha JS, Bramont WB, Leal IL, Druzian JI, Machado BAS. Caracterização de três cultivares de mangas de diferentes arranjos produtivos da Bahia. In: V Workshop de Pesquisa Tecnologia e Inovação (PTI) e I Simpósio Internacional de Inovação e Tecnologia (SIINTEC), Salvador, BA. Salvador, BA, Brazil: SENAI – CIMATEC; 2015

[49] Garritano G, Jorge CA, Gulias AP, Murici SM. In: Vieira RF, Agostinicosta TS, Silva DB, Sano SM, Ferreira FR, editors. Frutas nativas da Região Centro-Oeste do Brasil. Brasília, DF: Embrapa Informação Tecnológica; 2010

[50] Araujo ACMA, Menezes EGT, Terra AWC, Dias BO, De Oliveira ER, Queiroz F. Bioactive compounds and chemical composition of Brazilian Cerrado fruits' wastes: Pequi almonds, Murici, and sweet passionfruit seeds. Food Science and Technology. 2018;**38**(Suppl. 1):203-214. DOI: 10.1590/fst.19417

[51] Carvalho AV, Paracampo NENP, Mattietto RA, Do Nascimento WMO, Junior RAG, De Resende MDV. Avaliação Nutricional da Polpa de Frutos Provenientes de Clones de Muricizeiro. Belém, Pará: Boletim de Pesquisa e Desenvolvimento, Embrapa Amazônia Oriental; 2020

[52] Pires FCS, Silva APS, Salazara MAR, Da Costa WA, Da Costa HSC, Lopes AS, et al. Determination of process parameters and bioactive properties of the Murici pulp (*Byrsonima crassifolia*) extracts obtained by supercritical extracti. The Journal of Supercritical Fluids. 2019;**146**:128-113. DOI: 10.1016/j.supflu.2019.01.014

[53] Cordeiro MWS, Cavallieri ALF, Ferri PH, Naves MMV. Características físicas, composição químico-nutricional e dos óleos essenciais da polpa de Caryocar brasiliense nativo do estado de Mato Grosso. Revista Brasileira de Fruticultura. 2013;**35**(4):1127-1139

[54] Pereira DM, de Oliveira KÁR, Chantelle L, Sant'ana AMS, Guedes JPS, De Carvalho CT, et al. Characterization of nutritional composition and content of Pitanga pigments (*Eugenia uniflora* L.) in red and purple varieties. Brazilian Journal of Development. 2020;**6**(8):58026-58038. DOI: 10.34117/bjdv6n8-276

[55] Araújo FF, Neri-Numa IA, Farias DP, Cunha GRMC, Pastore GM. Wild Brazilian species of Eugenia genera (Myrtaceae) as an innovation hotspot for food and pharmacological purposes. Food Research International. 2019;**121**:57-72. DOI: 10.1016/j.foodres.2019.03.018

[56] Bagetti M, Facco EMP, Piccolo J, Hirsch GE, Rodriguez-Amaya D, Kobori CN, et al. Physicochemical characterization and antioxidant capacity of Pitanga fruits (*Eugenia uniflora* L.). Ciência e Tecnologia de Alimentos, Campinas. 2011;**31**(1):147-154

[57] Cabral FL, Bernardes VM, Passos DF, De Oliveira JS, Doleski PH, Silveira KL. *Astrocaryum aculeatum* fruit improves inflammation and redox balance in phytohemagglutinin-stimulated macrophages. Journal of Ethnopharmacology. 2020;**30**(247):12274. DOI: 10.1016/j.jep.2019.112274

[58] Matos KAN, Lima DP, Barbosa APP, Marcadante AZ, Chisté RC. Peels of Tucumã (*Astrocaryum vulgare*) and peach palm (Bactris gasipaes) are by-products classified as very high carotenoid sources. Food Chemistry. 2019;**272**:216-221. DOI: 10.1016/j.foodchem.2018.08.053

[59] Pereira E, Ferreira MC, Sampaio KA, Grimaldi R, de Meirelles AJ, Maximo GJ. Physical properties of Amazonian fats and oils and their blends. Food Chemistry. 2019;**278**:208-215. DOI: 10.1016/j.foodchem.2018.11.016

[60] Santos RCV, Sagrillo MR, Ribeiro EE, Cruz IBM. The Tucuma of Amazonas— Astrocaryum aculeatum. In: Exotic Fruits. London, UK: Academic Press; 2018. pp. 419-425. DOI: 10.1016/b978-0-12-803138-4.00056-3

[61] Roesler R, Malta LG, Carrasco LC, Holanda RB, Sousa CAS, Pastore GM. Atividade antioxidante de frutas do cerrado. Ciência e Tecnologia de Alimentos, Campinas. 2007;**27**(1):53-60

[62] Santos Filho AF, Toro MU, Ferreira IC. Estudo Fitoquíco, Enzimático e Capacidade Antioxidante da Polpa da Bacaba (*Oenocarpus bacaba* Mart). Científica Multidisciplinary Journal. 2020;**8**(2):1-17. DOI: 10.29247/2358-260x.2020v7i2.4558

[63] Vieira LM, Azevedo SCM, Da Silva GF, Albuquerque PM. Estudo do Potencial Antioxidante da Polpa do Tucumã (*Astrocaryum aculeatum*) in Natura Armazenada em Embalagens a Vácuo. The Journal of Engineering and Exact Sciences. 2017;**03**(4):0672-0677. DOI: 10.18540/2446941603042017O672

[64] Barreiros ML, Jesus RA, Barreiros ALBS, Sandres TS. Narendra Narain. Evaluation of the antioxidantactivity of eight tropical fruits by DPPH method. Acta Horticulturae. 2018;**1198**:185-192. DOI: 10.17660/ActaHortic.2018.1198.29

Section 3

Temperate Fruit Crops

Chapter 9

Increasing Fruit Set and Yield of Apple Trees Using Plant Growth Regulators

José Luiz Petri and Caroline Esperança

Abstract

One of the main factors for the abscission of flowers/fruits is lack of pollination (fertilization); however, numerous other factors contribute to low effective fruitings, such as environment, genetics, and orchard management. Some cultivars are more likely to have a low fruit set than others, especially when an adverse factor occurs. The spraying of growth regulators in flowering stages can increase the effective fruiting in adverse conditions. A treatment with a low dose of growth regulators can improve fruit set. After bloom, an initial increase in fruit set can be observed after treatment with Thiadizuron (TDZ), Proexadiona calcica (Pca), and Aminoetoxyvinylglycine (AVG). TDZ combined with PCa + AVG increases the fruit set of "Monalisa" apple trees and does not cause alternate bearing, however, it reduces the average fruit mass.

Keywords: *Malus domestica*, climate variability, increase fruit size, growth regulators, fruit set

1. Introduction

The flowering period, which defines the fruit set in the apple tree, is when productivity is also defined. The biological process of flower formation in the apple trees starts in the spring and continues until the next spring when the plants bloom and bear fruit. Therefore, the fruits are developing at the same time as the flowers for the next cycle. In general, apple plants produce many more flowers than necessary; between 5 and 10% of fertilized flowers are enough for satisfactory commercial yield. However, abundant flowering does not mean good productivity since there may be a high percentage of flower or fruit abscission, which occurs from flowering to 4 weeks after flowering. For the setting of the fruits, it is necessary to have enough compatible pollen, an agent to transfer that pollen (mainly bees), the germination of the pollen, the growth of the pollen tube through the style, the fertilization of the ovule, and the formation of the seed.

Also, the relationship between vegetative growth, flowering, and fruit set is one of the most complex processes. The quality of flower buds and the environmental conditions before, during, and after flowering are also extremely important factors. Fruit set can be severely impaired by insufficient chilling during the winter,

resulting in the asynchrony of the flowering period between cultivars [1], by rains and frosts during the flowering period. Consequently, the grower needs to explore all the possibilities that can contribute to a good fruit set, especially in years of adverse pollination conditions or with low flowering intensity [2]. These problems increase in mild winter conditions, mainly due to the great variability in temperatures during the period of effective fruiting. Under these conditions, flowering can occur along with vegetative growth, leading to competition for carbohydrates.

Several theories are formulated to explain the abscission of flowers and small fruits, such as the polar transport of auxins [3] and carbohydrate supplementation [4]. The polar transport of auxins theory suggests that auxins produced by seeds fertilized recently are transported outside the seed and, in the case of the apple tree, along the stem of the fruit. The continuous supplementation of auxin along the peduncle inhibits the activation of genes that induce the formation of the abscission zone, setting the fruit [3]. The carbohydrate supplementation theory of the growing fruit suggests that the growing fruit, after fertilization, requires continuous carbohydrate supplementation, supplied by photosynthetic production [4, 5]. In case of a carbohydrate deficit during this period, flowers/fruits fall. However, many other factors are associated with inducing fruit abscission, such as the auxin and ethylene interaction [6, 7]. In addition, the abscission process occurs together with an increase in abscisic acid [8].

Excessive flowering is a common feature of modern apple production systems. Orchards are planted with rootstocks that control the vigor of the plant in high-density systems, producing from 2000 to 2500 flowers, in addition to producing excessive flowering in the axillary buds, such as the cultivar Gala, making commercial production challenging, as 90% of flowers or fruits should be removed.

Among the environmental factors, the occurrence of negative temperatures during the flowering period favors the abscission of flowers and fruits. During flowering, the optimum temperature is 21–27°C; the temperature can also influence the growth of the pollen tube and consequently the abscission of flowers. Temperature variations can often explain why differences in the abscission of flowers and fruits occur between different places and years. Sudden variations in temperatures can cause stress and increase ethylene production even when temperatures do not reach the freezing level. Rainy periods during flowering that affect bee activity can also lower fruit set. The abscission of flowers and fruits varies between different orchards with different climatic conditions in the same year and also different in the same orchard in different years [9]. Conditions that vary between years and locations include winds, temperature, nutrients, water, insolation, and disease pressure [10].

Some cultivars are more likely to have a low fruit set than others, especially when an adverse factor occurs. Heo et al. [11] classify apple genotypes into three groups: those in which abscission does not occur, those in which abscission occurs after 30 days of flowering, and those in which only the central flower remains in abscission. In southern Brazil, problems of low fruit set are more frequent in "Monalisa" and "Gala" than in "Fuji," in which, under normal conditions, 5 fruits are maintained by inflorescence.

The spraying of growth regulators in flowering stages can increase the effective fruiting in adverse conditions. According to Amarante et al. [12] and Petri et al. [13], in the last decades, several studies have demonstrated the effectiveness of using

plant growth regulators to improve fruit set and fruit growth in temperate fruit trees. Synthetic cytokinins are known to have a remarkable ability to stimulate growth in tissue culture and, more recently, organs in the entire plant system [14]. Petri et al. [15] also observed that the application of Tidiazuron (TDZ) and Aminoetoxyvinylglycine (AVG) increases the fruit set in cultivars Monalisa and Gala.

2. Materials and methods

The experiments were carried out in southern Brazil, municipality of Caçador/SC (latitude 26°82′S, longitude 50°99′W, altitude 960 meters), during the cycle 2016/2017–2019/2020 in the "Monalisa"/M9 and another experiment with cultivars Gala/M9 and Monalisa/M9, in 2018/2019 and 2019/2020. The experimental design used was randomized blocks with five replications, the experimental unit being composed of one plant. The treatments and the application periods are shown in **Tables 1** and **2** and **Figure 1**.

Active ingredient	Products	Concentration	Time of application
1. Control		0.0 mg/L	—
2. Naftaleno acetico acid	ANA TECNIC	15 mg/L	Full bloom (FB)
3. Benziladenina (BA)	Maxcel	4000 mg/L	FB
4. Ethefon	Ethrel 240	1.50 ml/L	FB
5. Thidiazuron (TDZ) + Proexadiona calcica (Pca) + Aminoetoxyvinylglycine (AVG)	Dropp+ViViful+Retain	20 mg/ L + 400 mg/L L + 600 mg/L	FB
6. Giberelic ácid+Benzil adenina (GA + BA)	Promalin	50 mg/L	FB +7 + 7 days
7. ammonium thiosulfate	ATS	1.500 mg/L	FB
8. Thidiazuron (TDZ)	DROPP	40 mg/L	FB
9. Thidiazuron (TDZ)	DROPP	60 mg/L	FB

Table 1.
Treatments, products, concentration, and time of application in Monalisa apple tree.

Active ingredient	Products	Doses	Time of application
1. Control		0.0 mg/L	—
2. Amino toxivinilglicina (AVG)	Retain®[1]	103.7 mg/L	Between the phenological stage F2 and H
3. Amino toxivinilglicina	Retain®[2]	207.5 mg/L	Between the phenological stage F2 and H
4. Amino toxivinilglicina	Retain®[3]	311.2 mg/L	Between the phenological stage F2 and H
5. Amino toxivinilglicina	Retain®	415.0 mg/L	Between the phenological stage F2 and H
6. Amino toxivinilglicina	Retain®	622.5 mg/L	Between the phenological stage F2 and H

Table 2.
Treatments, products, concentration, and time of application in Monalisa and Gala apple trees.

Figure 1.
Phenological stages of apple; 1. Dormant; 2. Silver; 3. Green tip; 4. Half-inch green; 5. Tight cluster; 6. Pink; 7. Bloom; 8. Petal fall; 9. Fruit set.

The following evaluations were carried out:

- Fruit set: the relationship between the number of floral clusters counted during full bloom and the number of fruits, counted on a side branch previously selected on each plant.

- Fruit yield per plant: when the harvest date is reached, the fruits of each plant are harvested, and then the mass of the fruits per plant in kg.plant^{-1} and ha is determined.

- Number of fruits per plant: the fruits harvested per plant were counted.

- Average fruit mass: obtained through the relationship between fruit production per plant and the number of fruits harvested, expressed in g.fruit^{-1}.

The red skin color of fruits, the degree of russeting on fruits, and the classification of fruits by size.

The results obtained were subjected to analysis of variance, and the variables whose results revealed significance at a 5% probability of error had the means compared by the Scott-Knott test at 5% significance. Polynomial regression equations were also adjusted.

3. Results and discussion

The treatments TDZ 10 ppm + PCa 110 ppm + AVG 90 ppm, TDZ 20 ppm, and TDZ 30 ppm increased the fruit set of "Monalisa" apple trees in the 2016/2017 and 2018/2019 cycles compared to the other treatments, and in 2016/2017, the TDZ 30 ppm treatment showed the highest fruit set among all treatments, and in 2018/2019, there were no differences (**Table 3** and **Figure 2**). TDZ is a phenylurea that shows cytokinetic activity and is capable of increasing the fruit set of apple trees, especially when applied in full bloom [16]. In 2017/2018, the treatments TDZ 10 ppm + PCa 110 ppm + AVG 90 ppm, TDZ 30 ppm, and BA 80 ppm showed greater fruit sets compared to the others. In the following harvest, the ANA 15 ppm, BA 80 ppm, ethephon 360 ppm, and (BA + AG) 28.2 ppm treatments reduced the fruit set compared to the control and the other treatments. ANA, etefon, and BA are growth regulators frequently used in the chemical thinning of fruit trees [17], and the reduction of fruit set in their plants may be a result of the abscission caused by the products; the purpose of using these products was to reduce the flowering intensity for less competition for carbohydrates.

TDZ treatments reduced the return bloom of apple trees in the 2017/2018 cycle (**Table 3**). The alternating productive behavior is a problem of many cultivars and may have occurred due to the high fruit set of the plants in the previous harvest, as it is a consequence of an excessive number of fruits in the plant. In the 2018/2019 harvest, there were no differences in return bloom between treatments.

In the 2016/2017 and 2018/2019 crops, TDZ treatments with 20 and 30 ppm increased yield per plant compared to the other treatments (**Table 4**). In 2016/2017, TDZ treatments of 10 ppm + PCa 110 ppm + AVG 90 ppm did not differ from TDZ treatment alone, and, in 2018/2019, the treatment (BA + AG) of 28.2 ppm obtained a lower yield than the others. In 2017/2018, the ANA 15 ppm and BA 80 ppm treatments

Treatments	Fruit set (%)			Return bloom (%)	
	2016/17	2017/18	2018/19	2017/18	2018/19
1. Control	10.8 c	12.0 b	48.6 b	97.2 a	67.2 ^{ns}
2. ANA 15 ml/L	4.3 c	13.3 b	17.4 c	97.3 a	84.3
3. BA 400 mg/L	11.1 c	36.2 a	25.1 c	97.2 a	77.0
4. Etefon 1.5 ml/L	7.9 c	7.5 b	21.6 c	95.8 a	97.4
5. TDZ 20 mg/L + PCa 400 mg/L + AVG 600 mg/L	54.7 b	23.3 a	80.8 a	85.5 a	92.4
6. (BA + AG) 50 mg/L	6.6 c	16.9 b	19.2 c	98.8 a	88.1
7. ATS 1.500 mg/L	9.6 c	12.2 b	42.6 b	94.9 a	93.0
8. TDZ 40 mg/L	63.2 b	11.1 b	65.1 a	63.3 b	93.0
9. TDZ 60 mg/L	88.2 a	20.9 a	76.0 a	41.3 c	80.5
Mean	28.5	17.0	44.0	85.7	85.9
CV (%)	36.7	46.2	33.8	13.2	22.7

*Means followed by the same letter in the columns do not differ by the Scott-Knott test at 5% probability. ns = not significant (p > 0.05). *Fractional dose in 3 applications of 9.4 ppm.*

Table 3.
Fruit set and return bloom of "Monalisa" apple trees depending on the application of growth regulators during the PF stage, in three cycles.

Figure 2.
Effect of thidiazuron on the fruiting of apple Cv. Monalisa, left Thidiazuron 10 ppm, right control.

showed the highest yield per plant, and the others showed no differences. TDZ applied in full bloom increases the productivity of apple trees [16]. However, the high productivity of plants treated with TDZ in the 2016/2017 harvest induced greater alternate bearing, verified by the higher IAP in these treatments (**Table 4**).

The treatments TDZ 10 ppm + PCa 110 ppm + AVG 90 ppm, TDZ 20 ppm, and TDZ 30 ppm, which showed higher productivity in 2016/2017, reduced the average fruit mass in this cycle by more than 15%. In 2017/2018, Etefon 360 ppm treatment increased the average fruit mass by 14%. In the 2018/2019 harvest, TDZ 10 ppm + PCa 110 ppm + AVG 90 ppm treatment reduced the average fruit mass by 28%. TDZ applied at full bloom can delay fruit ripening [16] and consequently its growth.

Treatments	Yield per plant (kg)			PAI
	2016/17	2017/18	2018/19	
1. Control	11.3 b	12.9 b	18.2 b	0.14 b
2. ANA 15 ml/L	14.0 b	16.4 a	19.6 b	0.26 b
3. BA 400 mg/L	14.1 b	14.3 a	15.1 b	0.10 b
4. Etefon 1.5 ml/L	8.7 b	11.6 b	17.0 b	0.24 b
5. TDZ 20 mg/L + PCa 400 mg/L + AVG 600 mg/L	16.1 a	9.5 b	16.3 b	0.33 b
6. (BA + AG) 50 mg/L	7.3 b	11.4 b	9.3 c	0.25 b
7. ATS 1.500 mg/L	11.6 b	10.9 b	17.6 b	0.24 b
8. TDZ 40 mg/L	19.2 a	9.1 b	23.2 a	0.45 a
9. TDZ 60 mg/L	22.5 a	6.3 b	25.3 a	0.63 a
Mean	13.9	11.4	18.0	0.29
CV (%)	36.8	20.5	39.9	52.9

*Means followed by the same letter in the columns do not differ by the Scott-Knott test at 5% probability. *Fractional dose in 3 applications of 9.4 ppm.*

Table 4.
Yield by plant and productive alternation index (PAI) in "Monalisa" apple trees as a function of the application of growth regulators during the PF stage, in three cycles.

Even with higher rates of alternative bearing, treatments with TDZ 20 and 30 ppm showed higher accumulated productivity over the three cycles evaluated, not differing from the ANA treatment of 15 ppm, while the treatment (BA + AG) 28.2 ppm showed the lowest accumulated productivity (**Figure 3**). The thinning caused by BA + GA applications during the flowering and post-bloom periods may have reduced

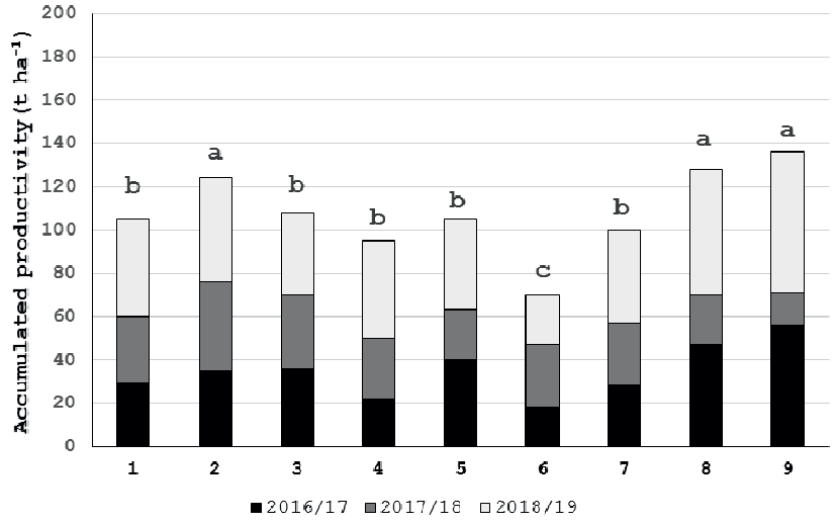

Figure 3.
Accumulated yield of "Monalisa" apples as a function of the application of growth regulators during the PF stage, in three cycles. 1. Control; 2. ANA 15 mg/L; 3. BA 4.000 mg/L; 4. Etefon 1.50 ml/L; 5. TDZ 20 mg/L + PCa 400 mg/L + AVG 600 mg/L; 6. (BA + AG) 50 mg/L; 7. ATS 1.500 mg/L; 8. TDZ 40 mg/L; 9. TDZ 60 mg/L.

apple productivity. ANA can increase fruit sets in several species when applied at full bloom [18], ensuring good productivity.

The fruit set on Cv. Monalisa increased significantly with Retain treatments of 207.5, 415, and 622.5 mg/L, which represented an increase of 89.1, 85.7, and 133.9%, respectively, compared to the control treatment. In Cvs., Gala and Daiane did not show any significant differences, showing an upward trend because in the best treatment, it was 575 and 371%, respectively, in comparison to the control treatment, which demonstrates the effect of Retain in the increase of the effective fruiting of the apple tree (**Table 5** and **Figure 4**).

The yield per plant and number of fruits per plant in Cv. Monalisa, with the exception of Retain's 207.5 mg/L treatment and all other treatments, were significantly superior to the control treatment (**Figure 5** and **Table 6**). At Cv. Gala, there were no

Treatments	**Fruit set (%)**	
	Monalisa	**Gala**
1. Control	20.3 b	5.8^{ns}
2. Retain 103.7 mg/L (F2-H)	18.2 b	18.8
3. Retain 207.5 mg/L (F2-H)	38.4 a	13.5
4. Retain 311.2 mg/L (F2-H)	25.7 b	22.7
5. Retain 415.0 mg/L (F2-H)	37.7 a	20.8
6. Retain 622.5 mg/L (F2-H)	47.5 a	33.4
Mean	31.3	19.2
CV (%)	24.3	51.1

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test ($P \leq 0.05$).
** ns: not significant ($P \geq 0.05$).*

Table 5.
Fruit set (%) of "Monalisa" and "Gala" apple trees subjected to different concentrations of Retain.

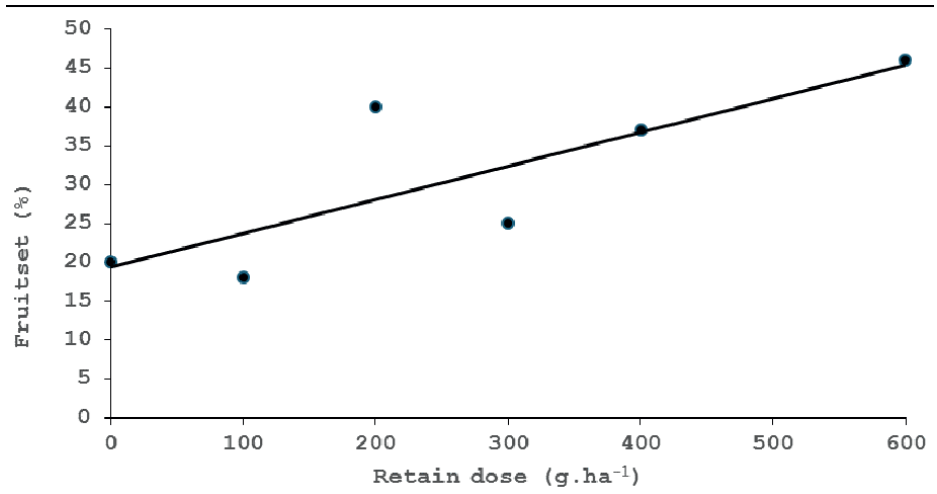

Figure 4.
Effect of Retain doses on the fruit set of the Cv apple tree Cv. Monalisa.

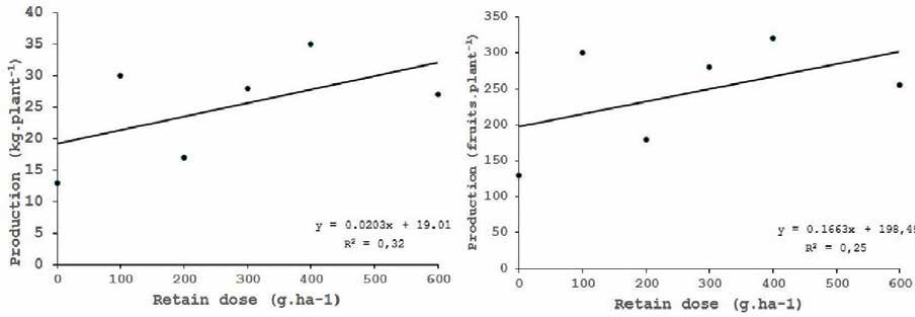

Figure 5.
Trend lines for yield (kg and fruits plant-1) of "Monalisa" apple trees, submitted to different treatments to increase fruit set, 2018/2019 cycle.

Treatments	Yield		Average fresh fruit mass (g)	Estimated productivity t/ha
	Kg plant^{-1}	Fruits per plant^{-1}		
1. Control	13.4 b	131.0 b	103.3^{ns}	33.5
2. Retain 103.7 mg/L (F2-H)	29.3 a	300.8 a	98.1	73.2
3. Retain 207.5 mg/L (F2-H)	16.4 b	179.8 b	91.8	41.0
4. Retain 311.2 mg/L (F2-H)	27.5 a	278.2 a	98.2	68.7
5. Retain 415.0 mg/L (F2-H)	34.4 a	321.8 a	109.1	86.0
6. Retain 622.5 mg/L (F2-H)	26.9 a	255.4 a	108.0	67.2
Mean	24.6	244.5	101.4	
CV (%)	36.0	35.5	13.0	

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test ($P \leq 0.05$).
**ns: not significant ($P \geq 0.05$).*

Table 6.
Yield (kg and fruits plant-1), number of fruits per plant, average fresh fruit mass, and estimated productivity from "Monalisa" apple trees, submitted to different treatments to increase fruit set.

significant differences, but all Retain treatments were numerically superior to the control treatment (**Table 7**). Regression analysis shows an increase in yield in both cultivars with an increase in Retain dosage (**Figure 3**). In the Cv., Gala the estimated yield (ton/ha) varied from 29.9 to 40.1 t/ha in the different concentrations of Retain, while the control treatment presented 20.8 t/ha. In the Cv. Monalisa, the estimated yield (ton/ha) varied from 41.0 to 86.0 t/ha among Retain treatments, with 33.5 t/ha in the control treatment (**Table 6**). The average fruit mass of the fruits did not present significant differences for the cultivars Monalisa and Gala even in the treatment of Retain with the highest productivity.

The classification of fruits in Cv. Monalisa showed significant differences. The percentage of fruits with larger caliber (+ 70 mm) in the control treatment and Retain, 415 and 622.5 mg/L, were superior to the other treatments, the same occurring in the class of smaller caliber (−55 mm) but with the same treatments having a lower percentage (**Table 8** and **Figure 6**). At Cv. Gala, there were no significant differences in the fruit size (**Table 9**). The russeting incidence did not present significant differences comparing cultivars Monalisa and Gala, which demonstrates that Retain applied in the flowering does not cause russeting (**Tables 10** and **11**).

Treatments	Yield per plant		Average mass (g)	Estimated productivity (t ha^{-1})
	Mass (kg)	N° of fruits		
1. Control	8.3 ns	73.0 ns	115.0 ns	20.8 ns
2. Retain 103.7 mg/L (F2-H)	16.0	137.4	116.9	40.1
3. Retain 207.5 mg/L (F2-H)	12.0	107.4	111.9	29.9
4. Retain 311.2 mg/L (F2-H)	15.5	145.6	106.5	38.9
5. Retain 415.0 mg/L (F2-H)	14.3	124.6	113.5	35.7
6. Retain 622.5 mg/L (F2-H)	15.9	155.8	102.7	39.8
Mean	13.7	124.0	111.1	34.2
CV (%)	36.8	35.8	9.5	36.8

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test (P ≤ 0.05).
**ns: not significant (P ≥ 0.05).*

Table 7.
Yield (kg and fruits plant^{-1}), number of fruits per plant, average fresh fruit mass, and estimated productivity from "Gala" apple trees, submitted to different treatments to increase fruit set.

Treatments	Caliber (%)		
	>70 mm	65 mm	<55 mm
1. Control	15.0 a	46.2^{ns}	38.8 b
2. Retain 103.7 mg/L (F2-H)	5.9 b	44.7	49.3 a
3. Retain 207.5 mg/L (F2-H)	2.3 b	35.5	62.2 a
4. Retain 311.2 mg/L (F2-H)	6.7 b	45.7	47.6 a
5. Retain 415.0 mg/L (F2-H)	9.3 a	52.0	38.7 b
6. Retain 622.5 mg/L (F2-H)	12.1 a	55.1	32.8 b
Mean	8.5	46.5	44.9
CV (%)	67.5	24.6	26.6

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test (P ≤ 0.05).
**ns: not significant (P ≥ 0.05).*

Table 8.
Caliber of fruits (%) (> 70 mm, 65 mm, and < 55 mm) from "Monalisa" apple trees, submitted to different treatments to increase fruit set.

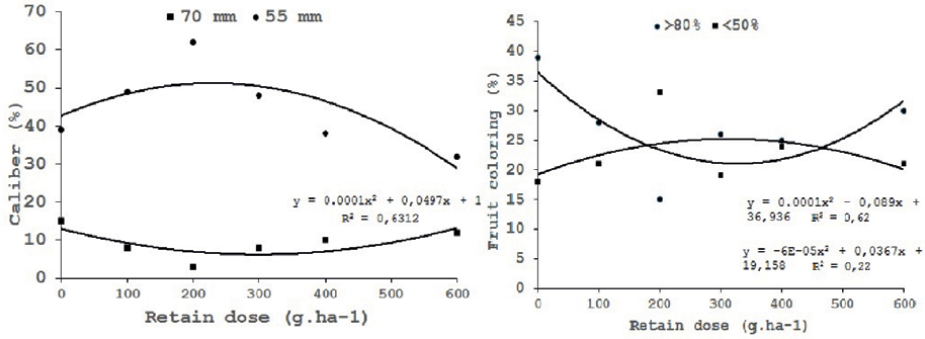

Figure 6.
Trend lines for fruit distribution in categories of fruit caliber and color (%), of "Monalisa" apple trees, submitted to different treatments to increase fruit set, 2018/2019 cycle.

Treatments	Caliber (%)		
	55 mm	50–80 mm	+70 mm
1. Control	27.9 ns	39.9 ns	32.1 ns
2. Retain 103.7 mg/L (F2-H)	20.5	49.9	29.6
3. Retain 207.5 mg/L (F2-H)	25.8	40.8	33.4
4. Retain 311.2 mg/L (F2-H)	22.2	55.6	22.1
5. Retain 415.0 mg/L (F2-H)	26.9	54.6	18.5
6. Retain 622.5 mg/L (F2-H)	31.1	48.8	20.2
Mean	25.8	48.3	26.0
CV (%)	14.3	13.7	25.0

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test ($P \leq 0.05$).
** ns: not significant ($P \geq 0.05$).*

Table 9.
Caliber of fruits (%) (> 70 mm, 65 mm, and < 55 mm) from "Gala" apple trees, submitted to different treatments to increase fruit set.

Treatments	Severity classes of "russeting"			
	1	2	3	4
1. Control	51.0	18.0	28.2	2.8
2. Retain 103.7 mg/L (F2-H)	55.1	19.5	22.9	2.5
3. Retain 207.5 mg/L (F2-H)	47.8	23.5	23.2	5.6
4. Retain 311.2 mg/L (F2-H)	50.5	19.6	26.9	3.0
5. Retain 415.0 mg/L (F2-H)	46.9	19.3	26.8	7.0
6. Retain 622.5 mg/L (F2-H)	47.2	18.1	29.4	5.4
Mean	49.7	19.7	26.2	4.4
CV (%)	16.0	25.5	20.4	93.2

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test ($P \leq 0.05$).
**ns: not significant ($P \geq 0.05$).*

Table 10.
Severity classes of russeting (1, 2, 3, and 4) of fruits from "Monalisa" apple trees, subjected to different treatments to increase fruit set, 2018/2019 harvest.

The red color of the fruits did not show significant differences in Cv. Gala. In Cv. Monalisa, in the class of higher coloration, only Retain's treatment with 103.7 mg/L ha showed a lower percentage of fruits in comparison to the other treatments. In the class of lower coloration, Retain's treatment presented a higher percentage, 207.5 mg/L (**Tables 12** and **13** and **Figure 6**). These results show that Retain applied in the flowering period does not interfere with the color of the fruits of Cvs. Gala and Monalisa.

The analysis of the set of variables showed that Retain had a strong effect in increasing the effective fruiting of the apple trees Gala, Monalisa, and Daiane, with a consequent increase in productivity, but did not influence the other variables that may interfere with the quality of the fruit (**Table 14**).

Treatments	Severity classes of "russeting" (%)			
	0	**1**	**2**	**3**
1. Control	21.5 ns	24.5 ns	30.2 ns	23.8 ns
2. Retain 103.7 mg/L (F2-H)	13.3	28.3	36.1	22.4
3. Retain 207.5 mg/L (F2-H)	19.6	34.4	29.2	16.8
4. Retain 311.2 mg/L (F2-H)	20.6	23.4	33.4	22.6
5. Retain 415.0 mg/L (F2-H)	16.6	27.1	34.0	22.3
6. Retain 622.5 mg/L (F2-H)	17.4	25.8	32.7	24.0
Mean	18.2	27.2	32.6	22.0
CV (%)	23.0	17.2	13.4	22.1

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test (P ≤ 0.05).
**ns: not significant (P ≥ 0.05).*

Table 11.
Severity classes of russeting (1, 2, 3, and 4) of fruits from "Gala" apple trees, submitted to different treatments to increase effective fruiting, 2018/2019 harvest.

Treatments	Red color of fruits (%)		
	>80	**50–80**	**<50**
1. Control	39.4 a	42.4 ns	18.2 b
2. Retain 103.7 mg/L (F2-H)	28.6 a	50.6	20.8 b
3. Retain 207.5 mg/L (F2-H)	15.7 b	51.8	32.6 a
4. Retain 311.2 mg/L (F2-H)	26.1 a	55.1	18.7 b
5. Retain 415.0 mg/L (F2-H)	25.2 a	50.3	24.6 b
6. Retain 622.5 mg/L (F2-H)	28.9 a	50.2	20.9 b
Mean	27.3	50.1	22.6
CV (%)	30.9	17.0	29.2

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test (P ≤ 0.05).
**ns: not significant (P ≥ 0.05).*

Table 12.
Red color of fruits (%) (> 80; 50–80; <50) of fruits from "Monalisa" apple trees, submitted to different treatments to increase fruit set, 2018/2019 harvest.

Treatments	Red color of fruits (%)		
	>80%	**50–80%**	**<50%**
1. Control	40.2 ns	40.0 ns	19.8 ns
2. Retain 103.7 mg/L (F2-H)	39.8	49.4	10.9
3. Retain 207.5 mg/L (F2-H)	39.9	42.5	17.6
4. Retain 311.2 mg/L (F2-H)	26.1	56.5	17.4
5. Retain 415.0 mg/L (F2-H)	27.0	48.0	25.0
6. Retain 622.5 mg/L (F2-H)	35.0	45.8	19.2
Mean	34.7	47.0	18.3
CV (%)	21.0	20.6	22.2

Means followed by the same letter in the column do not differ from each other by the Scott-Knott test (P ≤ 0.05).
**ns: not significant (P ≥ 0.05).*

Table 13.
Red color of fruits from "Gala" apple trees submitted to different treatments to increase fruit set, 2018/2019 harvest.

Source of variation	Yield		Average mass (g)	Caliber of fruits (%)			
	kg plant^{-1}	fruits plant^{-1}		>70 mm	65 mm	<55 mm	
	Mean square						
Doses of Retain	324.762987**/L	27467.820000**/L	220.237869^{ns}	104.675741*	228.011086^{ns}	547.464491**/Q	
Block	122.256745	14517.000000	189.222012	53.104838	142.422242	127.951122	
Error	78.538799	7545.220000	173.088938	33.308066	131.118778	142.997820	
Mean	24.6	244.5	101.4	8.5	46.5	44.9	
CV (%)	36.0	35.5	13.0	67.5	24.6	26.6	
Source of variation	**Red color of the fruits (%)**			**Severity classes of "russeting"**			
	>80	**50–80**	**<50**	**1**	**2**	**3**	**4**
	Mean square						
Doses of Retain	291.405629**/Q	86.806630^{ns}	143.446901*/Q	49.513118^{ns}	19.724645^{ns}	34.999749^{ns}	17.067539^{ns}
Block	169.475197	66.245828	75.049763	86.099187	38.374787	33.696755	30.407025
Error	71.422053	72.714688	43.564041	63.616995	25.267289	28.621191	16.574241
Mean	27.3	50.0	22.6	49.7	19.7	26.2	4.4
CV (%)	30.9	17.0	29.2	16.0	25.5	20.4	93.2

*Means followed by the same letter in the column do not differ from each other by the Scott-Knott test ($P \leq 0.05$). *ns: not significant ($P \geq 0.05$).*

Table 14.
Analysis table of the variation of "Monalisa" apple trees, submitted to different treatments to increase fruit set, 2018/2019 cycle.

4. Conclusion

TDZ increases the fruit set and productivity of "Monalisa" apple trees when applied in full bloom; however, it reduces return bloom and induces greater alternate bearing.

Applied in full bloom, ANA increases the productivity of "Monalisa" apple trees, while BA + GA reduces it.

TDZ combined with PCa + AVG increases the fruit set of "Monalisa" apple trees, and does not cause alternate bearing; however, it reduces the average fruit mass.

Author details

José Luiz Petri[1*] and Caroline Esperança[2]

1 Dr. Honoris Causa, Agronomy Course, Alto Vale do Rio do Peixe University – UNIARP, Caçador, SC, Brazil

2 Agronomist, MSc, Alto Vale do Rio do Peixe University – UNIARP, Caçador, SC, Brazil

*Address all correspondence to: petri@gegnet.com.br

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Fagundes E. Efeito da concentração e época de aplicação de tidiazurom na frutificação e qualidade de frutos da macieira 'Royal Gala'. In: Dissertação (mestrado) – Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Programa de Pós-Graduação em Produção Vegetal. Lages: UDESC; 2015

[2] Vercammen J, Gomand A, Denruyter L. Improvement of the fruit set of 'Jonagold'. Acta Horticulturae. 2010;**884**:357-362

[3] Bangerth FK. Internal regulation of fruit growth and abscission. Acta Horticulturae. 2004;**636**:235-248

[4] Lasko AN, Robison TL, Greene DW. Integration of environment, physiology and fruit abscission via carbono balance modeling – Implications for understanding growth regulator response. Acta Horticulturae. 2006;**727**:321-326

[5] Byers RE, Banden JA, AND CARBAUGH, D.H. Thinning of spur 'delicious' apples by shade, terbacil, carbaryl, and ethephon. Journal of the American Society for Horticultural Science. 1990;**115**:9-15

[6] Abebie B, Lers A, Philosoph-Hadas S, Goren R, Riov J, Meir S. Differential effects of NAA and 2,4-D in reducing floret abscission in cestrum (Cestrum elegans) cut flowers are associated with their differential activation of aux/IAA homologous genes. Annals of Botany. 2008;**101**:249-259

[7] Taylor JE, Whitelaw CA. Signals in abscission. The New Phytologist. 2001;**151**:323-340

[8] Giulia EB, Alexandro D, Mariano B, Andrea R, Benedetto R. Early induction of apple fruitlet abscissions is characterized by na increase of both isoprene emission and abscisic acid contente. Plant Physiology. 2013;**161**:1952-1969

[9] Basak A, Buczek M. The effectiveness of 3, 5, 6-TPA used againstpre-harvest fruit drop in apple. Proc. XIth IS plant bioregulators fruit prod. Acta Horticulturae. 2010;**884**:215-222

[10] Arseneault MH, Cline JA. A review of apple preharvest fruit drop and practices for horticultural management. Scientia Horticulturae. 2016;**211**:40-52

[11] Heo S, Hwanga JH, Kim D, Yu DJ, Lee HJ. Classification of apple genetic resources using early fruit abscission pattern. Journal of the American Pomological Society. 2015;**69**(2):102-108

[12] Amarante CVT, Ernani PR, Blum LEB, Megguer CA. Efeito do thidiazuron no crescimento, no florescimento, na frutificação e na nutrição em macieiras. Pesquisa Agropecuária Brasileira, Brasília. 2002;**37**(10):1365-1372

[13] Petri JL, Hawerroth FJ, Fazio G, Francescatto P, Leite GB. Advances in fruit crop propagation in Brazil and worldwide-apple trees. Revista Brasileira de Fruticultura. 2019;**41**(3):e-004

[14] Dal'sant, S. R. Reguladores vegetais na frutificação e produção da macieira 'Imperial Gala'. 48 f. In: Dissertação (Mestrado em Agronomia – Produção Vegetal). Curitiba: Universidade Federal do Paraná; 2013

[15] Petri JL, Leite GB, Couto M, Hawerroth FJ. Effect of growth regulators on 'gala' apple fructification. Acta Horticulturae, Leuven. 2010;**884**:531-536

[16] Petri JL, Schuck E, Leite GB. Efeito do thidiazuron (tdz) na frutificação de fruteiras de clima temperado. Revista Brasileira de Fruticultura. Jaboticabal. 2001;**23**(3):513-517

[17] Petri JL, Hawerroth FJ, Leite GB, Couto M. Raleio químico em macieiras 'Fuji Suprema' e 'Lisgala'. Revista Brasileira de Fruticultura. Jaboticabal. 2013;**35**(1):170-182

[18] Looney NE. Plant bioregulators in fruit production: An overview and outlook. Journal of Korean Society for Horticultural Science, Taejon. 1998;**39**(1):125-128

Chapter 10

Current Challenges of Strawberry Production in Spain

José Ángel Mercado-Hornos and Sara Posé

Abstract

Strawberry production in Spain is especially exposed to hotter summers and warmer autumns as part of climate change. In addition, most strawberry crop areas share water resources at the basin level with Guadalquivir marshes, a wetland of international importance, including the Doñana National Park, which is also threatened by a long drought period and the extensive use of its resources. Additionally, stricter regulations and consumer demands for eco-friendly and high-quality products are additional challenges for the sector. This scenario entails a serious risk for the future of strawberry production in the region and claims for the obtention of new varieties more resistant to abiotic stress, including extreme temperatures and water deficit. In response to this, current breeding programmes are pursuing "rustic" varieties defined by greater resilience to abiotic stress in general and less water demand in particular. This chapter will sum up the particular features of the cultivation of strawberries in Spain, as an example of a highly profitable fruit crop, evaluating impacts and challenges at the global and local levels. Finally, biotechnological approaches will be discussed as alternatives to fulfilling more sustainable strawberry production in Spain, as well as other producing countries worldwide.

Keywords: strawberry, production, climate change, water stress, sustainability, resilience, biotechnology

1. Introduction

Strawberry is a highly appreciated fruit for its flavour, fragrant aroma and health benefits [1], with a wide distribution in tropical, subtropical and temperate areas. The economic value of this crop, based on gross yield per hectare, is among the highest of all agricultural products. As a result, this seasonal fruit is a globally important crop, with production increasing steadily from 0.75 Mt (million tonnes) in 1965 to more than 9 Mt in 2021 [2], as shown during the last period from 2011 to 2021 (**Figure 1**).

Strawberry is a crop of great economic importance for Spain, being the sixth largest producer, only behind China, the USA, Turkey, Mexico and Egypt. In Spain, around 320,000 tonnes were produced on a cropping area of more than 7200 hectares in the last season of 2023/2024, and both values have remained stable over the last 20 years [3].

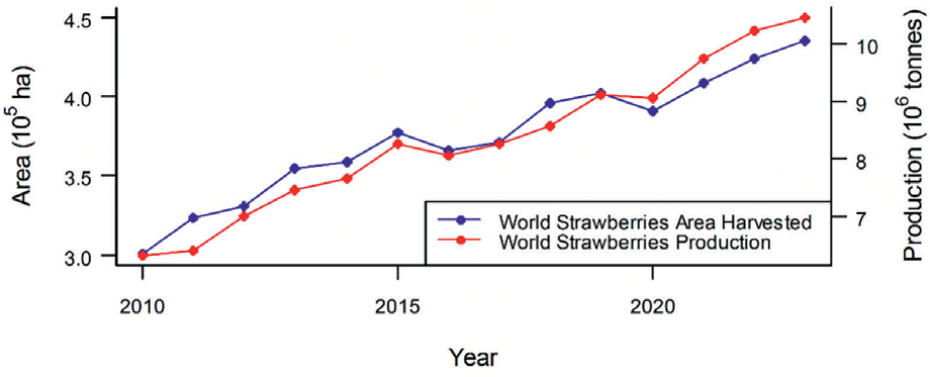

Figure 1.
World strawberry production in the period between 2010 and 2023. The crop area in hectares (ha) is shown with a blue line, and fruit production in tonnes is shown with a red line [2].

Almost the entire Spanish strawberry production comes from Andalusia, with the largest area devoted to strawberry production in Huelva province (SW Spain). During the 2021–2022 harvest season, Andalusia produced 97.3% of the Spanish strawberries and almost 30% of the strawberries in Europe (EU-27). This makes Spain the leading European producer (exporter) of strawberries, with an export value of around 587,000 euros in 2021/2022, with Germany, France and the United Kingdom among their main markets [4].

Strawberry production in Huelva began in the 1960s of the last century, thanks to the innovative initiative of founders such as Antonio Medina, whose farm 'Las Madres' served as a learning nucleus for other pioneers to become entrepreneurs in the cultivation and marketing of strawberries [5]. A few years later, in the 1980s–1990s, this crop began to expand nationally and internationally, with a production peak in the 1999/2000 season exceeding 8500 ha. Since then, the Spanish cropping area has stabilised at around 7000 ha at present and has been concentrated in Huelva. The latest data showed an estimated value of 407.83 million euros for the Huelva strawberry production, representing 3.5% of the vegetable production and 2.9% of Andalusia's agricultural production [6].

Strawberry is a seasonal crop, with its maximum production between March and April, localised in Huelva. From May onwards, Spanish strawberry production drops drastically because of the high temperatures in Andalusia, affecting the quality of the fruit and the competition from other Central European countries where the harvest season starts in May. Despite the good results of recent harvests, strawberry cultivation in Spain is under pressure due to the competition with other producing countries at certain times of the year. In fact, in the latest data published [3], there was a generalised drop in exports to our main destinations at the Intra-EU level compared to 2021/2022, such as Italy or Germany, with the Netherlands being the only market that increased. Extra-EU markets also recorded lower figures compared to the previous season, in particular, due to the anomalous behaviour of the United Kingdom as a result of BREXIT. In spite of this, the unit value (prices €/kg) of the Spanish strawberry increased in the 2021/2022 harvest, especially in Germany (+18.5%) and in the United Kingdom (+10.6%), which shows the competitiveness and relevance of this crop for Spain.

The advantages of Huelva for strawberry cultivation are based on its geographical location in the south of Spain, which determines the soil and climate conditions, as

well as the availability of good-quality water, which has led to a large expansion of this crop. The core of strawberry production and intensive horticulture in Huelva is located in the coastal area, which extends between the rivers Guadalquivir and Guadiana, with a length of more than 100 km. The province has a continental Mediterranean climate with Atlantic influences, classified as warm temperate, more continental towards the north areas and with maritime influences on the coast. The average annual temperature is 18.2°C, with monthly averages varying between 25.8°C for the warmest month and 11°C for the coldest. Minimum temperatures exceptionally fall below 0°C, which means that there is an almost total absence of frost. Huelva has more than 3000 hours of sunshine per year, which makes it the Spanish peninsular province with the highest annual sunshine index, together with Almería. Rainfall, although highly variable from year to year, can be estimated at between 500 and 700 mm per year, with an average of 525 mm in the period 1984–2010 [7].

1.1 Types of strawberry cultivation systems

Strawberry cultivation in Huelva has also been accompanied by a high level of technical expertise and management, with well-organised distribution structures and commercial dynamism, which have made strawberries a profitable crop for the province. In its beginnings during the 1960s, the development of this crop led to the take-off of a very economically depressed region. Today, its cultivation is highly technical, although it is still very labour-intensive. Currently, the cultivation systems adopted by farmers fall into three categories: conventional, organic (biological or ecological) and integrated [8–11]. The most relevant factors that distinguish these three systems are the use of agrochemicals (mineral fertilisers and pesticides), the implementation of organic matter as a source of nutrients and the impact on the environment of the cultivation practices employed.

The conventional system's main objective is to obtain the highest yield through the extensive use of chemical fertilisers, pesticides and intensive tillage practices to ensure the highest possible production [10]. In contrast, the organic farming system focuses on respect for the environment and is governed by the sustainability of the entire production system (use of organic materials and sustainability of the entire farming system, called agroecosystem) through the implementation of practices such as direct seeding (cultivation without ploughing to avoid disturbing the soil), crop rotation, the use of organic materials (e.g., compost) as a source of nutrients and soil amendment and biological methods for pest and disease control [8–11]. Finally, the integrated system is a compromise between the two cultivation practices described above, as it is based on both mineral and organic nutrient sources, as well as limited use of pesticides, fertilisers and water, which are implemented thanks to integrated management guidelines [12] based on previous knowledge. Integrated systems aim to achieve high production yields with good-quality products and generate the minimum amount of pesticide and agrochemical residues. Thus, the integrated farming system combines the structural elements of conventional farming, together with a more balanced and sustainable use of pesticides and fertilisers, minimum tillage practices and organic farming fundamentals (soil, ecosystem and human health), resulting in crops that are productive and more environmentally sustainable [9, 11].

The majority of strawberry production at Huelva (almost 80% of its surface area) follows a conventional and integrated production system, with a smaller percentage of production based on soilless cultivation (hydroponic) and barely 4% dedicated to organic production [6], thanks to the development and innovation that the sector

invests in this crop, which has resulted in a very profitable crop with low environmental impact. As we have indicated, the most widespread is the conventional integrated system, which is governed according to the legal obligations established in Spain (RD 1311/2012, 14 September, BOE-A-2012-11605), which include microclimatic adaptation using land that is structured in raised beds under tunnels, intensive cultivation in an annual cycle that starts with fresh plants from the nursery, and short-day varieties, as described later.

1.2 Annual cycle of intensive cultivation

Most of the strawberry crops in Spain are based on the use of new, fresh plants, mainly short-day varieties, following an annual cycle divided into two main stages: first, the production and propagation of new plants in high-altitude (800–1000 m) nurseries (north central Spain) and second, transplantation to the fruit production areas in southwest Spain. Strawberry plants are renewed every cropping season, beginning their vegetative development and propagation in high-altitude nurseries between April and October, where they accumulate the appropriate cold hours, known as vernalisation or 'winter requirements', which are crucial to induce flowering and promote a stronger development of plants. Nurseries, mainly located in Segovia (46%) and Ávila (42%) (Castilla León, NW Spain), produce the fresh plants, and at the beginning of autumn, the strawberry plants are transplanted from the northern nurseries to the fields of Huelva (SW Spain) to complete the fructification period between October and June [13].

There are 35 strawberry nurseries in Castilla y León (NW Spain), with a surface area of more than 1500 ha and a production of more than 900 million plants in 2023, according to data from the Castilla y León Regional Ministry of Agriculture, Livestock and Rural Development (**Figure 2A**). This sector generates around 120 to 130 million euros for the rural areas of the region and employs around 4500 people, both directly and indirectly. Most of the plants produced are sold in Huelva but also in other agro-environments with mild winters and sunny springs, including Greece, Italy, Portugal,

Figure 2.
Annual cycle of strawberry production in Spain. (A) Geographical distribution of strawberry cultivation in Spain begins with the production of mother plants in nurseries in northwestern regions of Castilla y León (marked in red) and the transfer of plants to the southwest region at Huelva (marked in yellow) for the fruit production stage. First stages at high-altitude nurseries: (B) Soil preparation at the beginning of the cycle; (C) nurseries with mother plants starting to set new stolons and seedlings; (D) final phase of nurseries with uprooting the plants between September and October. The second stage of fruit production takes place in Huelva with fresh plants rooted over raised beds of sandy soil covered by plastic mulches and protected under systems of macro-tunnels (E) and micro-tunnels (F). (G) Images of fruiting strawberry plants on top of raised beds covered by black plastic mulch, under macro-tunnels. Sources: [14–17].

Morocco, Algeria, Brazil and Tunisia, making Castilla y León the European leader in the production of mother plants of strawberries and Spain the second country after the US in the world ranking.

Planting in the high-altitude nurseries is carried out mechanised between April and May, using between 12,000 and 24,000 mother plants/ha, which until then have been kept in cold stores on previously disinfected soil (**Figure 2B**). Irrigation is started immediately to encourage rooting and is increased as the plant develops until maximum watering is achieved during July and August. During their development in these nurseries, the plants require many phytosanitary controls to prevent and treat diseases, nutritional deficiencies and weed growth. In addition, emerging flowers and dry leaves are removed manually to keep them free of decaying material that favours the appearance of diseases and pests. The production of daughter plants by stolons started with the long-day photoperiod at the beginning of summer (June) until they reach their maximum vegetative development in September (**Figure 2C**). By mid-October and early November, each mother plant gives rise to 15–30 daughter plants per stolon, physiologically mature and suitable for uprooting and planting in the fruiting fields of Huelva. Harvesting of the plants is mechanised (**Figure 2D**), with yields ranging between 650,000 and 700,000 commercial plants/ha.

As described above, the strawberry is a plant with complex handling and cultivation needs, and Castilla y León offers the best agro-climatic conditions for the development of mother plants. For the mother plants to achieve the desired physiological maturity and stolonate, the daughter plants need to accumulate certain hours of cold, known as vernalisation, to be taken to a different southern production area where they will be planted and bear fruit. This is achieved with temperatures below 7°C. The climate of the region meets the temperature and light requirements that the strawberry plant needs, as it is located between the 40° and 42° N parallels. Regarding the photoperiod, at the end of summer, there is a reduction in the hours of light, which induces their propagation by stolons [18]. Thus, low temperatures contribute to the first induction of flower buds and an earlier and more concentrated fruit set, so that in autumn planting in Huelva (September–October), the fruit harvest is early and more competitive in the export market [19]. In high-altitude nurseries, many tasks are carried out until the plants are uprooted in the nursery and transferred to Huelva (e.g., the development of the mother plant, the thinning of flowering, the pricking out of stolons and daughter plants, etc.). Each mother plant propagated by stolons produces 15–30 daughter plants.

The current challenge for high-altitude nurseries, as producers of mother plants, is to maintain the phytosanitary level of their plants due to the implementation of the Montreal Protocol on Ozone Depleting Substances in 1989, where many countries worldwide agreed on several control actions, including a reduction in the release of halogenated substances to mitigate global warming effects. Shortly after, the withdrawal of methyl bromide (MB) as a halogenated pesticide for soil disinfection (Reg. (EC) 1272/2008) led to a decline in yields in the strawberry sector, which was forced to increase cultivated area and plant higher densities to maintain productivity. Pesticide alternatives to methyl bromide, including 1,3-dichloropropene (1,3-D), chloropicrin (CP), dazomet (DAZ) and metam sodium (MS), were the main solutions to replace 100% of MB in strawberry crops since 2005 [19]. But the recent withdrawal of 1,3D and CP in 2022 (Reg. (EU) 2022/751), included in the list of chemical substances and their legal status at the 'EU pesticides database' (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances), imposes a new challenge to the sector in order to find treatments and/

or technologies that effectively replace them to guarantee their survival. At present, soil disinfection is essential to maintain the production levels of strawberry nurseries in Spain, by far the most important in Europe and the Mediterranean basin, with high levels of export of plants of high sanitary quality. The production of strawberry nursery plants is under strict EU and Spanish regulations on certification and control of nursery plants, where the requirements to obtain the phytosanitary certificate of the marketed plant include a very low tolerable percentage of infected plants, between 0.5% and 1%, depending on the pathogenic organism. The plant protection system is currently insufficient, and the sector is working to find effective alternatives for soil fumigation [20].

Once the new plants arrive in Huelva, one of their peculiarities is that they are cultivated on soil structured in mulched raised beds and protected under plastic micro or macro-tunnels (**Figure 2E–G**). Cultivation under tunnels offers protection against heavy rain and cold and creates a microclimate that favours early production in winter and its maintenance until spring [15, 21]. On the other hand, mulching with opaque plastic sheeting of the raised beds in autumn, just before planting new fresh plants, ensures a good winter survival percentage and optimal yield because it contributes to regulating soil temperature, preserving moisture by restricting evaporation losses, suppressing weed growth (being black/opaque), reducing the number of rotten fruits and increasing nutrient uptake and efficient water use [22, 23].

Strawberry roots develop better with soil temperatures above 12°C. Taking into account that soil temperature depends on its conductivity and thermal capacity, mulch of raised beds contributes to maintaining humidity in an optimal range for the development of the root system. Therefore, mulching is essential for the crop because it increases vegetative growth, plant flowering, yield and fruit quality [15, 24, 25].

Controlled irrigation management with fertirrigation systems is widespread in the strawberry crop yields in Huelva. In a large part of the province, strawberries are irrigated with very low salinity water and are grown in sandy soils with 90–95% sand, poor in organic matter (0.5%), low electrical conductivity (less than 0.8 dS/m), and therefore with low water and nutrient retention capacity. Therefore, although strawberries are irrigated with high frequency, the volumes of water applied can cause nutrients to be washed out due to the low retention capacity of the soils, so that, to a certain extent, strawberry cultivation in Huelva can almost be considered a 'soil hydroponic crop'. Therefore, it is considered a fertigated crop in which fertilisers are applied through the irrigation water, which allows the dosage control of the different nutrients to be adapted to the requirements of the crop during their different developmental stages [26], as is done in hydroponic cultivation.

The description of this conventional and integrated production system involves various techniques (high-altitude nurseries, short-day varieties, tunnels, mulched raised beds, sandy soils, fertirrigation...), showing the unique ways in which strawberries are cultivated in the most important area on the European continent. This conventional system is carried out in a continuous model year after year, with hardly any rotation with other crops, beginning in June–July with the removal of the remains of the previous crop and the preparation of the soil for a new crop, while the propagation, vegetative growth and first flower bud induction of the new plants takes place in high-altitude nurseries. Subsequently, planting in Huelva takes place between September and October to start production in November–December, which is maintained thanks to mulching and tunnels throughout the winter, to end the annual cycle in spring with the last harvests of the campaign, which usually coincides with the first week of June [13, 21].

1.3 Strawberry commercial cultivars in Spain

Most of the strawberry commercial varieties, also known as cultivars, grown in Huelva are short-day varieties, which require photoperiods of less than 14–15 hours of light to induce the development of their flower buds, with a facultative type of flowering, so that regardless of the short-day photoperiod, low temperatures (vernalisation) also induce the formation of flower buds. Thus, the inducing effect of the short-day photoperiod is optimal at 18–20°C, and flowering decreases progressively when temperatures are below 12°C and above 21°C [27, 28]. These short-day varieties are well suited for the mild winters of Huelva, but there are also some moderate or remontant day-neutral varieties, which have little need for cold in the greenhouse and are very early fruit producers, which makes them perfect complementary varieties capable of adapting to the markets of short-day varieties, as in the case of Huelva. Although Spanish strawberry cultivation in its first decades was dominated by a single main variety that covered more than 80–90% of the area, served by varieties coming from the breeding programmes of the University of California (cv 'Chandler' and 'Camarosa') and the University of Florida (cv 'Florida Fortuna') (**Figure 3**), cultivation has become more professional in Spain, and nowadays it has gone from 1 or 2 main varieties in the first decades to the 11 different varieties in the harvest season 22/23, which together represent more than 75% of the cultivated area. It should also be noted that breeding programmes have increased enormously, and today there are many varieties on the market obtained by Spanish companies or consortiums such as Fresas Nuevos Materiales (FNM) with 'Rociera' or 'Marisma' varieties or Masiá Ciscar with 'Palmerita' or 'Leticia' varieties (**Figure 3**) [29].

Specifically, the varietal composition of the 2022/2023 season is the most diverse, with 6 main varieties representing more than 50% of the total and another 16 varieties of low representativeness (between 5% and 1%) including many new varieties

Figure 3.
Commercial varieties cultivated in Huelva, including some of the classical cultivars: 'Chandler', 'Camarosa' or 'San Andreas' from the University of California and 'Florida Fortuna' from the University of Florida; and more recent cultivars considered "rustic": 'Rociera' and 'Marisma' from Fresas Nuevos Materiales (FMN), 'Palmerita' from Masiá Ciscar, or 'Red Sayra' from Planasa. Strawberry plants shown here are part of the "Fragaria Germplasm Bank" located at the IFAPA centre in Málaga, Spain (Photographs by Sara Posé).

in their first season, such as Planasa's latest variety (cv 'Red Sayra') (**Figure 3**), in addition to 7% made up of varieties with less than 1% representativeness. There is an increasing number of breeding companies interested in developing varieties adapted to Huelva, with up to 15 different breeders in the 2022–2023 harvest (e.g., University of Florida, Fresas Nuevos Materiales S.A., Masiá Ciscar S.A. and Fresh Forward Inc.), as is the fact that each of them is participating with a greater number of varieties registered or in the process of registration [29]. In terms of photoperiod, at least 17 of the 22 representative varieties in the 22/23 season were short-day, except for the 'San Andreas' variety from the University of California as the only one being day-neutral (**Figure 3**). Despite the release of new varieties from breeding programmes, classic varieties such as 'Florida Fortuna' are still being cultivated in Huelva in the current 2024/2025 season, with excellent yields.

2. Climate change as a global context

Today, climate change has become the most urgent global environmental problem as the concentration of greenhouse gases (GHGs) in the atmosphere increases. According to the 6th Assessment Report (AR6) of the Intergovernmental Panel on Climate Change (IPCC) [30], human activities, mainly due to emissions of GHGs, have unequivocally caused global warming, with global surface temperature increasing in just 10 years (2011–2020) by 1.1°C more than in the period 1850–1900. Depending on decisions and actions taken in the short term, future generations will face a world that will be warmer, but to a different degree.

According to the latest IPCC report [30], continued increases in emissions will further affect the main drivers of the climate system (temperature, precipitation, and soil moisture), and with each additional increase in global warming, extreme weather events will become more common. Global warming is projected to further alter the global water cycle, including its variability, global monsoon precipitation, wet or dry seasons and extreme weather events. With further warming, forecasts indicate that all regions will experience more frequent simultaneous changes in climatic factors, such as compound heatwaves and drought events. Moreover, Mediterranean countries are predicted to be especially affected by water stress in the future (**Figure 4**).

Global net anthropogenic GHG emissions in 2020 have been estimated to be 12% higher than in 2010 and 54% higher than in 1990 [30]. It is notable to note that historically, CO_2 emissions contributions vary substantially between regions in terms of total magnitude. While the top 10% of households with the highest per capita emissions contribute 34–45% of global consumption-based GHG emissions, the bottom 50% of countries contribute only 13–15%. Today, human-induced climate change is already affecting many extreme weather and climate events in all regions of the world, but the most vulnerable communities in the least developed countries, which have contributed the least to climate change, are disproportionately affected. The effects of climate change are therefore projected to increase the severity of its impact on natural and human systems in the future and to further accentuate regional differences [30].

2.1 Impact of agriculture on climate change: Options for improvement

In 2019, approximately 22% of global GHG emissions came from agriculture, forestry and other land use [30]. In turn, economic losses from climate change have

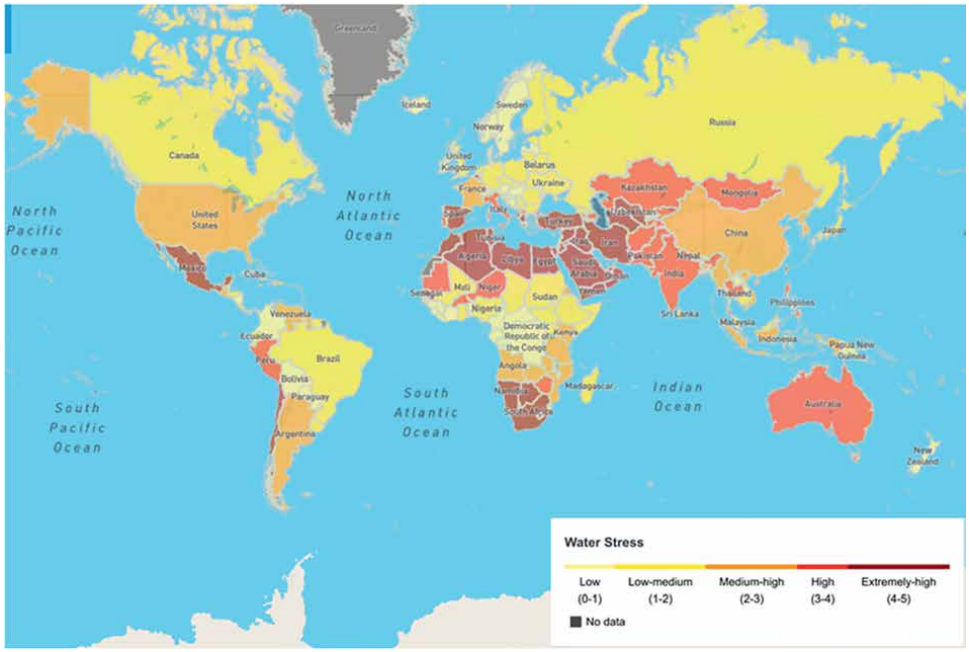

Figure 4.
Prediction of water stress risk in a 'scenario of business as usual' with temperatures increasing by 2.8–4.6°C by 2100. Mediterranean countries, where strawberry production has great importance, are predicted to be especially affected by water stress [31].

been detected in climate-exposed sectors such as agriculture, whereby climate change has reduced food security and affected water security, hampering efforts to achieve the Sustainable Development Goals. Although global agricultural productivity has increased, climate change has slowed this growth over the past 50 years worldwide, with related negative effects mainly in mid- and low-latitude regions, although positive in some high-latitude regions. Currently, almost half of the world's population suffers from severe water scarcity for at least part of the year due to a combination of climatic and non-climatic factors. **Figure 5** shows the uneven effect of climate change

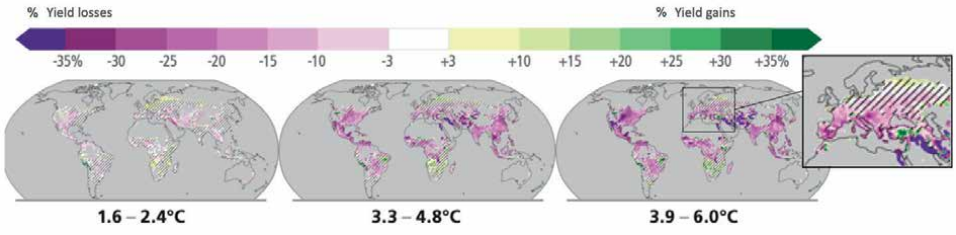

Figure 5.
Impact of climate change on global food production in different regions, taking as an example its effect on changes in % maize production (purple colours for losses and green colours for increases; areas with oblique stripes show areas with discordant data according to different models). Regional impacts are projected under three possible temperature increase scenarios, reflecting biophysical responses to changes in temperature, precipitation, solar radiation, humidity, wind, CO_2 increase and water scarcity in current cropping areas. The models assume that irrigated areas have no water scarcity. These models do not include the effects of diseases, pests, agro-technological developments and some extreme weather responses (modified from Ref. [30]).

on global food production, and it can be seen how the predictions for Spain show losses in production, taking maize as an example, which becomes more pronounced the greater the projected increase in global temperature.

Although the adverse impact of human-induced changes intensifies water scarcity and reduces food production, there is still some margin to try to reverse the negative effects, so all actions taken during this decade will have an impact not only now, but in the long term [30]. Although public opinion and political discussions often focus on the negative contributions of agriculture to global warming, agriculture also offers great potential for tackling climate change that is not possible in other systems or industries. One example is the ability of agriculture to sequester atmospheric carbon and act as a sink that stably stores CO_2 [32]. But for this to happen, development models must be applied that are resilient to climate change, that integrate both adaptation and mitigation measures, that ensure sustainable development based on international cooperation, whose policies make an economic effort commensurate with the problem, taking into account the most vulnerable regions, sectors and groups.

2.2 Climate change implications for agriculture: Food and water security

Food and water security implies that nutritious food and clean water are available regularly and accessible in sufficient quantities to enable them to lead a healthy and active life [33]. Climate change affects many food system processes directly and indirectly [34], but the primary effect is often associated with decreases in crop production, putting food security at risk (**Figure 5**).

Plants use environmental cues to regulate their growth, and their life cycles are seasonal [35]; therefore, climatic changes can have a large effect on many aspects of plant growth and development. Evidence from many crops (maize, raspberry, blackberry, strawberry, melon, tomato, potato, apple,...) shows how warm temperatures accelerate reproductive development, especially with less effect on vegetative development (leaf area, biomass, ...) [28, 36, 37]. Among the seasonally controlled developmental processes, known as phenological development, are the regulation of dormancy, pollination, flowering, and thus crop production, which are particularly sensitive to these changes. But the increase in temperature will not only affect production but will also negatively affect fruit quality by altering organoleptic characteristics such as size, colour, chemical composition or firmness [37].

Effects from global temperature rise, known as global warming, will be exacerbated when coupled with alterations in water availability (through deficiency or excess) due to climate change. Thus, it is crucial to understand the interaction of both variables, temperature and water, in plant development in order to investigate new biotechnological alternatives that produce resilient crops capable of facing the impact of extreme events associated with climate change [28], since forecasts point to an increase in the frequency of extreme weather events, aggravated by heat waves and droughts.

The IPCC's AR6 [30] showed a warmer and wetter climate in the future, but those changes will vary across geographic regions (**Figure 5**). For example, models indicate that temperature and precipitation will rise more in higher latitudes (e.g., northern Europe), while the Mediterranean region could become more arid due to recurrent periods of heat waves and drought, which would have a direct impact on several major strawberry-producing countries, including Turkey, Egypt, Spain, Morocco and Italy. It is therefore important to take into account the water resource in this crop, because many models do not include it in irrigated crops such as strawberries. Strawberry

production in the Mediterranean countries is already under pressure due to water scarcity for irrigation, and climate change is predicted to worsen the water deficit problem in these regions. It has also been highlighted above that among key solutions, a more efficient water use in agriculture is one of the proposed adaptation measures with great potential to combat climate change through better-managed, more environmentally sustainable agricultural systems.

2.3 The environmental impact of strawberry production

According to several studies about the environmental impact of strawberry production [38–42], this crop has a high impact on global warming (GWP100) due to its high energy consumption (machinery, production and use of plastics for mulching and protection systems by tunnels), high water demand, and the use of agrochemicals for both fertirrigation and phytosanitary control. Strawberries can be produced under low-input systems during their natural growing season (without tunnels, including long rotations and suitable soil conditions); however, the conventional protected system is the most profitable and productive method because it allows earlier and sequential production, although it involves a higher environmental cost [42, 43]. Organic systems showed the lowest environmental impacts in most categories per hectare, but their productivity was lower in comparison with protected systems, so these systems still need to optimise the use of environmentally friendly inputs, such as compost, to improve their profitability. Regarding the open-field strawberry production system, its impact on the environment was higher compared to the integrated and tunnel-protected systems, mainly due to the use of fertilisers, which turned out to be the major source of impact in most of the environmental categories and farming systems. Indeed, acidification, eutrophication and ecotoxicity were the categories with the highest impacts in all strawberry production systems evaluated [16, 39]. In Spain, the comparison of several strawberry cultivation systems showed that the integrated macro-tunnelling soilless (hydroponics) was the best system, offering the lowest impact per tonne of strawberry produced for all environmental impact categories assessed. Thus, several studies have indicated that optimising fertirrigation, using recycled and/or longer-lasting materials for mulching and protection, using renewable energy for machinery, and improving the efficiency of renewable inputs such as compost could reduce the environmental impacts of strawberry production in Spain [16], highlighting significant opportunities for enhancement regarding its environmental sustainability.

3. A sustainable future for Doñana and the Spanish strawberry

As described above, strawberries are an important crop for the province of Huelva, with Spain as the major exporter to European markets. However, most of the strawberry fields in Huelva are located in the coastal area and vicinity of Guadalquivir marshes (GM), which are protected wetlands, creating a problem between strawberry crops and marshes' sustainability in terms of resources, particularly water. Recently, this has been the subject of debate and concern, generating conflicts at the territorial and European levels.

Guadalquivir marshes are a wetland of international relevance that acts as a safeguarding water body for migratory waterbirds due to its strategic position between Africa and Europe in the East Atlantic Flyway. The exceptional waterbird diversity

and abundance of these marshes are well documented [44, 45] and recognised as a Ramsar and UNESCO World Heritage Site, including at least 25 waterbird species and more than 10% of the biogeographical population for six waterbird species at Doñana Natural Park [46, 47]. Doñana National Park was declared a World Heritage Site in 1994 for its variety of ecosystems and the great diversity of species that inhabit it, making it a unique enclave in Europe. The climate of Doñana is sub-humid Mediterranean, with an average annual rainfall of around 550 mm (EBD-CSIC). In recent years, precipitation levels have been below average, with the hydrological year 21/22 being one of the driest in the entire historical series, which covers almost 50 years of records since 1978, with the lowest precipitation levels of the last 10 years (283 mm), as can be observed by satellite images (**Figure 6**).

The rainfall situation has not improved during 2023, with a similar level (337 mm up to August). Regarding temperature, the last few years have been slightly above the reference temperature average, with the 21/22 and 22/23 seasons recording very high maximum temperatures and the highest annual average temperature (18.53°C) on record. However, climatic factors are not the only cause of the deterioration of the Doñana lagoon system. The intensive development of agriculture and tourism in this area has caused the degradation of its vegetation and soils, the drastic reduction of its wetlands and a serious decline in its flora and fauna diversity. Regarding the use of their natural water resources, their groundwater reserves have been overexploited for agriculture and tourism, which are considered the causes for desiccation of water bodies in Doñana National Park [48].

In spite of Guadalquivir marshes being partially protected under Doñana National Park (c. 30,000 ha), during the last century more than 80% of its surface area was converted into man-made wetlands dedicated to agriculture (mainly rice fields) and

Figure 6.
Images of Doñana on 12 March 2021 (left) and 2 March 2022 (right) taken by the European Union's Copernicus Sentinel-2 satellite.

aquaculture ponds, and its natural hydrological regime transformed to an almost entirely rain-dependent wetland.

Recent studies by the Doñana Biological Station, using data from the last 40 years, show that 59% of the largest lagoons in Doñana have disappeared. This phenomenon is significantly related to the high temperatures and the long period of low rainfall that Doñana is suffering, partly due to climate change, but also to the overexploitation of the aquifer that feeds this lagoon system [49]. Historically, the system of lagoons at Doñana has been a sanctuary for fauna, with only a few remaining with water all summer, offering refuge to the first waders migrating south after breeding in northern Europe and providing habitats for a good number of strictly aquatic species of flora and fauna. However, around 80% of these lagoons dried up earlier and were flooded less than would be expected with the last temperature and rainfall records, which shows that human activity is altering the natural balance of the lagoons and aggravating the problem. On this line, the drying up of Santa Olalla lagoon in summer is one of the most significant examples of the lagoon system deterioration (**Figure 7**) [50].

The decline of the Doñana National Park natural marshes and the recent events of the sudden disappearance of many of those natural and man-made wetlands over the last years in consecutive seasons have raised international concern about the uncertain future of these marshes and have damaged the Spanish strawberry brand in the international market. Thus, revaluation of the extensive transformation and overutilisation of this crucial wetland is urgent in order to preserve its outstanding and distinctive biodiversity and provide sustainable management of its natural resources [51–53].

In response, initiatives have emerged to restore the natural ecological processes of the marshes and to promote sustainable practices that ensure the valuable services provided by the marshes in the long term. One example is the 'Alianza Marismas del Guadalquivir-Doñana', an innovative coalition promoted by the NGO Fund for the Custody and Recovery of the Salt Marshes (SALARTE), which brings together landowners, farmers, fishermen, scientists, universities, public administrations, employers and non-governmental organisations [54]. Regarding strawberry fields in Huelva, this situation urges us to find solutions for a more sustainable and environmentally friendly production of the strawberry crop that can be applied not only in Spain but in other strawberry-producing areas, as climate change is a global challenge that affects us worldwide.

Figure 7.
Aerial views of the Santa Olalla Lagoon. The images show the state of the lagoon in the summer of 2017 (A) and the second consecutive complete drought of this lagoon, the last to be permanent, on 9 August 2023 (B). Source: Héctor Garrido (A) and Carlos Ruiz (B) from EBD-CSIC.

4. Current challenges and biotechnological alternatives

The relevance of the economic value of Spanish strawberries lies not only in their quantitative production but also in their qualitative aspects, which are also very important and have been decisive in maintaining their unit value despite market tensions and competition with other producing countries. The most important organoleptic quality parameters of the fruit are colour, aroma, flavour and texture. In particular, in fleshy fruits, the texture is of vital importance [55–58], and the case of strawberries is no exception because it softens a lot during ripening, and this results in this fruit being highly perishable and having a short postharvest life [59, 60]. The delicate texture of strawberries has been recorded to cause losses of 5–25% of production due to excessive softening, resulting in bruising and mechanical damage and promoting fruit infections during postharvest. Considering that more than 80% of the Spanish strawberry production is exported, control of premature or excessive softening is critical in strawberries, so its integrity during transport is crucial to reduce postharvest losses. An extensive body of research supports the relevance of pectin degradation at the cell wall on strawberry fruit texture at the molecular and genetic level. Transgenic and edited strawberry lines in pectinase genes have shown firmer fruits and extended shelf life without affecting the rest of their organoleptic features [61–64], and his transfer to a biotech crop in the market would contribute to controlling strawberry fruit softening and increasing its quality with economic benefits for producers, sellers and consumers, as well as contribute to reducing waste in the food chain [55, 57]. The last step to putting this knowledge in the EU market will be the production of these pectinase-edited strawberries through the use of editing tools not supported by transformation *via* Agrobacterium.

Currently, the bottleneck for the production of edited plants is the efficient delivery of CRISPR/Cas reagents to plant cells, because the rigid cell walls surrounding them are a physicochemical barrier. Several recent reviews about delivery mechanisms include Agrobacterium, viral vectors, biobalistic, protoplast, nanotechnological carriers or the new emerging morphogenetic tools [65–67].

Edited crops are a promising tool that will ease the translation of basic knowledge to improve and obtain new commercial varieties by the latest genome editing tools [68]. In this regard, the great potential of biotech crops produced using new genomic techniques (NGTs) is near to being accepted under EU legislation with the recent European Parliament legislative resolution of 24 April 2024 on the proposal for a regulation of the European Parliament and of the Council on plants obtained by certain new genomic techniques and their food and feed, and amending regulation (EU) 2017/625 (COM(2023)0411–C9-0238/2023–2023/0226(COD)). The gene-edited organisms are already exempt from GMO regulation in many countries as the US, Canada, Argentina or Japan, to name a few, and the first CRISPR-edited food was sold in Japan in 2021, being a gene-edited tomato containing higher levels of an amino acid expected to help lower blood pressure. These new techniques include CRISPR/Cas as the most successful approach that allows the modification at specific base pairs of genes with a known function. These NGTs have already shown their potential to improve crops and will help to improve not only fruit texture but also other interesting crop traits related to the current challenges of agriculture to feed the world under an unpredictable climate. Although the EU legislation is still in progress, the Parliament voted favourably in February 2024 in support of a simplified registration for plant varieties produced using NGTs similar to conventional varieties. The EU Parliament supported NGTs produced plants with the objective of making the food

system more sustainable and resilient by developing improved plant varieties that are climate resilient, pest resistant, and give higher yields or that require fewer fertilisers and pesticides.

On the other hand, conventional breeding techniques, although less precise than NGTs, have the advantage that they are not subject to legal GMOs constraints. Nowadays, in addition to the values of earliness, productivity, organoleptic quality and shelf life, breeding programmes include a new factor, known as 'rusticity', which refers to the ability of plants to adapt to different types of biotic and abiotic stress, including limited water resources, extreme temperatures or greater resistance to pathogens. Although fertirrigation is implemented in the case of Huelva strawberry crops, any improvement on a more efficient use of water will be a crucial feature to ensure the sustainability of this crop. Water use efficiency and resistance to water stress are complex traits that have not been taken into account in strawberry breeding programmes so far, so the range of improvement in this sense should be wide, especially if we compare the great resilience to water stress of wild species such as *Fragaria chiloensis*, from which the current commercial varieties of *Fragaria x ananassa* are derived (**Figure 3**) [69]. Biotic stress is also another source of pressure for this crop, due to the definitive ban in Spain and at the EU level, on the most effective agrochemicals for soil disinfection, which has put on the table the obligation to have new 'rustic' varieties, adapted to each production area [70, 71].

Due to climate change, summers and autumns on the Iberian Peninsula are getting hotter. Strawberry plants do not develop well because of this, both in nurseries and in the plantations in Huelva. In addition, the lack of manpower and restrictions in the application of phytosanitary products for disinfection tasks further limit the profitability of the strawberry sector. On the one hand, the lack of sustainable and effective alternatives for soil disinfection has increased the costs of personnel dedicated to crop maintenance, as the presence of weeds that must be removed manually has increased. Also, the poor management of water resources in Doñana and the high water demand for this crop jeopardise the

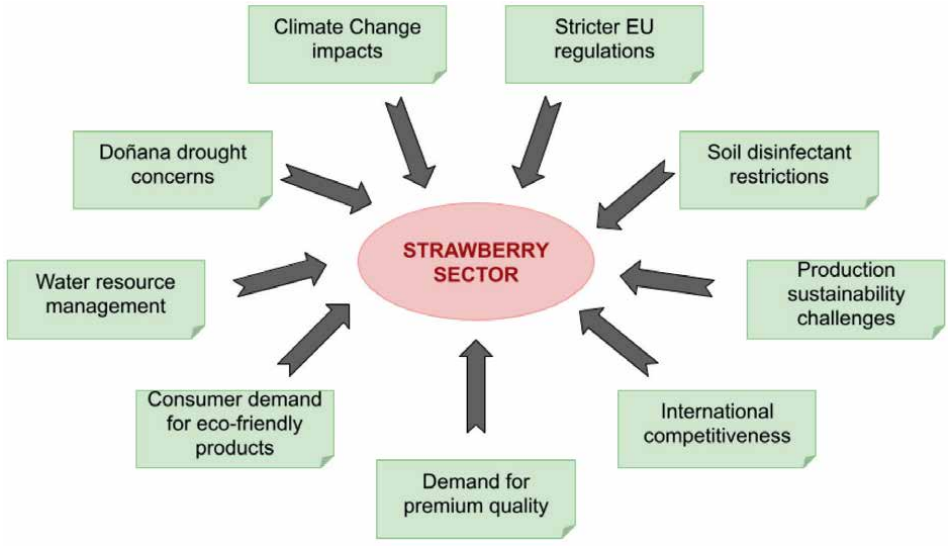

Figure 8.
Current problems and demands of the strawberry sector in Spain.

future sustainability of the sector in the region, as well as seriously damaging the Spanish strawberry brand in the international market.

5. Conclusions

Therefore, due to the current challenges of the strawberry sector in Spain, the future of this crop goes through edited plants by NGTs in the near future and conventional breeding of rustic varieties at present to improve the resistance to biotic and abiotic stress, with particular interest in water stress as a new breeding feature (**Figure 8**). The lack of alternatives to soil disinfection and the need for resilience to abiotic stress make these rustic varieties less demanding on water and more resistant to diseases, an option that responds to current climate challenges in the immediate future.

Acknowledgements

This work was supported by the Ministry of Science, Innovation and Universities (PID2023-149550OB-C31). Thanks to JF. Sánchez-Sevilla for its guidance and access to the Fragaria Germplasm Bank located at the IFAPA centre in Málaga, Spain.

Conflict of interest

The authors declare no conflict of interest.

Author details

José Ángel Mercado-Hornos and Sara Posé*
Department of Botany and Plant Physiology, Institute of Hortofruticulture Subtropical and Mediterranean 'La Mayora' (IHSM-UMA-CSIC), University of Málaga, Málaga, Spain

*Address all correspondence to: sarapose@uma.es

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Giampieri F, Forbes-Hernandez TY, Gasparrini M, Alvarez-Suarez JM, Afrin S, Bompadre S, et al. Strawberry as a health promoter: An evidence based review. Food & Function. 2015;**6**(5):1386-1398. DOI: 10.1039/c5fo00147a

[2] FAOSTAT [Internet]. 2023. Available from: https://www.fao.org/faostat/en/#home [Accessed: 8 December 2024]

[3] Ministry of Agriculture, Fisheries and Food, Spain. Season Analysis 2023/2024. Available from: https://www.mapa.gob.es/va/agricultura/temas/producciones-agricolas/analisisfrutosrojoscampana2023_2423deabril2024_tcm39-680918.pdf [Accessed: 8 December 2024]

[4] Observatorio Junta de Andalucía. Basic Data for 2021/22 Season of Strawberry. Available from: https://ws142.juntadeandalucia.es/agriculturaypesca/observatorio/servlet/FrontController?action=RecordContent&table=11113&element=4168384&subsector=& [Accessed: 30 November 2024]

[5] Voth A. El desarrollo comercial de la fresa de Huelva. In: Ciencia Regional en Andalucía: Identidad Regional y Globalización. Ed. Asociación Andaluza de Ciencia Regional, Huelva, España; 2002. p. 90. ISBN 84-607-3930-9

[6] Datos Básicos de Fresa. Campaña 2021/22. Observatorio de precios y Mercados. Conserjería de Agricultura, Pesca Agua y Desarrollo Rural. Junta de Andalucía, Sevilla, España. 2022

[7] Historical Records of Huelva Climate from Agencia Estatal de Meteorología (AEMET). Available from: https://www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos?l=4642E&k=undefined [Accessed: 15 November 2024]

[8] Bourn D, Prescott J. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. Critical Reviews in Food Science and Nutrition. 2002;**42**:1-34. DOI: 10.1080/10408690290825439

[9] Fernandes VC, Domingues VF, de Freitas V, Delerue-Matos C, Mateus N. Strawberries from integrated pest management and organic farming: Phenolic composition and antioxidant properties. Food Chemistry. 2012;**134**(4):1926-1931. DOI: 10.1016/j.foodchem.2012.03.130

[10] Jin P, Wang SY, Wang CY, Zheng Y. Effect of cultural system and storage temperature on antioxidant capacity and phenolic compounds in strawberries. Food Chemistry. 2011;**124**(1):262-270. DOI: 10.1016/j.foodchem.2010.06.029

[11] Akšić MF, Tosti T, Sredojević M, Milivojević J, Meland M, Natić M. Comparison of sugar profile between leaves and fruits of blueberry and strawberry cultivars grown in organic and integrated production system. Plants. 2019;**8**(7):205. DOI: 10.3390/plants8070205

[12] Martín Gil A, Bascón Fernández J, Alvarado Aldea P, Orta Cordero MS. Guía de Gestión Integrada de Plagas para el cultivo de Fresa y Fresón. In: Ministerio de Agricultura, Pesca y Alimentación (MAPA). Secretaría General Técnica Centro de Publicaciones Catálogo de Publicaciones de la Administración General del Estado, Gobierno de España, Madrid; 2019. ISBN 978-84-491-1471-7

[13] Márquez Domínguez JA. El marco geográfico de los campos de fresa. In: La fresa de Huelva. Junta de Andalucía, Sevilla, España. Conserjería de Agricultura y Pesca, Sevilla, España; 2008. pp. 49-102. ISBN:978-84-8474-222-7

[14] García Sinovas D, Andrade Benítez MA, Cal y Cortina A, Redondo Collado C, Becerril Polanco M, Martínez-Treceño A, et al. Los Viveros de Altura de Plantas de Fresa en Castilla y León. Horticultura global. Nova Ágora, España; 2012. Nº 302. p. 46. ISSN: 2173-5042

[15] Gavilán P, Gómez Mora JA, Molina F, Lozano D, Ruiz N, Miranda L. Optimización del Fertirriego en un Cultivo de Fresa. Córdoba: Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible. Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica; 2022. 1-16 pp

[16] Romero-Gámez M, Suárez-Rey EM. Huella ambiental producida por la fertilización en el cultivo de la fresa. VII Jornadas del grupo de Fertilización, Sociedad Española de Ciencias Hortícolas. Valencia, España: Acta Horticultura; 2019;**82**:63-66. ISBN 978-84-09-13217-1

[17] Ariza Fernández MT, Gómez Mora JA, Miranda Enamorado L, Gómez Moya JA, Medina Mínguez JJ, Martínez Ferri E, et al. Influencia del Sistema de Cultivo sobre Variedades de Fresa Campaña 2021/2022. II Calidad. Consejería de Agricultura, Pesca, Agua y Desarrollo Rural, Instituto de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica, Sevilla, España; 2022. 1-20 pp

[18] Caballero I. Castilla y León y Andalucía, la Combinación Perfecta para el Cultivo de Fresa [Internet]. 2021. Available from: https://archivo.revistaagricultura.com/fresa/frutales/castilla-y-leon-y-andalucia--la-combinacion-perfecta-para-el-cultivo-de-fresa_12837_168_16059_0_1_in.html [Accessed: 8 December 2024]

[19] Instituto Tecnológico Agrario de Castilla y León (ITACyL). Castilla y León Lidera el Cultivo de Planta de Fresa en Europa con la Exportación de Casi un Tercio de la Producción a más de una Decena de Países. 2024. Available from: https://www.itacyl.es/-/jornada-fresa-1 [Accessed: 8 December 2024]

[20] López-Aranda JM, Miranda L, Soria C, Pérez-Jiménez RM, Zea T, Talavera M, et al. Chemical alternatives to methyl bromide for strawberry in the area of Huelva (Spain): 2002-2007 results. In: VI International Strawberry Symposium. López-Medina J editor. Huelva, España. Vol. 842. 2008. pp. 957-960

[21] Neri D, Baruzzi G, Massetani F, Faedi W. Strawberry production in forced and protected culture in Europe as a response to climate change. Canadian Journal of Plant Science. 2012;**92**(6):1021-1036. DOI: 10.4141/cjps2011-276

[22] Darrow GM. The Strawberry. History, Breeding and Physiology. New York: Holt, Rinehart and Winston; 1996. 447 p

[23] Rannu RP, Ahmed R, Siddiky A, Ali ASMY, Ibn Murad KF, Sarkar PK. Effect of irrigation and mulch on the yield and water use of strawberry. International Journal of Agronomy. Wiley Online Library; 2018;**2018**:2903706, 10 pp. DOI: 10.1155/2018/2903706

[24] López-Aranda JM. El cultivo de la fresa en Huelva. In: La fresa de Huelva.

Junta de Andalucía. Conserjería de Agricultura y Pesca; 2008. p. 103. ISBN:978-84-8474-222-7

[25] Kumar S, Dey P. Effects of different mulches and irrigation methods on root growth, nutrient uptake, water-use efficiency and yield of strawberry. Scientia Horticulturae. 2011;**127**(3):318-324. DOI: 10.1016/j.scienta.2010.10.023

[26] Domínguez A, Muñoz MJ. Optimización de la fertirrigación en el cultivo de fresa en invernadero. Vida Rural. Ed. Ministerio de Agricultura, Pesca y Alimentación, España. 2011;**327**:28-32. ISSN: 1133-8938

[27] Taylor DR. The physiology of flowering in strawberry. Acta Horticulturae. 2002;**567**:245-251. DOI: 10.17660/ActaHortic.2002.567.50

[28] Heide OM, Sønsteby A. Climate-photothermographs, a tool for ecophysiological assessment of effects of climate warming in crop plants: Examples with three berry crops. Journal of Berry Research. 2020;**10**(3):411-418. DOI: 10.3233/JBR-190490

[29] Medina-Mínguez JJ, Miranda Enamorado L, Gómez Mora JA, Soria Navarro C. Estimación de la Distribución Varietal en el Cultivo de Fresa en Huelva Campaña 2022-2023. Consejería de Agricultura, Pesca, Agua y Desarrollo Rural, Instituto de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica, Sevilla, España; 2023. 1-13 pp

[30] AR6-IPCC. Intergovernmental Panel on Climate Change, 2023: Summary for policymakers. In: Core Writing Team, Lee H, Romero J, editors. Climate Change 2023: Synthesis Report. A Report of the Intergovernmental Panel on Climate Change. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland: IPCC; 2023. 36 p

[31] World Resources Institute [Internet]. 2024. Available from: https://www.wri.org/data [Accessed: 2 December 2024]

[32] Lal R. Soil carbon sequestration impacts on global climate change and food security. Science. 2004;**304**(5677):1623-1627. DOI: 10.1126/science.1097396

[33] Coleman-Jensen A, Gregory C, Singh A. Household food security in the United States in 2013. ERR-173. Ed. United States Department of Agriculture, Economic Research Service. Economic Research Report. Nº. 173. 2014. Available from: www.ers.usda.gov/publications/err-economic-research-report/err173.aspx

[34] Davis KF, Downs S, Gephart JA. Towards food supply chain resilience to environmental shocks. Nature Food. 2021;**2**(1):54-65. DOI: 10.1038/s43016-020-00196-3

[35] Taiz L, Zeiger E, Møller IM, Murphy A. Plant Physiology and Development. 6th ed. Sinauer Associates Incorporated; 2015. 761 p

[36] Hatfield JL, Prueger JH. Temperature extremes: Effect on plant growth and development. Weather and Climate Extremes. 2015;**10**:4-10. DOI: 10.1016/j.wace.2015.08.001

[37] Moretti CL, Mattos LM, Calbo AG, Sargent SA. Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review. Foodservice Research International. 2010;**43**(7):1824-1832. DOI: 10.1016/j.foodres.2009.10.013

[38] Eren O, Baran MF, Gokdogan O. Determination of greenhouse gas

emissions (GHG) in the production of different fruits in Turkey. Fresenius Environmental Bulletin. 2019;**28**(1):464-472

[39] Galafton C, Maga D, Sonnemann G, Thonemann N. Life cycle assessment of different strawberry production methods in Germany with a particular focus on plastic emissions. International Journal of Life Cycle Assessment. 2023;**28**:611-625. DOI: 10.1007/s11367-023-02167-9

[40] Gunady MG, Biswas W, Solah VA, James AP. Evaluating the global warming potential of the fresh produce supply chain for strawberries, romaine/cos lettuces (*Lactuca sativa*), and button mushrooms (*Agaricus bisporus*) in Western Australia using life cycle assessment (LCA). Journal of Cleaner Production. 2012;**28**:81-87. DOI: 10.1016/j.jclepro.2011.12.031

[41] Soode E, Lampert P, Weber-Blaschke G, Richter K. Carbon footprints of the horticultural products strawberries, asparagus, roses and orchids in Germany. Journal of Cleaner Production. 2015;**87**:168-179. DOI: 10.1016/j.jclepro.2014.09.035

[42] Warner DJ, Davies M, Hipps N, Osborne N, Tzilivakis J, Lewis KA. Greenhouse gas emissions and energy use in UK-grown short-day strawberry (Fragaria x ananassa Duch) crops. The Journal of Agricultural Science. 2010;**148**(6):667-681. DOI: 10.1017/S0021859610000493

[43] Girgenti V, Peano C, Baudino C, Tecco N. From "farm to fork" strawberry system: Current realities and potential innovative scenarios from life cycle assessment of non-renewable energy use and green house gas emissions. The Science of the Total Environment. 2014;**473-474**:48-53. DOI: 10.1016/j.scitotenv.2013.11.133

[44] Chapman A, Buck WJ. Wild Spain. London: Gurney and Jackson; 1893

[45] Mountfort G, Ferguson-Lees IJ. The birds of the Coto Doñana. Ibis. 1961;**103**(1):86-109

[46] Green AJ, Bustamante J, Janss GFE, Fernández-Zamudio R, Díaz-Paniagua C. Doñana wetlands (Spain). In: The Wetland Book. Springer Nature; 2016. pp. 1-14. DOI: 10.1007/978-94-007-6173-5_139-1

[47] Rendón MA, Green AJ, Aguilera E, Almaraz P. Status, distribution and long-term changes in the waterbird community wintering in Doñana, south–west Spain. Biological Conservation. 2008;**141**(5):1371-1388. DOI: 10.1016/j.biocon.2008.03.006

[48] de Felipe M, Aragonés D, Díaz-Paniagua C. Thirty-four years of Landsat monitoring reveal long-term effects of groundwater abstractions on a World Heritage Site wetland. Science of the Total Environment. 2023;**880**:163329. DOI: 10.1016/j.scitotenv.2023.163329

[49] Camacho C, Negro JJ, Elmberg J, Fox AD, Nagy S, Pain DJ, et al. Groundwater extraction poses extreme threat to Doñana World Heritage Site. Nature Ecology & Evolution. 2022;**6**(6):654-655. DOI: 10.1038/s41559-022-01763-6

[50] CSIC. Santa Olalla, la Laguna más Grande de Doñana, se Seca por Segundo año Consecutivo [Internet]. 2023. Available from: https://www.csic.es/sites/default/files/d7/noticias/10agosto2023_lagunasantaolalla_donana.docx.pdf [Accessed: 8 December 2024]

[51] Santamaría L, Martin-Ortega J. How Europe's most iconic wetland could be finished off by a strawberry farming bill. Nature Water. 2023;**1**(7):564-565

[52] Green AJ, Guardiola-Albert C, Bravo-Utrera MÁ, Bustamante J, Camacho A, Camacho C, et al. Groundwater abstraction has caused extensive ecological damage to the Doñana World Heritage Site, Spain. Wetlands. 2024;**44**(2):20

[53] de Felipe M, Amat JA, Arroyo JL, Rodríguez R, Díaz-Paniagua C. Habitat changes at the local scale have major impacts on waterfowl populations across a migratory flyway. Global Change Biology. 2024;**30**(12):e17600

[54] SALARTE. "Alianza Marismas del Guadalquivir-Doñana", Project for the Custody and Recovery of the Salt Marshes by NGO Fund (SALARTE) [Internet]. 2024. Available from: https://salarte.org/ [Accessed: 8 December 2024]

[55] Brummell DA, Bowen JK, Gapper NE. Biotechnological approaches for controlling postharvest fruit softening. Current Opinion in Biotechnology. 2022;**78**:102786. DOI: 10.1016/j.copbio.2022.102786

[56] Brummell DA, Harpster MH. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Molecular Biology. 2001;**47**:311-339

[57] Mercado JA, Matas AJ, Posé S. Fruit and Vegetable Texture: Role of Their Cell Walls. In: Encyclopedia of Food Chemistry. Melton L, Shahidi F, Varelis P, editors. Elsevier Inc.; 2019. pp. 1-7. DOI: 10.1016/B978-0-08-100596-5.21679-X

[58] Redgwell RJ, Fischer M. 3. Fruit texture, cell wall metabolism and consumer perceptions. In: Knee M, editor. Fruit Quality and Its Biological Basis. 1st ed. Sheffield: Sheffield Academic Press; 2002. pp. 46-88

[59] Posé S, García-Gago JA, Santiago-Doménech N, Pliego-Alfaro F, Quesada MA, Mercado JA. Strawberry fruit softening: Role of cell wall disassembly and its manipulation in transgenic plants. Genes, Genomes and Genomics. 2011;**5**(1):40-48

[60] Posé S, Paniagua C, Matas AJ, Gunning AP, Morris VJ, Quesada MA, et al. A nanostructural view of the cell wall disassembly process during fruit ripening and postharvest storage by atomic force microscopy. Trends in Food Science and Technology. 2019;**87**:47-58. DOI: 10.1016/j.tifs.2018.02.011

[61] Santiago-Doménech N, Jiménez-Bermúdez S, Matas AJ, Rose JKC, Muñoz-Blanco J, Mercado JA, et al. Antisense inhibition of a pectate lyase gene supports a role for pectin depolymerization in strawberry fruit softening. Journal of Experimental Botany. 2008;**59**(10):2769-2779. DOI: 10.1093/jxb/ern142

[62] Paniagua C, Ric-Varas P, García-Gago JA, López-Casado G, Blanco-Portales R, Muñoz-Blanco J, et al. Elucidating the role of polygalacturonase genes in strawberry fruit softening. Journal of Experimental Botany. 2020;**71**(22):7103-7117. DOI: 10.1093/jxb/eraa398

[63] López-Casado G, Sánchez-Raya C, Ric-Varas PD, Paniagua C, Blanco-Portales R, Muñoz-Blanco J, et al. CRISPR/Cas9 editing of the polygalacturonase FaPG1 gene improves strawberry fruit firmness. Horticulture Research. 2023;**10**(3):uhad011. DOI: 10.1093/hr/uhad011

[64] Ric-Varas P, Paniagua P, López-Casado G, Molina-Hidalgo FJ, Schückel J, Knox JP, et al. Suppressing the rhamnogalacturonan lyase gene FaRGLyase1 preserves RGI pectin degradation and enhances strawberry fruit firmness. Plant Physiology and

Biochemistry. 2024;**206**:108294. DOI: 10.1016/j.plaphy.2023.108294

[65] Laforest LC, Nadakuduti SS. Advances in delivery mechanisms of CRISPR gene-editing reagents in plants. Frontiers in Genome Editing. 2022;**4**:830178. DOI: 10.3389/fgeed.2022.830178

[66] Atkins PA, Voytas DF. Overcoming bottlenecks in plant gene editing. Current Opinion in Plant Biology. 2020;**54**:79-84. DOI: 10.1016/j.pbi.2020.01.002

[67] Maher MF, Nasti RA, Vollbrecht M, Starker CG, Clark MD, Voytas DF. Plant gene editing through de novo induction of meristems. Nature Biotechnology. 2020;**38**:84-89. DOI: 10.1038/s41587-019-0337-2

[68] Stokstad E. European Parliament votes to ease regulation of gene-edited crops. Science. ScienceInsider; 2024. DOI: 10.1126/science.zdjbra4

[69] VanDerZanden AM, Cameron JS. Effect of water deficit stress on 11 native Fragaria chiloensis clones selected as ornamental groundcovers. Scientia Horticulturae. 1996;**66**(3-4):241-253

[70] González PA, Parga-Dans E, Luzardo OP. Big sales, no carrots: Assessment of pesticide policy in Spain. Crop Protection. 2021;**141**:105428. DOI: 10.1016/j.cropro.2020.105428

[71] Lamichhane JR, Dachbrodt-Saaydeh S, Kudsk P, Messéan A. Toward a reduced reliance on conventional pesticides in European agriculture. Plant Disease. 2016;**100**(1):10-24. DOI: 10.1094/PDIS-05-15-0574-FE

Edited by Mateus Pereira Gonzatto and Júlia Scherer Santos

This book highlights advances in the science of fruit crops, with a focus on ecophysiology and horticultural aspects. In addition to general topics related to fruit crop production and ecophysiology, the book chapters address questions related to production, physiology, and nutraceutical aspects of tropical, subtropical, and temperate species, such as citrus, mango, palm trees, apple, strawberry, and Brazilian fruit species.

Maria Rosário Bronze, Food Science and Nutrition Series Editor

ISSN 2977-8174
ISBN 978-1-83634-420-9
9 781836 344209

Published in London, UK
© 2025 IntechOpen
© Servet Turan / iStock

IntechOpen