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New Insights into Phytohormones

Edited by Basharat Ali and Javed Iqbal

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Meet the editors

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Contents

Preface

It is with great pleasure and enthusiasm that I introduce this comprehensive book, *New Insights into Phytohormones*. As the editor, I am honored to present a collection of contributions from esteemed researchers and experts in the field of plant biology, each shedding light on the intricate and fascinating world of phytohormones. In recent decades, our understanding of plant physiology has experienced a profound transformation, largely due to advancements in molecular biology, genetics, and bioinformatics. This book captures the essence of these advancements, offering a synthesis of current knowledge and providing fresh perspectives on the roles played by phytohormones in the life of plants. Phytohormones, the signaling molecules orchestrating various aspects of plant growth, development, and response to environmental stimuli, serve as the protagonists of this exploration. Each chapter delves into the specific functions and mechanisms through which auxins, gibberellins, cytokinins, abscisic acid, ethylene, brassinosteroids, and jasmonic acid exert their influence on plant physiology. The contributors bring forth their expertise, presenting cutting-edge research findings and critical analyses that illuminate the nuanced interplay of these molecules within the cellular and molecular tapestry of plant life. This volume is designed to cater to a diverse audience, including researchers, educators, and students, providing a valuable resource for both the seasoned expert and the curious novice. I hope that this book will not only serve as a reference for those deeply engaged in the field but also inspire a new generation of scientists to explore the uncharted territories of phytohormonal regulation in plants. As we navigate through these pages, I encourage readers to contemplate the implications of our evolving understanding of phytohormones. The insights shared within these chapters have the potential to shape future research directions, influence agricultural practices, and contribute to our broader understanding of the interconnectedness of life on our planet. I extend my gratitude to the contributing authors for their dedication and scholarly contributions, as well as to the readers who embark on this journey of discovery. May this book be a beacon of knowledge, sparking curiosity and fostering a deeper appreciation for the remarkable role phytohormones play in the intricate dance of plant life.

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Chapter 1

Role of Abscisic Acid in Plant Stress

Rahul Sharma and Priyanka Sharma

Abstract

The multifaceted role of Abscisic acid (ABA) as a phytohormone of great repute cannot be overstated. ABA right after its synthesis within plastids embark on a quest to find specific receptors. On binding these receptors a complex signaling cascade is triggered that ultimately modulates gene expression and other cellular processes, responsible for normal growth and development processes of plants. Under abiotic and biotic stresses ABA levels change tremendously, triggering a cascade of physiological responses that help the plant adapt to its environment. A deeper understanding of ABA's mechanisms like understanding its metabolic pathways or its regulation at genetic and epigenetic levels hold the promise of enhancing crop productivity and resilience in the face of the daunting challenges posed by a changing climate. Use of gene editing techniques like CRISPER-Cas technology, regulating the ABA mediated stress responsive genes, using RNAi and modifying the intragenic and promoter regions of the genes involved in ABA biosynthesis are a few methods which can enhance the ABA production or ABA mediated response to tolerate the stress conditions. In essence, ABA is a paramount player in plant stress responses, and unlocking its mysteries holds the potential to revolutionize agriculture and safeguard food security.

Keywords: abscisic acid, phytohormone, abiotic stress, epigenetic changes, stress tolerance, transcription factor

1. Introduction

The continuously growing world population is expected to reach about nine billion by 2050. This situation may pose a significant challenge to meet the increasing food demands by our already struggling agriculture industry (**Figure 1**). Factors such as water scarcity, soil degradation, and a variety of biotic and abiotic stresses further worsen the situation, affecting agricultural productivity. These stresses have detrimental effects on plant growth, development, and reproductive processes, leading to substantial crop losses.

However, research on Abscisic acid (ABA) has emerged as a promising avenue to mitigate the detrimental effects of these stresses. ABA, a lipid hormone, find its role in regulating seed dormancy, leaf abscission, and the accumulation of nutrient reserves in seeds [1]. Studies have shown that ABA levels increase significantly during periods of water shortage, leading to stomatal closure and reduced water loss through transpiration [2]. This hormone helps plants to cope with drought, high salinity, oxidative stress, photo stress, low temperature, and other environmental stresses. Understanding various mechanisms and functions of ABA has the potential to enhance crop resilience and improve agricultural productivity in the face of abiotic stresses.

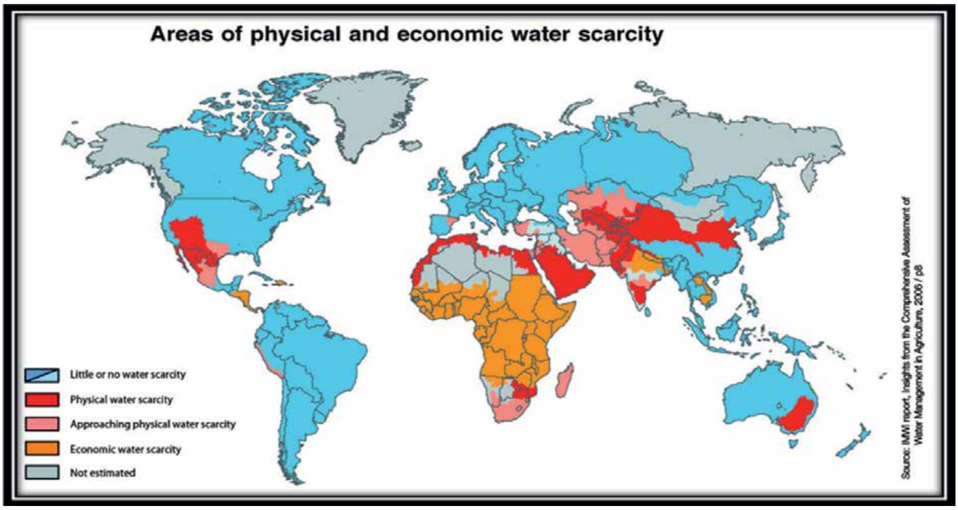

Figure 1.
Global map of physical and economic water scarcity areas. Image source: IWMI.

ABA is also known to interplay with other hormones, such as Gibberellins, Auxins, Cytokinins, Jasmonic acid, Salicylic acid etc. to influence plant growth and development. Jasmonic acid and Salicylic acid are the phytohormones which modulate the plant's defense against biotic stresses like action of pathogens, insects, pests and herbivores. By understanding the role of ABA and its interactions with other hormones, researchers can develop novel approaches to boost crop resilience and alleviate the detrimental effects of abiotic and biotic stresses [3].

By investigating the role of ABA and elucidating its mechanisms of action, researchers are paving the way for innovative strategies to mitigate the harmful effects of abiotic stresses and improve crop productivity. Thus, the study of ABA and its outcome on plant responses to environmental stresses ensure for addressing the challenges faced by agriculture. In the present chapter, various strategies employed by the plant to cope up with these stress situations will be discussed. Further, strategies to improve stress tolerance in plants will also be considered.

2. Abscisic acid (ABA): a crucial phytohormone

2.1 Background of ABA

Abscisic acid (ABA) was first discovered in the 1960s by a team of researchers led by Frederick Addicott at the University of California, Davis. The team, while studying the physiological processes involved in fruit abscission, found a compound that triggered this process [4]. Initially referred to as "abscisin II" as it was the second compound to be identified in the abscission process, it was later renamed "abscisic acid" due to its acidic properties and involvement in various physiological processes beyond fruit abscission [5]. Since its discovery, ABA has been extensively studied for its role in plant growth and stress responses [2]. Soon after its discovery, it was revealed that ABA levels increase significantly when plants wilt and that ABA causes stomatal closure. These two findings highlighted the utility of ABA in environmental stress mediating responses plants. Later, it was revealed that ABA is also associated with accumulation of seed nutrient reserves and attainment of desiccation tolerance in seeds. Regardless of its name, ABA is not a major regulator of abscission, which is mainly controlled by ethylene [6].

Plant stress response is a highly complicated process involving numerous genes to act in sync and interact with one another during the process. Stress induced gene products regulate the biosynthesis of the well known plant growth regulators like ABA, salicylic acid, and ethylene, which can then start the second round of signaling. In this mechanism, small molecules like ABA are crucial [7]. During the past 40 years, the core components of ABA biosynthesis and signaling have been identified through molecular, genetic, biochemical, and pharmacological approaches.

2.2 ABA structure

ABA is a sesquiterpenoid compound with a complex chemical structure, containing a 15-carbon isoprenoid chain and a carboxyl group at one end, which modulates its acidic properties [4]. ABA holds several functional groups, including hydroxyl (-OH), ketone (=O), and carboxylic acid (-COOH) groups, which contributes to its biological activity (**Figure 2**).

ABA's molecular structure has various important features that make its biological functions possible. The side chains with the two double bonds (**Figure 2**) and ABA's stereocenter are two such chief properties. It is the stereocenter of the ABA which is responsible for its chirality and existence of ABA in two mirror image forms called as enantiomers. Due to differing spatial orientations, enantiomers are engaged in various biological interactions and functions. Exposure to ultraviolet (UV) light changes ABA's conformation from active to inactive form. This can be due to due to photoisomerisation where rearrangement of atoms within molecule leads to different conformations. Exposure to UV radiations also lead to crosslinking of ABA with neighboring ABA molecule, reducing its conformational flexibility. However, prolonged UV exposure may lead to cleavage of chemical bonds within ABA molecules which may lead to its fragmentation or loss of Functional groups and loss of biological activity.

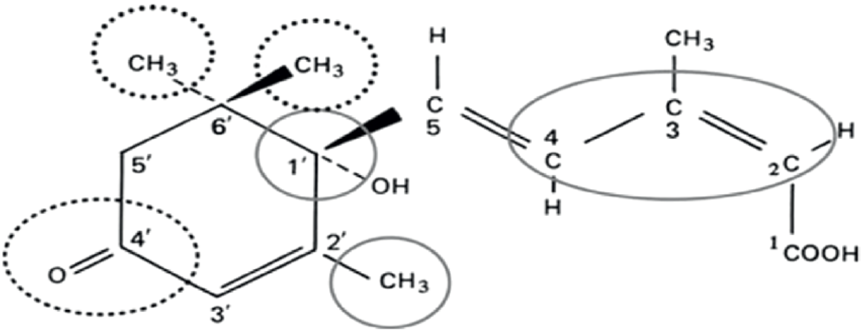

Figure 2.
Structure of abscisic acid (a phytohormone). Adapted from Finkelstein [6].

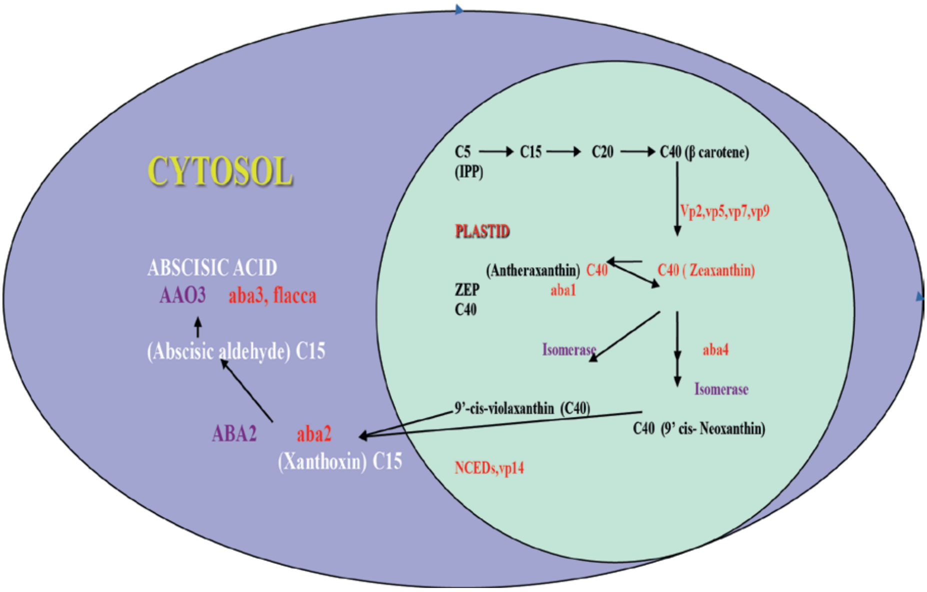

Figure 3.
Abscisic acid (ABA) biosynthetic pathway.

2.3 Biosynthetic pathway of ABA

ABA is synthesized mostly in plant chloroplast and plastid-containing cells [6]. However, it is also produced in animals including humans and fungi. It is a sesquiterpene (C15H24) manufactured from isopentenyl pyrophosphate (IPP) in the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway or Mevalonic acid pathway. IPP is firstly converted to Zeaxanthin through a serious of enzymatic reactions involving condensation of IPP with Dimethylallyl pyrophosphate (DMAPP) producing Geranylgeranyl pyrophospahte (GGPP). GGPP is further converted to Zeaxanthin through cyclization and hydroxylation reactions. Neoxanthin is created from IPP with intermediate products of zeaxanthin and violaxanthin via an intermediate (antheraxanthin) in plastids. All-trans-neoxanthin, all-trans-violaxanthin and 9-cisneoxanthin can proceed as precursors for xanthoxin in ABA synthesis. Subsequently, xanthoxin is oxidized to ABA aldehyde and then to ABA in the cytosol through various intermediate enzymatic reactions. The complex chemical structure of ABA and its involvement in stress responses have made it a subject of extensive research in the field of plant biology (**Figure 3**).

Understanding the mechanisms underlying ABA synthesis and signaling pathways can have significant implications for improving crop yield and stress tolerance in plants. In the subsequent sections, we will focus on the role of ABA in plant growth, development and response to stress.

3. Role of ABA in plant growth and development

ABA plays a vital role in the general growth and development of plants by regulating various physiological processes. Here are some of the key functions of ABA in plant growth and development.

3.1 Seed dormancy

Seed dormancy is a crucial and essential process for plant survival and adaptation [8]. Seed dormancy can be considered as a stress tolerance response of the plant. It ensures that seeds remain dormant until favorable conditions for germination are present, increasing the likelihood of successful seedling establishment. This precise timing of germination is regulated by a complex network of genes and proteins. However, ABA is a key regulator to this process [9]. ABA accumulation in seeds promotes and maintains dormancy, while a decline in ABA levels triggers dormancy release and germination. Transcription factors, including Phytochrome Interacting Factors (PIFs), contribute to dormancy regulation. PIF1 interacts with ABA signaling components and controls the expression of genes involved in ABA biosynthesis and signaling. This interaction influences the timing of dormancy release and germination [8, 10].

The process of ABA induced seed dormancy involves the recognition of ABA by receptors called PYR/PYL/RCAR (Pyrabactin Resistance/PYR1-like/Regulatory Component of ABA Receptor) in the plasma membrane [2]. When ABA binds to these receptors, it triggers a series of signaling events. One of the key outcomes is the inhibition of protein phosphatases called PP2C (Protein Phosphatase 2C), which normally suppress the dormancy pathway [11, 12]. By inhibiting PP2C, ABA allows SnRK2 (Sucrose Non-Fermenting 1-Related Protein Kinase 2) to become active. These kinases phosphorylate ABF/AREB (ABA-Responsive Element Binding/ABA Response Element Binding) transcription factors, which then move to the nucleus and bind to specific DNA elements namely ABA-responsive elements (ABREs), activating or repressing genes related to seed dormancy and stress responses [13]. Apart from this receptor-mediated signaling, ABA also induces the production of LEA (Late Embryogenesis Abundant) proteins that help protect seeds from desiccation and other stresses during development and storage [14].

The coordination of various genes and proteins, such as ABA, ABI (Abscisic Acid Insensitive) proteins, DOG (Delay Of Germination) proteins, LEC (Leafy Cotyledons) genes, PIFs, and WRI1 (Wrinkled1), are crucial for seed dormancy regulation. This means that at higher concentrations of ABA, the activated ABF/ABRE gets associated with the above mentioned proteins and activates genes responsible for dormancy. However, on the onset of favorable conditions like light, moisture etc. ABA levels decline resulting a decrease in its inhibitory effects on germination. This allows ABF/AREB to become more active and able to attach strongly to the specific parts of genes involved in germination. The modified ABF/AREB moves into the nucleus and binds to specific regions in the genes called ABA-responsive elements (ABREs). In this situation, the modified ABF/AREB turns on genes that are needed for seedling growth, including genes that help break down stored nutrients, promote cell growth, and carry out other important processes for germination. This process can be aided by certain co-activators like CBP (CREB binding proteins); transcription factors NAC (NAM, ATAF1/2, CUC2) and chromatin remodeling factors like SWI/SNF (Switch/Sucrose Non-Fermentable). Understanding these mechanisms has significant implications for crop improvement and seed management strategies. By manipulating these genes and proteins, it is possible to develop crops with optimized germination characteristics [8, 9].

3.2 Seed germination

ABA helps to maintain seed dormancy by inhibiting the growth of the embryo until environmental conditions are favorable for germination. When conditions are suitable, ABA levels decrease, and the embryo begins to grow. ABA also has a part in changeable seed dormancy and seed germination with its interface with another plant hormone called *gibberellins*. Gibberellins are the hormones liable for signaling and stimulating remarkable increases in plant size, particularly in fruits and stems of plants. Both ABA and Gibberellins have reverse effects; plant hormones with opposite effects contribute to the balance within a plant obligatory for homeostasis. ABA also prevents loss of seed dormancy. Several ABA-mutant *Arabidopsis thaliana* plants have been identified and are available from the Nottingham *Arabidopsis* Stock Centre– deficient in ABA production and with altered sensitivity to its action. Plants that are hypersensitive or insensitive to ABA show phenotypes in seed dormancy, germination, stomatal regulation, and some mutants show stunted growth and brown/yellow leaves. These mutants reflect the importance of ABA in seed germination and early embryo development.

3.3 Fruit ripening

ABA plays quintessential role in regulating the ripening of fruit by promoting the breakdown of chlorophyll and the synthesis of pigments that give fruit its characteristic color. The changes in the levels of ABA concentration from very low in unripe fruits to tremendously high during fruit ripening depicts the ABA role in regulating fruit ripening.

In climacteric fruit such as apples, the level of ABA increases from maturation to harvest, while in non-climacteric sweet cherries, the level of ABA increases before maturation and thereafter decreases until harvest. Endogenous signals and environmental factors might affect ethylene biosynthesis primarily through ABA biosynthesis [15]. The differing ABA levels suggest that the role of ABA may vary between fruits. The application of ABA on fruit might be an effective tool for improving fruit quality and increasing health benefits. Fruit ripening being a complex process, sees dramatic changes in color, texture, flavor, and aroma of a fruit. There is much evidence that shows the role of ABA in fruit ripening and its involvement with fruit quality.

3.4 Root growth

Roots are crucial for the normal growth and development of plants. However, the architecture of the roots is significantly modified at the onset of environmental stresses. ABA controls root growth by preventing cell division and elongation at the root tip. This helps in regulating the root system's depth and spread in response to environmental cues. Thus, it can be concluded that ABA influences root architecture by inhibiting root growth and promoting the formation of lateral roots, enabling plants to explore the soil for water and nutrients [16]. Depending on its dosage, ABA can either promote or hinder root growth. Even in well-watered environments, ABA can affect the root as well as shoot growth. ABA shows a biphasic effect on the growth and development of roots. Under favorable conditions, ABA is present in low concentrations, and it promotes root growth by enhancing cell division and elongation by acting in conjunction with auxin. However, under severe water stress conditions, the concentration of endogenous ABA rises and shows negative effects on the growth of roots. The modest biphasic effects of dry soil on root growth were enhanced by water deficit but hindered by severe water deficiency. Furthermore, Li et al. [17] showed that exogenous ABA had complex biphasic effects on root growth in well-watered settings also.

Changes in the root environment will consequently affect ABA-mediated responses on a local and systemic level [18]. Abiotic stress agents like drought, salt salt concentration and osmotic stressor lead to increased osmotic stress in roots. The fact that ABA increases water flow and ion flux in root tissues indicates that it modulates turgor by both boosting water influx into roots and decreasing transpiration [19].

4. Plant stress biology and role of ABA

Environmental changes are known to pose stress-like conditions for living organisms. Environmental stress can be classified as biotic or abiotic. The impact and intensity of the stress are two major criteria for living organisms to prompt the necessary response. Plants, being sessile, are readily prone to environmental stresses, which significantly affect plant growth and productivity in both natural environments and agricultural settings. Therefore, it is crucial to understand the different types of plant stresses and their underlying causes in order to develop effective strategies to mitigate their negative impacts.

4.1 Abiotic stress

Abiotic stress is the term used to describe the non-living components of environmental elements. These stressors can affect the plants negatively by impairing their growth, development and productivity. Plants must rapidly adapt to different abiotic stress conditions, such as drought, oxidative stress, salinity, cold and high temperature stress, as they are immobile. This sedentary life style makes them more vulnerable to effects of environmental stresses as compared to mobile organisms. To survive, plants have evolved various strategies to deal with these challenges [20]. As studied earlier ABA plays a significant role in facilitating plant responses to various abiotic stresses. By controlling physiological and molecular processes, ABA aids in plant adaptation and resilience in the face of challenging environmental conditions like drought, high temperatures, salinity, and extreme cold. Some major abiotic stresses where ABA has significant role are discussed in the subsections.

4.1.1 Drought stress

Drought stress is one of the most prevalent forms of plant stress, characterized by limited water availability. Drought can be defined as a situation when plant does not get sufficient water required for maximum growth and productivity [21, 22]. Water is an important component of plant's transport system, as it helps in transfer of metabolites from one part of the plant to other. It also helps in maintaining the turgor pressure in the plant that allows the plant to grow in upright direction [23]. Plants use many strategies of drought avoidance, like possessing deep roots to increase the area of root zone effect [24], conservation of water by decreasing the total number of stomata, leaf widening, decreasing the leaf size, etc. (**Figure 4**). When drought stress is applied, the root system has been observed to adjust to the stress by aligning toward the wet patches in the pot [25]. Plants use many strategies to deal with unfavorable drought stress but a complete reprogramming of the transcriptional system is mainly required to show tolerance [26]. Since plants do not have a nervous system, therefore, hormones act as intraorganismal messengers, impacting the signal transduction, to make plants to deal with environmental changes. Decrease in water content can activate a set of physiological processes and bring about change in the localization of phytohormones, as adaptive response to osmotic stress [27].

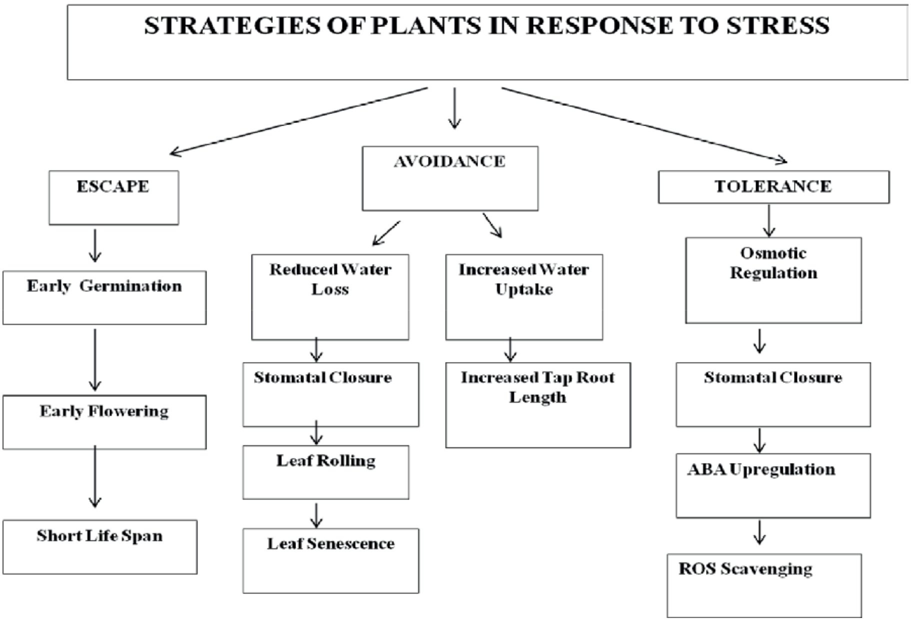

Figure 4.
Plants strategies in response to stress.

Abscisic acid is a well characterized plant hormone, which plays a very important role during environmental catastrophes. Transpiration can be problematic for the plant during the drought stress. Stomatal closure and leaf wilting are the ways to diminish the effect of transpiration and save water. Abscisic acid plays a crucial role in stomatal closure and reduction in photosynthesis which in turn is one of the most important plants' response against drought stress.

Abscisic acid accumulation has been a vital characteristic of drought tolerant plants as compared to drought sensitive plants [28]. Several studies have been conducted to check the endogenous level of ABA in stress affected plants. Exogenous spray of ABA was also found to enhance the drought tolerance in Bermuda grass [29].

4.1.1.1 ABA and stomatal closure

ABA helps to regulate the opening and closing of stomata, which are pores on the surface of leaves that allow for gas exchange. In response to drought or other environmental stresses, ABA triggers the closure of stomata, which helps to conserve water. This system regulates the absorption and release of certain gasses such as carbon dioxide, oxygen, and water vapor. Stomatal opening and closing occurs as a result of turgor pressure differences in the surrounding guard cells. ABA is formed in several plant parts, one of them being within the roots of plants. Roots produce ABA in response to identification of low or no moisture in the soil. ABA is then transferred from the roots to the leaves. ABA decreases the turgor of the guard cells causing them to close. This reduces plant transpiration, or the evaporation of water out of the stomata of leaves, thus prevents water loss and wilting in times of low moisture or drought. Furthermore, plants use stomata to absorb carbon dioxide out of the atmosphere which the plant needs to carry out photosynthesis. If the plant already has sufficient carbon dioxide, ABA signals the plant to close its stomata. The discovery and functional characterization of ABA-dependent candidate genes responding to drought was accelerated by recent developments in plant genomics. It was discovered that the MATE transporter gene AtDTX50 is implicated in ABA efflux and that mutants of dtx50 exhibit increased drought tolerance with decreased stomatal conductance in comparison to WT (wild type) plants [30].

4.1.1.2 Regulation of drought tolerance and ABA

Plants must consistently adjust ABA levels in response to shifting physiological and ecological situations. The synthesis of ABA is tightly regulated by various environmental factors, such as drought stress, which can induce the expression of genes involved in ABA biosynthesis [31]. The methods for raising ABA levels are still not fully understood. Both ABA-dependent and ABA-independent systems are involved in how plants respond to stress (**Figure 5**). To increase resistance to drought and salinity stress, a full understanding of how TF (transcription factors) pathways and ABA interact to generate stress responses is necessary. It is known that a number of transcription factors, including DREB2A/2B (Dehydration-Responsive Element Binding), AREB1, RD22BP1(Responsive to Dehydration 22 Binding Protein 1), MYC/MYB (Myelocytomatosis/ Myeloblastosis), and others, regulate the expression of the ABA-responsive genes by interacting with the corresponding cis-acting elements, DRE/CRT (Dehydration-Responsive Element/Cis-acting Regulatory Elements), ABRE (Abscisic Acid Responsive Element), and MYCRS/MYBRS (Myc-Responsive Cis-Element/Myb-Responsive Cis-Element), respectively. It's important to comprehend these systems to improve crop plants' ability to withstand stress.

Abscisic acid responsive elements (ABREs) are short DNA sequences (consensus sequence ACGTG (G/T) (G/T) C) found in the promoter regions of many genes that are involved in ABA signaling pathways. ABREs are recognized by transcription factors, such as ABRE-binding factors (ABFs), which can bind to and activate gene expression. The role of ABREs in ABA signaling is to mediate the transcriptional response of genes to ABA. When a plant is exposed to stress conditions, such

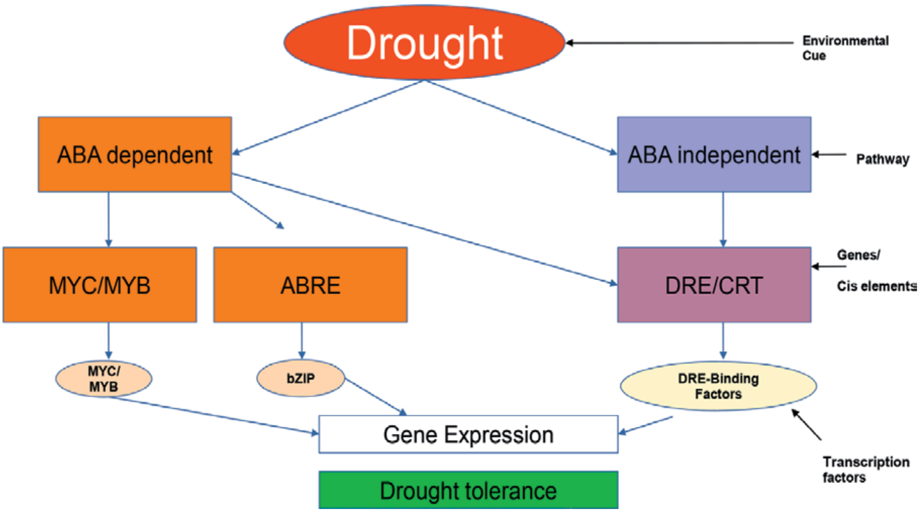

Figure 5.
ABA dependent and ABA independent signaling in plants.

as drought or high salinity, the concentration of ABA in the plant increases. This increase in ABA concentration leads to the activation of ABFs, which in turn bind to ABREs and induce the expression of genes that are involved in stress responses, such as those that encode for osmoprotectants and stress-related enzymes.

ABREs have been found to be involved in the regulation of many genes that are involved in stress responses in various crops, including rice, wheat, maize, and soybean. The discovery and characterization of ABREs have provided insight into the molecular mechanisms underlying ABA-mediated stress responses in plants. Studies in the promoter region of the *Arabidopsis thaliana* RD29A gene and rice OsRab16A gene contains contain one and three functional ABRE respectively, essential for the ABA-mediated induction of RD29A expression in response to dehydration stress and OsRab16A expression in response to salt stress [32, 33]. Similar studies in wheat have shown that the promoter region of the wheat TaERF1 gene contains multiple ABREs that are involved in the ABA-mediated induction of TaERF1 expression in response to drought stress [34].

Studies have further revealed that Farnesyl-transferase has some role in reducing the abscisic acid sensitivity of the plants; it shows negative correlation with the activity of abscisic acid. Therefore, it has been proposed that down regulation of farnesyl-transferase can help in increasing drought tolerance. In *Arabidopsis*, the down regulation of any subunit α- or β, of farnesyl-transferase, increases drought tolerance [35]. In canola, the down regulation of farnesyl transferase (α-subunit) by using drought responsive promoter of At HPR1 (*Arabidopsis* hydroxypyruvate reductase), driving an RNAi construct, resulted into enhanced closure of stomata thereby, minimizing the loss of water by transpiration and yield protection against drought in fields. The strategy can be used for enhancing drought tolerance in many crops [36].

4.1.2 Salinity stress

Salinity stress occurs when plants are exposed to high levels of salt in the soil or water, disrupting the osmotic balance within plant cells. This disrupts water uptake and causes ion toxicity, leading to reduced crop yield and limited cultivable lands [37]. According to a report by the Food and Agriculture Organization (FAO), approximately 20% of irrigated agricultural lands worldwide encounter salinity issues, leading to diminished crop productivity and economic setbacks (FAO, 2020) [38]. Salinity stress negatively impacts multiple facets of plant physiology, encompassing water balance, ion homeostasis, and gene expression [16]. To counteract the deleterious effects of salinity stress, plants have developed intricate mechanisms. Among these mechanisms, abscisic acid (ABA) emerges as a pivotal participant, acting as a phytohormone that regulates plant growth, development, and responses to stress. Salinity stress influences the biosynthesis of ABA, resulting in heightened ABA levels in various plant tissues, including roots and leaves [39].

Studies have shown that exogenous application of ABA can improve salt stress tolerance in various crops, such as wheat, maize, and rice. ABA application in Wheat plants exposed to salt stress, improved plant growth, reduced ion toxicity, and enhanced the activities of antioxidant enzymes, leading to reduced oxidative stress and improved salt stress tolerance [40]. Furthermore, genetic manipulation of ABA biosynthesis and signaling pathways has been used to enhance salt stress tolerance in crops. Over expression of genes involved in ABA biosynthesis, such as NCED (9-cis-epoxycarotenoid dioxygenase), and genes involved in ABA signaling, such as ABF (ABA-responsive element-binding factor), has been shown to enhance salt stress tolerance in *Solanum lycopersicum* L. [41].

Modulation of ABA levels and signaling pathways represents a promising approach for improving salt stress tolerance in crops. Elevated ABA levels serve as a signal for plants to initiate adaptive responses. ABA regulates stomatal closure, reducing water loss through transpiration, thereby aiding in water conservation and cellular hydration [4]. Moreover, it influences ion transport, facilitating the uptake of vital potassium (K+) ions while impeding the entry of toxic sodium (Na+) ions into plant cells, thus maintaining ion homeostasis [39].

At the molecular level, ABA acts as a transcriptional regulator, modulating the expression of stress-responsive genes. It activates genes encoding proteins such as late embryogenesis abundant (LEA) proteins and osmoprotectants, which play crucial roles in safeguarding plant cells against dehydration and preserving cellular integrity [16]. ABA regulates the expression of genes encoding antioxidant enzymes, counteracting the harmful effects of reactive oxygen species (ROS) generated during salinity stress [39]. ABA-mediated responses to salinity stress extend beyond cellular processes and encompass overall plant physiology and development. Additionally, ABA governs seed germination and dormancy, ensuring that germination occurs under favorable conditions while preventing premature seedling emergence in saline environments [39]. Thus it can be conclude ABA plays a central role in plants' response to salinity stress by regulating stomatal closure, ion transport, gene expression, root architecture, and seed physiology. Understanding the mechanisms underlying ABA-mediated responses is vital for developing strategies to enhance salinity tolerance in crop plants and mitigate the adverse impacts of salinity stress on agricultural productivity.

4.1.3 Heavy metal stress

Some micro-nutrients essential for plant growth and development are heavy metals (HM), which are naturally occurring elements with large atomic weight and density. Excessive HM concentrations, however, adversely impact plant development and survival. Because of anthropogenic activities like industrialization, smelting, extensive mining, fertilizer application, polluted water irrigation, fossil fuel combustion, and vehicle emitted gasses and pollutants, the intensity of some heavy metals like Cd, Cu, Pb, Hg, and Cr is skyrocketing in agricultural and other natural areas [42]. Abiotic stress, mostly brought on by heavy metal toxicity, has harmful impacts on humans, plants, and animals' health. Due to their high reactivity, they have a negative impact on the energy synthesis, growth and senescence processes. Elevated HM levels increase ROS generation, causing metabolic imbalance, disturbing ion homeostasis, disorganizing the antioxidant defense system, disrupting protein structure, pigment synthesis, enzyme activities and membrane integrity, increasing lipid peroxidation, and thus decreasing plant growth and productivity [43].

In essence, plants under HM stress evolve defense systems to mitigate its negative effects but not to prevent them. Yet, through boosting osmolyte accumulation and antioxidant machinery, exogenous ABA treatment is a strategy for improving plants' resistance to HM toxicity. ABA regulates a number of physiological functions to assist plants in surviving the detrimental effects of HM stress [44]. ABA modifies the transfer of hazardous metals from roots to shoots. It inhibits the long-distance transmission of HM by closing stomata and decreasing transpiration rate [45]. Some plant species under cadmium (Cd) stress, including *Oryza sativa L., Solanum tuberosum L., Brassica napus L., Triticum aestivum L.*, and others, activate genes involved in ABA production, increasing endogenous AB concentration [44, 45]. For instance, 10 and 20 mM foliar ABA spray increased growth, chlorophyll content, photosynthetic efficiency, catalase activity, and proline and protein levels in *Lactuca sativa* L. to reduce Cd inhibitory effects [46]. According to Deng et al. [47], treatment of 5 mM ABA reduced root damage caused by Cd by reducing cell death, hydrogen peroxide levels, and malondialdehyde concentrations. Moreover, through increasing chlorophyll content, osmolyte concentration, and antioxidant defense mechanisms, 10 mM foliar ABA action reduced Cd toxicity in *Vigna radiata* L. [48]. It is confirmed that ABA is essential for improving agricultural plant morphology, development, yield, and quality indices as well as reducing the risks associated with HM stress [42].

4.1.4 Temperature stress

Temperature stress encompasses both heat stress and cold stress. High temperatures can damage plant cells, impair photosynthesis, and accelerate water loss. Conversely, low temperatures can cause chilling injury, decrease metabolic activity, and affect nutrient uptake [49].

Cold stress presents a notable obstacle to the growth and development of plants, leading to decreased crop productivity and subsequent economic losses [50]. Low temperature causes cellular damage, disrupt vital physiological processes, and impair photosynthesis [51]. On the exposure to cold temperature, ABA synthesis and accumulation is triggered in plant tissues. ABA acts as a signaling molecule, initiating a cascade of physiological and molecular changes aimed at enhancing cold tolerance [52]. ABA regulates gene expression by activating specific stress-responsive genes involved in cold acclimation and the synthesis of protective proteins [53]. This activation helps plants adapt to the cold environment and mitigate cellular damage. One of the key roles of ABA in response to cold stress is the modulation of osmotic stress. ABA promotes the accumulation of cryoprotectants, such as soluble sugars and proteins, which safeguard cellular structures from freezing temperatures [54]. These cryoprotectants act as osmolytes, maintaining cellular hydration and preventing the formation of ice crystals that could damage cells.

Cold stress triggers ABA accumulation in guard cells, leading to stomatal closure and reducing water loss [55]. This process helps maintain cellular hydration, prevents excessive dehydration, and preserves cellular integrity. Furthermore, ABA interacts with other signaling molecules and hormones to coordinate the plant's response to cold stress. These interactions contribute to the enhancement of cold tolerance in plants. Understanding the molecular mechanisms underlying ABA's role in cold stress response is crucial for developing strategies to enhance plant tolerance and improve crop productivity. The identification of ABA signaling components and downstream target genes has provided valuable insights into the intricate regulatory networks involved in cold stress response [56].

High temperature is majorly associated with accelerated water loss and for such type of osmolytic stress, ABA accumulation has been observed as the key strategy of the plants to overcome its detrimental effects. Therefore, it can be concluded that temperature stresses namely heat stress and cold stress affects the osmotic balance of the plants in general. And, plants have evolved a common mechanism of ABA biosynthesis and signaling which leads as a plant's response to the temperature stresses.

4.1.5 Oxidative stress

Oxidative stress occur when there is an imbalance between the production of Reactive oxygen species (ROS) and the antioxidant defense system in plants. ROS, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are natural byproducts of various metabolic processes. Though ROS serve as signaling molecules in plant growth and development, their excess accumulation can lead to oxidative damage to cellular components [57]. The effects of oxidative stress on plants are diverse and can impact various physiological and biochemical processes. For instance, oxidative stress disrupts membrane integrity, resulting in lipid peroxidation and loss of cellular homeostasis [58]. It can also impair photosynthesis by damaging the photosynthetic machinery, including chlorophyll and photosystem proteins [59]. Moreover, oxidative stress can cause DNA damage and affect the stability and functionality of proteins [60].

To counteract the detrimental effects of oxidative stress, plants have evolved complex antioxidant defense systems. These systems include enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and peroxidases, as well as non-enzymatic antioxidants like ascorbic acid (vitamin C), glutathione, and tocopherols (vitamin E). These antioxidants scavenge ROS and protect cellular components from oxidative damage [61]. In relation to oxidative stress, abscisic acid (ABA) has been found to play a crucial role in plant responses. ABA treatment has been shown to enhance antioxidant defense mechanisms and mitigate oxidative damage, thus improving plant tolerance to oxidative stress [62]. ABA regulates the expression of antioxidant genes and increases the activities of antioxidant enzymes leading to reduced ROS accumulation and lipid peroxidation [63]. Additionally, ABA promotes the accumulation of non-enzymatic antioxidants, such as ascorbate and glutathione, which contribute to enhanced ROS detoxification and protection against oxidative stress [64]. Understanding the relationship between ABA and oxidative stress is crucial for developing strategies to enhance plant stress tolerance and improve agricultural productivity. Continued research in this field will provide further insights into the molecular mechanisms underlying plant responses to oxidative stress and aid in the development of innovative approaches to mitigate its negative impacts.

4.1.6 Photo-stress

Photo-stress refers to the harmful effects caused by excessive light exposure in plants, leading to physiological and biochemical imbalances that can impact plant growth, development, and survival. Plants have developed adaptive mechanisms to cope with photo-stress, and one important factor in this response is abscisic acid (ABA). ABA acts as a signaling molecule and stress hormone, helping plants adapt to high light intensity and counteract its adverse effects. Abscisic acid deficient plants may arise due to mutation or epigenetic regulation at any level, in the abscisic acid pathway. In *Arabidopsis*, BCH1/BCH2 (Brassinosteroid insensitive 3) mutants showed a decrease in the production of zeaxanthin which encompasses a small proportion of β-carotene pool in the leaves. Zeaxanthin is a photo protective material and increases in quantity in response to sudden high light intensity via xanthophyll cycle. Zeaxanthin epoxidase (ZEP), a plastid imported protein located at ABA1 (AT5G67030) locus of *Arabidopsis*, shows spatial regulation in different species. Drought induced ZEP expression was observed in case of roots but absent in leaves of tomato [29, 30]. In high light conditions, ABA triggers the closure of stomata, reducing water loss through transpiration and preventing dehydration. This adaptive response allows plants to conserve water and maintain cellular integrity during periods of intense light exposure. Moreover, ABA also plays a role in regulating the antioxidant defense systems in plants. Excessive light exposure during photo-stress can generate reactive oxygen species (ROS) that cause oxidative damage to plant cells. ABA enhances the activity of antioxidant enzymes and promotes the synthesis of antioxidants, enabling the scavenging and neutralization of ROS. This protective mechanism helps shield plant cells from oxidative stress caused by excessive light. Extensive research has been conducted to understand the involvement of ABA in plant responses to photo-stress.

4.2 Biotic stress

Plants, with their sedentary lifestyle, face unique challenges when it comes to coping with biotic stress compared to animals. While animals can move to escape threats or actively defend themselves, plants being rooted in place, making them vulnerable to various pests and pathogens. Biotic stress in plants, which includes the attack of pests, pathogens, and herbivores, can have far-reaching consequences on plant growth, development, and overall fitness. Biotic stress can disrupt ecological balance by affecting plant populations and interactions with other organisms. The loss of a dominant plant species due to pest infestation can lead to changes in community structure and species composition, impacting ecosystem functions and services.

Disruption of photosynthetic machinery leads to reduced photosynthetic efficiency which is one of the most common consequences of Biotic stress. Pests and pathogens may damage chloroplasts, interfere with photosynthetic pigments, or induce the production of reactive oxygen species (ROS), thereby impairing the plant's ability to convert sunlight into chemical energy. Insects and pathogens on the other hand can feed on plant tissues, leading to tissue loss, reduced fruit set, or even complete crop failure. The economic impact of yield losses due to biotic stress is substantial, affecting farmers' income and food security at both local and global scales. Certain pests like nematodes can cause root galling, affecting nutrient uptake, while fungal pathogens can colonize the root system and disrupt nutrient transport pathways. Nutrient deficiencies can further weaken the plants, making them more susceptible to additional stressors.

4.2.1 Biotic stress and phytohormones

In response to these stressors, plants have evolved refined defense mechanisms and signaling pathways to mitigate the damage caused by biotic stress. These mechanisms involve both physical barriers and intricate signaling networks. Interestingly, while biotic stress can have negative consequences, it can also trigger Induced Systemic Resistance (ISR) in plants. ISR is a defense mechanism where localized stress responses in one part of the plant activate systemic defense responses throughout the entire plant. This response helps plants to ward off subsequent attacks by pests or pathogens. Abscisic acid (ABA) is a key player in regulating plant responses to both biotic and abiotic stresses. ABA interacts with other signaling pathways, forming a complex network that fine-tunes plant responses to biotic stress. Abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) are three key phytohormones that play crucial roles in fine-tuning plant defense responses to biotic stressors. While ABA primarily regulates plant responses to abiotic stresses, it also interacts with JA and SA pathways to modulate defense against biotic stress agents.

This crosstalk is often fine tuned as per stress condition. Under certain severe abiotic stress conditions, increased ABA levels can suppress JA signaling and defense responses associated with herbivore attacks. ABA promotes the accumulation of protein phosphatases, which dephosphorylate and inactivate key components of the

JA signaling pathway, such as MYC2 transcription factors. This antagonistic interaction helps plants conserve resources by down-regulating defense mechanisms against herbivores when facing severe abiotic stress. However, while concurrently dealing with abiotic and pathogen attack sometime ABA can positively regulate Jasmonic acid responsive genes for action against necrotrophs [65].

Similarly, in case of salicylic acid there is a complex cross talk with ABA. ABA is generally known to suppress the activities of SA. Higher levels of ABA suppresses the expression of pathogenesis related (PR) genes which are in turn induced by SA. However it can act synergistic or independently to SA for making response against biotic stress more refined. ABA has been shown to negatively regulate SA-mediated defense responses against pathogens. Increased ABA levels can suppress the expression of pathogenesis-related (PR) genes that are typically induced by SA. This antagonistic interaction allows plants to allocate resources away from SA-mediated defenses and prioritize stress tolerance under severe abiotic stress conditions. However, under moderate stress conditions, ABA and SA pathways may act synergistically to enhance plant defense against pathogens. This synergy involves the coordination of various defense mechanisms, including the activation of PR genes, production of antimicrobial compounds, and reinforcement of physical barriers. ABA, JA, and SA signaling pathways converge on common transcription factors and regulatory elements, enabling coordinated gene expression. Transcription factors, such as MYC2, NPR1 (Non expressor of Pathogensis-Related gene), and WRKYs, act as hubs for integrating signals from ABA, JA, and SA pathways, regulating the expression of downstream defense-related genes. These transcription factors facilitate the synergistic or antagonistic interactions between the hormone pathways, determining the outcome of plant defense responses.

4.2.2 ABA and plant microbiome

The plant microbiome, composed of various microorganisms such as bacteria, fungi, and viruses, plays a critical role in plant growth, health, and adaptation to different environmental conditions [66]. Understanding the mechanisms that regulate the plant microbiome is essential for enhancing crop productivity and sustainability. One significant mechanism involved in modulating the plant microbiome is the influence of abscisic acid (ABA) [67]. ABA affects the plant microbiome through its influence on root exudation. It can modify the composition of root exudates, which are organic compounds released by plant roots into the soil. These exudates serve as an energy source for microorganisms and can attract or repel specific microbial taxa [68]. By altering the composition of root exudates, ABA indirectly affects the abundance and diversity of microbial communities in the rhizosphere. Furthermore, ABA can directly affect microbial growth and activity. Some studies have demonstrated that ABA can promote the growth of beneficial microbes, such as plant growth-promoting rhizobacteria (PGPR), while inhibiting the growth of pathogenic microorganisms [69]. This direct effect of ABA on microbial populations further contributes to the modulation of the plant microbiome.

Understanding the role of ABA in modulating the plant microbiome has significant implications for agriculture and plant health. Manipulating ABA levels in plants could potentially be used as a strategy to enhance beneficial microbial colonization, improve nutrient uptake, and confer resistance against pathogens. However, further research is needed to unravel the complex interactions between ABA, plants, and the microbiome, and to develop practical applications for agricultural systems.

5. ABA and crosstalk with other phytohormones

Phytohormones are chief controllers of plant growth, development, and stress response. ABA plays a role in the modulation of other phytohormones, such as auxin, cytokinins, and gibberellins. The interplay between these phytohormones is critical for maintaining proper balance and coordination of plant growth and development.

ABA and auxin have antagonistic effects on root growth, where ABA inhibits root growth, while auxin promotes it. ABA also inhibits the biosynthesis and transport of auxin, thereby reducing its concentration and activity in the plant [70]. In contrast, ABA and cytokinins have synergistic effects on stress responses, particularly in enhancing the antioxidant defense system in plants. Studies have shown that the application of ABA and cytokinins together can increase the activity of antioxidant enzymes and reduce oxidative damage under stress conditions [62]. Moreover, ABA and gibberellins have been found to interact in the regulation of seed germination and dormancy. ABA promotes seed dormancy, while gibberellins promote germination. The balance between ABA and gibberellins is critical for proper seed development and germination.

As discussed earlier, the ABA can act synergistic, antagonistic or independent to Salicylic acid and Jasmonic acid regulating their effects in biotic stress responses. It is this crosstalk or interplay between these phytohormones that makes plant to recognize and differentiate the microorganisms as symbiotic or pathogenic partners. Overall, the modulation of other phytohormones by ABA is a complex and dynamic process that contributes to the regulation of plant growth, development, and stress responses.

6. Strategies to develop stress tolerant varieties

6.1 ABA, crop breeding and genetic engineering

Crop breeding has been a traditional approach to develop stress-tolerant varieties of crops. Recent advances in molecular biology and genetic engineering have allowed for more targeted and efficient approaches to crop breeding, including the use of ABA signaling pathways. A recent study published in the Journal Plant Physiology in 2018 reported the successful development of a stress-tolerant tomato (*Solanum lycopersicum*) variety using a gene-editing approach to enhance ABA signaling. The resulting plants showed improved drought and salt tolerance, as well as increased yield under stress conditions [71].

In another work, researchers reported the successful development of stress-tolerant rice (*Oryza sativa*) using a combination of conventional breeding and molecular breeding approaches. The resulting rice varieties showed improved tolerance to drought, salinity, and submergence. The molecular breeding approach involved the identification of genes involved in ABA signaling and the development of molecular markers for these genes to enable more efficient selection of stress-tolerant plants during breeding [72]. Generally, the use of ABA signaling pathways in crop breeding is a promising approach to developing stress-tolerant varieties of crops, and ongoing research in this area is likely to lead to further advances in the coming years. Overall, the multifaceted roles of ABA in plant growth, development, and stress responses make it a crucial component of plant physiology and a key target for research aimed at improving crop productivity and stress tolerance.

6.2 Epigenetic changes and crop improvement

Term Epigenetics was coined by Waddington [73] referring to inherited changes, meiotic or mitotic [74] in terms of chromatin structure, DNA cytosine methylation and histone modifications. All these modifications create various global and locus specific epialleles [75]. These changes can be passed on from generation to generation and can be influenced by environmental factors, including stress. Better understanding of epigenetic mechanisms and modifying the epigenetic framework of the plant could help us in developing better varieties. Drought stress or ABA treatment is known to transform the pattern of histone epigenetic marks which directly influence the genes action [76]. H3K14 acetylation has been observed in *Arabidopsis* and tobacco cultured cells when exposed to salinity stress or ABA treatment [77].

According to recent research, plants' responses to abiotic stressors such drought, salinity, heat, and cold are significantly influenced by epigenetic alteration leading to changes in plant's epigenome [78]. In this chapter, we will discuss the epigenetic control of stress signaling networks associated with ABA. One approach for developing stress-tolerant crops using CRISPR-Cas9 is epigenome editing. This technology allows for precise modifications to be made to the epigenetic marks on specific genes to enhance stress tolerance.

A recent study has compiled all the aspects of CRISPR-Cas9 mediated epigenetic regulations in various plants [79]. Another approach is through the use of stress-induced epigenetic changes. For example, a study published in the Journal Plant Cell in 2019 explained that exposing maize (*Zea mays*) plants to mild drought stress during seed development resulted in changes to DNA methylation patterns that enhanced drought tolerance in the resulting plants [80]. Mostly, the use of epigenetic changes to develop stress-tolerant crops is still a comparatively new field of research, and further studies are needed to fully understand the potential of this approach. However, early studies have shown promising results and suggest that epigenetic editing could be a valuable tool in developing crops that are better able to cope with abiotic stresses.

7. ABA quantification techniques

The amount of ABA concentration present in different plant tissues can be quantified using a wide variety of techniques. Researchers can understand the dynamics of ABA levels and how they affect plant growth and response by quantifying ABA.

Different types of methods executed for ABA measurement are:

- Enzyme-linked immunosorbent assay (ELISA): This technique makes use of specific antibodies that bind to ABA. An indirect measurement of the concentration of ABA is provided by the binding, which is measured using colorimetric or fluorescent signals. Dubas et al. [81] measured ABA in microspores of *Brassica napus* using indirect ELISA. The MAC 252 was used as antibody (Babraham Technix, Cambridge, UK). Absorbance was calculated by microplate reader Model 680 (Bio-Rad Laboratories, Inc.) at the wavelength of 405 nm.

- Gas chromatography-mass spectrometry (GC-MS): GC-MS uses gas chromatography to isolate ABA from other components in the sample and mass spectrometry to identify it. With its exceptional sensitivity and specificity, this approach produces accurate measurements.

- High-performance liquid chromatography (HPLC): HPLC uses liquid chromatography to extract ABA from other chemicals. With the help of methods like UV absorption or fluorescence, the separated ABA is detected, enabling accurate measurement.

- Immunoassays: Immunoassay techniques rely on the particular binding of antibodies to ABA, as in RIA (Radioimmunoassay) and FIA (Fluorescence Immunoassay). To characterize the binding and calculate the concentration of ABA, radioactive or fluorescent markers are used. RIA has an effective range of 0.165–13.2 ng ABA and has been used to find out ABA levels in extracts of leaves of tomato, *Lycopersicon esculentum* Mill. cv. *Ailsa Craig* (wild type) and three wilty mutants, notabilis, flacca and sitiens [82].

- Biosensors: To transform the binding of ABA into quantifiable signals, biosensors combine biological components with transducers. It is possible to quantify the concentration of ABA by detecting the interaction between it and the biological component. Typically biosensors are used for detecting analytes in low concentrations. There are two types of biosensors that have been developed for finding ABA viz., Localized surface plasmon resonance (LSPR) and Förster resonance energy transfer (FRET) sensors. LSPR sensors develop the interaction of light with metal nanoparticles to identify substances, whereas FRET sensors depend on energy transfer between a donor and acceptor molecule. LSPR sensors possess a remarkable sensitivity that enables the detection of substances without the need for labels, and they also have the capability to simultaneously detect multiple targets. On the other hand, FRET sensors exhibit exceptional specificity and can be customized for various applications. In the field of biosensors, aptamers have emerged as a cost-effective and sensitive substitute for antibodies. Recently, scientists have developed genetically encoded FRET sensors for the purpose of detecting ABA concentration and uptake in plants. These sensors demonstrate a high signal-to-noise ratio and enable precise measurements of ABA levels in living tissue [83].

- Fluorescence resonance energy transfer (FRET): Many of the sensor variants did not respond to the addition of ABA; however, FRET pair variants with improved dimerization fluorescent protein versions consistently responded to the addition of ABA. Understanding the pertinent metabolic fluxes can be gained from the kinetics of FRET sensor responses. ABI1aid and PYL1 domain interaction results in a "closed" state after ABA binding, which brings the fluorophores closer together and boosts FRET effectiveness. When the sensor closes, fluorescent proteins with improved dimerization propensities will improve the sensor's closed state through dimerization. Additionally, a high FRET efficiency is anticipated as a result of the heterodimer state's parallel orientation [83].

Among these methods, the choice depends on factors such as sensitivity, specificity, ease of use, and available resources. GC-MS is often considered the gold standard method due to its high sensitivity and specificity, allowing for accurate quantification. However, ELISA and HPLC are also widely used due to their simplicity and availability of commercial kits. Immunoassays offer high sensitivity and have been extensively used in research. Biosensors, while still developing, hold promise for rapid and on-site analysis.

8. Conclusion

Plants are absolute necessity for life on earth serving as cornerstone for survival and sustenance of mankind on this planet. Cultivated plants also called as crops not only serve as source of food and nutrition but also make the foundation of a big agricultural industry. However, plants face numerous pressures on a constant basis including biotic and abiotic elements, which offer serious obstacle to their growth, development and productivity. Plants have developed a variety of defense mechanisms that cooperate to lessen the detrimental effects of these stimuli. ABA has been found as a fundamental regulator of plant stress tolerance. ABA supports the Plant's ability to adapt to the challenging environmental cues and preserve cellular homeostasis by activating a specific signaling pathways.

Improved crop resilience and productivity under numerous stresses have been made possible by better understanding the role of ABA in stress tolerance. The introduction or modification of genes involved in ABA production, signaling, and subsequent stress response is possible due to genetic engineering techniques. Scientists are working to create crops that are more resistant to stress by increasing the synthesis of ABA or changing the expression of ABA-responsive genes. For instance, it has been demonstrated that many crops may tolerate stress better when genes associated with ABA biosynthesis or transcription factors involved in ABA signaling are over-expressed. Promoter region of various genes involved in ABA biosynthesis and stress responses are modified to regulate the expression of these specific genes. Site specific mutagensis or gene editing using CRISPER-Cas9 technology has been serving as key members in the race for crop improvement in terms of stress avoidance, resilience or tolerance. Though stress is a complex process but its response is even more complex where many pathways crosstalk with one another. Still ABA acts as Hub molecule modulating other stress responsive molecules as spokes making it overall a hub and spoke model for stress tolerance.

Another tactic is cross-breeding or hybridization, where hybrid plants with improved stress tolerance are selected utilizing the inherent genetic diversity found in crop species. Breeders can add advantageous features associated to ABA-mediated stress responses by mating several kinds or species. Using this method, crops with enhanced resistance to environmental stresses have been successfully developed.

The plant's ability to cope with stress is influenced by both genetic and epigenetic alterations. Plants' gene expression and stress responses can be affected by epigenetic alterations such DNA methylation and histone modifications. Discovering these epigenetic mechanisms and how they interact with ABA signaling pathways is opening up new possibilities for creating crops that can withstand stress. Editing the epigenome of a plant using CRISPER-Cas technology with guide RNA targeted to a specific gene or epigenetic modification is nowadays a commonly used technique to enhance crop stress resistance.

Researchers from Seoul National University in South Korea found that ABA receptors in *Arabidopsis thaliana* which were modified via agrochemical engineering were more responsive to ABA, increasing the plants' tolerance to salt and drought [84, 85]. The agrochemical engineering strategy, according to the researchers, might be used to increase other crops' tolerance to salt and drought. Since salt stress and drought are becoming more prevalent due to climate change, this could aid in addressing the growing issue of food poverty.

Plant stress biology is a wide and complicated field with numerous posed threats and unlimited opportunities. Future developments in cross-breeding methods, epigenetic research, agrochemical and genetic engineering hold considerable promise for creating crops with increased resistance to Environmental stresses. The potential ecological and ethical ramifications of these strategies must be carefully considered, though. To ensure the safe and responsible use of genetically modified crops, thorough risk analyzes and regulatory frameworks are required. Furthermore, the scientific community is not the only one who must address the impacts of Environmental stresses on crop production. The demand for sustainable agriculture practices and policies that put climate resilience and food security first is growing as the world's population expands. To assist farmers in implementing climate-smart agricultural methods and stress-reduction techniques, investments in R&D, infrastructure, and education are crucial. In order to secure food security in the face of shifting climatic conditions, it is essential to strike a balance between technological breakthroughs and ethical issues.

9. Future perspectives

Various points can be kept in mind for ABA future research:

- Future study on biotic and abiotic plant stress will deepen our grasp of the complex mechanisms underpinning plant stress tolerance. The hormone ABA, which is crucial in modulating stress reactions, is one compound whose production is of special interest. Despite extensive research has been carried out on various aspects of ABA, insights into how plants experience and react to stress will be gained from more research into the biosynthetic pathways of ABA and the control of its synthesis.

- The manipulation of genes involved in ABA production, signaling, and subsequent stress responses will be the main focus of research. Researchers can improve the expression of ABA-responsive genes or increase ABA production by precisely altering these genes, which will increase the ability of crops to withstand stress. The creation of stress-tolerant cultivars will also be accelerated by improvements in gene-editing technologies like CRISPR-Cas9. These technologies allow for more precise and focused alterations.

- Crop breeding presently is and will be a key factor in improving stress tolerance, especially through cross-breeding and hybridization. Breeders can selectively integrate ABA-mediated stress response features into commercial varieties by taking use of the inherent genetic diversity seen among crop species. Advanced phenotyping tools and genomic selection strategies shall be used in conjunction with this strategy to help breeders more effectively identify and select plants with desired stress tolerance features.

- Stress tolerance studies are increasingly focussing on epigenetic changes, such as variations in DNA methylation and histone acetylation. The epigenetic changes connected to ABA signaling networks and stress responses will open up new possibilities for enhancing crop resilience. By examining how alterations in the epigenome affect gene expression and stress-related metabolic pathways, researchers will pave the road for novel methods to improve stress tolerance through epigenetic adjustments.

- Modifications of promoter regions of ABA biosynthesis genes and ABA-responsive components will be another topic of future study. Researchers can fine-tune the ABA signaling system and improve stress tolerance by finding and modifying particular promoter sequences and cis-regulatory regions that regulate the production of stress-responsive genes. This strategy will make it possible to precisely control how genes are expressed in response to stressful situations, enabling crops to employ more potent stress mitigation techniques.

- Additionally, a thorough understanding of plant stress reactions will be possible through clarifying the interactions between ABA signaling and other hormone pathways. The complex relationships between ABA and other hormones, including salicylic acid, jasmonate, and ethylene, can be uncovered by researchers in order to pinpoint critical regulatory sites and create plans for improving crop stress responses. The ability of plants to tolerate stress more generally could be increased by manipulating the interactions between hormone pathways.

- Future developments in omics technologies, like as genomes, transcriptomics, proteomics, and metabolomics, will considerably increase our understanding of the mechanisms underlying stress tolerance. With the use of these technologies, scientists will be able to thoroughly examine the molecular alterations brought on by stress on plants. Combining data from several omics levels will enable the identification of critical targets for enhancing stress tolerance and offer a comprehensive perspective of stress responses.

- Overall, a combination of genetic engineering, crop breeding, epigenetic changes, and a better comprehension of ABA signaling networks and their interactions will enable the development of crops that can withstand stress. Using this potential and turning it into workable solutions for sustainable agriculture would require continued study and cooperation among scientists, breeders, and policymakers. We can secure food security and agricultural sustainability in the face of a changing climate and an expanding global population by tackling the problems caused by biotic and abiotic pressures.

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References

[1] Finkelstein RR, Gampala SS, Rock CD. Abscisic acid signaling in seeds and seedlings. The Plant Cell. 2002;**14**(suppl_1):S15-S45

[2] Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR. Abscisic acid: Emergence of a core signaling network. Annual Review of Plant Biology. 2010;**61**:651-679

[3] Acharya BR, Jeon BW, Zhang W, Assmann SM. Open stomata 1 (OST 1) is limiting in abscisic acid responses of Arabidopsis guard cells. New Phytologist. 2013;**200**(4):1049-1063

[4] Davis LA, Addicott FT. Abscisic acid: Correlations with abscission and with development in the cotton fruit. Plant Physiology. 1972;**49**(4):644-648

[5] Zeevaart JA. Abscisic acid metabolism and its regulation. In: New Comprehensive Biochemistry. Netherlands: Elsevier; 1999. pp. 189-207

[6] Finkelstein R. Abscisic acid synthesis and response. The Arabidopsis Book/American Society of Plant Biologists. 2013;**11**:e0166

[7] Tuteja N. Abscisic acid and abiotic stress signaling. Plant Signaling & Behavior. 2007;**2**(3):135-138

[8] Finch-Savage WE, Leubner-Metzger G. Seed dormancy and the control of germination. New Phytologist. 2006;**171**(3):501-523

[9] Finkelstein R, Reeves W, Ariizumi T, Steber C. Molecular aspects of seed dormancy. Annual Review of Plant Biology. 2008;**59**:387-415

[10] Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L. The gibberellic acid signaling repressor RGL2 inhibits *Arabidopsis* seed germination by stimulating abscisic acid synthesis and ABI5 activity. The Plant Cell. 2008;**20**(10):2729-2745

[11] Melcher K, Ng LM, Zhou XE, Soon FF, Xu Y, Suino-Powell KM, et al. A gate–latch–lock mechanism for hormone signalling by abscisic acid receptors. Nature. 2009;**462**(7273):602-608

[12] Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, et al. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science. 2009;**324**(5930):1068-1071

[13] Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park SY, et al. In vitro reconstitution of an abscisic acid signalling pathway. Nature. 2009;**462**(7273):660-664

[14] Battaglia M, Covarrubias AA. Late embryogenesis abundant (LEA) proteins in legumes. Frontiers in Plant Science. 2013;**4**:190

[15] Setha S. Roles of abscisic acid in fruit ripening. Walailak Journal of Science and Technology (WJST). 2012;**9**(4):297-308

[16] Munns R, Tester M. Mechanisms of salinity tolerance. Annual Review of Plant Biology. 2008;**59**:651-681

[17] Li X, Chen L, Forde BG, Davies WJ. The biphasic root growth response to abscisic acid in Arabidopsis involves interaction with ethylene and auxin signalling pathways. Frontiers in Plant Science. 2017;**8**:1493

[18] Puértolas J, Conesa MR, Ballester C, Dodd IC. Local root abscisic acid (ABA)

accumulation depends on the spatial distribution of soil moisture in potato: Implications for ABA signalling under heterogeneous soil drying. Journal of Experimental Botany. 2015;**66**(8):2325-2334

[19] Sah SK, Reddy KR, Li J. Abscisic acid and abiotic stress tolerance in crop plants. Frontiers in Plant Science. 2016;**7**:571. Available from: https://bitly/2M9bmU8

[20] Bharath P et al. Abscisic acid-induced stomatal closure: An important component of plant defense against abiotic and biotic stress. Frontiers in Plant Science. 2021;**12**:615114. DOI: 10.3389/fpls.2021.615114

[21] Deikman J, Petracek M, Heard JE. Drought tolerance through biotechnology: Improving translation from the laboratory to farmers' fields. Current Opinion in Biotechnology. 2012;**23**(2):243-250

[22] Jones HG, Serraj R, Loveys BR, Xiong L, Wheaton A, Price AH. Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. Functional Plant Biology. 2009;**36**(11):978-989

[23] Des Marais DL, Juenger TE. Pleiotropy, plasticity, and the evolution of plant abiotic stress tolerance. Annals of the New York Academy of Sciences. 2010;**1206**(1):56-79

[24] Blum A. Plant Breeding for Stress Environments. Boca Raton: CRC Press; 2018

[25] Wang L, De Kroon H, Bögemann GM, Smits AJ. Partial root drying effects on biomass production in Brassica napus and the significance of root responses. Plant and Soil. 2005;**276**:313-326

[26] Omidvar V, Fellner M. DNA methylation and transcriptomic changes in response to different lights and stresses in 7B-1 male-sterile tomato. PLoS One. 2015;**10**(4):e0121864

[27] Chaves MM, Maroco JP, Pereira JS. Understanding plant responses to drought—from genes to the whole plant. Functional Plant Biology. 2003;**30**(3):239-264

[28] Thameur A, Ferchichi A, López-Carbonell M. Quantification of free and conjugated abscisic acid in five genotypes of barley (Hordeum vulgare L.) under water stress conditions. South African Journal of Botany. 2011;**77**(1):222-228

[29] Lu S, Su W, Li H, Guo Z. Abscisic acid improves drought tolerance of triploid Bermuda grass and involves H2O2-and NO-induced antioxidant enzyme activities. Plant Physiology and Biochemistry. 2009;**47**(2):132-138

[30] Zhang H, Zhu H, Pan Y, Yu Y, Luan S, Li L. A DTX/MATE-type transporter facilitates abscisic acid efflux and modulates ABA sensitivity and drought tolerance in Arabidopsis. Molecular Plant. 2014;**7**(10):1522-1532

[31] Ma Y, Cao J, He J, Chen Q, Li X, Yang Y. Molecular mechanism for the regulation of ABA homeostasis during plant development and stress responses. International Journal of Molecular Sciences. 2018;**19**(11):3643

[32] Yamaguchi-Shinozaki K, Shinozaki K. A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. The Plant Cell. 1994;**6**(2):251-264. DOI: 10.1105/tpc.6.2.251

[33] Zhou Z, Fan J, Zhang J, Yang Y, Zhang Y, Zan X, et al. OsMLP423 is

a positive regulator of tolerance to drought and salt stresses in rice. Plants. 2022;**11**(13):1653

[34] Yu Y, Yu M, Zhang S, Song T, Zhang M, Zhou H, et al. Transcriptomic identification of wheat AP2/ERF transcription factors and functional characterization of TaERF-6-3A in response to drought and salinity stresses. International Journal of Molecular Sciences. 2022;**23**(6):3272

[35] Thompson AJ, Jackson AC, Parker RA, Morpeth DR, Burbidge A, Taylor IB. Abscisic acid biosynthesis in tomato: Regulation of zeaxanthin epoxidase and 9-cis-epoxycarotenoid dioxygenase mRNAs by light/dark cycles, water stress and abscisic acid. Plant Molecular Biology. 2000;**42**:833-845

[36] Wang Y, Beaith M, Chalifoux M, Ying J, Uchacz T, Sarvas C, et al. Shoot-specific down-regulation of protein farnesyltransferase (α-subunit) for yield protection against drought in canola. Molecular Plant. 2009;**2**(1):191-200

[37] Munns R, Gilliham M. Salinity tolerance of crops–what is the cost? New Phytologist. 2015;**208**(3):668-673

[38] Food and Agriculture Organization (FAO). Salinity Management for Sustainable Irrigation: FAO Land and Water Development Technical Brief. 2020. Available from: http://www.fao.org/3/ca8911en/CA8911EN.pdf

[39] Kesawat MS, Satheesh N, Kherawat BS, Kumar A, Kim HU, Chung SM, et al. Regulation of reactive oxygen species during salt stress in plants and their crosstalk with other signaling molecules—current perspectives and future directions. Plants. 2023;**12**(4):864

[40] Chen G, Zheng D, Feng N, Zhou H, Mu D, Zhao L, et al. Physiological mechanisms of ABA-induced salinity tolerance in leaves and roots of rice. Scientific Reports. 2022;**12**(1):1-26

[41] Martínez-Andújar C, Martínez-Pérez A, Ferrández-Ayela A, Albacete A, Martínez-Melgarejo PA, Dodd IC, et al. Impact of overexpression of 9-cis-epoxycarotenoid dioxygenase on growth and gene expression under salinity stress. Plant Science. 2020;**295**:110268

[42] Kumar S, Shah SH, Vimala Y, Jatav HS, Ahmad P, Chen Y, et al. Abscisic acid: Metabolism, transport, crosstalk with other plant growth regulators, and its role in heavy metal stress mitigation. Frontiers in Plant Science. 2022;**13**:972856

[43] Jalmi SK, Bhagat PK, Verma D, Noryang S, Tayyeba S, Singh K, et al. Traversing the links between heavy metal stress and plant signaling. Frontiers in Plant Science. 2018;**9**:12

[44] Shen G, Niu J, Deng Z. Abscisic acid treatment alleviates cadmium toxicity in purple flowering stalk (Brassica campestris L. ssp. chinensis var. purpurea Hort.) seedlings. Plant Physiology and Biochemistry. 2017;**118**:471-478

[45] Hu B, Deng F, Chen G, Chen X, Gao W, Long L, et al. Evolution of abscisic acid signaling for stress responses to toxic metals and metalloids. Frontiers in Plant Science. 2020;**11**:909

[46] Tang Y, Wang L, Xie Y, Yu X, Li H, Lin L, et al. Effects of exogenous abscisic acid on the growth and cadmium accumulation of lettuce under cadmium-stress conditions. International Journal of Environmental Analytical Chemistry. 2020;**100**(6):720-731

[47] Deng B, Zhang W, Yang H. Abscisic acid decreases cell death in Malus hupehensis Rehd. Under Cd stress by

reducing root Cd 2+ influx and leaf transpiration. Journal of Plant Growth Regulation. 2021;**41**:1-8

[48] Leng Y, Li Y, Ma YH, He LF, Li SW. Abscisic acid modulates differential physiological and biochemical responses of roots, stems, and leaves in mung bean seedlings to cadmium stress. Environmental Science and Pollution Research. 2021;**28**(5):6030-6043

[49] Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: An overview. Environmental and Experimental Botany. 2007;**61**(3):199-223

[50] Sanghera GS, Wani SH, Hussain W, Singh NB. Engineering cold stress tolerance in crop plants. Current Genomics. 2011;**12**(1):30

[51] Zhao Y, Han Q, Ding C, Huang Y, Liao J, Chen T, et al. Effect of low temperature on chlorophyll biosynthesis and chloroplast biogenesis of rice seedlings during greening. International Journal of Molecular Sciences. 2020;**21**(4):1390

[52] Lata C, Prasad M. Role of DREBs in regulation of abiotic stress responses in plants. Journal of Experimental Botany. 2011;**62**(14):4731-4748

[53] Nakashima K, Ito Y, Yamaguchi-Shinozaki K. Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. Plant Physiology. 2009;**149**(1):88-95

[54] Janská A, Maršík P, Zelenková S, Ovesná J. Cold stress and acclimation–what is important for metabolic adjustment? Plant Biology. 2010;**12**(3):395-405

[55] Osakabe Y, Yamaguchi-Shinozaki K, Shinozaki K, Tran LS. ABA control of plant macroelement membrane transport systems in response to water deficit and high salinity. New Phytologist. 2014;**202**(1):35-49

[56] Guo X, Liu D, Chong K. Cold signaling in plants: Insights into mechanisms and regulation. Journal of Integrative Plant Biology. 2018;**60**(9):745-756

[57] Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science. 2002;**7**(9):405-410

[58] Møller IM, Sweetlove LJ. ROS signalling–specificity is required. Trends in Plant Science. 2010;**15**(7):370-374

[59] Anjum SA, Tanveer M, Hussain S, Shahzad B, Ashraf U, Fahad S, et al. Osmoregulation and antioxidant production in maize under combined cadmium and arsenic stress. Environmental Science and Pollution Research. 2016;**23**:11864-11875

[60] Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany. 2012;**2012**:1-26

[61] Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 2010;**48**(12):909-930

[62] Li S, Liu S, Zhang Q, Cui M, Zhao M, Li N, et al. The interaction of ABA and ROS in plant growth and stress resistances. Frontiers in Plant Science. 2022;**13**:1-16

[63] Hasanuzzaman M, Raihan MR, Masud AA, Rahman K, Nowroz F, Rahman M, et al. Regulation of reactive oxygen species and antioxidant defense in plants under salinity. International Journal of Molecular Sciences. 2021;**22**(17):9326

[64] Hasanuzzaman M, Bhuyan MHMB, Anee TI, et al. Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants (Basel). 2019;**8**(9):384. DOI: 10.3390/antiox8090384

[65] Anderson JP, Badruzsaufari E, Schenk PM, Manners JM, Desmond OJ, Ehlert C, et al. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. The Plant Cell. 2004;**16**(12):3460-3479

[66] Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. The importance of the microbiome of the plant holobiont. New Phytologist. 2015;**206**(4):1196-1206

[67] Lopes LD, Futrell SL, Bergmeyer E, Hao J, Schachtman DP. Root exudate concentrations of indole-3-acetic acid (IAA) and abscisic acid (ABA) affect maize rhizobacterial communities at specific developmental stages. FEMS Microbiology Ecology. 2023;**99**(3):fiad019

[68] Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology. 2006;**57**:233-266

[69] Ma Y, Rajkumar M, Freitas H. Inoculation of plant growth promoting bacterium Achromobacter xylosoxidans strain Ax10 for the improvement of copper phytoextraction by Brassica juncea. Journal of Environmental Management. 2009;**90**(2):831-837

[70] Qin H, Wang J, Zhou J, Qiao J, Li Y, Quan R, et al. Abscisic acid promotes auxin biosynthesis to inhibit primary root elongation in rice. Plant Physiology. 2023;**191**(3):1953-1967

[71] Thompson AJ, Andrews J, Mulholland BJ, McKee JM, Hilton HW, Horridge JS, et al. Overproduction of abscisic acid in tomato increases transpiration efficiency and root hydraulic conductivity and influences leaf expansion. Plant Physiology. 2007;**143**(4):1905-1917

[72] Kumar A, Dixit S, Ram T, Yadaw RB, Mishra KK, Mandal NP. Breeding high-yielding drought-tolerant rice: Genetic variations and conventional and molecular approaches. Journal of Experimental Botany. 2014;**65**(21):6265-6278

[73] Waddington CH. Epigenetics and evolution. Symposia of the Society for Experimental Biology. 1953;**7**:186-199

[74] Russo VE, Martienssen RA, Riggs AD. Epigenetic Mechanisms of Gene Regulation. United Kingdom: Cambridge University Press; 1996

[75] Manning K, Tör M, Poole M, Hong Y, Thompson AJ, King GJ, et al. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nature Genetics. 2006;**38**(8):948-952

[76] Yuan L, Liu X, Luo M, Yang S, Wu K. Involvement of histone modifications in plant abiotic stress responses. Journal of Integrative Plant Biology. 2013;**55**(10):892-901

[77] Sokol A, Kwiatkowska A, Jerzmanowski A, Prymakowska-Bosak M. Up-regulation of stress-inducible genes in tobacco and Arabidopsis cells in response to abiotic stresses and ABA treatment correlates with dynamic changes in histone H3 and H4 modifications. Planta. 2007;**227**:245-254

[78] King GJ, Amoah S, Kurup S. Exploring and exploiting epigenetic variation in crops. Genome. 2010;**53**(11):856-868

[79] Jogam P, Sandhya D, Alok A, Peddaboina V, Allini VR, Zhang B. A review on CRISPR/Cas-based epigenetic regulation in plants. International Journal of Biological Macromolecules. 2022;**219**:1261-1271

[80] Wang Q, Xu J, Pu X, Lv H, Liu Y, Ma H, et al. Maize DNA methylation in response to drought stress is involved in target gene expression and alternative splicing. International Journal of Molecular Sciences. 2021;**22**(15):8285

[81] Dubas E, Janowiak F, Krzewska M, Hura T, Żur I. Endogenous ABA concentration and cytoplasmic membrane fluidity in microspores of oilseed rape (Brassica napus L.) genotypes differing in responsiveness to androgenesis induction. Plant Cell Reports. 2013;**32**:1465-1475

[82] Whitford PN, Croker SJ. An homogeneous radioimmunoassay for abscisic acid using a scintillation proximity assay technique. Phytochemical Analysis. 1991;**2**(3):134-136

[83] Narang J, Mohan H. Biosensors for phytohormone abscisic acid and its role in humans: A review. Sensors International. 2023;**4**:100234

[84] Jones AM, Danielson JÅ, Manoj Kumar SN, Lanquar V, Grossmann G, Frommer WB. Abscisic acid dynamics in roots detected with genetically encoded FRET sensors. eLife. 2014;**3**:e01741

[85] Park SY, Peterson FC, Mosquna A, Yao J, Volkman BF, Cutler SR. Agrochemical control of plant water use using engineered abscisic acid receptors. Nature. 2015;**520**(7548):545-548

Chapter 2

Perspectives of Phytohormones Application to Enhance Salinity Tolerance in Plants

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Abstract

Plants undergo a wide range of morphological, cellular, anatomical, and physiological changes in response to salinity stress. However, plants produce some signaling molecules, usually known as phytohormones, to combat stress conditions. Salinity tolerance is a complex mechanism, whereas phytohormones have a central role in it. Phytohormone-mediated plant responses improve nutrient uptake, the source-sink relationship, and plant growth and development. Phytohormones triggers the specific gene expressions which are responsible for the modification of various plant mechanisms under salinity stress. This review summarized the most recent research findings about plant responses to salinity stress at physiological and molecular levels and discussed the probable function of several (abscisic acid, indole acetic acid, cytokinins, gibberellic acid, salicylic acid, brassinosteroids, ethylene, and triazoles) phytohormones and their interaction in modulating salinity stress. Further, the understanding of specific genes involved in phytohormonal regulation toward salinity tolerance is a key to developing breeding and transgenic approaches for meeting food demand under sustainable crop production.

Keywords: salt stress, plant growth, phytohormones, salinity tolerance, future perspectives, abiotic stress, hormonal regulation

1. Introduction

The world's population is continuously increasing, and it is expected to reach 9.1 billion in 2050 that would require 70% more food to feed to world [1–4]. The changing climate is endangering the productivity and sustainability of agricultural production systems [5]. It would be the biggest challenge to meet the food requirement of growing population in near future [5]. Climate change exerts a range of severe abiotic stressors including high salt, drought, cold, and heat stress, which significantly impede crop yields [6]. Among these, salt stress is the most widely spread abiotic stress that covered the 1/3rd area of arable land globally [7] and is increasing by 10% annually due to global climate change [8]. Salt stress lowers seed germination, seedling establishment, enzymatic production, and protein synthesis in plants [9]. Salt stress is detrimental to crop growth, yield, and overall agricultural productivity [10]. Salinity develops osmotic stress and ion toxicity that affect the metabolic processes of plants [11]. The antagonism in essential nutrient uptake by plant roots from saline soil leads to the deficiency of Ca^{2+}, K^+, Fe^{++}, and Zn^{++} ions and water scarcity in plant tissues [12]. Paliwal et al. [13] noticed shrinkage of leaf area, less stomatal conductance, and chlorophyll concentration, alongside the ROS development, which lowers the seed germination, plant height, and biomass [13]. Due to this, the average agricultural yield was reduced by 50% [2, 14].

However, crop plants exhibit complex alterations to mitigate the negative effect of salt stress for survival [15]. These alterations may include both morphological and developmental patterns (growth plasticity) and physiological and biochemical adaptations [16]. Further, plants build up bioactive endogenous compounds, also known as phytohormones, which accelerate the plant growth and development under stress conditions [17]. Phytohormones produce various organic solutes such as sugars, polyols, betaines, and proline to protect the cell structure, ionic balance, reactive oxygen species (ROS) scavenging, protein regulation, and expression of related genes to enhance crop growth and developmental processes under stress conditions [17, 18]. Further, enhancing stress tolerance has emerged as a significant area of focus in the agricultural sector that improves the crop performance under stress, environmental sustainability, horticulture, and economic viability.

The primary emphasis of this chapter is to develop a comprehensive understanding of phytohormones application in modulating the biochemical and physiological processes of plants exposed to salinity-induced stress. Several studies highlighted various detrimental effects of salinity on plants [19]. Contrarily, the salinity tolerance can be regulated through various kinds of phytohormones including auxin, cytokinin, gibberellic acid, abscisic acid, and ethylene [20]. Further, salicylic acid, brassinosteroids, and triazoles are also found imperative to enhance salinity stress tolerance in plants [20]. Plants produce different proteins under stress condition that act as phytohormones to regulate the plant's normal functions [21]. However, poor hormonal regulation can lead to reduced germination and emergence and limited plant growth and development under salinity stress [22]. Moreover, the exogenous application of phytohormones found a promising practical strategy to deal with salt stress. The exogenous application of phytohormones exhibited a rapid increase in plant growth under stress conditions [17, 23–25]. Therefore, understanding the function of specific phytohormones will provide essential insights of plant mechanism to adapt salinity tolerance.

2. Soil salinization: a threat to global food production

Soil salinization is the most significant global abiotic stress that reduced the soil fertility of approximately 1100 Mha (7% of total Earth land) area [26]. Soil salinization is increased with natural geochemical processes (primary salinity) and different human activities (secondary salinity) [27]. Primary salinization is caused by various atmospheric depositions, sea-level rise, saltwater intrusion into freshwater aquifers, and rising temperatures, while excessive fertilizer use, poorly managed practices, and intensified agriculture activities increase the secondary salinization that lowered the productivity of 30% cultivated land [28, 29]. The limited availability of freshwater resources for irrigation purposes, coupled with the continuous deterioration of agricultural fields due to salt stress, leads to significant reductions in agricultural productivity of arid and semiarid regions.

Soil salinization began from hundreds to thousands of years ago that degraded the arable land globally [30]. About 30% of irrigated land and 6% of the total land is degraded by soil salinity that caused an estimated $12 billion loss to agricultural production [15, 31]. The rising salinity in arable areas highlights the necessity for the understanding of the plant-salinity-tolerance mechanisms to maintain crop productivity. A significant reduction in plant growth and development, photosynthesis, respiration, and protein synthesis is reported due to salinity stress [32, 33]. The production of reactive oxygen species (ROS), including superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($HO^{\bullet}$) injuring the chloroplasts and mitochondria, is a significant indicator of oxidative damage in salinity [34]. These ROS disrupt the membranous structure and its cell permeability; thus, nutrient availability is reduced under salinity stress [35]. Therefore, plants use antioxidant enzymes as a defensive mechanism to safeguard nucleic acids, proteins, and membrane lipids to avoid the detrimental impacts of ROS [36].

2.1 Causes of soil salinization

The buildup of water-soluble salts (Na^+, K^+, Cl^-, and SO_4^{2-}) inside the root zone raises the salinity. This accumulation leads to osmotic fluctuations that impede the capacity of plant roots to uptake water [37–39]. The presence of high salt ions develops hyper-ionic salt stress. Soluble sodium and chloride ions are the primary ions responsible for developing soil salinity. In addition, the high Na^+ ions develop a sodicity problem in soil [40]. Ancient cells were built to survive salinity since the early evolution of life originated in primeval oceans with similar or even more salt than contemporary oceans [41]. Therefore, numerous terrestrial plants have the ability to endure low to moderate levels of salinity. In contrast, naturally existing salt-tolerant plants (halophytes) exhibit a stringent salinity tolerance mechanism. However, a majority of growing crop species belong to the glycophyte group and therefore exhibit a poorly adapted mechanism to thrive in saline soils. Rice and tomato cultivars are highly susceptible to developing phytotoxic ions due to osmotic damage in saline stress conditions [42]. Salt-induced osmotic stress is observed during the early phase of salt exposure as a result of the gradual absorption of salts and consequent decrease in water potential in the vicinity of the root zone. This reduction in water conductivity within plant cells predominantly hampers plant growth [43, 44]. However, prolonged salinity stress leads to the accumulation of Na^+, Cl^-, and SO_4^{2-} ions, which cause ion toxicity and hinder nutrient absorption, hence being detrimental to the growing plant cells and tissues [45]. The adverse effects of salt stress on plants can be observed in their morphology, physiology, and biochemical properties. Morphologically, plants experience stunted growth, chlorosis, and impaired seed germination. Physiologically, salt stress inhibits photosynthesis and disrupts nutrient balance. Further, plants undergo various oxidative stresses, electrolyte leakage, and membrane disorganization during biochemical processes [46, 47]. Moreover, salinity negatively affected the reproductive stage of the crop [48]. Therefore, a comprehensive understanding of salinity-induced injuries should be known, highlighting the need for salinity tolerance in plants for sustainable food production around the world.

2.2 Effects of salinity on plants: a challenge to agriculture

2.2.1 Plant growth and development

Salt stress decreases plant growth, and the rate of the decreasing trend depends on the plant's growth stage and the intensity of the stress [49]. Researchers discovered that the stunted growth of plants acts as an adaptive mechanism for their survival against salt stress [50]. Salinity stress inhibits gene expression (cyclin and cyclin-dependent kinase) necessary for cell growth and number in the plant meristem, nutrient and water absorption, and plant stability. Numerous plants react quickly to stress and consequently stop their growth. Contrarily, some plants face salinity stress while maintaining their normal growth and start dying [51]. In moderate salinity, plants with quick responses activate the dehydration that results in cell shrinkage that recovers later [52–54]. However, salinity caused cell injury and decreased cell elongation and division, which resulted in less root and shoot growth in plants [55]. Under salinity, soil water component alteration in the rhizosphere disturbs the cell-water relationship of plants that develop osmotic injury [56]. Further, the osmotic injury lowers water uptake, causing inhibition or reduction in photosynthesis and respiration and carbohydrate and protein synthesis, leading to decreased plant growth and development [57, 58].

2.2.2 Photosynthesis

Photosynthesis is one of the most fundamental physiological attributes of plants, wherein solar energy is transformed into chemical energy. There may be a drop in photosynthesis caused by salt because of poor biosynthesis of chlorophyll [59], changes in enzyme activity [60], the closing of stomata [61], less carbon dioxide supplementation [62], and a damaged photosystem [63]. A significant decrease in chlorophyll levels in plants exposed to high salt concentrations could be due to the enhanced oxidation and degradation of chlorophyll owing to high ROS accumulation [63]. Further, ROS production under salinity stress inhibits the electron transport chain (ETC) that produces pseudocyclic electron transport chains [64]. As a result, modifications in photosynthetic proteins and disruptions in the assembly of photosystems occur [65]. Furthermore, the swelling of the thylakoid membrane under high salinity stress was observed to destroy the chloroplast ultrastructure [66]. Consequently, substantial photosynthesis resulted in less plant growth, which led to a decrease in plant production of *Jatropha curcas*, *O. sativa*, *P. oleracea*, and *Solanum melongena* in saline conditions [47, 67–69].

2.2.3 Nutrient balance

Plant growth and development in optimum soil conditions is commonly represented by the "generalized dose-response curve" [70]. Either nutrient-induced deficiency or nutrient toxicity can hinder plant growth due to suboptimal nutrient uptake [26]. The primary cause of less mineral acquisition under salt stress is the interactive effect of Na^+ and Cl^- with calcium (Ca^{2+}), potassium (K^+), and magnesium (Mg^{2+}) ions [71]. Many researchers have reported a complex interaction between salt ions and vital mineral nutrients like potassium, phosphorus, and nitrogen [72–74]. Nitrogen is a vital mineral element that serves as a fundamental part of various cellular components within plants. Due to Cl^-/NO_3^- antagonism, greater Cl^- uptake and accumulation under saline conditions reduce total nitrogen uptake [75]. Phosphorous is needed for photosynthesis, storage, and energy transfer, whereas its uptake reduces in the presence of high Cl^- and SO_4^{2-} concentrations and due to the low solubility of the Ca ± P minerals [76]. Though potassium is essential for protein synthesis and water balance, high Na^+ ions reduce K^+ availability in saline environments [77]. Thus, the maintenance of cellular equilibrium between sodium and potassium is crucial for the survival of plants in saline soil. Although sodium cannot replace potassium in cellular functions, they both share a chemical resemblance that leads to potassium substitution by sodium. Na^+ and K^+ compete for root uptake sites and lower the K^+ and Ca^{2+} intake during salinity stress [78]. Similarly, a significant decrease in K^+ absorption by plants is due to high Na^+ ions, regardless of whether Na^+, chloride, or sulfate ions predominate in soil solutions [71]. Thus, salinity creates nutritional imbalances and lowers crop productivity in a saline environment [79, 80].

2.2.4 Water relations

The rapid uptake of ions results in the buildup of ions within the cellular structures of plants, hence exerting a detrimental impact on plant water relations [81]. The osmotic potential of plant cells decreased under salinity conditions that created an osmotic gradient; consequently, water moved out, causing a loss in plant cell turgidity [82]. Less water intake and transpiration rate of *C. olitorius* is noticed from saline soil [83, 84]. Further, these aforementioned findings are supported by recent investigations [85, 86]. However, the maintenance of turgor pressure at a constant level in plants is achieved by decreasing their osmotic potential relative to the overall water potential during salt stress [87]. Moreover, the hydrostatic potential gradient has immense importance as it controls the water transport during transpiration from soil through root xylem cells in an apoplastic pathway. However, the saline stress alters the water intake primarily through the cell-to-cell pathway, thus affecting the water relations more significantly [88, 89].

2.2.5 Yield

Salinity affects energy metabolism, cell signaling, and the synthesis of proteins necessary for plant growth, development, and achieving high yields. Hence, it ultimately impedes agricultural production by impeding plant growth and plant adaptation to stress responses, resulting in an overall reduction in yield [90]. The rate of salt absorption and salt-induced osmotic stress showed cellular and membrane injury leads to a significant loss in crop biomass [91, 92]. Different researchers reported the yield loss of different field crops is affected by salt stress [93]. They suggested that a thorough comprehension of the salinity of plants has the potential to help further enhance agricultural crop productivity in salt-affected areas [26].

3. Role of phytohomones to alleviate salt stress on plants

Plants produce various kinds of phytohormones through biosynthetic pathways that can act locally or be transported to any part of the plant to maintain normal functioning in normal or stressful conditions [94]. These phytohormones, including auxins (IAA), cytokinins, gibberellins (GA), ethylene, abscisic acid (ABA), and brassinosteroids (BRs), regulate numerous physiological and biochemical processes to improve the growth and development of plants in saline conditions [19]. Moreover, strigolactone, nitric oxide (NO), and polyamines also act as phytohormones and regulate responses to environmental stimuli; therefore, phytohormones play a crucial role in salinity stress [95]. Salt stress-induced plants' survival and avoidance mechanisms hinder their growth and normal functioning [96]. However, phytohormones regulate morphological, physiological, and other biochemical processes under stressful conditions [97]. Eyidogan et al. [98] observed continued changes in the synthesis, distribution, and signal transduction of phytohormones used for plant defensive mechanisms. Further, the salt stress signal transduction triggers the release of different phytohormones that act as baseline transducers [99]. Moreover, the details of the different phytohormones acting against salt stress are given below:

3.1 Abscisic acid (ABA)

ABA is a primitively important phytohormone that regulates the plant stress response through the expression of salt-responsive genes under salinity stress [100]. ABA showed promising results at different plant developmental stages of the crops under stress conditions [101]. Therefore, it is also known as stress hormone as internal ABA signaling activates under stress conditions to survive the plant through various adaptations [102]. Many scientists observed ABA production under various abiotic stresses [103]. ABA production regulates the water potential that tends to decrease in salinity stress [104]. Plants under salinity stress caused ABA generation that influenced the movement of guard cell and leaf water potential and thus considered as an important growth hormone under stress conditions [105]. However, a different response of ABA was observed for plant roots that increase their growth under stress conditions [106]. A contributory effect of soil pH was observed for the redistribution of ABA under stress conditions that control the stomatal opening and closure during salinity stress [107].

ABA and other phytohormones regulate root growth, stress-induced gene and protein expression (dehydrins and late embryogenesis abundant proteins), and compatible solute accumulation [15]. Salinity accelerates ABA buildup, which protects plants [108] and activates salt-induced genes in roots [109]. Another study found that salt-induced ABA helps leaves to restrict Na^+ and Cl^- accumulation [110]. The ABA regulates stomatal closure to reduce transpiration and water loss [111]. Salinity causes stomatal closure due to ABA-induced cytoplasmic Ca^{++} increases. Further, succeeding plasmalemma ion channel activation and guard cell turgor losses are also linked to ABA-induced H_2O_2 generation, an intermediate signal of ABA in stomatal closure [112].

The synthesis and accumulation of osmoprotectants (proline) and dehydrins in response to ROS formation during salt-stress-induced dehydration depend on phytohormone [19]. ABA-mediated H_2O_2 buildup generates NO, which activates MAPK and upregulates ROS-scavenging antioxidant enzyme genes [113]. ABA reduces Na^+ accumulation and is translocation in shoot to augment salinity adaptation [114]. ABA increased barley (*Hordeum vulgare* L.) root vacuolar Na^+ while inhibiting xylem transport and plasmalemma influx [115]. Exogenous ABA prevented harmful Cl^{-1} ions from accumulating in citrus leaves, decreasing ethylene release and leaf abscission under salt stress [116]. Exogenously applied ABA increased K^+/Na^+ ratio that enhanced the salinity tolerance in rice [117]. Similarly, [118] found that exogenous ABA prevented Na^+ and Cl^- ions and high Na^+/K^+ ratio in rice grains. Thus, ABA boosted rice grain production *via* boosting proline, soluble sugars, and K^+ and Ca^{++} homeostasis [118]. Moreover, [109] noticed high accumulation of Ca^{2+} ions and membrane stability in plants due to ABA concentration under salt stress conditions. High

ABA contents were recorded from salt-resistant maize hybrid leaves [119]. Further, a significant amount of ABA contents were noticed from tomato genotypes showing salinity tolerance [24].

In addition, the exogenously applied ABA at 100 μM increased the expression of OsP5CS1 responsible for proline accumulation that augment 20% more survival ratio under salt stress [120]. Mahajan et al. [103] indicated that multiple transcription factors regulate ABA-responsive gene expression. Salinity stress increases ABA and induces salt and osmotic relief genes [121]. ABA and salt stress regulate AtNHX1 expression and tissue distribution as ABA influences the HVP1 and HVP10 for vacuolar H^+ inorganic pyrophosphatase and HvVHA-A for the catalytic subunit (subunit A) of ATPase [122]. They quantified the transcript levels and identified that ABA is responsible for salt-stress-adaptable gene expression in barley. Keskin et al. [102] found that ABA treatment induced TIP1 and GLP1 genes expression faster in wheat [123].

3.2 Auxin (IAA)

IAA is commonly known as the first plant hormone based on its discovery [2]. IAA controls cell elongation, vascular tissue development, and apical dominance [121]. Although IAA has been widely recognized for its effects on plant growth and development, it can regulate stress response or coordinate growth under stress [98]. Many researchers found a contributory role of IAA in plants during salinity stress [19, 124]. They proposed that a membrane-bound transcription factor (NTM2) includes auxin signal and modulates seed germination under salt stress. Further, NTM2 IAA30 gene overexpression mediates salt signaling pathway [124]. However, the mechanism of IAA to control salt stress is still unclear [123]. Further, salt alters the IAA metabolism and distribution, homeostasis, and its response against ROS production [125]. Many studies showed that IAA contents varied similar to ABA under salinity [126, 127]. High production of IAA lowers plant growth suggested the imbalance in stress-induced hormonal regulation plants [128]. However, the exogenous application of auxin augments plant growth and development.

IAA as a seed-priming agent also reduced the deleterious effects of salt stress on salt-sensitive wheat cultivar *via* regulating ionic homeostasis and auxin-induced leaf salicylic acid production [129, 130]. Auxin-response genes increase transcription of many genes in soybean, Arabidopsis, and rice [131]. Auxin/indoleacetic acid (Aux/IAA), GH_3, and SAUR gene families contain these sensitive genes. Auxin reduces rice tiller bud growth by decreasing node OsIPT expression and cytokinin production [132]. However, the discovery of novel salt stress genes allows researchers to develop genetic engineering tools for stress tolerance [133]. From the perspectives of phytohormones, it is interesting to know that salinity considerably lowered the IAA production level in maize, while salicylic acid application increased IAA production. These findings show that hormonal homeostasis and cross talk are crucial for stress response signal perception, transduction, and mediation [126].

3.3 Cytokinins

Plants produce N6-substituted adenine derivatives commonly known as cytokinins that have either an aromatic or an isoprenoid side chain. Cytokinin affects cell division, chloroplast production, apical dominance, leaf senescence, vascular differentiation, nutrient mobilization, shoot differentiation, anthocyanin production, and photomorphogenic development in plants. Cytokinin is important to reduce the negative effects of salt stress on plant growth [134]. Cytokinin seed priming increased salinity stress tolerance by lowering ABA production in wheat [135]. Cytokinin also counteracts water stress-induced leaf and fruit abscission and seed dormancy under stress conditions. They observed cytokinin as ABA antagonists and IAA antagonists/synergists in plant processes [19]. The increased regulation in plant growth as salt stress adaption was noticed due to the cytokinin production [136].

Ref. [137] found that exogenous cytokinin boosted proline content and salt resistance in egg plants. Cytokinin is an intermediary in the protective action of epibrassinolide and methyl jasmonate in wheat under salt stress [109]. Cytokinin production increased the K-shuttle under salinity. Interestingly, cytokinin production decreased in the salt-resistant cultivar of barley under 65 mM NaCl. However, salinity negatively affected the growth of salt-sensitive cultivars [138]. Salinity lowers the contents of zeatin, zeatin riboside, isopentenyl adenine, and isopentenyl adenine in root and shoot of salt-sensitive barley genotype [139]. Further, the benzyl adenine production during salt stress hinders the growth parameters of barley, while the high cytokinin production improved growth rate and shoot/root ratio [139]. Kinetin acts as a direct free radical scavenger or part of the antioxidant process that protects purine degradation in stress conditions [140].

Moreover, functional investigations of cytokinin receptor mutants showed that all three Arabidopsis cytokinin receptors negatively regulate ABA signaling and osmotic stress responses due to cytokinin-dependent CRE1/AHK4 [141]. Cytokinin receptor genes are regulated by osmotic conditions, suggesting that their function in the osmotic stress response may be similar but poorly known [2].

3.4 Gibberellic acid (GA)

Gibberellic acid concentration increases under salinity stress that helps plants to regulate their mechanism through sugar production and other antioxidant enzyme metabolism [19]. GA substantially affects seed germination, leaf expansion, stem elongation, flower and trichome initiation, and fruit development [142]. The release of photosynthetic enzymes to improve plant photosynthetic efficiency by enlarging the leaf-area index and light interception [143]. GA increases photosynthate source potential and redistribution that helps in food storage [143]. GA3 reverses the morphological and stress-protective effects of triazoles (TR), demonstrating a close link between GA3 and plant stress protection [144]. Gibberellic acid improves plant water interactions and water usage efficiency (WUE) under salinity stress [142]. Maggio et al. [145] found that GA reduced stomatal resistance and improved WUE in tomatoes. The exogenously applied GA restores metabolic activity of plants [19].

Further, GA also regulates other phytohormones that helps in soybean development and higher yield production under salinity stress [146]. This improvement is due to the increased level of bioactive GA1 and GA4 that decreased the ABA, and SA; RNA maintenance and higher protein synthesis in soybean and mustard crops [146, 147]. Salt stress reduced the enzymatic activity, whereas GA signaling improves source-sink interaction under adverse environmental conditions [19]. GA enlarged the leaf area, root growth by increasing the nitrogen and magnesium uptake under salinity stress [148]. Moreover, GA3 boosted reducing sugars, antioxidant production, and protein synthesis and lowered ribonuclease and polyphenol oxidase in salt-stressed mung bean seedlings [149].

GA3-priming reduces ions (Na^+, Cl^-) uptake and their partitioning in plant root and shoot under salinity stress that improved wheat germination [150]. Salt stress disturbs the hormonal balance that impairs plant development, whereas the use of phytohormone provides an interesting stress-reduction strategy in barley cultivar [151]. Achard et al. [152] noticed the function of DELLA protein for plant survival in salt toxicity. GA production is responsible for salt-inducible DDF1 (dwarf and delayed flowering 1) gene that increases seed germination and growth responses under high saline environments. Further, GA production is stimulated by the influence of IAA in different plant species [153]. Additionally, GA increases ABA catabolism and enhances ethylene that affects its signaling mechanism, suggesting a cross talk between these phytohormones to improve salinity tolerance in crops [154].

3.5 Salicylic acid (SA)

Salicylic acid regulates the plant growth, development, and defense mechanisms against abiotic stresses [101]. SA improves stomatal conductance, transpiration, photosynthetic rate, fruit production, glycolysis, and ion uptake and transport in plants [155].

Though, SA is well-known for its response to biotic stresses, new studies suggested a significant effect of SA to regulate plant functions in salt stress conditions [2]. SA boosts photosynthetic capability by increasing Rubisco activity and pigments [156]. Moreover, SA treatment improved soybean pigments, photosynthesis, and glucose metabolism, thus improving salt tolerance in plants [157].

SA enhanced IAA and lowered ABA in maize that resulted in higher root growth and decreased antioxidant enzyme production while under SS conditions [158]. Bastam et al. [155] found that exogenous SA application enhanced salt tolerance in pistachio seedlings, beans [159], wheat [160], barley [161], mung bean, and mustard [162, 163]. The soil application of SA reduced the salt ion accumulation in maize and mustard crops [164]. SA improves the proline and glycine betaine accumulation in plants, which produce more antioxidants that helps to tolerate the salt stress [164]. Further, the decreased lipid peroxidation and membrane permeability improves during salinity stress in plants [165]. Nazar et al. [163] recorded the antioxidant metabolism and ATP sulfurylase and nitrate reductase activity with SA treatment and improved photosynthesis in mustard growing under saline soils. Additionally, SA pre-treatment of Arabidopsis prevented salt-induced membrane depolarization and guard cell outward rectifying channel K^+ loss [166].

The use of SA as a stress management hormone provides a significant improvement in salinity tolerance to agricultural crops and resulted in higher yields. However, SA interaction with other phytohormones is important to open new avenues in the field of crop science [167].

3.6 Brassinosteroids (BRs)

Brassinosteroids are the novel phytohormone that promote plant growth under stress conditions [168]. Plants modify their response in the presence of BRs during stress [169]. The study showed that Arabidopsis' CPD gene encodes CYP90, which was a steroid hydroxylase-like cytochrome P450 protein [123]. BRs regulate stress response by activating or suppressing important enzymatic pathways, inducing protein synthesis, and producing other hormones [170]. BRs increased the ear number, length, kernel number, and weight of maize crop [171]. Exogenously applied BRs augment the pod formation and seed yield in legumes [172]. Moreover, cotton and rapeseed growth and seed yield also increased with BR application [173].

Additionally, BRs restored chlorophyll and enhanced nitrate reductase that is essential for nitrogen uptake; thus, seed germination and seedling growth of rice (*Oryza sativa* L.) improved under salinity stress [170]. The experiment showed that 0.5 M NaCl damages the cellular nuclei and chloroplast, whereas the use of BRs protects the cellular structure of barley [174]. For rice, the addition of BRs in salt solution (150 nM NaCl) reduced the Na^+ and Cl^- ion uptake, which promotes seed germination that might be due to the high soluble protein synthesis [175]. The previous studies suggested the positive response of BRs to cope salinity, however, salt stress tolerance mechanism still demands a further study.

3.7 Ethylene

Unlike other phytohormones, ethylene is found in a gaseous form and controls plant growth and development [176, 177]. Researchers called ethylene as a stress hormone that is generated by several stimuli during stress conditions [178]. Plants with high salinity tolerance produce less ethylene level [179]. Contrarily, [180] reported the high ethylene production as a marker of salt tolerance in rice. Pierik et al. [181] observed larger crop growth stimulated with ethylene production. Salinity tolerance in Arabidopsis is due to the ethylene production [152]. In addition, Cao et al. [182] reported ethylene is vital for salt tolerance as the disruption in ethylene receptor activity caused salt sensitivity in plants.

The interaction and/or balance between the receptor and ethylene determines how a plant responds to salt stress. The plant exhibits large rosette with late flowering when receptor signaling is prominent in absence of ethylene signaling, whereas the abundant ethylene signaling triggers the early flowering of tiny rosette. In this, the plant faces two different extreme conditions and needs an adjustment [182]. Fine-tuning at different levels with active homeostasis can aid in plant survival and normal growth against stress conditions [15].

The mutant analysis of triple responses of etiolated seedlings treated with ethylene revealed an Arabidopsis ethylene signal transduction pathway involved in ethylene receptors, CTR1, EIN2, and EIN3, and other components [183]. Based on the structure, the five receptor genes of Arabidopsis are divided into two subfamilies as ETR1 and ERS1 in subfamily I and ETR2, EIN4, and ERS2 in subfamily II. Ethylene binds to all these receptors, and ETR1 kinases ethylene [184]. Both the ethylene receptors and signaling regulate plant development and stress responses. In Arabidopsis, salt stress lowered the ethylene receptor ETR1 expression, which mediates plant responses to abiotic stressors. Further, Khan et al. [20] observed a complex relationship between ethylene genes with GB-mediated salinity tolerance in *T. aestivum*.

3.8 Triazoles (TRs)

Triazoles are the plant growth regulators that can also be used as a fungicides [15]. Fletcher et al. [185] reported a noteworthy role of TRs against biotic and abiotic stressors in plants. Uniconazole was the most efficient TR that helps to boost the salinity tolerance, but its residual action in plant tissues and soil limits its usage in agriculture to prevent salinity [15]. TR increased net photosynthesis, intercellular CO_2 concentration, and dry biomass of radish [185] and pigeon pea growing in saline soils [186]. The seed priming of wheat with paclobutrazol showed less Na^+ accumulation and more water-soluble carbohydrates and reducing sugars under salt stress [187]. TR application increased nitrate reductase, protease, POD, SOD, and polyphenol oxidase activity under salt stress [186]. Propiconazole (TR-containing chemical) improved root development (length and biomass) and enzymatic antioxidant activity in Madagascar periwinkle plants under NaCl stress [188]. Though TR has a significant effect on plants, few studies have examined its role in relieving salt stress in diverse crops [185].

4. Conclusion

The global impact of salinity and the trend of a growing population are alarming for food security. Crop yield and productivity is hindered in saline soil due to osmotic and ionic damage. Salt-responsive gene regulation, the antioxidant defense system, nutrient transport, osmolyte synthesis, and salt compartmentalization are badly affected under salinity stress. Plants regulate antioxidant enzymes, calcium-mediated responses, and hormonal signaling against ROS production during salinity stress to maintain homeostasis for optimal growth. However, the production of phytohormones during salinity stress regulates plant growth, seed germination, metabolism, and physiological activities. Plants use phytohormones as phytoprotectants against salinity stress. Exogenously applied phytohormones improved the stomata regulation, gas exchange mechanism that positively affected plant–water relations, and nutrient uptake, and subsequently, plant growth and productivity increased. Moreover, the hormonal priming of seeds that improves the seed germination in saline soil that results in utmost plant population. Plants integrate exogenous and endogenous cues during stressful and non-stressful situations, linking their stress response by hormonal route. Thus, hormones and their interactions with other plant components are vital to develop appropriate management strategies and phytoprotectants against salinity stress.

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References

[1] Food and Agricultural Organization (FAO). Land and Plant Nutrition Management Service. Rome, Italy: FAO; 2009. Available from: www.fao.org/wsfs/forum2050/

[2] Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, et al. Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: Consequences for changing environment. Environmental Science and Pollution Research. 2014;**3**:1-15. DOI: 10.1007/s11356-014-3754-2

[3] He W, Goodkind D, Kowal PR. An aging world. In: International Population Reports. Chapter 1. 2015. Washington DC: U.S. Government Printing Office; 2016. pp. 2-3

[4] Falcon WP, Naylor RL, Shankar ND. Rethinking global food demand for 2050. Population and Development Review. 2022;**48**:921-957. DOI: 10.1111/padr.12508

[5] Alrteimei HA, Ash'aari ZH, Muharram FM. Last decade assessment of the impacts of regional climate change on crop yield variations in the Mediterranean region. Agriculture. 2022;**12**:1787. DOI: 10.3390/agriculture12111787

[6] Saud S, Chen Y, Baowen L, Fahad S, Arooj S. The different impact on the growth of cool season turf grass under the various conditions on salinity and drought stress. International Journal of Agricultural Science Research. 2013;**3**:77-84

[7] Biswas S, Seal P, Majumder B, Biswas AK. Efficacy of seed priming strategies for enhancing salinity tolerance in plants: An overview of the progress and achievements. Plant Stress. 2023;**9**:100186. DOI: 10.1016/j.stress.2023.100186

[8] Farhat F, Ashaq N, Noman A, Aqeel M, Raja S, Naheed R, et al. Exogenous application of Moringa leaf extract confers salinity tolerance in sunflower by concerted regulation of antioxidants and secondary metabolites. Journal of Soil Science and Plant Nutrition. 2023;**23**:1-17. DOI: 10.1007/s42729-023-01301-8

[9] Kumar A, Singh S, Gaurav AK, Srivastava S, Verma JP. Plant growth-promoting bacteria: Biological tools for the mitigation of salinity stress in plants. Frontiers in Microbiology. 2020;**11**:1216. DOI: 10.3389/fmicb.2020.01216

[10] Khan A, Khan AL, Muneer S, Kim YH, Al-Rawahi A, Al-Harrasi A. Silicon and salinity: Crosstalk in crop-mediated stress tolerance mechanisms. Frontiers in Plant Sciences. 2019;**10**:1429. DOI: 10.3389/fpls.2019.01429

[11] Rahman MM, Mostofa MG, Rahman MA, Islam MR, Keya SS, Das AK, et al. Acetic acid: A cost-effective agent for mitigation of seawater-induced salt toxicity in mung bean. Scientific Reports. 2019;**9**:15186. DOI: 10.1038/s41598-019-51178-w

[12] Khan WUD, Aziz T, Maqsood MA, Farooq M, Abdullah Y, Ramzani PMA, et al. Silicon nutrition mitigates salinity Stress in maize by modulating ion accumulation, photosynthesis, and antioxidants. Photosynthetica. 2018;**56**:1047-1057. DOI: 10.3390/w15040739

[13] Paliwal S, Tripathi MK, Tiwari S, Tripathi N, Payasi DK, Tiwari PN, et al.

Molecular advances to combat different biotic and abiotic stresses in linseed (*Linum usitatissimum* L.): A comprehensive review. Genes. 2023;**14**:1461. DOI: 10.3390/genes14071461

[14] Akram R, Fahad S, Masood N, Rasool A, Ijaz M, Ihsan MZ, et al. Plant growth and morphological changes in rice under abiotic stress. In: Handbook of Advances in Rice Research for Abiotic Stress Tolerance. Sawston, Cambridge, United Kingdom: Woodhead publishing; 2019. pp. 69-85. DOI: 10.1016/B978-0-12-814332-2.00004-6

[15] Fahad S, Hussain S, Matloob A, Khan FA, Khaliq A, Saud S, et al. Phytohormones and plant responses to salinity stress: A review. Plant Growth Regulation. 2015;**75**:391-404. DOI: 10.1007/s10725-014-0013-y

[16] Saud S, Li X, Chen Y, Zhang L, Fahad S, Hussain S, et al. Silicon application increases drought tolerance of Kentucky bluegrass by improving plant water relations and morphophysiological functions. The Scientific World Journal. 2014;**2014**:368694. DOI: 10.1155/2014/368694

[17] Muchate NS, Nikalje GC, Rajurkar NS, Suprasanna P, Nikam TD. Plant salt stress: Adaptive responses, tolerance mechanism and bioengineering for salt tolerance. The Botanical Review. 2016;**82**:371-406. DOI: 10.1007/s12229-016-9173-y

[18] Sharma I, Chin I, Saini S, Bhardwaj R, Pati PK. Exogenous application of brassinosteroid offers tolerance to salinity by altering stress responses in rice variety Pusa Basmati-1. Plant Physiology and Biochemistry. 2013;**69**:17-26. DOI: 10.1016/j.plaphy.2013.04.013

[19] Iqbal N, Umar S, Khan NA, Khan MIR. A new perspective of phytohormones in salinity tolerance: Regulation of proline metabolism. Environmental and Experimental Botany. 2014;**100**:34-42. DOI: 10.1016/j.envexpbot.2013.12.006

[20] Khan MIR, Iqbal N, Masood A, Per TS, Khan NA. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. Plant Signaling & Behavior. 2013;**8**:e26374. DOI: 10.4161/psb.26374

[21] Khan M, Ali S, Manghwar H, Saqib S, Ullah F, Ayaz A, et al. Melatonin function and crosstalk with other phytohormones under normal and stressful conditions. Genes. 2022;**13**:1699. DOI: 10.3390/genes13101699

[22] Chen J, Pang X. Phytohormones unlocking their potential role in tolerance of vegetable crops under drought and salinity stresses. Frontiers in Plant Science. 2023;**14**:1121780. DOI: 10.3389/fpls.2023.1121780

[23] Iqbal N, Masood A, Khan NA. Phytohormones in salinity tolerance: Ethylene and gibberellins cross talk. In: Khan NA, Nazar R, Iqbal N, Anjum NA, editors. Phytohormones and Abiotic Stress Tolerance in Plants. Berlin: Springer; 2012. pp. 77-98. DOI: 10.1007/978-3-642-25829-9_3

[24] Amjad M, Akhtar J, Anwar-ul-Haq M, Yang A, Akhtar SC, Jacobsen E. Integrating role of ethylene and ABA in tomato plants adaptation to salt stress. Scientia Horticulturae. 2014;**172**:109-116. DOI: 10.1016/j.scienta.2014.03.024

[25] Kaur Y, Das N. Roles of polyamines in growth and development of the Solanaceous crops under normal and

stressful conditions. Journal of Plant Growth Regulation. 2022;**2**:1-22. DOI: 10.1007/s00344-022-10841-9

[26] Balasubramaniam T, Shen G, Esmaeili N, Zhang H. Plants' response mechanisms to salinity stress. Plants. 2023;**12**:2253-2267. DOI: 10.3390/plants12122253

[27] Singh A. Soil salinity: A global threat to sustainable development. Soil Use and Management. 2022;**38**:39-67. DOI: 10.1111/sum.12772

[28] Machado RMA, Serralheiro RP. Soil salinity: Effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. Horticulturae. 2017;**3**:30-37. DOI: 10.3390/horticulturae3020030

[29] Hopmans JW, Qureshi AS, Kisekka I, Munns R, Grattan SR, Rengasamy P, et al. Critical knowledge gaps and research priorities in global soil salinity. Advances in Agronomy. 2021;**169**:1-191. DOI: 10.1016/bs.agron.2021.03.001

[30] Chang AC, Brawer SD. Salinity and Drainage in San Joaquin Valley, California. Berlin/Heidelberg, Germany: Springer; 2016. pp. 6-21. DOI: 10.1007/978-94-007-6851-2

[31] Shabala S. Learning from halophytes: Physiological basis and strategies to improve abiotic stress tolerance in crops. Annals of Botany. 2013;**112**:1209-1221. DOI: 10.1093/aob/mct205

[32] Hussain S, Khaliq A, Matloob A, Wahid MA, Afzal I. Germination and growth response of three wheat cultivars to NaCl salinity. Soil and Environment. 2013;**32**:36-43

[33] Mustafa Z, Pervez MA, Ayyub CM, Matloob A, Khaliq A, Hussain S, et al. Morpho-physiological characterization of chilli genotypes under NaCl salinity. Soil and Environment. 2014;**33**:133-141

[34] Ul Islam SN, Asgher M, Khan NA. Hydrogen peroxide and its role in abiotic Stress tolerance in plants. In: Gasotransmitters Signaling in Plant Abiotic Stress: Gasotransmitters in Adaptation of Plants to Abiotic Stress. Cham: Springer International Publishing; 2023. pp. 167-195. DOI: 10.1007/978-3-031-30858-1_9

[35] Shakya R. Markers of oxidative Stress in plants. In: Ecophysiology of Tropical Plants. Boca Raton, Florida, United States: CRC Press; 2024. pp. 298-310

[36] Singh A, Rajput VD, Sharma R, Ghazaryan K, Minkina T. Salinity stress and nanoparticles: Insights into antioxidative enzymatic resistance, signaling, and defense mechanisms. Environmental Research. 2023;**235**:116585. DOI: 10.1016/j.envres.2023.116585

[37] Munns R, Tester M. Mechanisms of salinity tolerance. The Annual Review of Plant Biology. 2008;**59**:651-681. DOI: 10.1146/annurev.arplant.59.032607.092911

[38] Kamran M, Parveen A, Ahmar S, Malik Z, Hussain S, Chattha MS, et al. An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. International Journal of Molecular Sciences. 2019;**21**:148. DOI: 10.3390/ijms21010148

[39] Stavi I, Thevs N, Priori S. Soil salinity and sodicity in drylands: A review of causes, effects, monitoring, and restoration measures. Frontiers in Environmental Science. 2021;**330**:712831. DOI: 10.3389/fenvs.2021.712831

[40] Foronda DA. Reclamation of a saline-sodic soil with organic

amendments and leaching. Environmental Sciences Proceedings. 2022;**16**:56-67. DOI: 10.3390/environsciproc2022016056

[41] Knauth LP. Salinity history of the Earth's early ocean. Nature. 1998;**395**:554-555

[42] Ullah A, Bano A, Khan N. Climate change and salinity effects on crops and chemical communication between plants and plant growth-promoting microorganisms under stress. Frontiers in Sustainable Food Systems. 2021;**5**:618092. DOI: 10.3389/fsufs.2021.618092

[43] Munns R. Genes and salt tolerance: Bringing them together. New Phytologist. 2005;**167**:645-663. DOI: 10.1111/j.1469-8137.2005.01487.x

[44] Abbasi H, Jamil M, Haq A, Ali S, Ahmad R, Malik Z, et al. Salt stress manifestation on plants, mechanism of salt tolerance and potassium role in alleviating it: A review. Zemdirbyste-Agriculture. 2016;**103**:229-238. DOI: 10.13080/z-a.2016.103.030

[45] Isayenkov SV, Maathuis FJ. Plant salinity stress: Many unanswered questions remain. Frontiers in Plant Science. 2019;**10**:80-88. DOI: 10.3389/fpls.2019.00080

[46] Ji X, Tang J, Zhang J. Effects of salt stress on the morphology growth physiological parameters of *Juglans microcarpa* L. Seedl. Plants. 2022;**11**:2381. DOI: 10.3390/plants11182381

[47] Hannachi S, Steppe K, Eloudi M, Mechi L, Bahrini I, Van Labeke MC. Salt stress induced changes in photosynthesis and metabolic profiles of one tolerant ('Bonica') and one sensitive ('black beauty') eggplant cultivars (*Solanum melongena* L.). Plants. 2022;**11**:590-601. DOI: 10.3390/plants11050590

[48] Rafaliarivony S, Ranarijaona HLT, Rasoafalimanana M, Radanielina T, Wissuwa M. Evaluation of salinity tolerance of lowland rice genotypes at the reproductive stage. bioRxiv. 2022;**2**:1-25. DOI: 10.1101/2022.08.22.504861

[49] Yadav SP, Bharadwaj R, Nayak H, Mahto R, Singh RK, Prasad SK. Impact of salt stress on growth, productivity and physicochemical properties of plants: A review. International Journal of Chemical Studies. 2019;**7**:1793-1798

[50] Munns R. Comparative physiology of salt and water stress. Plant, Cell and Environment. 2002;**25**:239-250. DOI: 10.1046/j.0016-8025.2001.00808.x

[51] Chinnusamy V, Zhu JK. Plant salt tolerance. In: Plant Responses to Abiotic Stress. Berlin/Heidelberg, Germany: Springer; 2003. pp. 241-270. DOI: 10.1007/978-3-540-39402-0_10

[52] Budagovskaya NV. Rapid response reactions of buckwheat plant shoots on changes in sodium chloride concentration at the root zone and blockage of calcium channels. The European Journal of Plant Science and Biotechnology. 2010;**4**:128-130

[53] Ma Y, Dias MC, Freitas H. Drought and salinity stress responses and microbe-induced tolerance in plants. Frontiers in Plant Science. 2020;**11**:591911. DOI: 10.3389/fpls.2020.591911

[54] Tufa KN. Review on effects, mechanisms and managements of plants water stress. Irrigation and Drainage Systems. 2022;**11**:2-7. DOI: 10.37421/2168-9768.2022.11.357

[55] Ansari ZG, Rao GR, Aparna K. Physiological and molecular aspects of salinity stress tolerance in crop plants. Plant Stress Biology. 2019;**3**:47-59

[56] Alkharabsheh HM, Seleiman MF, Hewedy OA, Battaglia ML, Jalal RS, Alhammad BA, et al. Field crop responses and management strategies to mitigate soil salinity in modern agriculture: A review. Agronomy. 2021;**11**:2299. DOI: 10.3390/agronomy11112299

[57] Petretto GL, Urgeghe PP, Massa D, Melito S. Effect of salinity (NaCl) on plant growth, nutrient content, and glucosinolate hydrolysis products trends in rocket genotypes. Plant Physiology and Biochemistry. 2019;**141**:30-39. DOI: 10.1016/j.plaphy.2019.05.012

[58] Denaxa NK, Nomikou A, Malamos N, Liveri E, Roussos PA, Papasotiropoulos V. Salinity effect on plant growth parameters and fruit bioactive compounds of two strawberry cultivars, coupled with environmental conditions monitoring. Agronomy. 2022;**12**:2279-2288. DOI: 10.3390/agronomy12102279

[59] Qin C, Ahanger MA, Zhou J, Ahmed N, Wei C, Yuan S, et al. Beneficial role of acetylcholine in chlorophyll metabolism and photosynthetic gas exchange in *Nicotiana benthamiana* seedlings under salinity stress. Plant Biology. 2020;**22**:357-365. DOI: 10.1111/plb.13079

[60] Al Hinai MS, Ullah A, Al-Rajhi RS, Farooq M. Proline accumulation, ion homeostasis and antioxidant defence system alleviate salt stress and protect carbon assimilation in bread wheat genotypes of Omani origin. Environmental and Experimental Botany. 2022;**193**:104687. DOI: 10.1016/j.envexpbot.2021.104687

[61] Orzechowska A, Trtílek M, Tokarz KM, Szymanska R, Niewiadomska E, Rozpadek P, et al. Thermal analysis of stomatal response under salinity and high light. International Journal of Molecular Sciences. 2021;**22**:4663. DOI: 10.3390/ijms22094663

[62] Zahra N, Al Hinai MS, Hafeez MB, Rehman A, Wahid A, Siddique KH, et al. Regulation of photosynthesis under salt stress and associated tolerance mechanisms. Plant Physiology and Biochemistry. 2022;**178**:55-69. DOI: 10.1016/j.plaphy.2022.03.003

[63] Seleiman MF, Aslam MT, Alhammad BA, Hassan MU, Maqbool R, Chattha MU, et al. Salinity stress in wheat: Effects, mechanisms and management strategies. Phyton. 2022;**91**:667-694. DOI: 10.32604/phyton.2022.017365

[64] Zahra N, Wahid A, Shaukat K, Hafeez MB, Batool A, Hasanuzzaman M. Oxidative stress tolerance potential of milk thistle ecotypes after supplementation of different plant growth-promoting agents under salinity. Plant Physiology and Biochemistry. 2021;**166**:53-65. DOI: 10.1016/j.plaphy.2021.05.042

[65] Huihui Z, Yue W, Xin L, Guoqiang H, Yanhui C, Zhiyuan T, et al. Chlorophyll synthesis and the photoprotective mechanism in leaves of mulberry (Morus alba L.) seedlings under NaCl and $NaHCO_3$ stress revealed by TMT-based proteomics analyses. Ecotoxicology and Environmental Safety. 2020;**190**:110164. DOI: 10.1016/j.ecoenv.2020.110164

[66] Goussi R, Manaa A, Derbali W, Cantamessa S, Abdelly C, Barbato R. Comparative analysis of salt stress, duration and intensity, on the chloroplast ultrastructure and photosynthetic apparatus in *Thellungiella salsuginea*. Journal of Photochemistry and Photobiology B: Biology. 2018;**183**:275-287. DOI: 10.1016/j.jphotobiol.2018.04.047

[67] Hnilickova H, Kraus K, Vachova P, Hnilicka F. Salinity stress affects photosynthesis, malondialdehyde formation, and proline content in *Portulaca oleracea* L. Plants. 2021;**10**:845-852. DOI: 10.3390/plants10050845

[68] Wang X, Wang W, Huang J, Peng S, Xiong D. Diffusional conductance to CO_2 is the key limitation to photosynthesis in salt-stressed leaves of rice (*Oryza sativa*). Physiologia Plantarum. 2018;**163**:45-58. DOI: 10.1111/ppl.12653

[69] Silva END, Ribeiro RV, Ferreira-Silva SL, Viégas RA, Silveira JAG. Salt stress induced damages on the photosynthesis of physic nut young plants. Scientia Agricola. 2011;**68**:62-68. DOI: 10.1590/S0103-90162011000100010

[70] Berry W, Wallace A. Toxicity: The concept and relationship to the dose response curve. Journal of Plant Nutrition. 1981;**3**:13-19. DOI: 10.1080/01904168109362814

[71] Grattan SR, Grieve CM. Mineral element acquisition and growth response of plants grown in saline environments. Agriculture, Ecosystems & Environment. 1992;**38**:275-300. DOI: 10.1016/0167-8809(92)90151-Z

[72] Kumar A, Kumar A, Bihari B, Qasmi M. Soil fertility and mineral nutrition of plants. Current Research in Soil Fertility. 2020;**65**:23-35

[73] Aslam MT, Imran K, Chattha MU, Maqbool R, Ziaulhaq M, Lihong W, et al. The critical role of nitrogen in plants facing the salinity stress: Review and future prospective. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2023;**51**:13347-13347. DOI: 10.15835/nbha51313347

[74] Janaki D. Nutrient transformation in salt affected soil. In: Current Research in Soil Fertility. Vol. 24. AkiNik Publications; 2022. pp. 83-100

[75] Munns R, Termaat A. Whole-plant responses to salinity. Functional Plant Biology. 1986;**13**:143-160. DOI: 10.1071/PP9860143

[76] Hao D, Li X, Kong W, Chen R, Liu J, Guo H, et al. Phosphorylation regulation of nitrogen, phosphorus, and potassium uptake systems in plants. The Crop Journal. 2023;**3**:45-56. DOI: 10.1016/j.cj.2023.06.003

[77] Turcios AE, Papenbrock J, Tränkner M. Potassium, an important element to improve water use efficiency and growth parameters in quinoa (*Chenopodium quinoa*) under saline conditions. Journal of Agronomy and Crop Science. 2021;**207**:618-630. DOI: 10.1111/jac.12477

[78] Ran X, Huang X, Wang X, Liang H, Wang Y, Li J, et al. Ion absorption, distribution and salt tolerance threshold of three willow species under salt stress. Frontiers in Plant Science. 2022;**13**:969896. DOI: 10.3389/fpls.2022.969896

[79] Hasana R, Miyake H. Salinity stress alters nutrient uptake and causes the damage of root and leaf anatomy in maize. KnE Life Sciences. 2017;**3**:219-225. DOI: 10.18502/kls.v3i4.708

[80] Cruz JL, Coelho EF, Coelho Filho MA, Santos AAD. Salinity reduces nutrients absorption and efficiency of their utilization in cassava plants. Ciência Rural. 2018;**48**:e20180351. DOI: 10.1590/0103-8478cr20180351

[81] Hailu B, Mehari H. Impacts of soil salinity/sodicity on soil-water relations and plant growth in dry land areas: A review. Journal of Natural Sciences Research. 2021;**12**:1-10. DOI: 10.7176/JNSR/12-3-01

[82] Betzen BM, Smart CM, Maricle KL, MariCle BR. Effects of increasing salinity on photosynthesis and plant water potential in Kansas salt marsh species. Transactions of the Kansas Academy of Science. 2019;**122**:49-58. DOI: 10.1660/062.122.0105

[83] Taneenah A, Nulit R, Yusof UK, Janaydeh M. Tolerance of Molokhia (*Corchorus olitorius* L.) seed with dead sea water, sea water, and NaCl: Germination and anatomical approach. Advances in Environmental Biology. 2015;**9**:106-116

[84] Chaudhuri K, Choudhuri MA. Effects of short-term NaCl stress on water relations and gas exchange of two jute species. Journal of Agricultural Research. 1997;**40**:373-380. DOI: 10.1023/A:1001013913773

[85] Álvarez S, Sánchez-Blanco MJ. Long-term effect of salinity on plant quality, water relations, photosynthetic parameters and ion distribution in *Callistemon citrinus*. Plant Biology. 2014;**16**:757-764. DOI: 10.1111/plb.12106

[86] Sheldon AR, Dalal RC, Kirchhof G, Kopittke PM, Menzies NW. The effect of salinity on plant-available water. Plant and Soil. 2017;**418**:477-491. DOI: 10.1007/s11104-017-3309-7

[87] Rajasekaran LR, Aspinall D, Jones GP, Paleg LG, Stress metabolism. IX. Effect of salt stress on trigonelline accumulation in tomato. Canadian Journal of Plant Science. 2001;**81**:487-498. DOI: 10.4141/P00-079

[88] Shaheen S, Naseer S, Ashraf M, Akram NA. Salt stress affects water relations, photosynthesis, and oxidative defense mechanisms in *Solanum melongena* L. Journal of Plant Interaction. 2013;**8**:85-96. DOI: 10.1080/17429145.2012.718376

[89] Mirfattahi Z, Karimi S, Roozban MR. Salinity induced changes in water relations, oxidative damage and morpho-physiological adaptations of pistachio genotypes in soilless culture. Acta Agriculturae Slovenica. 2017;**109**:291-302

[90] Munns R, Gilliham M. Salinity tolerance of crops–what is the cost? New Phytologist. 2015;**208**:668-673. DOI: 10.1111/nph.13519

[91] Maas EV, Hoffman GJ. Crop salt tolerance-current assessment. Journal of the Irrigation and Drainage Division. 1977;**103**:115-134. DOI: 10.1061/JRCEA4.0001137

[92] Volkmar KM, Hu Y, Steppuhn H. Physiological responses of plants to salinity: A review. Canadian Journal of Plant Science. 1998;**78**:19-27. DOI: 10.4141/P97-020

[93] Amombo E, Ashilenje D, Hirich A, Kouisni L, Oukarroum A, Ghoulam C, et al. Exploring the correlation between salt tolerance and yield: Research advances and perspectives for salt-tolerant forage sorghum selection and genetic improvement. Planta. 2022;**255**:71-88. DOI: 10.1007/s00425-022-03847-w

[94] Peleg Z, Blumwald E. Hormone balance and abiotic stress tolerance in crop plants. Current Opinion in Plant Biology. 2011;**14**:290-295. DOI: 10.1016/j.pbi.2011.02.001

[95] Davies PJ. Plant Hormones: Biosynthesis, Signal Transduction, Action. Kluwer, Dordrecht: Springer Science & Business Media; 2004

[96] Skirycz A, Inze´ D. More from less: Plant growth under limited water. Current Opinion in Biotechnology. 2010;**21**:197-203. DOI: 10.1016/j.copbio.2010.03.002

[97] Kosakivska IV, Vedenicheva NP, Babenko LM, Voytenko LV, Romanenko KO, Vasyuk VA. Exogenous phytohormones in the regulation of growth and development of cereals under abiotic stresses. Molecular Biology Reports. 2022;**49**:617-628. DOI: 10.1007/s11033-021-06802-2

[98] Eyidogan F, Oz MT, Yucel M, Oktem HA. Signal transduction of phytohormones under abiotic stresses. In: Khan NA, Nazar R, Iqbal N, Anjum NA, editors. Phytohormones and Abiotic Stress Tolerance in Plants. Berlin: Springer; 2012. pp. 1-48. DOI: 10.1007/978-3-642-25829-9_1

[99] Harrison MA. Cross-talk between phytohormone signaling pathways under both optimal and stressful environmental conditions. In: Khan NA, Nazar R, Iqbal N, Anjum NA, editors. Phytohormones and Abiotic Stress Tolerance in Plants. Berlin: Springer; 2012. pp. 49-76. DOI: 10.1007/978-3-642-25829-9_2

[100] Sharma N, Abrams SR, Waterer DR. Uptake, movement, activity, and persistence of an abscisic acid analog (80 acetylene ABA methyl ester) in marigold and tomato. Journal of Plant Growth Regulation. 2005;**24**:28-35. DOI: 10.1007/s00344-004-0438-z

[101] Devinar G, Llanes A, Masciarelli O, Luna V. Different relative humidity conditions combined with chloride and sulfate salinity treatments modify abscisic acid and salicylic acid levels in the halophyte *Prosopis strombulifera*. Plant Growth Regulation. 2013;**70**:247-256. DOI: 10.1007/s10725-013-9796-5

[102] Keskin BC, Sarikaya AT, Yuksel B, Memon AR. Abscisic acid regulated gene expression in bread wheat. Australian Journal of Crop Science. 2010;**4**:617-625. DOI: 10.3316/INFORMIT.857732547077080

[103] Mahajan S, Tuteja N. Cold, salinity and drought stresses: An overview. Archives of Biochemistry and Biophysics. 2005;**444**:139-158. DOI: 10.1016/j.abb.2005.10.018

[104] Yildiz M, Poyraz İ, Çavdar A, Özgen Y, Beyaz R. Plant responses to salt stress. In: Plant Breeding-Current and Future Views. London, UK: IntechOpen; 2020. DOI: 10.5772/intechopen.93920

[105] Zhang J, Jia W, Yang J, Ismail AM. Role of ABA in integrating plant responses to drought and salt stresses. Field Crops Research. 2006;**97**:111-119. DOI: 10.1016/j.fcr.2005.08.018

[106] Jia W, Wang Y, Zhang S, Zhang J. Salt -stress-induced ABA accumulation is more sensitively triggered in roots than in shoots. Journal of Experimental Botany. 2002;**53**:2201-2206. DOI: 10.1093/jxb/erf079

[107] Hartung W, Sauter A, Hose E. Abscisic acid in the xylem: Where does it come from, where does it go to? Journal of Experimental Botany. 2002;**53**:27-32. DOI: 10.1093/jexbot/53.366.27

[108] Shakirova FM, Avalbaev AM, Bezrukova MV, Kudoyarova GR. Role of endogenous hormonal system in the realization of the antistress action of plant growth regulators on plants. Plant Stress. 2010;**4**:32-38

[109] Parida AK, Das AB. Salt tolerance and salinity effects on plants: A review. Ecotoxicology & Environmental Safety. 2005;**60**:324-349. DOI: 10.1016/j.ecoenv.2004.06.010

[110] Cabot C, Sibole JV, Barcelo J, Poschenrieder C. Abscisic acid decreases leaf Na+ exclusion in salt treated *Phaseolus vulgaris* L. Journal of Plant Growth Regulation. 2009;**28**:187-192. DOI: 10.1007/s00344-009-9088-5

[111] Wilkinson S, Davies WJ. Drought, ozone, ABA and ethylene: New insights from cell to plant to community. Plant Cell and Environment. 2010;**33**:510-525. DOI: 10.1111/j.1365-3040.2009.02052.x

[112] Kim TW, Wang ZY. Brassinosteroid signal transduction from receptor kinases to transcription factors. Annual Review of Plant Biology. 2010;**61**:681-704. DOI: 10.1146/annurev.arplant.043008.092057

[113] Lee BR, La VH, Park SH, Mamun MA, Bae DW, Kim TH. H_2O_2-responsive hormonal status involves oxidative burst signaling and proline metabolism in rapeseed leaves. Antioxidants. 2022;**11**:566. DOI: 10.3390/antiox11030566

[114] Khadri M, Tejera NA, Lluch C. Sodium chloride-ABA interaction in two common bean (*Phaseolus vulgaris*) cultivars differing in salinity tolerance. Environmental and Experimental Botany. 2007;**60**:211-218. DOI: 10.1016/j.envexpbot.2006.10.008

[115] Behl R, Jeschke WD. Influence of abscisic acid on unidirectional fluxes and intracellular compartmentation of K^+ and Na^+ in excised barley root segments. Physiologia Plantarum. 1981;**53**:95-100. DOI: 10.1111/j.1399-3054.1981.tb04116.x

[116] Gomez CA, Arbona V, Jacas J, Primomillo E, Talon M. Abscisic acid reduces leaf abscission and increases salt tolerance in citrus plants. The Journal of Plant Growth Regulation. 2002;**21**:234-240. DOI: 10.1007/s00344-002-0013-4

[117] Bohra JS, Dorffling H, Dorffling K. Salinity tolerance of rice (*Oryza sativa* L.) with reference to endogenous and exogenous abscisic acid. Journal of Agronomy and Crop Science. 1995;**174**:79-86. DOI: 10.1111/j.1439-037X.1995.tb00197.x

[118] Gurmani AR, Bano A, Ullah N, Khan H, J-ahangir M, Flowers TJ. Exogenous abscisic acid (ABA) and silicon (Si) promote salinity tolerance by reducing sodium (Na^+) transport and bypass flow in rice (*Oryza sativa* indica). Australian Journal of Crop Science. 2013;**7**:1219-1226. DOI: 10.3316/INFORMIT.619232089838636

[119] Zorb C, Geilfusb CM, Muhlingb KH, Ludwig-Muller J. The influence of salt stress on ABA and auxin concentrations in two maize cultivars differing in salt resistance. Journal of Plant Physiology. 2014;**170**:220-224. DOI: 10.1016/j.jplph.2012.09.012

[120] Sripinyowanich S, Klomsakul P, Boonburapong B, Bangyeekhun T, Asami T, Gu H, et al. Exogenous ABA induces salt tolerance in indica rice (Oryza sativa L.): The role of OsP5CS1 and OsP5CR gene expression during salt stress. Environmental and Experimental Botany. 2013;**86**:94-105. DOI: 10.1016/j.envexpbot.2010.01.009

[121] Wang Y, Mopper S, Hasentein KH. Effects of salinity on endogenous ABA, IAA, JA, and SA in Iris hexagona. Journal of Chemical Ecology. 2001;**27**:327-342. DOI: 10.1023/A:1005632506230

[122] Shi HZ, Zhu JK. Regulation of expression of the vacuolar Na^+/H^+ antiporter gene AtNHX1 by salt stress and abscisic acid. Plant Molecular Biology. 2002;**50**:543-550. DOI: 10.1023/A:1019859319617

[123] Javid MG, Sorooshzadeh A, Moradi F, Sanavy SAMM, Allahdadi I. The role of phytohormones in alleviating salt stress in crop plants. Australian Journal of Crop Science. 2011;**5**:726-734

[124] Jung J, Park C. Auxin modulation of salt stress signaling in arabidopsis seed germination. Plant Signaling

and Behavior. 2011;**6**:1198-1200. DOI: 10.4161/psb.6.8.15792

[125] Schopfer P, Liszkay A, Bechtold M, Frahry G, Wagner A. Evidence that hydroxyl radicals mediate auxin-induced extension growth. Planta. 2002;**214**:821-828. DOI: 10.1007/s00425-001-0699-8

[126] Fahad S, Saud S, Chen Y, Wu C, Wang D. Abiotic Stress in Plants. London, UK: BoD–Books on Demand; 2021. DOI: 10.5772/intechopen.91549

[127] El Sabagh A, Islam MS, Hossain A, Iqbal MA, Mubeen M, Waleed M, et al. Phytohormones as growth regulators during abiotic stress tolerance in plants. Frontiers in Agronomy. 2022;**4**:765068. DOI: 10.3389/fagro.2022.765068

[128] Ribaut JM, Pilet PE. Water stress and indole-3ylacetic acid content of maize roots. Planta. 1994;**193**:502-507. DOI: 10.1007/BF02411554

[129] Iqbal M, Ashraf M. Seed treatment with auxins modulates growth and ion partitioning in salt-stressed wheat plants. Journal of Integrated Plant Biology. 2007;**49**:1003-1015. DOI: 10.1111/j.1672-9072.2007.00488.x

[130] Iqbal M, Ashraf M. Salt tolerance and regulation of gas exchange and hormonal homeostasis by auxin-priming in wheat. Pesquisa Agropecuaria Brasileira. 2013;**48**:1210-1219. DOI: 10.1590/S0100-204X2013000900004

[131] Hagen G, Guilfoyle T. Auxin-responsive gene expression: Genes, promoters and regulatory factors. Plant Molecular Biology. 2002;**49**:373-385. DOI: 10.1023/A:1015207114117

[132] Liu Y, Xu J, Ding Y, Wang Q, Li G, Wang S. Auxin inhibits the outgrowth of tiller buds in rice (*Oryza sativa* L.) by downregulating OsIPT expression and cytokinin biosynthesis in nodes. Australian Journal of Crop Science. 2011;**5**:169-174

[133] Zhu JK. Salt and drought stress signal transduction in plants. Annual Review of Plant Biology. 2002;**53**:247-273. DOI: 10.1146/annurev.arplant.53.091401.143329

[134] Barciszewski J, Siboska G, Rattan SIS, Clark BFC. Occurrence, biosynthesis and properties of kinetin (N6-furfuryladenine). Plant Growth Regulation. 2000;**32**:257-265. DOI: 10.1023/A:1010772421545

[135] Iqbal M, Ashraf M, Jamil A. Seed enhancement with cytokinins: Changes in growth and grain yield in salt stressed wheat plants. Plant Growth Regulation. 2006;**50**:29-39. DOI: 10.1007/s10725-006-9123-5

[136] Hadiarto T, Tran LS. Progress studies of drought-responsive genes in rice. Plant Cell Reports. 2011;**30**:297-310. DOI: 10.1007/s00299-010-0956-z

[137] Wu X, He J, Chen J, Yang S, Zha D. Alleviation of exogenous 6-benzyladenine on two genotypes of egg-plant (*Solanum melongena* mill.) growth under salt stress. Protoplasma. 2013;**251**:169-176. DOI: 10.1007/s00709-013-0535-6

[138] Kuiper D, Kuiper PJC, Lambers H, Schuit J, Staal M. Cytokinin concentration in relation to mineral nutrition and benzyladenine treatment in Plantago major ssp. Pleiosperma. Physiologia Plantarum. 1989;**75**:511-517. DOI: 10.1111/j.1399-3054.1989.tb05617.x

[139] Kuiper D, Schuit J, Kuiper PJC. Actual cytokinin concentrations in plant tissue as an indicator for salt resistance in cereals. Plant and Soil. 1990;**123**:243-250. DOI: 10.1007/BF00011276

[140] Chakrabarti N, Mukherji S. Alleviation of NaCl stress by pretreatment with phytohormones in Vigna radiata. Plant Biology. 2003;**46**:589-594. DOI: 10.1023/A:1024827931134

[141] Tran LSP, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, et al. Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in Arabidopsis. National Academy of Sciences of the United States of America. 2007;**104**:20623-20628. DOI: 10.1073/pnas.0706547105

[142] Yamaguchi S. Gibberellin metabolism and its regulation. Annual Review of Plant Biology. 2008;**59**:225-251. DOI: 10.1146/annurev.arplant.59.032607.092804

[143] Khan NA, Singh S, Nazar R, Lone PM. The source–sink relationship in mustard. Asian and Australasian Journal of Plant Science and Biotechnology. 2007;**1**:10-18

[144] Gilley A, Flecher RA. Gibberellin antagonizes paclobutrazole induced stress protection in wheat seedlings. Journal of Plant Physiology. 2007;**103**:200-207. DOI: 10.1016/S0176-1617(98)80066-7

[145] Maggio A, Barbieri G, Raimondi G, de Pascale S. Contrasting effects of GA3 treatments on tomato plants exposed to increasing salinity. Journal of Plant Growth Regulation. 2010;**29**:63-72. DOI: 0.1007/s00344-009-9114-7

[146] Hamayun M, Khan SA, Khan AL, Shin JH, Ahmad B, Shin DH, et al. Exogenous gibberellic acid reprograms soybean to higher growth and salt stress tolerance. Journal of Agricultural and Food Chemistry. 2010;**58**:7226-7232. DOI: 10.1021/jf101221t

[147] Siddiqui MH, Khan MN, Mohammad F, Khan MMA. Role of nitrogen and gibberellic acid (GA3) in the regulation of enzyme activities and in osmoprotectant accumulation in *Brassica juncea* L. under salt stress. Journal of Agronomy and Crop Science. 2008;**194**:214-224. DOI: 10.1111/j.1439-037X.2008.00308.x

[148] Tuna AL, Kaya C, Dikilitas M, Higgs D. The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environmental and Experimental Botany. 2008;**62**:1-9. DOI: 10.1016/j.envexpbot.2007.06.007

[149] Mohammed AHMA. Physiological aspects of mungbean plant (Vigna radiata L. Wilczek) in response to salt stress and gibberellic acid treatment. Research Journal of Agriculture and Biological Sciences. 2007;**3**:200-213

[150] Radi AF, Shaddad MAK, El-Enany AE, Omran FM. Interactive effects of plant hormones (GA3 or ABA) and salinity on growth and some metabolites of wheat seedlings. In: Plant Nutrition: Food Security and Sustainability of Agro-Ecosystems Through Basic and Applied Research. Vol. 92. Springer Science & Business Media; 2006. pp. 436-437. DOI: 10.1007/0-306-47624-X_211

[151] Vettakkorumakankav NA. Crucial role for gibberellin in stress protecting of plants. Plant and Cell Physiology. 1999;**40**:542-548. DOI: 10.1093/oxfordjournals.pcp.a029575

[152] Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, et al. Integration of plant responses to environmentally activated phytohormonal signals. Science. 2006;**311**:91-94. DOI: 10.1126/science.111864

[153] Wolbang CM, Chandler PM, Smith JJ, Ross JJ. Auxin from the developing inflorescence is required for the biosynthesis of active gibberellins in barley stems. Plant Physiology. 2004;**134**:769-776. DOI: 10.1104/pp.103.030460

[154] Gonai T, Kawahara S, Tougou M, Satoh S, Hashiba T, Hirai N, et al. Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. Journal of Experimental Botany. 2004;**55**:111-118. DOI: 10.1093/jxb/erh023

[155] Bastam N, Baninasab B, Ghobadi C. Improving salt tolerance by exogenous application of salicylic acid in seedlings of pistachio. Plant Growth Regulation. 2013;**69**:275-284. DOI: 10.1007/s10725-012-9770-7

[156] Mimouni H, Wasti S, Manaa A, Gharbi E, Chalh A, Vandoorne B, et al. Does salicylic acid (SA) improve tolerance to salt stress in plants? A study of SA effects on tomato plant growth, water dynamics, photosynthesis, and biochemical parameters. Omics: a Journal of Integrative Biology. 2016;**20**:180-190. DOI: 10.1089/omi.2015.0161

[157] Khodary SEA. Effect of salicylic acid on growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. International Journal of Agriculture and Biology. 2004;**6**:5-8

[158] Fahad S, Bano A. Effect of salicylic acid on physiological and biochemical characterization of maize grown in saline area. Pakistan Journal of Botany. 2012;**44**:1433-1438

[159] Azooz MM. Salt stress mitigation by seed priming with salicylic acid in two faba bean genotypes differing in salt tolerance. International Journal of Agriculture and Biology. 2009;**11**:343-350

[160] Sakhabutdinova AR, Fatkhutdinova DR, Bezrukova MV, Shakirova FM. Salicylic acid prevents the damaging action of stress factors on wheat plants. Bulgarian Journal of Plant Physiology. 2003;**29**:314-319

[161] El-Tayeb MA. Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regulation. 2005;**45**:215-225. DOI: 10.1007/s10725-005-4928-1

[162] Khan NA, Syeed S, Masood A, Nazar R, Iqbal N. Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mungbean and alleviates adverse effects of salinity stress. International Journal of Plant Biology. 2010;**1**:11-21. DOI: 10.4081/pb.2010.e1

[163] Nazar R, Iqbal N, Syeed S, Khan NA. Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. Journal of Plant Physiology. 2011;**168**:807-815. DOI: 10.1016/j.jplph.2010.11.001

[164] Gunes A, Inal A, Alpaslan M, Eraslan F, Bagci EG, Cicek N. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (Zea mays L.) grown under salinity. Journal of Plant Physiology. 2007;**164**:728-736. DOI: 10.1016/j.jplph.2005.12.009

[165] Horvath E, Szalai G, Janda T. Induction of abiotic stress tolerance by salicylic acid signaling. Journal of Plant Growth Regulation. 2007;**26**:290-300. DOI: 10.1007/s00344-007-9017-4

[166] Jayakannan M, Bose J, Babourina O, Rengel Z, Shabala S.

Salicylic acid improves salinity tolerance in Arabidopsis by restoring membrane potential and preventing salt-induced K+ loss via a GORK channel. Journal of Experimental Botany. 2013;**64**:2255-2268. DOI: 10.1093/jxb/ert085

[167] Pieterse CM, Leon-Reyes A, Van Der Ent S, Van Wees SCM. Networking by small molecule hormones in plant immunity. Nature Chemical Biology. 2009;**5**:308-316

[168] Bhardwaj R, Arora HK, Nagar PK, Thukral AK. Brassinosteroids-a novel group of plant hormones. In: Trivedi PC, editor. Plant Molecular Physiology-Current Scenario and Future Projections. Jaipur, India: Aavishkar Publishers; 2006. pp. 58-84

[169] Yang J, Miao W, Chen J. Roles of jasmonates and brassinosteroids in rice responses to high temperature stress–a review. The Crop Journal. 2021;**9**:977-985. DOI: 10.1016/j.cj.2021.02.007

[170] Bajguz A, Hayat S. Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiology and Biochemistry. 2009;**47**:1-8. DOI: 10.1016/j.plaphy.2008.10.002

[171] Ali Q, Athar HR, Ashraf M. Modulation of growth, photosynthetic capacity and water relations in salt stressed wheat plants by exogenously applied 24-epibrassinolide. Plant Growth Regulation. 2008;**56**:107-116. DOI: 10.1007/s10725-008-9290-7

[172] Rao AAR, Vardhini BV, Sujatha E, Anuradha S. Brassinosteroids a new class of phytohormones. Current Science. 2002;**82**:1239-1245

[173] Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: A review. Environmental and Experimental Botany. 2010;**68**:14-25. DOI: 10.1016/j.envexpbot.2009.08.005

[174] Krishna P. Brassinosteroid-mediated stress responses. Journal of Plant Growth Regulation. 2003;**22**:289-297. DOI: 10.1007/s00344-003-0058-z

[175] Anuradha S, Rao SSR. Effect of brassinosteroids on salinity stress induced inhibition of seed germination and seedling growth of rice (*Oryza sativa* L.). Plant Growth Regulation. 2001;**33**:151-153. DOI: 10.1023/A:1017590108484

[176] Bakshi A, Shemansky JM, Chang C, Binder BM. History of research on the plant hormone ethylene. Journal of Plant Growth Regulation. 2015;**34**:809-827. DOI: 10.1007/s00344-015-9522-9

[177] Kwon TH, Park CG, Lee BH, Jeong IH, Lee SE. A new approach: Ethyl formate fumigation to control *Bemisia tabaci* (Hemiptera: Aleyrodidae) in a yellow melon vinyl house. Applied Sciences. 2022;**12**:5173. DOI: 10.3390/app12105173

[178] Wang B, Zhang J, Xia X, Zhang WH. Ameliorative effect of brassinosteroid and ethylene on germination of cucumber seeds in the presence of sodium chloride. Plant Growth Regulation. 2011;**65**:407-413. DOI: 10.1007/s10725-011-9595-9

[179] El-Iklil Y, Karrou M, Benichou M. Salt stress effect on epinasty in relation to ethylene production and water relations in tomato. Agronomie. 2000;**20**:399-406. DOI: 10.1051/agro:2000136

[180] Khan AA, Akbar M, Seshu DV. Ethylene as an indicator of salt tolerance in rice. Crop Science. 1987;**27**:1242-1248. DOI: 10.2135/cropsci1987.0011183X0027 00060031x

[181] Pierik R, Tholen D, Poorter H, Visser EJW, Voesenek LACJ. The janus face of ethylene: Growth inhibition and stimulation. Trends in Plant Science. 2006;**11**:176-183. DOI: 10.1016/j.tplants.2006.02.006

[182] Cao WH, Liu J, He XJ, Mu RL, Zhou HL, Chen SY, et al. Modulation of ethylene responses affects plant salt-stress responses. Plant Physiology. 2007;**143**:707-719. DOI: 10.1104/pp.106.094292

[183] Guo H, Ecker JR. The ethylene signaling pathway: New insights. Current Opinion in Plant Biology. 2004;**7**:40-49. DOI: 10.1016/j.pbi.2003.11.011

[184] Hall AE, Findell JL, Schaller GE, Sisler EC, Bleecker AB. Ethylene perception by the ERS1 protein in arabidopsis. Plant Physiology. 2000;**123**:1449-1457. DOI: 10.1104/pp.123.4.1449

[185] Fletcher RA, Gill A, Davis TD, Sankhla N. Triazoles as plant growth regulators and stress protectants. Horticultural Reviews. 2000;**24**:55-138

[186] Karikalan L, Rajan SN, Gopi R, Sujatha BM, Pannersevalam R. Induction of salt tolerance by triadimefon in pigeon pea (*Cajanus cajan* L.) mill sp. Indian Journal of Experimental Biology. 1999;**37**:825-829

[187] Hajihashemi S, Kiarostami K, Enteshari S, Saboora A. Effect of paclobutrazol on wheat salt tolerance at pollination stage. Russian Journal of Plant Physiology. 2009;**56**:251-257. DOI: 10.1134/S1021443709020149

[188] Jaleel CA, Gopi R, Manivannan P, Gomathinayagam M, Murali PV, Panneerselvam R. Soil applied propiconazole alleviates the impact of salinity on *Catharanthus roseus* by improving antioxidant status. Pesticide Biochemistry and Physiology. 2008;**90**:135-139. DOI: 10.1016/j.pestbp.2007.11.003

Chapter 3

Phytohormone-Producing Rhizobacteria and Their Role in Plant Growth

Tekalign Kejela

Abstract

Phytohormone-producing rhizobacteria are a group of beneficial bacteria residing in the rhizosphere that have the unique ability to produce, release, and also modulate phytohormones such as auxins, cytokinins, gibberellins, ethylene, and jasmonic acid (JA). This work explores a diverse group of rhizobacteria that possess the ability to synthesize and secrete phytohormones and their effects on the growth of different plants. Indole-3-acetic acid (IAA) is a commonly produced hormone by many rhizobacteria that include *Azospirillum brasilense*, *Pseudomonas putida,* and *Pseudomonas fluorescens*. IAA producers promote plant growth through multiple mechanisms. Gibberellic acid (GA3) produced by certain species of rhizobacteria, which include *Serratia marcescens* and *Bacillus licheniformis*, enhances plant height and biomass in different crops. Cytokinins are produced by rhizobacteria, including *Bacillus, Pseudomonas*, and *Azospirillum*. Few rhizobacteria strains also produce abscisic acid (ABA). For example, *A. brasilense* produces abscisic acid, which can regulate the plant water status and enhance drought tolerance in different crops. Several rhizobacteria, including *P. fluorescens*, *P. putida*, and *Pseudomonas aeruginosa*, have been reported to induce JA production in plants, promoting defense responses against pathogens. Overall, this work indicates that rhizobacteria produce key phytohormones, enabling them to promote plant growth through multifarious ways, and hence phytohormone-producing rhizobacteria are potential input in agricultural production.

Keywords: rhizobacteria, auxins, cytokinins, gibberellins, jasmonic acid, ethylene

1. Introduction

Rhizosphere is the soil volume under the physical and biological influence of the root, including the root tissues colonized by microorganisms. Rhizosphere bacteria (rhizobacteria) are a group of bacteria competent in colonizing the root environment. About 2–5% of rhizobacteria, when reintroduced by plant inoculation in a soil containing competitive microflora, exert a beneficial effect on plant growth and are termed as plant growth-promoting rhizobacteria (PGPR) [1]. Plant growth-promoting rhizobacteria display a wide array of mechanisms to promote the development of plants. Among the different mechanisms, diverse PGPR can alter root architecture and promote plant development due to their ability to synthesize and secrete plant hormones (phytohormones) that include indole-3-acetic acid (IAA), gibberellins (GAs), cytokinins, and certain volatiles, hence they are termed phytostimulators [2]. The capacity of rhizobacteria to produce phytohormones is strain specific [3].

The PGPR stimulatory effect comes from a manipulation of the complex and balanced network of plant hormones that are directly involved in growth or stimulation of the root formation. For instance, the biosynthesis of IAA by various PGPR has been demonstrated to enhance root proliferation [4]. Bacteria use this phytohormone to interact with plants as part of their colonization strategy, including phytostimulation and avoidance of basal plant defense mechanisms. Moreover, it has recently been indicated that IAA can also be a bacterial signaling molecule and can therefore have a direct effect on bacterial physiology. Similarly, PGPB-synthesized gibberellins can increase plant stem growth, alter the dormancy of germinating seeds, and increase leaf and fruit senescence [5]. In the same way, PGPB-produced cytokinins are important for the establishment of interaction between plants and bacteria and also alleviate the damage engendered by different abiotic stresses [6, 7]. PGPR, which acquire the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, facilitate plant growth and development by decreasing ethylene levels, inducing salt tolerance, and reducing drought stress in plants [8].

So far, there are many research findings and reviews focused on specific types of phytohormones produced by a specified species or different species of rhizobacteria and its growth effects on specific plants [9–20]. However, this chapter provides a comprehensive highlight of the biosynthesis of major phytohormones in diverse groups of rhizobacteria and their effects on the growth of different plant species. Hence, this chapter is tremendously important to enhance our mechanistic understanding of the diversity of phytohormone-producing rhizobacteria and their effects on plant growth for potential application in agriculture.

2. Phytohormone production in rhizobacteria

The majority of PGPR can synthesize, or modulate the concentrations of, several phytohormones. The major phytohormones produced by the diverse rhizobacteria are discussed below.

2.1 Indole-3-acetic acid (IAA)

Indole-3-acetic acid (IAA), a key plant hormone involved in regulating various physiological processes, plays a crucial role in plant growth and development. It is also a commonly produced hormone by many rhizobacteria. IAA is primarily produced via tryptophan-dependent and tryptophan-independent pathways by rhizobacteria. The study of the different IAA biosynthetic pathways has revealed that there is a high degree of similarity between bacterial and plant pathways. Six different IAA biosynthetic bacterial pathways have been identified, five of them tryptophan-dependent [21]. It is important to note that different bacteria may employ different pathways for IAA biosynthesis, and the presence of specific enzymes can vary among bacterial species or strains. Additionally, bacteria can use multiple pathways simultaneously or switch between different pathways depending on the environmental conditions and nutrient availability. The major pathways of IAA biosynthesis in bacteria are:

2.1.1 Indole-3-pyruvic acid (IPA) pathway

The indole-3-pyruvic acid (IPA) pathway in bacteria involves the conversion of tryptophan to IPA, followed by the decarboxylation of IPA to indole-3-acetaldehyde, and the subsequent oxidation of indole-3-acetaldehyde to indole-3-acetic acid (IAA). IAA biosynthesis by the indole-3-pyruvic acid (IPA) pathway in bacteria involves the following steps:

i. The first step involves the conversion of the amino acid tryptophan to indole-3-pyruvic acid (IPA) by the enzyme tryptophan aminotransferase. This enzyme transfers an amino group from tryptophan to a ketone group, resulting in the formation of IPA.

ii. The enzyme tryptophan aminotransferase catalyzes the reaction by transferring the amino group from tryptophan onto a molecule called α-ketoglutarate, forming glutamate and IPA.

iii. Indole-3-pyruvic acid (IPA) can be further converted to indole-3-acetaldehyde by a decarboxylation reaction. This conversion is catalyzed by the enzyme indole-3-pyruvate decarboxylase. The decarboxylation reaction involves the removal of a carboxyl group from IPA, resulting in the formation of indole-3-acetaldehyde.

iv. The final step in the biosynthesis of IAA via the IPA pathway is the conversion of indole-3-acetaldehyde to indole-3-acetic acid (IAA) by the enzyme indole-3-acetaldehyde dehydrogenase. This enzyme catalyzes the oxidative conversion of indole-3-acetaldehyde into IAA, with the addition of a molecule of nicotinamide adenine dinucleotide (NAD^+) as a coenzyme.

Several bacteria have been reported to produce indole-3-acetic acid (IAA) through the indole-3-pyruvic acid (IPA) pathway. One example is *Azospirillum brasilense*, a well-known plant growth-promoting rhizobacterium (PGPR), which synthesizes IAA via the IPA pathway [10]. Another bacterium of interest is *Bacillus amyloliquefaciens*, which produces IAA through the IPA pathway and is associated with promoting plant growth in various crops [15, 22]. Additionally, bacteria belonging to the genus *Pseudomonas*, such as *P. putida* and *Pseudomonas fluorescens*, have been reported to produce IAA via the IPA pathway and show positive effects on plant growth [21]. Other rhizobacteria, such as *Azospirillum lipoferum*, *Enterobacter amnigenus*, *Enterobacter cloacae*, *Klebsiella oxytoca*, and *Bacillus cereus*, are also known to produce IAA through the IPA pathway [23]. It should be noted that the ability to produce IAA may vary among strains within a bacterial species.

2.1.2 Indole-3-acetamide (IAM) pathway

This pathway involves the conversion of tryptophan to indole-3-acetamide by enzymes such as tryptophan monooxygenase. Indole-3-acetamide is then further metabolized to IAA through various enzymatic reactions. IAA biosynthesis by the indole-3-acetamide (IAM) pathway in bacteria involves the following steps:

1. Tryptophan uptake: Bacteria take up tryptophan, an amino acid, from their surroundings.

2. Conversion of tryptophan to indole-3-acetamide (IAM): Tryptophan is enzymatically converted to indole-3-acetamide (IAM) inside the bacterial cell. This conversion is facilitated by enzymes such as tryptophan monooxygenase.

3. Conversion of IAM to IAA: The produced indole-3-acetamide (IAM) is further transformed into indole-3-acetic acid (IAA) by the action of the enzyme indole-3-acetamide hydrolase.

4. Regulation of IAA production: The production of IAA via the IAM pathway is regulated by various factors, including environmental conditions and the presence of plant signaling molecules.

5. Release of IAA: The synthesized IAA can be released by the bacteria into the surrounding environment through diffusion or active secretion. Once released, IAA can have diverse effects on plant growth and development.

There are several bacteria that have been reported to produce indole-3-acetic acid (IAA) through the indole-3-acetamide (IAM) pathway. One such bacterium is *E. cloacae*, which synthesize IAA from tryptophan via the IAM pathway and have positive effects on plant growth promotion. Another bacterium of interest is *Enterobacter asburiae*, which produce IAA through the IAM pathway and are associated with increased root elongation and improved nutrient uptake in plants. Additionally, bacteria belonging to the genus *Klebsiella*, such as *Klebsiella pneumoniae* and *K. oxytoca*, were reported to produce IAA via the IAM pathway and show beneficial effects on plant growth, including enhanced root development and biomass accumulation [23]. Other rhizobacteria that produce IAA through IAM pathway include *A. brasilense*, *P. fluorescens*, *Bacillus subtilis*, and *Rhizobium leguminosarum* [21, 23].

2.1.3 Indole-3-acetonitrile (IAN) pathway

The indole-3-acetonitrile (IAN) pathway is another route through which bacteria can produce indole-3-acetic acid (IAA). IAA biosynthesis by the IAN pathway in bacteria involves the following steps:

1. Tryptophan uptake: Bacteria acquire tryptophan, an amino acid, either from their environment or through *de novo* synthesis.

2. Conversion of tryptophan to indole-3-acetonitrile (IAN): Inside the bacterial cell, tryptophan is enzymatically converted to indole-3-acetonitrile (IAN) through the activity of an enzyme called tryptophan aminotransferase. This involves the removal of the carboxyl group from tryptophan and the addition of a nitrile group.

3. Conversion of IAN to indole-3-acetaldoxime (IAOx): The indole-3-acetonitrile (IAN) is then converted to indole-3-acetaldoxime (IAOx) by the action of a

nitrilase enzyme. This step involves the hydrolysis of the nitrile group to form an aldoxime group.

iv. Conversion of IAOx to IAA: The indole-3-acetaldoxime (IAOx) is further converted to indole-3-acetic acid (IAA) through a series of enzymatic steps. These steps involve the oxidation of IAOx to an intermediate called indole-3-acetamide (IAM) and subsequent hydrolysis of IAM to IAA by an amidase enzyme.

v. Regulation of IAA production: The biosynthesis of IAA via the IAN pathway is regulated by various factors, including environmental conditions and the presence of plant signaling molecules.

vi. Release of IAA: Finally, the synthesized IAA may be released by the bacteria into the surrounding environment through diffusion or active secretion.

There are several bacteria that have been reported to produce indole-3-acetic acid (IAA) through the indole-3-acetonitrile (IAN) pathway. One of the prominent bacteria known for IAN pathway-mediated IAA production is *P. putida*. *P. putida* strains have been reported to possess the nitrilase enzyme that converts indole-3-acetonitrile (IAN) to IAA, contributing to plant growth promotion. Additionally, other bacteria, such as *Alcaligenes faecalis* and *Herbaspirillum seropedicae*, were also identified for their ability to produce IAA through the IAN pathway [23].

2.1.4 The tryptamine (TAM) pathway

The tryptamine (TAM) pathway is another route through which bacteria can produce indole-3-acetic acid (IAA). The IAA biosynthesis via the TAM pathway in bacteria involves the following steps:

i. Tryptamine synthesis: The first step in the TAM pathway involves the synthesis of tryptamine, an intermediate compound. Bacteria possess specific enzymes, such as tryptophan decarboxylase, which catalyze the decarboxylation of tryptophan into tryptamine.

ii. Tryptamine oxidation: After the synthesis of tryptamine, the next step involves the oxidation of tryptamine to produce indole-3-acetaldehyde (IAld). This reaction is facilitated by an enzyme called tryptamine 2-monooxygenase or tryptophan 2-monooxygenase. Molecular oxygen (O_2) acts as a co-substrate in this reaction, assisting in the conversion of tryptamine to IAld.

iii. Conversion of IAld to indole-3-pyruvic acid (IPA): Indole-3-acetaldehyde (IAld) is subsequently converted into indole-3-pyruvic acid (IPA) through the action of a specific enzyme known as indole-3-acetaldehyde transaminase. This enzyme catalyzes the transamination of IAld with a keto acid, usually pyruvate, resulting in the formation of IPA.

iv. Conversion of IPA to indole-3-acetamide (IAM): Indole-3-pyruvic acid (IPA) can be further transformed into indole-3-acetamide (IAM) by the action of indole-3-pyruvate decarboxylase or indole-3-pyruvate lyase. These enzymes catalyze the decarboxylation of IPA, leading to the formation of IAM.

v. Conversion of IAM to IAA: The final step in the TAM pathway involves the conversion of indole-3-acetamide (IAM) into indole-3-acetic acid (IAA). IAM hydrolase, also known as amidase, is the enzyme responsible for this conversion. IAM is hydrolyzed by IAM hydrolase to release a free carboxylic acid group, resulting in the formation of IAA.

Bacteria that produce indole-3-acetic acid (IAA) through the tryptamine (TAM) pathway have been reported in various studies. One of the well-known bacteria utilizing the TAM pathway is *E. cloacae* strains that possess tryptophan decarboxylase enzyme, which converts tryptophan into tryptamine and subsequently converts tryptamine to IAA, contributing to plant growth promotion [21]. Another bacterium identified for its IAA production through the TAM pathway is *B. subtilis*. *B. subtilis* strains produce IAA by the action of tryptophan decarboxylase, followed by the conversion of tryptamine to IAA [23].

It is important to note that the capacity to produce IAA through the TAM pathway can vary among bacterial strains within each species. Additionally, the production of IAA may also be influenced by various environmental factors and growth conditions. Therefore, further research is necessary to fully understand the diversity and functional significance of IAA production by rhizobacteria via the TAM pathway.

2.1.5 The tryptophan side-chain oxidase (TSO) pathway

The biosynthesis of indole-3-acetic acid (IAA) in rhizobacteria can also occur through the tryptophan side-chain oxidase pathway. The IAA biosynthesis via the tryptophan side-chain oxidase pathway in bacteria involves the following steps:

i. Tryptophan uptake: Rhizobacteria take up tryptophan from their surrounding environment.

ii. Tryptophan side-chain oxidation: The first enzymatic step involves the oxidation of tryptophan's side chain. This reaction is catalyzed by tryptophan side-chain oxidase (TSO). TSO converts tryptophan into indole-3-acetaldoxime (IAOx).

iii. Indole-3-acetaldoxime (IAOx) reduction: In the next step, IAOx is reduced to indole-3-acetaldehyde (IAAld) by the enzyme IAOx reductase (IAOR). This conversion involves the transfer of electrons from a redox cofactor such as nicotinamide adenine dinucleotide phosphate hydrogen (NAD(P)H).

iv. Indole-3-acetaldehyde conversion to IAA: The final step in the tryptophan side-chain oxidase pathway is the conversion of IAAld to IAA. This reaction is mediated by the enzyme indole-3-acetaldehyde dehydrogenase (IAAD). IAAD oxidizes IAAld, resulting in the formation of IAA.

Bacteria that produce indole-3-acetic acid (IAA) through the tryptophan side-chain oxidase (TSO) pathway have been identified in various studies. Examples of bacteria known for utilizing the TSO pathway are *P. putida* strains, and *A. brasilense*

[23, 24]. It is important to note that the ability to produce IAA via the tryptophan side-chain oxidase pathway can vary among strains and species within genera.

2.1.6 Tryptophan-independent pathways of IAA biosynthesis

Tryptophan-independent pathways have been proposed, for both bacteria and plants, but have yet further to be studied and proven. The tryptophan-independent pathways provide alternative routes for IAA synthesis in bacteria, allowing them to produce IAA, even under conditions where tryptophan availability may be limited. Further research is required to fully understand the regulation and significance of these tryptophan-independent pathways in bacterial IAA synthesis.

Understanding the biosynthesis of IAA in rhizobacteria is crucial, as it plays a significant role in plant-microbe interactions and can contribute to plant growth promotion and disease resistance. Further research is needed to explore the regulation, genetic determinants, and functional outcomes of IAA production through the different pathways in rhizobacteria.

2.2 Gibberellin (GA3)

Some rhizobacteria produce gibberellins, including gibberellic acid (GA3). One such bacterium is *Serratia marcescens*. Studies have shown that *S. marcescens* can produce GA3 and promote plant growth, particularly in the case of rice plants [25]. Another bacterium known for its ability to produce GA3 is *Bacillus licheniformis*. *B. licheniformis* that produce GA3 stimulate seed germination and enhance plant height and biomass in crops such as wheat and maize [26].

The biosynthesis pathway of gibberellin involves several enzymatic steps, including the conversion of geranylgeranyl diphosphate (GGDP) to ent-kaurene, which is then converted to GA12. GA12 is subsequently converted to GA3 through the action of various enzymes. Based on available research, the following are generalized steps of gibberellin biosynthesis pathway in bacteria:

i. Precursor molecule: The first step is the formation of a precursor molecule called geranylgeranyl diphosphate (GGDP). This molecule serves as the building block for the synthesis of the gibberellin skeleton.

ii. Conversion to ent-kaurene: The next step involves the conversion of GGDP to ent-kaurene through the action of a specific enzyme called ent-copalyl diphosphate synthase (CPS) or kaurene synthase.

iii. Conversion to GA12: In the subsequent step, ent-kaurene is converted to GA12 through the action of the enzyme ent-kaurene oxidase (KO). This enzyme catalyzes the oxidation of ent-kaurene, resulting in the formation of GA12.

iv. Conversion to active gibberellins: GA12 can be further converted to various active gibberellins, including gibberellic acid (GA3). The specific enzymes involved in this conversion step in bacteria are not well characterized. However, in plants and fungi, the conversion of GA12 to GA3 is regulated by a series of enzymatic reactions, such as oxidations, reductions, and rearrangements.

It is important to note that the gibberellin biosynthesis pathway in bacteria may vary depending on the specific bacterial strain and environmental conditions. Further research is needed to elucidate the specific enzymes and genes involved in the gibberellin biosynthesis pathway in PGPR and to understand their regulation and significance in plant growth promotion.

2.3 Cytokinin

Cytokinins are primarily synthesized in plant tissues, but recent studies have revealed that certain rhizobacteria can also produce cytokinins. These bacteria possess specific enzymes called cytokinin synthases, which catalyze the conversion of precursor molecules into active cytokinins. Different types of rhizobacteria, including *Bacillus, Pseudomonas*, and *Azospirillum*, have been found to produce various forms of cytokinins, such as isopentenyladenine (iP), trans-zeatin (tZ), and cis-zeatin (cZ). *P. fluorescens* strain G20–18 has been shown to produce various forms of cytokinins, such as isopentenyladenine (iP) and zeatin (Z), which can positively impact plant growth and development [21]. Certain species of *Rhizobium* also produce cytokinins, including tZ and iP, which can promote root and shoot growth, enhance nutrient uptake, and improve the overall productivity of leguminous plants [18]. *B. subtilis* strains produce cytokinins, particularly kinetin (K) and zeatin (Z). These cytokinins have demonstrated positive effects on seed germination, root development, and overall plant growth [17].

The production of cytokinin by rhizobacteria is a complex process influenced by various factors. Some rhizobacteria have the ability to synthesize cytokinins via *de novo* pathways, while others are capable of modifying existing cytokinins found in the environment. The *de novo* synthesis pathway involves the conversion of isopentenyl pyrophosphate (IPP) and adenosine monophosphate (AMP) into different cytokinin forms through the action of cytokinin synthases [6]. Additionally, rhizobacteria can produce cytokinins by cleaving N^6-(Δ2-isopentenyl) adenine ribonucleosides into active cytokinins through the action of cytokinin oxidases and nucleosidases.

Based on available research, cytokinin biosynthesis pathway in PGPR involves the following generalized steps:

i. The precursor molecule for cytokinin biosynthesis in rhizobacteria is isopentenyl diphosphate (IPP). Rhizobacteria can synthesize IPP through either the mevalonate or non-mevalonate pathways.

ii. In rhizobacteria, the conversion of AMP (adenosine monophosphate) to adenosine is carried out by adenosine kinase.

iii. Adenosine is then converted to isopentenyladenosine (iP) by the enzyme tRNA isopentenyltransferase (Taim1), which adds an isopentenyl group to the N^6 position of adenosine. This step is analogous to the conversion of iAMP to iP in the plant pathway.

iv. In some rhizobacteria, iP can undergo further modifications to form different types of cytokinins, such as N^6-(Δ2-isopentenyl) adenine (2iP) or trans-zeatin (tZ). These modifications are catalyzed by enzymes such as cytokinin oxidase.

The final steps of cytokinin biosynthesis in rhizobacteria are not as well understood. It is believed that additional modifications and enzymatic reactions may occur to produce different forms of cytokinins, but further research is needed to fully elucidate these steps in rhizobacteria.

It is important to note that the cytokinin biosynthetic pathway in rhizobacteria may vary among different species and strains. Additionally, the specific enzymes and genes involved in cytokinin biosynthesis in rhizobacteria are still being characterized, and there may be variations and additional steps yet to be discovered.

2.4 Abscisic acid (ABA)

Abscisic acid (ABA) is primarily synthesized in higher plants and is involved in various physiological processes, including seed dormancy, drought tolerance, and stomatal regulation. However, few PGPR strains were also reported to produce ABA. For example, *A. brasilense* was reported to produce ABA, which can regulate plant water status and enhance drought tolerance in different crops [27]. Certain species of *Arthrobacter* are also reported to produce ABA, contributing to improved water-use efficiency and stress adaptation in plants [28]. *B. subtilis* strains were reported to produce ABA and enhance plant stress tolerance, such as drought and salinity stress [20]. In addition, PGPR can also indirectly influence ABA levels and signaling in plants through their interactions with the plant microbiome and modulation of plant stress responses. PGPR strains reported to potentially influence ABA levels in plants are *P. fluorescens*, *B. subtilis*, *Azospirillum* spp., *Azotobacter* sp., *Burkholderia* spp., *Rhizobium* sp., and *Enterobacter* sp. [29].

The specific mechanisms and effectiveness of ABA production by these PGPR strains are still not well understood and require further investigation. Additionally, the production of ABA by PGPR can vary depending on environmental conditions and strain-specific factors. Research on the specific interactions between PGPR and ABA in plant-microbe interactions and further studies are needed to fully understand the role of PGPR in ABA production and signaling in plants.

2.5 Ethylene

Ethylene is a gaseous plant hormone that plays a significant role in many physiological processes, including seed germination, root initiation, flowering, fruit ripening, and response to biotic and abiotic stresses. Ethylene is predominantly produced by plants and also, certain bacteria have been reported to produce ethylene as well [30]. In *Escherichia coli*, *Cryptococcus albidus*, and a variety of other bacteria, ethylene is spontaneously produced at trace amounts via oxidation of 2-keto-4-methylthiobutyric acid (KMBA), a transaminated derivative of methionine produced in an NADH:Fe(III)EDTA oxidoreductase-mediated reaction that is enhanced under ammonia limitation (C/N = 20) [31, 32]. Formation of KMBA is proposed as a means to recover amino nitrogen from methionine, resulting in the spontaneous production of ethylene from KMBA. Another type of ethylene pathway found in *Pseudomonas syringae* utilizes α-ketoglutarate (AKG) and arginine as substrates in a reaction catalyzed by an ethylene-forming enzyme (EFE) [33].

In PGPR, the production of ethylene can be beneficial for plant growth promotion under certain conditions. However, many PGPR strains possess the enzyme ACC deaminase, which can mitigate the negative effects of ethylene by breaking down ACC, the precursor of ethylene, into α-ketobutyrate and ammonia. By reducing ACC levels and subsequently ethylene production in the plant, ACC deaminase-producing PGPR can alleviate ethylene-induced stress and enhance plant growth.

Some common examples of bacteria that produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase are *Pseudomonas* spp.(*P. fluorescens*, *P. putida*, *Pseudomonas aeruginosa*) [34], *E. cloacae* [35], *Azospirillum* spp., *Rhizobium* spp., *K. pneumoniae*, *S. marcescens*, *B. subtilis*, and *Burkholderia phytofirmans* [19].

2.6 Jasmonic acid (JA)

Jasmonic acid (JA) is a plant hormone that is primarily synthesized in plants as a response to stress, such as herbivory or pathogen attack. Many plant growth-promoting rhizobacteria (PGPR) have been reported to induce jasmonic acid (JA) production in plants. PGPR stimulate JA production or exhibit JA-related effects in plants. Several species of *Pseudomonas*, including *P. fluorescens*, *P. putida*, and *P. aeruginosa*, were reported to induce JA production in plants, promoting defense responses against pathogens and insects [36]. Certain strains of *B. subtilis*, *B. amyloliquefaciens*, and *Bacillus pumilus* have been shown to enhance JA production in plants, contributing to induce systemic resistance against pathogens [37]. Some strains of *Burkholderia* exhibit JA-inducing activity in plants, contributing to enhanced disease resistance and plant growth promotion. *Serratia plymuthica* was reported to induce JA production in plants, promoting systemic resistance and plant growth. Certain strains of *Azospirillum*, such as *A. brasilense* and *Azospirillum lipoferum*, have been observed to stimulate JA synthesis in plants, resulting in enhanced growth, nutrient uptake, and tolerance to stresses [27].

It is important to note that the ability of PGPR to induce JA production can vary depending on the specific strain and plant species.

3. The role of phytohormone-producing rhizobacteria in plant growth

Generally, phytohormone-producing rhizobacteria promote plant growth directly through modulating endogenous plant hormone levels, increasing nutrient uptake, and enhancing plant tolerance to stresses. Based on the type of phytohormones produced by the rhizobacteria, the mechanisms of growth promotion also vary. The role of rhizobacteria producing the different types of phytohormones in plant growth is discussed.

3.1 The role of IAA-producing rhizobacteria in plant growth

The promotion of plant growth by IAA-producing rhizobacteria occurs through a range of mechanisms. IAA acts as a signaling molecule, participating in plant-microbe crosstalk and triggering plant responses. Rhizobacterial IAA stimulates the expression of genes involved in root elongation, nutrient uptake, and stress tolerance [21]. IAA promotes lateral root formation, making plants more efficient in accessing water and nutrients in the soil. IAA-producing rhizobacteria can enhance seed germination, increase seedling vigor, and improve plant tolerance to abiotic stresses such as drought, salinity, and heavy metal toxicity [38]. The intricate interplay between rhizobacteria-produced IAA and the host plants' physiological responses contributes to improved crop productivity and quality.

Several IAA-producing rhizobacteria have been demonstrated to show positive growth effects on plant growth (**Table 1**). *Rhizobium* spp., including

Kinds of phytohormones	Phytohormone-producing rhizobacteria	Observed effect on plant	References
Indole-3-acetic acid (IAA)	*Pseudomonas* spp.	Increased the length of potato tubers and sprouting capacity	[39]
	Pseudomonas fluorescens	increased the length of coleoptiles of avena	[40]
	Pseudomonas fluorescens and *Bacillus subtilis*	increase root length, shoot length, root and shoot fresh and dry weight, on bacterial inoculated onion seeds	[41]
	Klebsiella strains significantly	increased root and shoot length of inoculated wheat seedlings	[42]
	Bacillus megaterium, Lactobacillus casei, Bacillus subtilis, Bacillus cereus, and *Lactobacillus acidophilus,*	increased growth and yield of wheat and maize	[43]
	Enterobacter spp.	increased plant biomass and enhanced phytoextraction of metals (Ni, Zn, and Cr)	[44]
	Gluconacetobacter azotocaptans DS1, *Pseudomonas putida* CQ179, and *Azospirillum lipoferum* N7	increased root and shoot weight of corn when compared to uninoculated plants	[45]
	Microbacterium sp., *Mycobacterium* sp., *Bacillus* sp., and *Rhizobium* sp., *Sphingomonas* sp., *Rhodococcus* sp., *Cellulomonas* sp., *Pseudomonas* sp., and *Micrococcus luteus*	treatment of kidney bean cuttings with bacterial culture liquid promoted formation of a “root brush” with a location height 7.4- to 13.4-fold greater than the one in the control samples	[46]
	Pontibacter niistensis	a significant increase in the growth of cowpea	[47]
Cytokinins	*Bacillus megaterium*	growth promotion and root developmental responses in *Arabidopsis thaliana* and *Proteus vulgaris* seedlings	[48]
	Paenibacillus pofymyxa	promoted the growth of plants	[37]
	Rhizobium leguminosarum	promoted early seedling root growth of the nonlegumes such as canola (*Brassica campestris* cv. Tobin, *Brassica napus* cv. Westar) and lettuce (*Lactuca sativa* cv. Grand Rapids)	[49]
	Micrococcus luteus-chp37	increases in the number of leaves, shoot length, root length, and dry weight g-1 fresh weight in maize plants	[50]

Kinds of phytohormones	Phytohormone-producing rhizobacteria	Observed effect on plant	References
Gibberellin	*Bacillus cereus* MJ-1	growth of red pepper plants (root and shoot) was enhanced	[51]
	Bradyrhizobium sp.	*Phaseolus lunatus* plants inoculated with the bacterium showed a marked internodal elongation	[52]
	Rhizobium leguminosarum	The *Rhizobium*-rice combination promotes root and shoot growth, thereby improving seedling vigor and increasing grain yield.	[53]
	Azospirillum sp. *and Bacillus* sp.	increased nitrogen (N) uptake seen after inoculation of wheat roots	[53]
	Azospirillum lipoferum	promoted the growth of both roots and shoots of maize seedlings under drought	[53]
	Acetobacter diazotrophicus	increased total carbohydrate accumulation in shoots of *Sorghum bicolor*	[53]
	Bacillus pumilus and *Bacillus licheniformis*	Both have strong growth-promoting activity (dwarf phenotype induced in *Alnus glutinosa* L. Gaertn. seedlings by Paclobutrazol (an inhibitor of gibberellin biosynthesis)) was effectively reversed by applications of extracts from medium incubated with both bacteria	[53]
	Bacillus licheniformis and *Bacillus pumilus*	enhanced growth of *Pinus pinea* plants	[53]
Abscisic acid (ABA)	*Alcaligenes* sp. and *Bacillus pumilus*	enhanced growth of sunflower (*Helianthus annuus*L.)	[54]
	Pseudomonas fluorescens	enhanced root growth, nutrient uptake, and overall wheat growth	[55]
	Bacillus subtilis	promoted a rapid recovery of the growth rates of shoots, as well as the wet and dry mass of roots potato (*Solarium tuberosum* L.)	[56]
	Azospirillum brasilense	treatment in maize plants enhanced plant growth, nitrogen fixation, and improved drought resistance	[28]
	Azotobacter chroococcum	induced stomatal closure, improved water-use efficiency, and enhanced plant growth in maize plants	[57]

Kinds of phytohormones	Phytohormone-producing rhizobacteria	Observed effect on plant	References
Jasmonic acid (JA)	*Alcaligenes* sp., *Bacillus pumilus*	enhanced growth of sunflower (*Helianthus annuus* L.)	[54]
	Azospirillum brasilense	treatment increased JA synthesis in maize plants, leading to improved root growth, nutrient uptake, and overall plant growth	[58]
	Bacillus pumilus	enhanced disease resistance and overall plant growth in rice plants	[59]
	Pseudomonas fluorescens	enhanced plant growth and stress tolerance in *Arabidopsis* plants	[60]
Inhibition of ethylene biosynthesis (ACC)) deaminase production)	*Serratia* and *Aerococcus* strains	most effective strains in improving the growth of wheat seedlings in water stress conditions	[61]
	Pseudomonas fluorescens biotype G (ACC-5)	eliminated the inhibitory effects of drought stress on the growth of peas and promoted growth	[62]
	Burkholderia caryophylli	increased the wheat growth and yield under an axenic condition	[63]
	Bradyrhizobium	promoted root and shoot growth as well as nodulation in a mung bean plant.	[64]

Table 1.
The effects of inoculation of some phytohormone-producing rhizobacteria on the growth of different plants.

R. leguminosarum, have been found to produce IAA and promote root elongation, nutrient uptake, and overall plant growth [65]. Another example is *Azospirillum* spp., such as *A. brasilense*, which produces IAA and has been associated with enhanced root elongation, lateral root formation, and increased biomass in various crops [21, 66]. Additionally, bacteria from the genus *Pseudomonas*, such as *P. putida* and *P. fluorescens*, have been identified as IAA producers and have shown positive effects on plant growth, including increased shoot and root growth, improved nutrient uptake, and disease resistance [16, 67]. Therefore, IAA-producing rhizobacteria promote plant growth in multiple ways and they are potential bio inoculant.

3.2 The role of GA3-producing rhizobacteria in plant growth

GA3-producing rhizobacteria play a significant role in promoting plant growth. The production of GA3 by rhizobacteria positively influences plant growth by stimulating seed germination, root development, stem elongation, flowering, and fruit development [68]. There are several rhizobacteria that have been reported to produce gibberellic acid (GA3) and have shown effects on plant growth (**Table 1**). One example is the bacterium *B. licheniformis*, which has been found to produce GA3 and promote plant growth by stimulating seed germination, increasing stem elongation, and enhancing overall biomass production [52]. Another example is *A. brasilense*, a well-known plant growth-promoting rhizobacterium (PGPR), which produces GA3 and has been shown to improve plant growth characteristics, including increased shoot and root growth, nutrient uptake, and yield in various crops [14]. Additionally, GA3-producing species from the genus *Pseudomonas*, including *P. putida* and *P. fluorescens*, have been reported to produce GA3 and exhibit effects on plant growth, such as enhanced shoot and root development, improved flowering, and increased crop yield [9, 13]. These studies highlight the potential of GA3-producing rhizobacteria in promoting plant growth and provide insights for their utilization in agriculture.

3.3 The role of cytokinin-producing rhizobacteria in plant growth

The role of cytokinin-producing rhizobacteria in plant growth is multifaceted and can have several positive effects. Cytokinins are known to stimulate cell division and growth in plants. Rhizobacteria that produce cytokinins can enhance this process in plant roots, leading to increased root mass and overall plant growth [55]. Cytokinins produced by rhizobacteria can improve nutrient uptake in plants, particularly essential minerals like nitrogen, phosphorus, and potassium [56, 57]. This is because cytokinins influence root architecture and development, leading to increased surface area and absorption capacity. Cytokinins play a crucial role in regulating plant responses to various abiotic and biotic stresses [69]. Rhizobacteria that produce cytokinins can help enhance the plant's stress tolerance by promoting antioxidant activity, reducing oxidative damage, and stimulating defense mechanisms (**Table 1**). For instance, *Bacillus subtilis* strain has been shown to promote growth in lettuce plants by producing cytokinins [70]. Similarly, *P. fluorescens* strain G20–18 has been found to stimulate shoot and root growth in *Arabidopsis* through its cytokinin production [71]. *Azospirillum* spp., including *A. brasilense*, have been reported to promote germination, root and shoot development in corn (*Zea mays* L.) and soybean (*Glycine max* L.) through cytokinin synthesis and other growth hormones [72]. Furthermore, certain strains of *Rhizobium* bacteria produce cytokinins that facilitate nodule formation and growth in leguminous plants [59]. From these findings, one can conclude that cytokinin-producing bacteria enhance plant growth, although further research is needed to understand the mechanisms by which these bacteria interact with plants and modulate cytokinin levels.

It is important to note that the effectiveness of cytokinin-producing rhizobacteria can vary depending on the specific plant species, environmental conditions, and the overall microbial community present in the rhizosphere.

3.4 The role of abscisic acid (ABA)-producing rhizobacteria in plant growth

The role of abscisic acid (ABA)-producing rhizobacteria in plant growth is multifaceted and can have several positive effects. PGPR strains that produce ABA or enhance its production in plants may help improve the plant's ability to withstand periods of water stress and drought [29]. Treatment of different plants with *P. fluorescens*, which are known to produce ABA, enhanced root growth, nutrient uptake, and overall plant growth [58]. Another study by Sorokan *et al.* [60] showed that *B. subtilis* 26D in plant tissues promoted a rapid recovery of the growth rates of shoots, as well as the wet and dry mass of roots potato (*Solanum tuberosum* L.) after the pest attack, which we associate with the maintenance of a high level of IAA, ABA, and cytokinins in their tissues. Similarly, a study by Zeffa *et al.* [66] demonstrated that *A. brasilense* treatment enhanced plant growth, nitrogen fixation, and improved drought resistance in maize plants. *Azotobacter chroococcum* is another example of rhizobacteria-producing ABA and its treatment induced stomatal closure, improved water-use efficiency, and enhanced plant growth in maize plants [73].

It is important to note that the effect of plant growth-promoting rhizobacteria (PGPR) producing abscisic acid (ABA) on plant growth can vary depending on several factors, including the specific strain of PGPR, the plant species, environmental conditions, and the overall balance of plant hormones.

Further research is needed to fully understand and optimize the use of ABA-producing PGPR for enhancing plant growth and stress tolerance.

3.5 The role of ACC deaminase-producing rhizobacteria on plant growth

The role of 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing plant growth-promoting rhizobacteria (PGPR) in plant growth is significant. By reducing the levels of ACC in plants, ACC deaminase-producing PGPR can modulate ethylene biosynthesis and the ethylene response in plants. Ethylene is a plant hormone that can inhibit plant growth and development, particularly under stress conditions. The reduction in ethylene promotes plant growth and development by enhancing plant stress tolerance and improving overall plant performance under stressful conditions. ACC deaminase-producing bacteria can trigger systemic resistance in plants, making them more resistant to various pathogens. This is thought to be mediated by the modulation of ethylene signaling and the activation of defense mechanisms in plants.

Several reports indicated rhizobacteria-producing ACC deaminase promote plant growth (**Table 1**). ACC deaminase-producing strains of *P. putida* have been shown to improve plant growth in various crops by promoting root development, nutrient uptake, and stress tolerance [11, 34]. Another example is *E. cloacae*, a beneficial rhizobacterium, which produces ACC deaminase and has demonstrated positive effects on plant growth, particularly under stressful conditions including drought and salinity [39, 40]. Additionally, species from the genus *Rhizobium*, including *R. leguminosarum* and *Rhizobium meliloti*, have also been reported to produce ACC deaminase and exhibit beneficial effects on plant growth, especially in leguminous crops, by enhancing nodulation, nitrogen fixation, and overall yield [41, 59].

3.6 The role of jasmonic acid (JA)-producing rhizobacteria in plant growth

Rhizobacteria that can induce or enhance JA production in plants promote growth through different mechanisms. Rhizobacteria that enhance JA production in plants can induce systemic resistance in plants, making them more resistant to diseases and pests. For instance, a study by Kumar *et al*. [42] demonstrated that *B. pumilus* treatment increased JA levels in rice plants, leading to enhanced disease resistance and overall plant growth. A study by Creus *et al*. [43] demonstrated that *A. brasilense* treatment increased JA synthesis in maize plants, leading to improved root growth, nutrient uptake, and overall plant growth. Jasmonic acid stimulates the production of secondary metabolites, such as phenols, flavonoids, and defense-related compounds. These metabolites contribute to plant defense and other physiological processes. Rhizobacteria-produced JA can enhance the production of these beneficial compounds, thereby improving plant health and resilience [44].

It is important to note that the effects of rhizobacteria-induced JA production on plant growth can vary depending on the specific plant species, environmental conditions, and the concentration of JA produced. Additionally, the indirect modulation of JA by rhizobacteria may involve complex signaling pathways in plants.

4. Conclusions

Diverse group of rhizobacteria can synthesize, or modulate the concentrations, of several phytohormones and promote plant growth in different ways. Indole-3-acetic acid (IAA) is a commonly produced hormone by many rhizobacteria primarily via five tryptophan-dependent pathways. The promotion of plant growth by IAA-producing rhizobacteria occurs through a range of mechanisms. Exogenously produced IAA by rhizobacteria acts as a signaling molecule, participating in plant-microbe crosstalk and triggering plant responses. IAA-producing rhizobacteria can enhance seed germination, increase seedling vigor, and improve plant tolerance to abiotic stresses such as drought, salinity, and heavy metal toxicity. The complex interaction between rhizobacteria-produced IAA and the host plants' physiological responses contributes to improved crop productivity, hence, IAA-producing rhizobacteria are a potential biostimulant used as biofertilizer. Gibberellin (GA3) is produced by certain group of rhizobacteria through biosynthesis pathway that involves several enzymatic steps, including the conversion of geranylgeranyl diphosphate (GGDP) to ent-kaurene, which is then converted to GA12 and which is subsequently converted to GA3 through the action of various enzymes. The inoculation of GA3-producing rhizobacteria in plants positively influences plant growth by stimulating seed germination, root development, stem elongation, flowering, and fruit development. Cytokinins are produced in certain rhizobacteria that possess specific enzymes called cytokinin synthases, which catalyze the conversion of precursor molecules (isopentenyl pyrophosphate (IPP) and adenosine monophosphate (AMP)) into active cytokinins. Rhizobacteria that produce cytokinins can enhance this process in plant roots, leading to increased root mass and overall plant growth. Abscisic acid (ABA) is also produced by certain PGPR strains while other strains enhance its production in plant. PGPR that produce or enhance ABA production in plants improve the plant's ability to withstand periods of water stress and drought. Many PGPR strains possess the enzyme ACC deaminase, which can mitigate the negative effects of ethylene and has been shown to improve plant growth in various crops by promoting root development, nutrient uptake, and stress tolerance. Rhizobacteria that enhance jasmonic acid (JA) production in plants can induce systemic resistance in plants, making them more resistant to diseases and pests.

Overall, the production and modulation of phytohormones by PGPR have a profound impact on plant growth, development, and stress responses. Harnessing the potential of phytohormone-producing PGPR presents a promising avenue for sustainable agriculture, as it reduces the reliance on synthetic inputs and promotes eco-friendly plant growth promotion. Further research in understanding the mechanisms by which PGPR produce and functionally modulate phytohormones will contribute to the development of effective strategies for maximizing crop yields, enhancing plant resilience, and ensuring global food security.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saudi University. 2014;**26**(1):1-20. DOI: 10.1016/j.jksus.2013.05.001

[2] Lugtenberg B, Kamilova F. Plant-growth-promoting Rhizobacteria. Annual Review of Microbiology. 2009;**63**(1):541-556. DOI: 10.1146/annurev.micro.62.081307.162918

[3] Boiero L, Perrig D, Masciarelli O, Penna C, Cassán F, Luna V. Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Applied Microbiology and Biotechnology. 2007;**74**(4):874-880

[4] Khalid A, Arshad M, Zahir ZA. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. Journal of Applied Microbiology. 2004;**96**(3):473-480. DOI: 10.1046/j.1365-2672.2003.02161.x

[5] Lee KE, Radhakrishnan R, Kang SM, You YH, Joo GJ, Lee IJ, et al. *Enterococcus faecium* LKE12 cell-free extract accelerates host plant growth via gibberellin and indole-3-acetic acid secretion. Journal of Microbiology and Biotechnology. 2015;**25**(9):1467-1475

[6] Uniyal S, Bhandari M, Singh P, Singh RK, Tiwari SP. Cytokinin biosynthesis in cyanobacteria: Insights for crop improvement. Frontiers in Genetics. 2022;**13**:1-12

[7] Bryksová M, Hybenová A, Hernándiz AE, Novák O, Pěnčík A, Spíchal L, et al. Hormopriming to mitigate abiotic stress effects: A case study of N9-substituted Cytokinin derivatives with a fluorinated carbohydrate moiety. Frontiers in Plant Science. 2020;**2020**:11

[8] Zahir ZA, Munir A, Asghar HN, Shaharoona B, Arshad M. Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (Pisum sativum) under drought conditions. Journal of Microbiology and Biotechnology. 2008;**18**(5):958-963

[9] del Orozco-Mosqueda M, Santoyo G, Glick BR. Recent advances in the bacterial phytohormone modulation of plant growth. Plants. 2023;**12**(3):606. DOI: 10.3390/plants12030606

[10] Cassán F, Vanderleyden J, Spaepen S. Physiological and agronomical aspects of phytohormone production by model plant-growth-promoting Rhizobacteria (PGPR) belonging to the genus *Azospirillum*. Journal of Plant Growth Regulation. 2014;**33**(2):440-459

[11] Jalili F, Khavazi K, Pazira E, Nejati A, Rahmani HA, Sadaghiani HR, et al. Isolation and characterization of ACC deaminase-producing fluorescent pseudomonads, to alleviate salinity stress on canola (*Brassica napus* L.) growth. Journal of Plant Physiology. 2009;**166**(6):667-674

[12] Fukami J, Ollero FJ, de la Osa C, Valderrama-Fernández R, Nogueira MA, Megías M, et al. Antioxidant activity and induction of mechanisms of resistance to stresses related to the inoculation with *Azospirillum brasilense*. Archives of Microbiology. 2018;**200**(8):1191-1203

[13] Egamberdieva D, Wirth SJ, Alqarawi AA, Abd-Allah EF, Hashem A. Phytohormones and beneficial microbes:

Essential components for plants to balance stress and fitness. Frontiers in Microbiology. 2017;**8**:1-14

[14] Keswani C, Singh SP, García-Estrada C, Mezaache-Aichour S, Glare TR, Borriss R, et al. Biosynthesis and beneficial effects of microbial gibberellins on crops for sustainable agriculture. Journal of Applied Microbiology. 2022;**132**:1597-1615

[15] Zhang P, Jin T, Sahu SK, Xu J, Shi Q, Liu H, et al. The distribution of tryptophan-dependent indole-3-acetic acid synthesis pathways in bacteria unraveled by large-scale genomic analysis. Molecules. 2019;**24**(7):1-14

[16] Malik DK, Sindhu SS. Production of indole acetic acid by Pseudomonas sp.: Effect of coinoculation with Mesorhizobium sp. Cicer on nodulation and plant growth of chickpea (*Cicer arietinum*). Physiology and Molecular Biology of Plants. 2011;**17**(1):25-32

[17] Kudoyarova GR, Melentiev AI, Martynenko EV, Timergalina LN, Arkhipova TN, Shendel GV, et al. Cytokinin producing bacteria stimulate amino acid deposition by wheat roots. Plant Physiology and Biochemistry. 2014;**1**(83):285-291

[18] Taller BJ, Sturtevant DB. Cytokinin Production by Rhizobia. Dordrecht: Springer; 1991. pp. 215-221

[19] Sethi M, Kaur C, Hagroo RP, Singh MP. Endophyte mediated plant health via phytohormones and biomolecules. In: Microbial Endophytes and Plant Growth. Cambridge: Academic Press; 2023. pp. 151-166. DOI: 10.1016/B978-0-323-90620-3.00017-9

[20] Wang H, Wang S, He X, Xie M, Cai M, Zhu Y, et al. A promising product: Abscisic acid-producing bacterial agents for restricting cadmium enrichment in field vegetable crops. Food Chemisty X. 2023 Oct;**30**(19):100795

[21] Spaepen S, Vanderleyden J, Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiology Reviews. 2007;**31**(4):425-448. DOI: 10.1111/j.1574-6976.2007.00072.x

[22] Shao J, Li S, Zhang N, Cui X, Zhou X, Zhang G, et al. Analysis and cloning of the synthetic pathway of the phytohormone indole-3-acetic acid in the plant-beneficial Bacillus amyloliquefaciens SQR9. Microbial Cell Factories. 2015;**14**(1):130

[23] Duca DR, Glick BR. Indole-3-acetic acid biosynthesis and its regulation in plant-associated bacteria. Applied Microbiology and Biotechnology. 2020;**104**(20):8607-8619

[24] Spaepen S, Vanderleyden J. Auxin and plant-microbe interactions. Cold Spring Harbor Perspectives in Biology. 2011;**3**:1-14

[25] Devi KA, Pandey P, Sharma GD. Plant growth-promoting endophyte Serratia marcescens AL2-16 enhances the growth of *Achyranthes aspera* L., a medicinal plant. HAYATI Journal of Bioscience. 2016;**23**(4):173-180. DOI: 10.1016/j.hjb.2016.12.006

[26] Silpa D, Rao PB, Kumar GK, Ram MR. Studies on gibberellic acid production by *Bacillus licheniformis* DS3 isolated from Banana field soils. International Journal of Science Research and Science Technology. 2018;**4**(5):1106-1112

[27] Miransari M. Soybeans, stress, and plant growth-promoting rhizobacteria. In: Environmental

Stresses in Soybean Production: Soybean Production. Cambridge: Academic Press; 2016. pp. 177-203. DOI: 10.1016/B978-0-12-801535-3.00008-5

[28] Vanissa TTG, Berger B, Patz S, Becker M, Turečková V, Novák O, et al. The response of maize to inoculation with arthrobacter sp. and *Bacillus* sp. in phosphorus-deficient, salinity-affected soil. Microorganisms. 2020;**8**(7):1-20

[29] Porcel R, Zamarreño ÁM, García-Mina JM, Aroca R. Involvement of plant endogenous ABA in Bacillus megaterium PGPR activity in tomato plants. BMC Plant Biology. 2014;**14**:36

[30] Weingart H, Ullrich H, Geider K, Völksch B. The role of ethylene production in virulence of Pseudomonas syringae pvs. glycinea and phaseolicola. Phytopathology. 2001;**91**(5):511-518

[31] Mansouri S, Bunch AW. Bacterial ethylene synthesis from 2-oxo-4-thiobutyric acid and from methionine. Journal of Genetic Microbiology. 1989;**135**(11):2819-2827

[32] Shipston N, Bunch AW. The physiology of L-methionine catabolism to the secondary metabolite ethylene by *Escherichia coli*. Journal of General Microbiology. 1989;**135**(6):1489-1497

[33] Takahira Ogawa K, Fujii T, Tazaki M, Tanase S, et al. Purification and properties of an ethylene-forming enzyme from *Pseudomonas syringae* pv. phaseolicola PK2. Journal of General Microbiology. 1991;**137**:2281-2286

[34] Glick BR. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research. 2014;**169**(1):30-39

[35] Mayak S, Tirosh T, Glick BR. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Science. 2004;**166**(2):525-530

[36] Sah S, Krishnani S, Singh R. Pseudomonas mediated nutritional and growth promotional activities for sustainable food security. Current Research in Microbiological Science. 2021;**2**:100084. DOI: 10.1016/j.crmicr.2021.100084

[37] Miljaković D, Marinković J, Balešević-Tubić S. The significance of *Bacillus* spp. In disease suppression and growth promotion of field and vegetable crops. Microorganisms. 2020;**8**:1-19

[38] Kang SM, Shahzad R, Bilal S, Khan AL, Park YG, Lee KE, et al. Indole-3-acetic-acid and ACC deaminase producing Leclercia adecarboxylata MO1 improves *Solanum lycopersicum* L. growth and salinity stress tolerance by endogenous secondary metabolites regulation. BMC Microbiology. 2019;**19**(1):1-14

[39] Zafar-Ul-Hye M, Hussain NM, Danish S, Aslam U, Zahir ZA. Multi-strain bacterial inoculation of enterobacter cloacae, serratia ficaria and burkholderia phytofirmans with fertilizers for enhancing resistance in wheat against salinity stress. Pakistan Journal of Botany. 2019;**51**(5):1839-1846

[40] Maqbool S, Amna MA, Mehmood S, Suhaib M, Sultan T, et al. Interaction of acc deaminase and antioxidant enzymes to induce drought tolerance in enterobacter cloacae 2wc2 inoculated maize genotypes. Pakistan Journal of Botany. 2021;**53**(3):893-903

[41] Ma W, Guinel FC, Glick BR. Rhizobium leguminosarum biovar viciae 1-aminocyclopropane-1-carboxylate deaminase promotes nodulation of pea plants. Applied and Environmental Microbiology. 2003;**69**(8):4396-4402

[42] Kumar A, Singh S, Mukherjee A, Rastogi RP, Verma JP. Salt-tolerant plant growth-promoting *Bacillus pumilus* strain JPVS11 to enhance plant growth attributes of rice and improve soil health under salinity stress. Microbiological Research. 2021;**242**:126616

[43] Creus CM, Sueldo RJ, Barassi CA. Water relations and yield in Azospirillum- inoculated wheat exposed to drought in the field. Canadian Journal of Botany. 2004;**82**(2):273-281. DOI: 10.1139/b03-119

[44] Glick BR, Penrose DM, Li J. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. Journal of Theoretical Biology. 1998;**190**(1):63-68

[45] Postic D, Starovic M, Popovic T, Bosnic P, Stanojkovic-Sebic A, Pivic R, et al. Selection and RAPD analysis of Pseudomonas ssp. isolates able to improve biological viability of potato seed tubers. Genetika. 2013;**45**(1):237-249

[46] Kapoor R, Kumar A, Kumar A, Patil S, Pratush A, Kaur M. Indole acetic acid production by fluorescent Pseudomonas isolated from the rhizospheric soils of Malus and Pyrus. Recent Research in Science and Technology. 2012;**4**(1):6-9

[47] Reetha S, Bhuvaneswari G, Thamizhiniyan P, Mycin TR. Isolation of indole acetic acid (IAA) producing rhizobacteria of *Pseudomonas fluorescens* and *Bacillus subtilis* and enhance growth of onion (*Allium cepa* L). International Journal of Current Microbiology and Applied Science. 2014;**3**(2):568-574

[48] Sachdev DP, Chaudhari HG, Kasture VM, Dhavale DD, Chopade B. Isolation and characterization of indole acetic acid (IAA) producing *Klebsiella pneumoniae* strains from rhizosphere of wheat (*Triticum aestivum*) and their effect on plant growth. Indian Journal of Experimental Biology. 2009;**47**:993-1000

[49] Mohite B. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. Journal of Science and Plant Nutrition. 2013;**13**(3):638-649

[50] Kumar KV, Singh N, Behl HM, Srivastava S. *Influence* of plant growth promoting bacteria and its mutant on heavy metal toxicity in *Brassica juncea* grown in fly ash amended soil. Chemosphere. 2008;**72**(4):678-683

[51] Mehnaz S, Lazarovits G. Inoculation effects of Pseudomonas putida, Gluconacetobacter azotocaptans, and Azospirillum lipoferum on corn plant growth under greenhouse conditions. Microbial Ecology. 2006;**51**(3):326-335

[52] Gutiérrez-Mañero FJ, Ramos-Solano B, Probanza A, Mehouachi J, Tadeo FR, Talon M. The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiologia Plantarum. 2001;**111**(2):206-211

[53] Tsavkelova E, Cherdyntseva T, Netrusov I. Auxin production by bacteria associated with orchid roots. Microbiology. 2005;**74**(1):46-53

[54] Dastager SG, Deepa CK, Pandey A. Plant growth promoting potential of Pontibacter niistensis in cowpea (*Vigna unguiculata* (L.) Walp.). Applied Soil Ecology. 2011;**49**:250-255. DOI: 10.1016/j.apsoil.2011.04.016

[55] Akhtyamova Z, Martynenko E, Arkhipova T, Seldimirova O,

Galin I, Belimov A, et al. Influence of plant growth-promoting Rhizobacteria on the formation of Apoplastic barriers and uptake of water and potassium by wheat plants. Microorganisms. 2023;**11**(5)

[56] Timmusk S, Nicander B, Granhall U, Tillberg E. Cytokinin production by Paenibacillus polymyxa. Soil Biology and Biochemistry. 1999;**31**(13):1847-1852

[57] Singh SK, Wu X, Shao C, Zhang H. Microbial enhancement of plant nutrient acquisition. Stress Biology. 2022;**2**:1-14. DOI: 10.1007/s44154-021-00027-w

[58] Saranraj P, Sayyed RZ, Kokila M, Sudha A, Sivasakthivelan P, Devi MD, et al. Plant growth-promoting and biocontrol metabolites produced by Endophytic Pseudomonas fluorescence. In: Secondary Metabolites and Volatiles of PGPR in Plant-Growth Promotion [Internet]. Cham: Springer International Publishing; 2022. pp. 349-381

[59] Fahde S, Boughribil S, Sijilmassi B, Amri A. Rhizobia: A promising source of plant growth-promoting molecules and their non-legume interactions: Examining applications and mechanisms. Agriculture. 2023;**13**(7):1279

[60] Sorokan A, Veselova S, Benkovskaya G, Maksimov I. Endophytic strain *Bacillus subtilis* 26D increases levels of phytohormones and repairs growth of potato plants after Colorado potato beetle damage. Plants. 2021;**10**(5)

[61] Ortíz-Castro R, Valencia-Cantero E, López-Bucio J. Plant growth promotion by Bacillus megaterium involves cytokinin signaling. Plant Signaling & Behavior. 2008;**3**:263-265

[62] Noel TC, Sheng C, Yost CK, Pharis RP, Hynes MF. Rhizobium leguminosarum as a plant growth-promoting rhizobacterium: Direct growth promotion of canola and lettuce. Canadian Journal of Microbiology. 1996;**42**(3):279-283

[63] Raza FA, Faisal M. Growth promotion of maize by desiccation tolerant micrococcus luteus-cp37-chp37 isolated from Cholistan desert, Pakistan. Australian Journal of Crop Science. 2013;**7**(11):1693-1698

[64] Joo G-J, Kim Y-M, Kim J-T, Rhee I-K, Kim J-H, Lee I-J. Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of red peppers. Journal of Microbiology. 2005;**43**(6):510-515

[65] Jaiswal SK, Mohammed M, Ibny FYI, Dakora FD. Rhizobia as a source of plant growth-promoting molecules: Potential applications and possible operational mechanisms. Frontier in Sustainable Food System. 2021;**4**:1-14

[66] Zeffa DM, Perini LJ, Silva MB, de Sousa NV, Scapim CA, Oliveira ALM, et al. *Azospirillum brasilense* promotes increases in growth and nitrogen use efficiency of maize genotypes. PLoS One. 2019;**14**(4):e0215332. DOI: 10.1371/journal.pone.0215332

[67] Glick BR. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica (Cairo). 2012;**2012**:963401

[68] Pandya ND, Desai PV. Screening and characterization of GA3 producing *Pseudomonas monteilii* and its impact on plant growth promotion. International Journal of Current Microbiological and Applied Science. 2014;**3**(5):110-115

[69] Cortleven A, Leuendorf JE, Frank M, Pezzetta D, Bolt S, Schmülling T. Cytokinin action in response to abiotic and biotic stresses in plants. Plant, Cell & Environment. 2019;**42**(3):998-1018

[70] Arkhipova TN, Veselov SU, Melentiev AI, Martynenko EV, Kudoyarova GR. Ability of bacterium Bacillus subtilis to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. Plant and Soil. 2005;**272**(1-2):201-209

[71] Großkinsky DK, Tafner R, Moreno MV, Stenglein SA, De Salamone IEG, Nelson LM, et al. Cytokinin production by *Pseudomonas fluorescens* G20-18 determines biocontrol activity against *Pseudomonas syringae* in Arabidopsis. Scientific Reports. 2016;**6**: 1-11. DOI: 10.1038/srep23310

[72] Cassán F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V. *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). European Journal of Soil Biology. 2009;**45**(1):28-35

[73] Correa PA, Nosheen A, Yasmin H, Ali MA. Regulatory role of microbial inoculants to induce salt stress tolerance in horticulture crops. In: Sustainable Horticulture: Microbial Inoculants and Stress Interaction. Cambridge: Academic Press; 2022. pp. 125-155. DOI: 10.1016/B978-0-323-91861-9.00002-1

Chapter 4

Insights on Mechanism of Plant Related Bacteria Producing Phytohormones

Vidya Niranjan, Pooja Sureshkumar, Likitha Shankara, Gulab Khedkar and Jitendra Kumar

Abstract

Phytohormones are chemicals released by plants for several mechanism which includes growth and development such as cell divisions, cell elongation and tissue differentiation, it also helps in stress tolerance and senescence. Major phytohormone groups include auxin, cytokinin, gibberellin, ethylene, abscisic acid, brassinosteroids and jasmonates. Phytohormones are naturally produced in low concentration. Certain naturally available soil microorganisms produce phytohormones, the current approach of plant growth regulators to crops improve yield by dual activity and genetic modifications is highly beneficial. The pilot study on metagenomic analysis on commercially important crops helped us to expand the study on identifying the nitrogen fixing bacteria also promoting phytohormone production. Expected outcome: *Agrobacterium*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Proteus*, *Klebsiella and Mycorrhizal* are microorganisms that play dual activity. All these growth-promoting bacteria are proven to be involved in indole-3-acetic acid pathways which help in the biosynthesis of auxin and cytokinin. The dual benefit of the plant-growth promoting bacteria is that it can act as a diazotroph which helps in nitrogen fixation as well as the biosynthesis of phytohormones. Several microorganisms play crucial role in plants as nitrogen-fixing bacteria, phytohormone production, etc. they play multiple function in plant growth and development. These are essential microbes in application field of agriculture and biotechnology.

Keywords: Phytohormones, Diazotroph, auxin, cytokinin, gibberellin, ethylene, abscisic acid, brassinosteroids and jasmonates

1. Introduction

Phytohormones are well known as Plant hormones. They play a vital role in regulating various physiological processes in plants. These chemical substances act as signaling molecules, coordinating the growth and development of plants [1]. Phytohormones are produced in one part of the plant and then transported to different sites where they elicit specific responses [2].

Phytohormones play a distinct role in plant growth and development. Auxins are responsible for cell elongation and root formation. They help plants bend towards light sources and facilitate the development of lateral roots [3, 4]. Cytokinins, on the other hand, promote cell division and delay senescence, allowing plants to maintain their youthful vigor. They are essential for proper shoot and leaf growth [5, 6].

Gibberellins are phytohormones that stimulate stem elongation and seed germination. They also regulate various developmental processes, such as flowering and fruit development [7]. Abscisic acid is another important hormone that regulates seed dormancy and plays a crucial role in plant responses to stress conditions, such as drought and high salinity [8].

Ethylene, often referred to as the "aging hormone," influences fruit ripening and senescence. It is involved in the abscission (shedding) of leaves, flowers, and fruits [9]. Brassinosteroids, on the other hand, are involved in cell expansion and differentiation, contributing to overall plant growth and development [10].

In addition to these well-known phytohormones, there are other plant growth regulators worth mentioning. Polyamines (PAs) and karrikins (KARs) are small chemical molecules with less understood production and receptors. Although their precise roles are still being explored, they have been found to influence plant growth, development, and responses to environmental stimuli [2].

The long-term evolution of microorganisms in plant and its surrounding environment has acquired numerous beneficial strategies in enhancing the growth and development. The interaction of microbes with plants modulates in biotic and abiotic stresses. These microbes indirectly have a control over the phytohormone production [11].

Piriformospora indica is a root fungus that interferes with the synthesis and signaling of plant hormones that promote growth, the onset of flowering, differentiation, and systemic immunological responses. In reaction to the microbes, plants modify the hormone levels in their roots to regulate colonization and fungal growth [12].

Advance studies have proven the existence of microorganisms such as *Pseudomonas*, *Enterobacter*, Azospirillum and *Klebsiella* can stimulate the phytohormone production [13, 14].

Therefore, the chapter aims to explain beneficial microorganism and their mechanism in producing phytohormone as well as nitrogen fixation in plants, this will act as dual benefit to plants and reduce additional burden to farmers. The study of these microbes has extensive advantages in agricultural biotechnology.

2. Historical background

The study of phytohormones has a rich and interesting historical background. The concept of plant hormones emerged in the late 19th and early 20th centuries when scientists began to observe and investigate the remarkable growth and developmental processes in plants. These scientists sought to understand the underlying factors that influenced plant growth and responses to various environmental stimuli.

The initial breakthrough in understanding phytohormones came with the discovery of auxins. In the late 19th century, Charles Darwin and his son Francis, observed that the tips of coleoptiles in grass seedlings showed bending towards a light source. This phenomenon, known as phototropism, led to the identification of a substance that was responsible for this growth response [15, 16]. In the 1920s, the Dutch scientist Frits Went isolated and characterized this substance, which he named auxin. This discovery marked the beginning of the era of plant hormone research [17, 18].

In 1930s, Japanese scientist Eiichi Kurosawa identified a group of compounds produced by fungi that caused excessive elongation of rice plants. These compounds were later named gibberellins, and their role in promoting stem elongation and flowering [19]. In the 1950s, another group of hormones, cytokinins, was discovered by Folke Skoog and colleagues, who found that these compounds stimulated cell division and played a crucial role in plant tissue culture [20, 21].

The identification of other plant hormones continued with the discovery of abscisic acid (ABA) in the 1960s, which was found to regulate plant responses to stress and dormancy. Ethylene, a gaseous hormone involved in fruit ripening and senescence, was discovered in the 20th century as well [22]. In more recent years, other classes of plant hormones, such as brassinosteroids, jasmonates, and strigolactones, have been identified and extensively studied [10, 23].

The understanding of plant hormones and their roles in plant growth and development has revolutionized the field of plant biology and agriculture. It has provided insights into how plants respond and adapt to their environment, and has led to the development of applications in crop production, horticulture, and plant biotechnology.

3. Classification of phytohormones and their mechanism

Phytohormones are extensively studied to understand their function and mechanism in plant physiology. Recent research has investigated on their biosynthesis, transport, perception and signal transduction pathways.

Additionally, there are different pathways in which the phytohormones play a key role which includes: Cell Division and Growth, Apical Dominance, Root Development, Tropisms, Seed Germination and Dormancy, Flowering, Fruit Ripening and Senescence and Stress responses. (**Figure 1** and **Table 1**) [24].

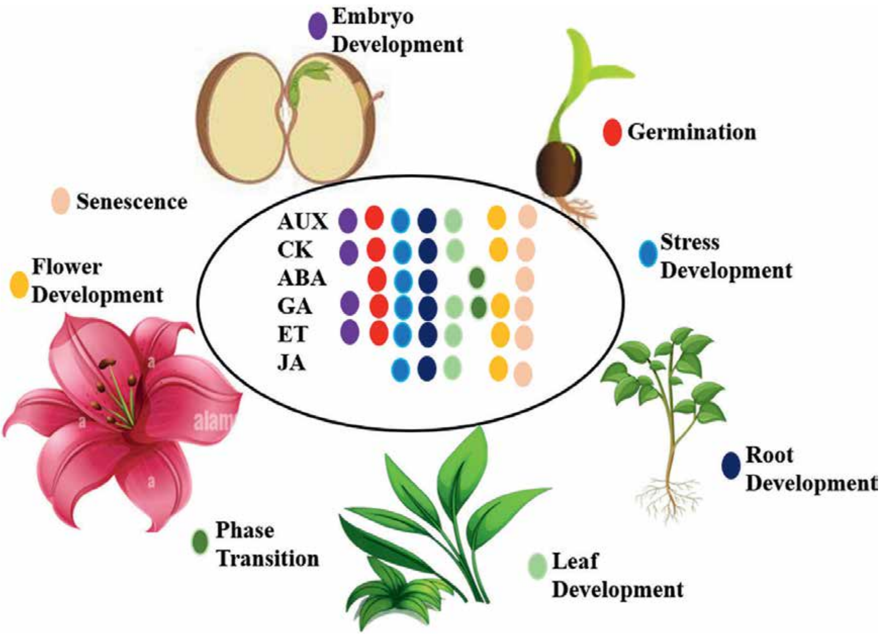

Figure 1.
Representation of phytohormones and their function. The six classes of phytohormones and the plant development processes.

Title	Phytohormone and their mechanism
Cell division and growth	Auxin and Gibberellins promote stem elongation and cell division [25]. Cytokinins stimulate the cell division and also promote shoot and root growth [5].
Apical dominance	Auxin stimulate the dominance of apical meristem, over lateral buds, controlling the growth and branching pattern [18]
Root development	Auxin initiate root growth, Cytokinin initiate lateral root formation [25].
Tropisms	Auxin regulate phototropism and gravitropism, Ethylene influences the triple response in plants, causing change in growth and bending during gravitropism and phototropism [26]
Seed germination and dormancy	Gibberellin promotes seed germination and break seed dormancy [7]
Flowering	Gibberellins are involved in regulation of flowering, particularly in transition from vegetative to reproductive growth [27]
Fruit ripening and senescence	Ethylene is key regulator of fruit ripening and senescence processes, while Abscisic acid influences the timing of seed dormancy and germination and promote leaf and fruit senescence [28]
Stress responses	Abscisic acid is involved in response to drought stress, regulating stomatal closure and promoting water conservation [8] Jasmonates are involved in plant defense responses against insects, pathogens and other biotic stresses [29]

Table 1.
Phyto hormones and their mechanism.

4. Exploiting microbial potential in production of phytohormones

The advanced research shows that plant-microbes plays a vital role in inducing plant growth promoters i.e., phytohormones (**Figure 2**). In addition to the production of hormones by plants these microorganisms also produce, stimulate or involve in the mechanism of converting compounds that are similar to phytohormones. These microorganisms are naturally present in the fertile soil which usually involved in supplying nutrition to the plants. Due to the high nutritional content of root exudates, plant roots are extensively populated with microorganisms. They also produce antifungal substances, enzymes, and suitable solutes. These microbial metabolites are essential for plant development, growth, and nourishment. In response to drought, salt, high heat, and heavy metal toxicity, microbial phytohormones play a critical role in altering root shape. They also have an impact on the metabolism of endogenous growth regulators in plant tissue [30, 31].

The pilot study on various commercially important crops such as wheat, rice, maize, soyabean, peanut etc. has helped us to conclude that certain microorganisms can do multi-function in enhancing the plant as it can improve the plant health, provide nutrient efficiency and resistance to biotic and abiotic stresses [32–34]. These

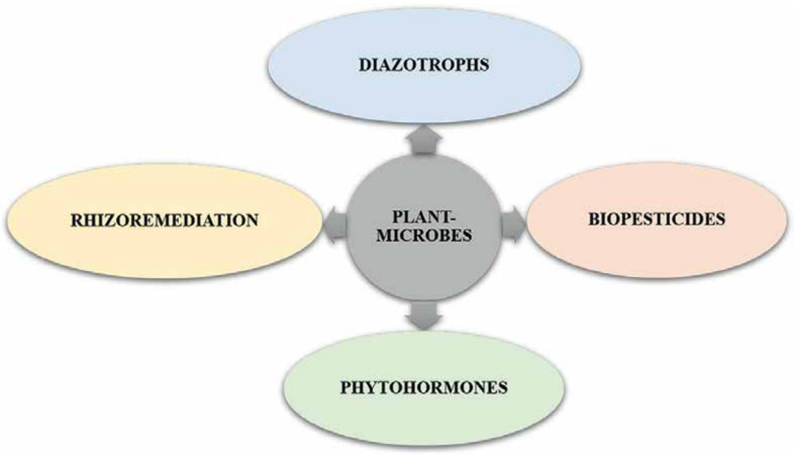

Figure 2.
Classification of plant-growth promoting microbes. The plant microbes play multiple role that includes diazotrophs, biopesticides, phytohormones and rhizoremediation which are highlighted.

can also facilitate the symbiotic relationship between the nitrogen fixation by rhizobia or mycorrhizal associations which also mediate the production of phytohormones.

Benefit of using microorganism to produce phytohormones:

- Cost-effective: Bacteria and fungi are used as efficient and low-cost production systems for phytohormones. They can be grown in large-scale fermentation processes, which are often more economically viable than the extraction or synthesis of natural or synthetic hormones [35–37].

- Sustainable and eco-friendly: Phytohormones produced by microbes are sustainable alternative to the hormones produced by the plant or the artificial-hormones supplemented to plants. It reduces the need for plant material collection, minimizing the impact on natural resources. Moreover, microbial production can be carried out in controlled environments, reducing the use of chemical solvents and minimizing the release of pollutants [38].

- Increased availability and scalability: Microbial production allows for consistent and scalable production of phytohormones. With appropriate cultivation and optimization techniques, large quantities of phytohormones can be generated, ensuring a steady and reliable supply for agricultural applications [39].

- Manipulation and customization: Microorganisms can be genetically engineered or modified to produce specific phytohormones or variants with enhanced properties are the advanced technique to optimized characteristics, such as increased stability, activity, or specificity.

- Reduced dependence on scarce resources: Some phytohormones, especially rare or complex natural compounds, may be challenging to obtain from plant sources due to their low abundance or difficulty in extraction. Microbial production provides an alternative source for these compounds, reducing the dependence on scarce plant resources.

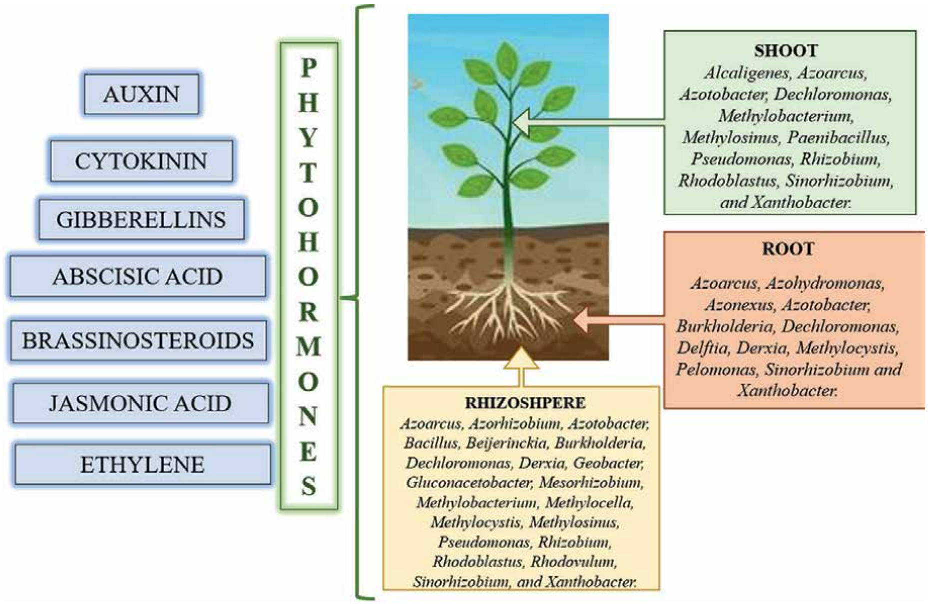

Figure 3.
Illustration of microbes and phytohormones. Right side of the image shows microbes which is present in shoot, root and rhizosphere. And left side of the image shows phytohormones produced by the microbes.

- Quality control and purity: Microbial production allows for precise control over the production process, ensuring consistent quality and purity of the phytohormones. This is particularly important for agricultural applications, where the effectiveness and reliability of the hormones play a crucial role in crop growth and development.

Understanding the plant-microbes and their mechanism in producing the phytohormones which offers promising avenues for sustainable agriculture, biocontrol strategies and improving plant performance in various ecosystem (**Figure 3**).

5. Biosynthesis of phytohoromones

Biosynthesis of phytohormones refers to the natural process by which plants produce these vital chemical compounds. The biosynthesis of phytohormones occurs within specific cells or tissues of plants, where enzymes and biochemical pathways catalyze the production of these hormones' molecules [40–42]. The microbial biosynthesis of phytohormones holds great promise for various applications in agriculture and biotechnology [43]. Using microorganisms are sustainable and eco-friendly approaches to enhance the crops productivity, improve stress tolerance and optimize plant growth.

5.1 Auxin (AUX)

Auxins are one of the most essential plant hormones it plays curial role in growth and development of the plants. Auxin has an impact on a variety of leaf development processes, including initiation, growth ax specification, morphogenesis, and marginal patterning [4, 18]. Although it is evident that some of the processes by which auxin exerts significantly various across plant species.– Modification in auxin distribution has an impact on leaf and leaflet initiation, adaxial-abaxial polarity, and marginal patterning in many plant species. The placement of serrations and the initiation of leaflets and lobes from the border of leaf primordia are determined by auxin, which also coordinates the phyllotaxis of leaf initiation from the shoot apical meristem (SAM). Auxin and cytokinin, which is crucial for SAM maintenance, are both involved in leaf initiation [6].

Auxin usually obtained as natural and synthetic form. The natural form of auxins is Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and 4-chloroindole-3-acetic acid (4-Cl-IAA). The synthetic form of auxins is Naphthaleneacetic acid (NAA), 2,4-Dichlorophenoxyacetic acid (2,4-D), 1-Naphthaleneacetamide (NAD) and 2-Naphthoxyacetic acid (NOA) [18].

Recent studies have shown certain genes and biochemical reactions interfere in the auxin biosynthesis. Auxin are essential for the following processes; gametogenesis, embryogenesis, seedling growth, vascular patterning and flower development. Auxin biosynthesis has the most complexity as it contributes to several pathways to de novo auxin production. Indole-3-acetic acid (IAA) can also be released from IAA conjugates by hydrolytic cleavage of IAA-amino acids, IAA-sugars and IAA methyl ester. The evolutionarily conserved core mechanisms for auxin biosynthesis may differ from plant species which can have unique strategies and modification to optimize their IAA biosynthesis [44]. To overcome the cost burden and to promote dual efficiency certain bacteria are also involved in producing phytohormones such as *Agrobacterium* and *Pseudomonas* are known as Tryptophan-2-monooxygenase (iaaM) these convert the tryptophan to indole-3-acetamide (IAM) which is subsequently hydrolyzed into IAA by the hydrolase iaaH. IAA can also be produced by try through indole-3-pyruvate pathways (IPA).

The IPA decarboxylase has been cloned from *Enterobacter cloacae* and *Azospirillum brasilense*, but the genes that catalyze the conversion of tryptophan to IPA and the enzymes that catalyze the conversion of indole-3-acetaldehyde to IAA have not been definitively identified in microorganisms. The function of IPA decarboxylase produced by microbes in plants are yet to be investigation [3, 18]. Thus, plant growth promotor microbes are highly essential for the production of phytohormones to reduce the chemicals or artificial hormones which are supplemented.

5.2 Cytokinins (CK)

Cytokinin is another crucial plant-hormones which regulate the development of cell division and cytokinesis in roots and shoots. Cytokinin might be thought of as a “juvenility” element in leaf development, increasing morphogenesis and postponing differentiation and senescence. The plant hormone cytokinin (CK) participates in orchestrating a variety of growth-related activities in the plant, including the constructive control of SAM size and activity. Major cell specification processes are regulated by auxin and cytokinin during embryogenesis [6]. A brief antagonistic interaction between auxin and cytokinin is indicated as the role of cytokinin in early development.

Cytokinin are divided into two groups adenine-type and phenylurea-type cytokinins. The adenine cytokinin groups includes natural and synthetic compounds such as zeatin, isopentenyl adenine, kinetin, and 6-benzylaminopurine, while phenylurea-type includes synthetic compounds such as diphenylurea and thidiazuron all these molecules exhibit biological activity on plant tissues [45]. Several physiological and biological processes, such as the development of roots, shoots, leaves, controlling apical dominance in the shoot, chloroplast development, and organ formation in a variety of tissues are all impacted by the stimulatory or repressive actions of cytokinins. Senescence is also delayed by an increase in chlorophyll [21].

Different rhizobacterial strains that produce cytokinins as phytohormones include *Halomonas desiderata*, *Proteus mirabilis*, *P. vulgaris*, *Klebsiella pneumoniae*, *Bacillus megaterium*, *B. cereus*, *B. subtilis*, and *Escherichia coli* [45].

The loss of cytokinins during a drought may actually harm drought-stressed plants. Inoculating well-watered plants with Bacillus IB-22 efficiently increases shoot zeatin and its riboside and strengthens dependability, shorter root length and increased root mass in the inoculated cabbage revealed that the root axes had swollen, most likely as a result of a post-transcriptional acceleration of ethylene production. It was also indicated that the majority of cytokinin accumulation occurred in the shoot rather than the roots [46].

The cytokinin route and auxin function are closely related, and both hormones are known to regulate the beginning of lateral organs from stem cell niches, a behavior that is well-described during root development and apical dominance in plants. To balance cell division and differentiation, auxin and cytokinin work in opposition to one another. In contrast to cytokinin, which encourages cell differentiation towards the elongation zone by suppressing polar auxin transport, auxin maintains stem cell division in the root apical meristem.

5.3 Gibberellins (GA)

Biologically active gibberellins (bioactive GAs) regulate many different aspects of plant growth and development, such as seed germination, stem lengthening, leaf enlargement, and the production of flowers and seeds [27]. The four main bioactive GAs: GA1, GA3, GA4, and GA7.

In plants, terpene synthases (TPSs), cytochrome P450 monooxygenases (P450s), and 2-oxoglutarate-dependent dioxygenases (2ODDs) are necessary for the production of bioactive Gibberellins from trans-geranylgeranyl diphosphate (GGDP). There are multiple possible ways that Gibberellins are metabolically degraded. The 2-hydroxylation reaction, which is catalyzed by a family of 2ODDs called Gibberellins 2-oxidases (GA2oxs), is the best understood deactivation process. Gibberellins are created in the embryo and subsequently transferred to the aleurone cells during the post germinative development of cereal grains. In these cells, −amylase gene expression is stimulated to facilitate the hydrolysis of endosperm starch [7].

Rhizobium spp enhance nitrogen fixation and also influence in plant hormonal balance which includes gibberellin metabolism [47]. *Mycorrhizal* fungi enhance the nutritional uptake and also plays a role in gibberellin biosynthesis [48]. *Bacillus* and *Pseudomonas* which are plant growth promoting rhizobacteria stimulate the plant growth and development and also enhance the hormonal signaling pathways which can indirectly affect gibberellins production and metabolism in plants [49].

5.4 Abscisic acid (ABA)

Abscisic acid (ABA) is a phytohormone which is essential for adapting to stress conditions like drought and excessive salt. Under water stress, it builds up in plant cells, encourages stomatal closure in guard cells, and controls the production of several genes whose by-products may shield vegetative tissues from dehydration or excessive osmotic pressure. Furthermore, abscisic acid is crucial for numerous developmental phases, including seed maturation and dormancy [8].

Abscisic acid has the ability to modify the biosynthesis of ethylene and auxin, it also influences in related gene expression which altogether regulates fruit ripening in climacteric species [28].

The hormone may slow (late effect in immature fruit) or hasten (early effect in more mature fruit) ripening depending on fruit developmental stage and, consequently, on flesh texture and physiological status, as shown by repression or enhancement of ethylene-, cell wall-, and auxin-related ripening induced gene expression, respectively. By influencing ethylene biosynthesis at a certain developmental stage, which affects metabolic and signaling cross-talk, abscisic acid may either weaken or augment ethylene/auxin action [50].

5.5 Brassinosteroids (BRs)

Brassinosteroids (BRs) are plant-specific steroidal hormones that play a variety of roles in monitoring a wide range of plant growth and developmental processes, including seed germination, cell elongation, cell division, senescence, vascular differentiation, reproduction, root development, sex differentiation, seedling gravitropism, fruit ripening and photomorphogenesis and also responding to various biotics and abiotic stresses [51]. They are distinguished by their polyhydroxylated sterol structure. *Catharanthus roseus* cell lines in culture were originally used to unravel the biosynthesis routes of Brassinosteroids. Bioactive brassinosteroids, such as brassinolide, bind to an island domain of the BRI1 receptor kinase, which is located on the plasma membrane, to initiate BR signaling. The synthetic auxin 2, 4-dichlorophenoxyacetic acid (2,4-D) greatly enhanced DWF4 expression, notably in the root tips, according to physiological tests utilizing a DWF4pro: GUS transgenic line. Auxin induces DWF4 expression, which in turn raises the number of brassinosteroids via the auxin signaling pathway. DWF4 is a crucial flux-determining enzyme that regulates the endogenous level of Brassinosteroids [52].

Studies showed that the BR biosynthesis route is a triterpenoid pathway after extensive research. The triterpenoid process uses evalonic acid as a precursor. It is compressed and changed into 2,3-oxidosqualene, which then goes through further modifications to create important plant sterols including sitosterols and campesterols. In order to create brassinolide (BL), the most active form of BR, campesterol is transformed to campestenal [53, 54].

The main receptor, BRI1, has a widespread expression pattern, whereas the expression of its two homologs only affects the vasculature and has a more restricted impact on growth. High BR activity promotes SAM cell differentiation and proliferation, which can be induced outside the border zone [55].

A complicated interaction between BRs and GA has been suggested as the cause of BRs' opposing influence on cell elongation in the leaf sheath in rice. By increasing GA production, physiological levels of BRs cause cell elongation in the leaf sheath. High BR concentrations, on the other hand, prevent cell elongation by inducing GA catabolism when administered exogenously to the leaf sheath [56].

5.6 Jasmonic acid (JA) and ethylene (ET)

Plant defense responses against a variety of diseases, pests, and abiotic stressors such wounding and exposure to ozone are known to be significantly regulated by three phytohormones, SA, JA, and ET. When plants are exposed to pathogens, their salicylic acid levels rise, and exogenous applications lead to the activation of genes associated with pathogenesis (PR) and improved resistance to a wide variety of infections. The defensive signaling network that the plant activates and uses depends on the kind of pathogen and its mechanism of pathogenicity. JA and ET are often involved with defense against necrotrophic diseases and herbivorous insects. JAs are also engaged in a variety of processes, including fruit ripening, leaf senescence, stomatal opening, tendril coiling, root development, tuber formation, and tendril coiling. Coronatine insensitive 1 (COI1), jasmonate resistant 1 (JAR1), and Jasmonate insensitive 1/MYC2 (JIN1/MYC2) are the three primary JA-signaling components [29].

6. Microbes associated in production of phytohormones

Microorganisms in plants do multi-function and these are well known as plant growth-promoting rhizobacteria (PGPR), which is a synonym for PGRs, to describe helpful, free-living bacteria that colonize roots. The term "PGPR" refers to any bacteria that live in plant roots and impact plant growth favorably through a variety of methods, including direct influences like enhanced nutrient absorption and indirect influences like disease suppression (biocontrol). Bacteria from the genera *Azotobacter*, *Azospirillum*, *Klebsiella*, *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Enterobacter*, *and Serratia* are included in the well-known PGPR (**Table 2**) [67].

Diazotrophic bacteria are also members of the PGPR due to their capacity to transform nitrogen into usable ammonia for plants [68]. Therefore, apart from nitrogen fixation they also contribute in synthesis of phytohormone. There have been reports

Microbes	Function/mechanism
Azoarcus, *Azorhizobium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Dechloromonas*, *Derxia*, *Geobacter*, *Gluconacetobacter*, *Mesorhizobium*, *Methylobacterium*, *Methylocella*, *Methylocystis*, *Methylosinus*, *Pseudomonas*, *Rhizobium*, *Rhodoblastus*, *Rhodovulum*, *Sinorhizobium*, *and Xanthobacter.*	Present in rhizosphere of the plant which helps in nitrogen fixation [57]
Azoarcus, *Azohydromonas*, *Azonexus*, *Azotobacter*, *Burkholderia*, *Dechloromonas*, *Delftia*, *Derxia*, *Methylocystis*, *Pelomonas*, *Sinorhizobium and Xanthobacter.*	Present in root of the plant which helps in nitrogen fixation and distributing the nutrition to the entire plant [57]
Alcaligenes, *Azoarcus*, *Azotobacter*, *Dechloromonas*, *Methylobacterium*, *Methylosinus*, *Paenibacillus*, *Pseudomonas*, *Rhizobium*, *Rhodoblastus*, *Sinorhizobium*, *and Xanthobacter.*	Present in shoot region of the plant and help in distributing the nutrient and absorbing the essential components [57]
Rheinheimera sp. J3-AN42	It is isolated from younger lateral roots. VFR5–3 (vegetative state of soil fertilized with N roots) has the ability to fix nitrogen and produce IAA and moderately produce cellulase and pectinase [58].
Burkholderia vietnamiensis	They show increased N-fixing as its marked with GUS gene and higher IAA production [59]
Corynebacterium sp	Produced the highest IAA in presences of tryptophan, among the isolates from seven types of rice soils [60]

Microbes	Function/mechanism
Azospirillum amazonense	increased dry matter accumulation of rice grain, the number of panicles and nitrogen accumulation at grain maturation [61]
Azospirillum brasilense	Increase yield of rice and also promote cell elongation, root development and tropic response [62]
Klebsiella sp. SS2	Increased root length [63]
Lysinibacillus sphaericus	They showed highest acetylene reduction (ARA) activity and significant biocontrol activity with complete inhibition of sclerotium germination compared to control and other test isolates. In the quantitative estimation of GA endophytic isolate L1 showed the highest value [64].
Klebsiella pneumoniae and Bacillus cereus	Highest IAA production [64]
Arthrobacter sp. M6	Detected with highest protease activity and highest IAA production [65]
A.tumefaciens, *Klebsiella sp.*, *Ochrobactrum anthropic*, *P. stutzeri*, *Pseudomonas sp.*	Bacteria's has ability to exhibit catalase activity and produce ammonia, synthesize IAA Phosphate solubilizing activity, nitogenase activity, ACC deminase activity, significant increase in growth and salt tolerant capacity compared to positive control [66]

Table 2.
Function/mechanism of the microbes.

of diazotrophs in a number of bacterial genera, including Acetobacter, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Paenibacillus*, *and Pseudomonas*, which may be loosely or more closely (endophytes) associated with plants [69].

Endophytic diazotrophs can be discovered in the roots, stems, leaves, and even seeds of plants. Through processes like chemotaxis, biofilm formation, and the secretion of compounds that stimulate plant growth, they are able to colonize these tissues. Tropical plants including sugarcane, maize, and rice have been shown to contain exceptionally high concentrations of endophytic diazotrophs. They are crucial in these plants for encouraging plant development and improving soil nitrogen fixation.

Di-nitrogenase and Di-nitrogen reductase, two proteins that work together to catalyze the conversion of atmospheric nitrogen to ammonia, make up the complex enzyme system known as nitrogenase. A wide range of diazotrophs are capable of converting atmospheric nitrogen into a form that may be used by plants and other living things. They are essential components of the global nitrogen cycle, supplying the biosphere with nitrogen and preserving life as we know it [70]. *Piriformospora indica* is an endophytic root fungus which stimulate the production of phytohormones. The mechanism of drought and salt tolerance mediated by fungi in plants involves in the production of cytokinin and abscisic acid. This fungus also stimulate lateral root growth and increase root surface area by producing auxin and gibberellin, this helps the plant to take up more water and minerals during stresses, thereby enhancing the yield [12].

Azospirillum, *Rhizobium*, *and Klebsiella* species are some of the diazotrophs that are most frequently utilized as bio-fertilizers. The process of transforming atmospheric nitrogen gas into a form that plants can utilize is known as nitrogen fixation, and it is promoted by these bacteria's ability to colonize the roots of plants and develop symbiotic partnerships. Diazotrophs can boost plant growth and production in a number of crops, including rice, maize, and wheat, according to studies on their usage as bio-fertilizers. They have also been demonstrated to increase the sustainability of agricultural practises, increase soil fertility, and decrease environmental pollution from synthetic fertilizers [38, 71, 72]. These bacteria are carefully studied to understand their mechanism as diazotrophs and phytohormone synthesizer. *Azospirillum*, *Rhizobium* and *Klebsiella* are essential in root promotion and increase IAA production in plants, therefore they contribute in dual benefits.

The most prevalent diazotrophs identified in rice plants are individuals from the genera *Azospirillum*, *Herbaspirillum*, *and Burkholderia*. It is known that these organisms colonize the roots of rice plants and encourage nitrogen fixation by activating nitrogenase enzymes [71, 73].

The concentration of the most significant plant hormones generated by *Azospirillum* species, such as auxins (IAA), cytokinins (Z), and gibberellins (GA3), rises during the exponential and/or stationary growth phase. By changing the homeostasis of the phytohormones, co-inoculation with rhizobacteria that produce them might affect the result of the symbiotic relationship. Researchers have focused on this genus to create particular strains as inoculants for use in agriculture since *Azospirillum sp*. has beneficial impacts on the development of various plant species [74–77].

An experiment was conducted on the availability of tryptophan as precursors in which IAA concentration produced by Azospirillum was monitored. The amount of IAA generated is significantly influenced by the pH of the culture medium. After the carbon source in the medium used for batch culture has been depleted, the stationary phase of the bacterium cells controls the release of huge quantities of IAA by Azospirillum spp. cultures [78]. Several PGPR have the ability to enhance the plant development, heavy metal stress such as Arsenic (As) adversely affects certain cascade pathways and phytohormonal signaling stimulations [79]. An experiment was conducted on wheat and sunflower plant to examine the arsenic toxicity level. The results conclude, plants produced more biomass when *Bradyrhizobium japonicum CB1809*, was present in the medium which was also quite tolerant of various arsenic concentration [80].

1-aminocyclopropane-1-carboxylate (ACC) deaminase-producing rhizobacteria that promote plant growth help plants grow and develop by lowering ethylene levels, increasing salt tolerance, and lessening drought stress. A large number of rhizobacteria consume the ethylene precursor ACC and transform it into 2-oxobutanoate and NH3. Plant roots elongate, shoot development is encouraged, rhizobia nodulation and N, P, and K intake are improved, and mycorrhizal colonization occurs in a variety of crops as a result of seed/root inoculation with ACC deaminase-producing rhizobacteria [81–83].

Thus, bacteria are the most crucial component which play multiple function in plant's growth and development. Azospirillum is the most widely studied organism concerning nitrogen fixation and phytohormone production such as auxin. There are several such organism which play multi-function in plant which are described in Table-2. Microorganisms are equally important in plant, research has proven they functions as biofertilizers, biopesticides, natural decomposers, nitrogen fixation bacteria, plant growth promotors and many more [84]. Genetic engineering is an operation tool to enhance the mechanism of these microbes to produce the desirable products [85]. Therefore, their great potential for the application field of agriculture and biotechnology.

7. Application of phytohormones

- Plant Growth Regulation: Phytohormones are used as plant growth regulators (PGRs) to manipulate plant growth and development. They can control plant height, enhance branching, promote flowering, regulate fruit development, and improve overall plant architecture [86].

- Seed Treatment and Germination: Phytohormones are applied to seeds to enhance germination rates, overcome dormancy, and promote uniform seedling emergence. This is particularly useful for improving the establishment of crops with low germination rates or challenging environmental conditions.

- Fruit Ripening and Shelf-Life Extension: Phytohormones, such as ethylene, are involved in fruit ripening and senescence. By regulating ethylene levels using inhibitors or ethylene-releasing compounds, the post-harvest shelf life of fruits can be extended, reducing spoilage and maintaining product quality.

- Flowering Induction: Phytohormones can be used to induce flowering in certain crops. By applying specific hormones at the appropriate stage, flowering can be promoted, leading to synchronized and increased flower production.

- Crop Yield Improvement: Phytohormones play a crucial role in optimizing crop yields. By understanding the hormonal regulation of plant growth and development, farmers can apply phytohormones strategically to maximize productivity and improve crop quality [39].

- Stress Tolerance: Phytohormones, such as abscisic acid (ABA), play a role in plant responses to various stresses, including drought, salinity, and temperature extremes. By applying ABA or other stress-related hormones, plants can exhibit improved tolerance and resilience in challenging environmental conditions [37].

- Plant Tissue Culture: Phytohormones are essential in plant tissue culture techniques for in vitro propagation and regeneration of plants. They stimulate cell division, callus formation, and differentiation, allowing for the production of large numbers of uniform plantlets [87].

- Weed and Pest Control: Phytohormones can be used as part of integrated pest management strategies to control weeds and pests. By manipulating hormone levels, it is possible to disrupt their growth and reproductive processes, offering an alternative approach to chemical pesticides [39].

- Root Development and Nutrient Uptake: Phytohormones, particularly auxins, play a crucial role in root development and nutrient uptake. By applying auxins, root growth can be stimulated, leading to enhanced nutrient absorption and overall plant vigor [39].

- Environmental Stimulation: Phytohormones can be applied to induce specific responses in plants, such as stimulating bud break, promoting lateral shoot development, or enhancing rooting. These applications are commonly used in horticulture and landscaping practices [86].

8. Conclusion

Phytohormones regulate the physiological processes in plants. Their ability to influence plant growth, development and responses to environment stimuli. The use of phytohormones which is synthesized by microbes holds higher application in research field. The regulatory power of phytohormones can optimize the plant growth, improve yields, overcome challenges and ensure sustainable and efficient crop production. Auxin, Cytokinin and Gibberellin are used as the plant growth regulator as it helps in enhancing the plant height, widening of branch, promote flowering and also regulate fruits development. It has also been found that it promotes seed treatment and germination which is most challenging environmental conditions. Phytohormones are naturally produced by plants but in low concentration. To enhance the plant growth regulator certain artificially synthesized plant-hormones are supplemented which in-turn affects the plant in long-term. The pilot study of nitrogen fixing bacteria in several commercially important crops has helped us to expand the study on phytohormone producing bacteria. The plant-growth promoting bacteria plays dual benefit for the plants which not only help in nitrogen fixation but also helps in producing phytohormones.

Agrobacterium, *Pseudomonas*, *Enterobacter cloacae* and *Azospirillum brasilense* stimulate in auxin production. *Halomonas desiderata*, *Proteus mirabilis*, *P. vulgaris*, *Klebsiella pneumoniae*, *Bacillus megaterium*, *B. cereus*, *B. subtilis*, and *Escherichia coli* influence in cytokinin production while *Bacillus*, *Pseudomonas and Mycorrhizal* fungi enhance the nutritional uptake and also plays a role in gibberellin biosynthesis. Nevertheless, Azospirillum is well studied in the production of auxin i.e., IAA productions. There are similar microorganisms which play multiple function in plant growth and development whose mechanism is yet to be discovered.

Genetic Engineering in modification of the microorganism to enhance the yield and productivity has contributed with respect to application in agricultural and biotechnology field. Therefore, these advanced approaches improve resources efficiency, minimizes environmental impacts and maximizes crop yield and quality. This benefits the farmers and society to increase the economic production.

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Conflict of interest

The authors declare there is no conflict of interest.

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References

[1] Altmann M, Altmann S, Rodriguez PA, Weller B, Elorduy Vergara L, Palme J, et al. Extensive signal integration by the phytohormone protein network. Nature. 2020;**583**(7815):271-276

[2] Zhao B, Liu Q, Wang B, Yuan F. Roles of Phytohormones and their Signaling pathways in leaf development and stress responses. Journal of Agricultural and Food Chemistry. 2021;**69**(12):3566-3584

[3] Korasick DA, Enders TA, Strader LC. Auxin biosynthesis and storage forms. Journal of Experimental Botany. 2013;**64**(9):2541-2555

[4] Michniewicz M, Brewer PB, Friml JÍ. Polar auxin transport and asymmetric auxin distribution. Arabidopsis Book. 2007;**5**:e0108

[5] Cortleven A, Leuendorf JE, Frank M, Pezzetta D, Bolt S, Schmülling T. Cytokinin action in response to abiotic and biotic stresses in plants: The role of cytokinin in stress responses. Plant, Cell & Environment. 2019;**42**(3):998-1018

[6] Shwartz I, Levy M, Ori N, Bar M. Hormones in tomato leaf development. Developmental Biology. 2016;**419**(1):132-142

[7] Yamaguchi S. Gibberellin metabolism and its regulation. Annual Review of Plant Biology. 2008;**59**(1):225-251

[8] Umezawa T, Nakashima K, Miyakawa T, Kuromori T, Tanokura M, Shinozaki K, et al. Molecular basis of the Core regulatory network in ABA responses: Sensing, Signaling and transport. Plant and Cell Physiology. 2010;**51**(11):1821-1839

[9] Schaller GE. Ethylene and the regulation of plant development. BMC Biology. 2012;**10**(1):9

[10] Oh MH, Honey SH, Tax FE. The control of cell expansion, cell division, and vascular development by Brassinosteroids: A historical perspective. IJMS. 2020;**21**(5):1743

[11] Yan L, Zhu J, Zhao X, Shi J, Jiang C, Shao D. Beneficial effects of endophytic fungi colonization on plants. Applied Microbiology and Biotechnology. 2019;**103**(8):3327-3340. DOI: 10.1007/s00253-019-09713-2

[12] Xu L, Wu C, Oelmüller R, Zhang W. Role of Phytohormones in Piriformospora indica-induced growth promotion and stress tolerance in plants: More questions than answers. Frontiers in Microbiology. 2018;**9**:1646

[13] Laird TS, Flores N, Leveau JHJ. Bacterial catabolism of indole-3-acetic acid. Applied Microbiology and Biotechnology. 2020;**104**(22):9535-9550

[14] Fukami J, Cerezini P, Hungria M. Azospirillum: Benefits that go far beyond biological nitrogen fixation. AMB Express. 2018;**8**(1):73

[15] Kutschera U, Niklas KJ. Evolutionary plant physiology: Charles Darwin's forgotten synthesis. Die Naturwissenschaften. 2009;**96**(11):1339-1354

[16] Hurný A, Benková E. Methodological advances in auxin and Cytokinin biology. In: Dandekar T, Naseem M, editors. Auxins and Cytokinins in Plant Biology [Internet]. New York, NY: Springer New York; 2017 [cited June 17, 2023]. p. 1, 29 (Methods

in Molecular Biology; vol. 1569). Available from: http://link.springer.com/10.1007/978-1-4939-6831-2_1

[17] Pennazio S. The discovery of the chemical nature of the plant hormone auxin. Rivista di Biologia. 2002;**95**(2):289-308

[18] Enders TA, Strader LC. Auxin activity: Past, present, and future. American Journal of Botany. 2015;**102**(2):180-196

[19] Gray RA. Alteration of LEAF size and shape and other changes caused by gibberellins IN plants. American Journal of Botany. 1957;**44**(8):674-682

[20] Eckardt NA. A new classic of Cytokinin research: Cytokinin-deficient Arabidopsis plants provide new insights into Cytokinin biology. The Plant Cell. 2003;**15**(11):2489-2492

[21] Kamínek M. Tracking the story of Cytokinin research. Journal of Plant Growth Regulation. 2015;**34**(4):723-739

[22] Bakshi A, Shemansky JM, Chang C, Binder BM. History of research on the plant hormone ethylene. Journal of Plant Growth Regulation. 2015;**34**(4):809-827

[23] Tamogami S, Agrawal GK, Rakwal R. Jasmonates to Jasmolites in plants. In: Advances in Botanical Research [Internet]. Netherlands: Elsevier; 2011 [cited May 24, 2023]. pp. 309-348. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780123858511000068

[24] Curaba J, Singh MB, Bhalla PL. miRNAs in the crosstalk between phytohormone signalling pathways. Journal of Experimental Botany. 2014;**65**(6):1425-1438

[25] Takatsuka H, Umeda M. Hormonal control of cell division and elongation along differentiation trajectories in roots. Journal of Experimental Botany. 2014;**65**(10):2633-2643

[26] Nziengui H, Lasok H, Kochersperger P, Ruperti B, Rébeillé F, Palme K, et al. Root gravitropism is regulated by a crosstalk between *Para* -Aminobenzoic acid, ethylene, and auxin. Plant Physiology. 2018;**178**(3):1370-1389

[27] Martins AO, Nunes-Nesi A, Araújo WL, Fernie AR. To bring flowers or do a runner: Gibberellins make the decision. Molecular Plant. 2018;**11**(1):4-6

[28] Zhang M, Yuan B, Leng P. The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. Journal of Experimental Botany. 2009;**60**(6):1579-1588

[29] Bari R, Jones JDG. Role of plant hormones in plant defence responses. Plant Molecular Biology. 2009;**69**(4):473-488

[30] Egamberdieva D, Wirth SJ, Alqarawi AA, EF Abd_Allah, Hashem A. Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. Frontiers in Microbiology. 2017;**8**:2104

[31] Fahad S, Hussain S, Matloob A, Khan FA, Khaliq A, Saud S, et al. Phytohormones and plant responses to salinity stress: A review. Plant Growth Regulation. 2015;**75**(2):391-404

[32] Niranjan V, Rao A, Janaki B, Uttarkar A, Setlur AS, K C, et al. Molecular docking and interaction studies of identified abscisic acid receptors in Oryza sativa: An In-silico perspective on comprehending stress tolerance mechanisms. Current Genomics 2021;**22**(8):607-619.

[33] Sujitha D, Kumar H, Thapliayal G, Pal G, Vanitha A, Uttarkar A, et al. Transcription factors controlling the expression of oxidative stress associated genes in rice (Oryza sativa L.). Plant Biotechnology Reports. 2023:1-17. DOI: 10.1007/s11816-023-00819-8

[34] Pal G, Bakade R, Deshpande S, Sureshkumar V, Patil SS, Dawane A, et al. Transcriptomic responses under combined bacterial blight and drought stress in rice reveal potential genes to improve multi-stress tolerance. BMC Plant Biology. 2022;**22**(1):349

[35] Shi TQ, Peng H, Zeng SY, Ji RY, Shi K, Huang H, et al. Microbial production of plant hormones: Opportunities and challenges. Bioengineered. 2017;**8**(2):124-128

[36] Chiaranunt P, White JF. Plant beneficial Bacteria and their potential applications in vertical farming systems. Plants. 2023;**12**(2):400

[37] Ali S, Xie L. Plant growth promoting and stress mitigating abilities of soil born microorganisms. FNA. 2020;**11**(2):96-104

[38] Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World Journal of Microbiology and Biotechnology. 2012;**28**(4):1327-1350

[39] Bishnoi U. PGPR interaction. In: Advances in Botanical Research [Internet]. Netherlands: Elsevier; 2015 [cited May 24, 2023]. pp. 81-113. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0065229615000671

[40] Costacurta A, Vanderleyden J. Synthesis of Phytohormones by plant-associated Bacteria. Critical Reviews in Microbiology. 1995;**21**(1):1-18

[41] Novák O, Napier R, Ljung K. Zooming In on plant hormone analysis: Tissue- and cell-specific approaches. Annual Review of Plant Biology. 2017;**68**(1):323-348

[42] Kende H, Jad Z. The five "classical" plant hormones. The Plant Cell. 1997;**9**:1197-1210

[43] Stirk WA, van Staden J. Potential of phytohormones as a strategy to improve microalgae productivity for biotechnological applications. Biotechnology Advances. 2020;**44**:107612

[44] Woodward AW. Auxin: Regulation, action, and interaction. Annals of Botany. 2005;**95**(5):707-735

[45] Gupta S, Seth R, Sharma A, Seth R, Sharma A. Plant growth-promoting Rhizobacteria play a role as Phytostimulators for sustainable agriculture. In: Choudhary DK, Varma A, Tuteja N, editors. Plant-Microbe Interaction: An Approach to Sustainable Agriculture [Internet]. Singapore: Springer Singapore; 2016 [cited May 7, 2023]. pp. 475-493. Available from: http://link.springer.com/10.1007/978-981-10-2854-0_22

[46] Arkhipova TN, Prinsen E, Veselov SU, Martinenko EV, Melentiev AI, Kudoyarova GR. Cytokinin producing bacteria enhance plant growth in drying soil. Plant and Soil. 2007;**292**(1-2):305-315

[47] Shanmugam KT, O'Gara F, Andersen K, Valentine RC. Biological nitrogen fixation. Annual Review of Plant Physiology. 1978;**29**(1):263-276

[48] Bottini R, Fulchieri M, Pearce D, Pharis RP. Identification of gibberellins a $_1$, a $_3$, and Iso-a $_3$ in cultures of *Azospirillum lipoferum*. Plant Physiology. 1989;**90**(1):45-47

[49] Ryu CM, Hu CH, Locy RD, Kloepper JW. Study of mechanisms for plant growth promotion elicited by rhizobacteria in Arabidopsis thaliana. Plant and Soil. 2005;**268**(1):285-292

[50] Soto A, Ruiz KB, Ravaglia D, Costa G, Torrigiani P. ABA may promote or delay peach fruit ripening through modulation of ripening- and hormone-related gene expression depending on the developmental stage. Plant Physiology and Biochemistry. 2013;**64**:11-24

[51] Gruszka D. The Brassinosteroid Signaling pathway—New key players and interconnections with other Signaling networks crucial for plant development and stress tolerance. IJMS. 2013;**14**(5):8740-8774

[52] Saini S, Sharma I, Pati PK. Versatile roles of brassinosteroid in plants in the context of its homoeostasis, signaling and crosstalks. Frontiers in Plant Science. 2015;**6**:950. DOI: 10.3389/fpls.2015.00950

[53] Chung Y, Choe S. The regulation of Brassinosteroid biosynthesis in Arabidopsis. Critical Reviews in Plant Sciences. 2013;**32**(6):396-410

[54] Fujioka S, Yokota T. Biosynthesis and metabolism of Brassinosteroids. Annual Review of Plant Biology. 2003;**54**(1):137-164

[55] Singh AP, Savaldi-Goldstein S. Growth control: Brassinosteroid activity gets context. Journal of Experimental Botany. 2015;**66**(4):1123-1132

[56] Tong H, Xiao Y, Liu D, Gao S, Liu L, Yin Y, et al. Brassinosteroid regulates cell elongation by modulating gibberellin metabolism in Rice. The Plant Cell. 2014;**26**(11):4376-4393

[57] Roesch LFW, Camargo FAO, Bento FM, Triplett EW. Biodiversity of diazotrophic bacteria within the soil, root and stem of field-grown maize. Plant and Soil. 2008;**302**(1-2):91-104

[58] Prakamhang J, Minamisawa K, Teamtaisong K, Boonkerd N, Teaumroong N. The communities of endophytic diazotrophic bacteria in cultivated rice (Oryza sativa L.). Applied Soil Ecology. 2009;**42**(2):141-149

[59] Govindarajan M, Balandreau J, Kwon SW, Weon HY, Lakshminarasimhan C. Effects of the inoculation of Burkholderia vietnamensis and related endophytic Diazotrophic Bacteria on grain yield of Rice. Microbial Ecology. 2008;**55**(1):21-37

[60] Naher UA, Radziah O, Shamsuddin ZH, Halimi MS, Razi IM. Isolation of Diazotrophs from different soils of Tanjong Karang Rice growing area in Malaysia. International Journal of Agriculture and Biology. 2009;**11**(5):1560-8530

[61] Rodrigues EP, Rodrigues LS, De Oliveira ALM, Baldani VLD, Teixeira KRDS, Urquiaga S, et al. Azospirillum amazonense inoculation: Effects on growth, yield and N2 fixation of rice (Oryza sativa L.). Plant and Soil. 2008;**302**(1-2):249-261

[62] Thakuria D, Talukdar NC, Goswami C, Hazarika S, Boro RC, Khan MR. Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. Current Science. 2004;**86**(7):978-985

[63] Rangjaroen C, Rerkasem B, Teaumroong N, Noisangiam R, Lumyong S. Promoting plant growth in a commercial rice cultivar by endophytic diazotrophic bacteria isolated from rice landraces. Annales de Microbiologie. 2015;**65**(1):253-266

[64] Shabanamol S, Divya K, George TK, Rishad KS, Sreekumar TS, Jisha MS. Characterization and in planta nitrogen fixation of plant growth promoting endophytic diazotrophic Lysinibacillus sphaericus isolated from rice (Oryza sativa). Physiological and Molecular Plant Pathology. 2018;**102**:46-54

[65] Patel JK, Archana G. Diverse culturable diazotrophic endophytic bacteria from Poaceae plants show cross-colonization and plant growth promotion in wheat. Plant and Soil. 2017;**417**(1-2):99-116

[66] Sharma S, Kulkarni J, Jha B. Halotolerant Rhizobacteria promote growth and enhance salinity tolerance in Peanut. Frontiers in Microbiology. 2016;7:1600. DOI: 10.3389/fmicb.2016.01600/full

[67] Frankenberger WT, Arshad M. Phytohormones in Soils: Microbial Production and Function. New York: M. Dekker; 1995. 503 p (Books in soils, plants, and the environment)

[68] Hu J, Richwine JD, Keyser PD, Li L, Yao F, Jagadamma S, et al. Nitrogen fertilization and native C4 grass species Alter abundance, activity, and diversity of soil Diazotrophic communities. Frontiers in Microbiology. 2021;**12**:675693

[69] Dobbelaere S, Vanderleyden J, Okon Y. Plant growth-promoting effects of Diazotrophs in the rhizosphere. Critical Reviews in Plant Sciences. 2003;**22**(2):107-149

[70] González PJ, Correia C, Moura I, Brondino CD, Moura JJG. Bacterial nitrate reductases: Molecular and biological aspects of nitrate reduction. Journal of Inorganic Biochemistry. 2006;**100**(5-6):1015-1023

[71] Döbereiner J, Baldani VLD, Reis VM. Endophytic occurrence of Diazotrophic Bacteria in non-leguminous crops. In: Fendrik I, del Gallo M, Vanderleyden J, de Zamaroczy M, editors. Azospirillum VI and Related Microorganisms [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 1995 [cited June 16, 2023]. p. 3-14. 10.1007/978-3-642-79906-8_1

[72] Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University - Science. 2014;**26**(1):1-20

[73] Bally R, Thomas-Bauzon D, Heulin T, Balandreau J, Richard C, Ley JD. Determination of the most frequent N_2 -fixing bacteria in a rice rhizosphere. Canadian Journal of Microbiology. 1983;**29**(8):881-887

[74] Cassán F, Diaz-Zorita M. Azospirillum sp. in current agriculture: From the laboratory to the field. Soil Biology and Biochemistry. 2016;**103**:117-130

[75] Strzelczyk E, Kampert M, Li CY. Cytokinin-like substances and ethylene production by Azospirillum in media with different carbon sources. Microbiological Research. 1994;**149**(1):55-60

[76] Cohen AC, Bottini R, Piccoli PN. Azospirillum brasilense Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. Plant Growth Regulation. 2008;**54**(2):97-103

[77] Cassán F, Maiale S, Masciarelli O, Vidal A, Luna V, Ruiz O. Cadaverine production by Azospirillum brasilense and its possible role in plant growth promotion and osmotic stress mitigation. European Journal of Soil Biology. 2009;**45**(1):12-19

[78] Costacurta A, Keijers V, Vanderleyden J. Molecular cloning and sequence analysis of an Azospirilium brasilense indole-3-pyruvate decarboxylase gene. Molecular Genetics and Genomics. 1994;**243**(4):463-472

[79] Aymen U, Khan M, Singh R, Parihar P, Pandey N. Role of Phytohormones in regulating arsenic-induced toxicity in plants. In: Srivastava PK, Singh R, Parihar P, Prasad SM, editors. Arsenic in Plants [Internet]. 1st ed. Canada: Wiley; 2022 [cited June 17, 2023]. pp. 198-222. DOI: 10.1002/9781119791461.ch11

[80] Reichman SM. Probing the plant growth-promoting and heavy metal tolerance characteristics of Bradyrhizobium japonicum CB1809. European Journal of Soil Biology. 2014;**63**:7-13

[81] Nadeem SM, Zahir ZA, Naveed M, Arshad M. Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt-affected fields. Canadian Journal of Microbiology. 2009;**55**(11):1302-1309

[82] Nadeem SM, Zahir ZA, Naveed M, Arshad M. Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Canadian Journal of Microbiology. 2007;**53**(10):1141-1149

[83] Arshad M, Saleem M, Hussain S. Perspectives of bacterial ACC deaminase in phytoremediation. Trends in Biotechnology. 2007;**25**(8):356-362

[84] Prakash O, Sharma R, Rahi P, Karthikeyan N. Role of microorganisms in plant nutrition and health. In: Rakshit A, Singh HB, Sen A, editors. Nutrient Use Efficiency: From Basics to Advances [Internet]. New Delhi: Springer India; 2015 [cited June 17, 2023]. pp. 125-161. DOI: 10.1007/978-81-322-2169-2_9

[85] Liu Y, Feng J, Pan H, Zhang X, Zhang Y. Genetically engineered bacterium: Principles, practices, and prospects. Frontiers in Microbiology. 2022;**13**:997587

[86] Basra AS, editor. Plant Growth Regulators in Agriculture and Horticulture: Their Role and Commercial Uses. New York: Food Products Press; 2000. p. 264

[87] Gaspar T, Kevers C, Penel C, Greppin H, Reid DM, Thorpe TA. Plant hormones and plant growth regulators in plant tissue culture. In Vitro Cellular & Developmental Biology – Plant. 1996;**32**(4):272-289

Chapter 5

Phytohormones and Biomolecules Produced by *Trichoderma* Strains as Eco-Friendly Alternative for Stimulation of Plant Growth

Abdenaceur Reghmit

Abstract

The increase in agricultural food demand during the last years has caused the expansion of cultivated areas. As a result, more chemical fertilizers are used in agriculture to fulfill the demand of the increasing population worldwide. Pesticides and chemical fertilizers are not recommended because they lead to environmental pollution, the development of resistant pests, and cause serious health problems. Thus, the reduction in the use of synthetic products is highly recommended. In this regard, alternative strategies for sustainable agriculture should be implemented. One of these strategies is the use of biofertilizers, specifically biofertilizer fungi that are widely applied in agriculture. *Trichoderma* seems to be the best candidate for use in green technologies due to its wide biofertilization and biostimulatory potential. Most *Trichoderma* species increase nutrient availability and uptake in plants. They are considered as plant growth-promoting fungi (PGPF). This genus colonizes the root systems of plants and promotes their growth. It can increase nutrient availability and uptake in plants by fixing nitrogen and solubilizing phosphorus. Moreover, they help plants tolerate environmental stresses such as drought, salinity, and stimulate plant growth due to their capacity to produce phytohormones such as indole-3-acetic acid (IAA) and gibberellins (GAs). Phytohormones play an important role in agriculture; they enhance plant growth through several processes.

Keywords: *Trichoderma* spp., biofertilizer, pesticides, phytohormones, green technologies

1. Introduction

Food security and intensive crop production have led to increased use of chemical fertilizers in agriculture [1, 2]. The use of chemical fertilizers has negative effects on the environment, soil microbiomes, terrestrial and aquatic ecosystems, as well as human and animal health [3]. Furthermore, the application of chemical pesticides in agriculture increases the resistance of microorganisms to pesticides and contaminates soil and groundwater [4, 5]. Thus, alternative measures should be taken instead of using synthetic chemicals. Plant growth-promoting microbes offer promising strategies to enhance plant growth [6–8]. Numerous researchers have studied the functions of plant growth-promoting fungi (PGPF). Many *Trichoderma* strains are capable of producing compounds as growth regulators that stimulate plant growth through several processes [9]. The effectiveness of *Trichoderma* species in agriculture is related to their metabolic activity and their type of interaction with plants and other microorganisms. They colonize the root systems of plants and promote their growth. *Trichoderma* species can increase nutrient availability and uptake in plants by fixing nitrogen, mineralizing organic matter, and producing several biomolecules and phytohormones [10, 11]. Phytohormones play an important role in agriculture [12]. They are synthesized by many rhizosphere microorganisms, including *Trichoderma* spp. They can enhance plant growth through several processes, such as modifying the physiological functions of plants to accelerate their growth by intensive cell division in callus tissue, promoting phloem development, enhancing lateral root development, stimulating plant growth, and preventing leaf aging by slowing down the breakdown of chlorophyll pigments in plants, as well as improving metabolism [13–15]. Other beneficial effects of *Trichoderma* are related to the stimulation of root development due to their ability to increase the solubilization of insoluble forms of phosphorus (P) in the soil, as well as acting as P-mobilizing microorganisms for plants [16]. Thus, the utilization of effective plant growth-promoting fungi to enhance crop production represents an eco-friendly alternative for sustainable crop production [17]. This review highlights the benefits of *Trichoderma* species for sustainable crop production providing a sustainable alternative to agrochemical products. Besides, this review provides environment friendly approach by exploring effective *Trichoderma* species with beneficial effects such as nutrient uptake, nitrogen fixation, siderophore and phytohormone production as well as phosphorus solubilizing. Therefore, these effective strains could be applied in crops production to increase their productivity.

2. *Trichoderma* as a biofertilizer

2.1 Characteristics of the *Trichoderma spp.*

Trichoderma genus is a group of filamentous fungi classified as anamorphic Hypocreales. Many strains are widespread around the world, typically colonizing rotting wood and other forms of organic plant matter [18]. They inhabit various ecological niches, including farmland, salt marshes, forests, and deserts in all climatic zones, encompassing temperate and tropical regions, Antarctica, and the tundra [3, 19]. *Trichoderma* genus is recognized as a cosmopolitan group of saprotrophic fungi, often existing as endophytes in woody plants [19]. Furthermore, *Trichoderma* strains produce diffusible pigments that can range from greenish-yellow to reddish tinges, and colorless strains may also be available. Conidia can exhibit different hues, ranging from dull to various shades of green or brown [20]. Microscopic identification criteria for *Trichoderma* are as follows: septate and translucent hyphae; conidiophores are short, translucent, and branched, often giving a pyramidal appearance, not verticillate; phialides are attached at right angles to the conidiophores. Spores produced are translucent and ovoid in shape, borne in small terminal clusters at the tips of phialides. Some species can produce globose chlamydospores, which are intercalary or terminal. These chlamydospores are usually unicellular [21] (**Figure 1**).

Figure 1.
Macroscopic and microscopic appearance of colonies representing phenotypic groups of Trichoderma *isolated from rhizosphere of olive trees: (A): T. koninji, (B): T. aureoviride [22].*

2.2 Plant growth-promoting properties of *Trichoderma*

Trichoderma species are often associated with the rhizosphere of host plants. They are typically known as symbiotic and saprotrophic fungi that invade the roots and stimulate plant growth through several mechanisms [23]. They exhibit beneficial effects on plants, such as promoting their growth, elongating lateral root growth, enhancing seed germination, as well as increasing photosynthesis efficiency, flowering, and yield quality [24]. The synthesis of phytohormones and phytoregulators is the most important stimulating factor at almost all stages of plant growth and development [12, 25].

2.3 Competition and plant root colonization

Rapid growth of *Trichoderma* strains makes them competitive candidates for space and nutrients because the growth of antagonistic microorganisms will be restricted, especially when nutrients become a limiting factor [26]. Importantly, *Trichoderma* can also colonize space by producing chelators such as siderophores, which increase the absorption and concentration of certain nutrients (copper, iron, phosphorus, manganese, and sodium). As a result, iron becomes less available for pathogens. Therefore, competition in the soil between microorganisms is considered an indirect mechanism for stimulating plant growth [27]. *Trichoderma* plays a role in stimulating plant growth by reducing oxidative stress. Additionally, studies conducted by [28] showed the role of siderophores produced by *Trichoderma asperellum* T34 in controlling *Fusarium oxysporum*, resulting in a reduction in tomato infestation and stimulation of root plant growth. Moreover, *Trichoderma* species can colonize the root surfaces of olive trees, leading to changes in metabolism. It has been previously reported that these fungi can invade olive roots [22]. Furthermore, the interaction between plants and *Trichoderma* offers a valuable source of nutrients released into root exudates for the benefit of *Trichoderma* [29, 30].

2.4 Phytohormones as inducers of systemic resistance

The recognition between the plant and *Trichoderma* leads to the synthesis of phytoalexins (fungitoxic molecules) [31, 32]. Phytohormones contribute to the regulation of complex and interrelated plant immune signaling pathways, providing a rapid defense response and adaptation to various environmental conditions [33]. *Trichoderma* species are capable of eliciting plant defense mechanisms through the synthesis of the enzyme ACC deaminase (1-aminocyclopropane-1-carboxylate) and indole-3-acetic acid. The biosynthesis of ethylene increases in response to ACC deaminase. In fact, plant development and defense systems are interconnected through a network of hormonal signaling pathways [34, 35].

2.5 The synthesis of phytohormones and metabolites influencing the phytohormonal balance

Phytohormones produced by fungi play crucial roles in agriculture, with a growing interest in their industrial production [36]. Many *Trichoderma* species are able to produce phytoregulators and phytohormones such as auxin and gibberellin, including the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which regulates the ethylene biosynthetic pathway [33, 37]. The enhancement of rhizosphere and root colonization by *Trichoderma* occurs through the synthesis of indole-3-acetic acid [38]. Moreover, *Trichoderma* species isolated from the rhizosphere soil of olive trees were able to produce a complex of growth hormones, IAA and gibberellin [39].

2.5.1 Production of auxin phytohormone indole-3-acetic acid

Several strains of *Trichoderma* are capable of producing auxin phytohormones, including IAA, which can enhance plant growth through various processes [40]. IAA regulates plant development; this phytohormone is responsible for the division, extension, and differentiation of plant cells and tissues. IAA stimulates seed and tuber germination, increases the rate of xylem and root formation, and controls processes of vegetative growth, tropism, fluorescence, and fructification of plants. It affects photosynthesis, pigment formation, resistance to stress factors, and the biosynthesis of various metabolites [41, 42]. The production of IAA usually depends on the presence of its precursor, L-tryptophan. This production is influenced by abiotic factors such as temperature and pH [12, 43]. IAA at low concentrations stimulates root elongation, while high concentrations are responsible for the proper formation of lateral and adventitious roots [12]. Moreover, several *in planta* studies have reported that *Trichoderma* species affect and increase plant growth in various crops such as olive [39], tomato [44], sorghum [45], bean [46], and wheat [47], due to the action of IAA produced by *Trichoderma*.

2.5.2 The production of gibberellin phytohormones

Gibberellins (GAs) are phytohormones that can enhance plant growth through seed germination, flowering, stem extension, aging, and stimulation of hydrolytic enzyme formation in germinating cereal grains [48, 49]. These properties are responsible for the extensive application of GAs in agriculture to improve the quality of agricultural and horticultural crops [50]. Several scientific reports have revealed the ability of numerous *Trichoderma* species to synthesize GAs [12, 51, 52]. After the inoculation of *T. koningiopsis* isolates, tomato growth was significantly improved, suggesting that GAs affect tomato growth [51]. Moreover, the production of GA by *Trichoderma* strains isolated from the rhizosphere of olive trees was confirmed previously [39]. Furthermore, the accumulation of GA3 produced by *T. harzianum* in combination with IAA was found to increase the plant growth-promoting effect [52].

2.5.3 The production of the ACC-deaminase enzyme

Trichoderma species are able to produce the enzyme 1-aminocyclopropane-1-carboxylate-deaminase (ACC deaminase), which is involved in promoting plant growth. This enzyme reduces the levels of ethylene (ET) in plants by cleaving the ET precursor ACC into ketobutyrate and ammonia [53]. The ACC deaminase enzyme affects the levels of ethylene (ET) when *Trichoderma* interacts with the plant roots [54]. This phytohormone (ET) affects the regulation of various physiological processes, in part through complex interactions with other phytohormones [55]. However, high levels of ET can inhibit root elongation and cause plant death [56, 57]. Several species of *Trichoderma* fungi have a considerable ability to produce ACC deaminase [12, 58, 59]. *T. asperellum* T203 produces ACC deaminase that regulates the endogenous ACC level and stimulates root elongation [34], enhancing plant tolerance to abiotic stress. Besides, ACC deaminase is able to modify the plant physiological functions and growth, enhancing plant tolerance to salinity stress [60].

2.6 Enhancement bioavailability and nutrient solubilization

Trichoderma plays an important role in enhancing plant growth by providing nutrient availability (phosphates, Fe3+, Cu2+, Mn4+, ZnO) and offering the necessary elements to the plant, mainly nitrogen, potassium, phosphorus, and microelements [61–63]. Phosphorus is an important element present in the soil with limited bioavailability to plants. It can be found in two forms: organic phosphorus and inorganic phosphorus, which is usually combined with calcium, aluminum, or manganese [64, 65]. Phosphorus solubilization in the soil is influenced by several factors such as microbial activity, pH, soil type, and organic matter availability [66]. The application of *Trichoderma* strains in the soil has been experimentally demonstrated to increase inorganic phosphate solubilization due to extracellular phytase activity [67] and the acidification of the soil environment through the production of acetic, butyric, citric, and fumaric acids [68]. *Trichoderma* spp. competes for the limited available phosphorus through various processes, such as solubilization, precipitation, absorption, and desorption. Inorganic phosphate and organic phosphorus can be mineralized through enzymatic action [66]. In previous studies, [69] reported that *Trichoderma atroviride* LBM 112 and *T. stilbohypoxyli* LBM 120 revealed positive results for phosphate solubilization with the formation of a halo-zone on the solid medium containing an insoluble inorganic phosphorus source. In addition, *Trichoderma harzianum* T11 (OL587563) isolated from the rhizosphere soil of olive trees has several plant growth-promoting traits, such as phosphate-solubilizing ability and the production of siderophores [39]. Furthermore, the *T. asperellum* CHF

78 strain showed increased nutrient uptake (P, K, Mg, and Zn) by tomato plants after pre-inoculation [70].

2.7 Nitrification and nitrogen fixation

Trichoderma species are among the beneficial rhizosphere microorganisms that are sustainably used for plant growth [71, 72]. The nitrogen fixation processes have significant ecological importance in various ecosystems, including those of agricultural interest. *Trichoderma* sp. is one of the important fungi that can colonize and solubilize various nutrients such as N, Zn, Fe, Cu, and Mn in soils [73]. Nitrogen fixation by microorganisms plays a key role in promoting plant growth. It has been suggested that the promotion effect on plant growth might be mediated by providing nitrogen through biological nitrogen fixation [74, 75]. The production of ammonia and nitrogen-fixing ability by *Trichoderma* strains is reported in previous findings. It has reported that ammonia is useful for plants. Ammonia production by the *Trichoderma* isolates may influence plant growth indirectly. ACC synthesized in plant tissues by ACC synthase is released from plant roots and taken up by neighboring microorganisms. Then, *Trichoderma* may hydrolyze 1-aminocyclopropane-1-carboxylic acid to ammonia [76]. *Trichoderma harzianum* T22 revealed nitrogen utilization efficiency in maize. Besides, it has been reported that *Trichoderma* species isolated from rhizosphere soil of olive were able to produce ammonia [39]. Furthermore, a previous study showed that among 20 *Trichoderma* spp. isolated from chili rhizosphere, 13 isolates were able to produce ammonia [77]. Due to the wide range of effects on plant growth and yields, *Trichoderma* applications have been widely extended over chemical fertilizers in the agriculture sector [78] (**Figure 2** and **Table 1**).

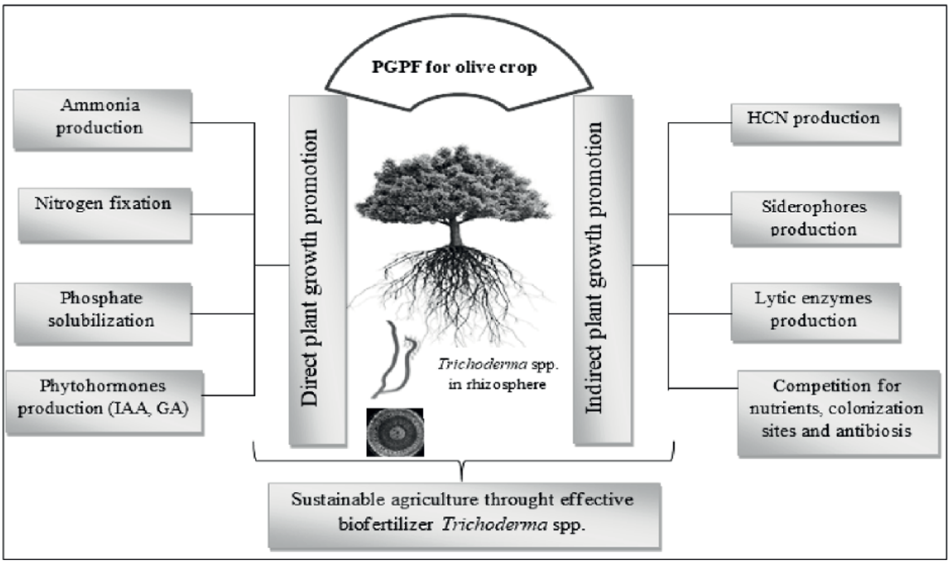

Figure 2.
Schematic description of the main mechanisms used by plant growth-promoting fungi to enhance crop production [39].

Compound	**Strain**	**Crops**	**Application mode**	**Beneficial outcome**	**References**
Biofertilizer	*T. asperellum T42*	Tomato	Seed treatment	Improves nutrient uptake (enhance nitrogen utilization efficiency, increase Phosphorus uptake	[79]
	T. harzianum	Most crops	Compost	Improves the rate of Residue decomposition resulting in greater availability of soil nutrients	[80]
	T. asperellum strain GDFS1009	Maize	On soil as granules	Increased yields	[81]
	Trichoderma azevedoi	Lettuce	Simple exposure	Increases carotenoids and chlorophyll with reduction in the white mold attack to about 78.83%	[82]
	Trichoderma afroharzianum	Tomato	Seed inoculation or treatment	Helps in the secretion of Phytohormones like homeostasis, antioxidant activity, phenylpropanoid biosynthesis and glutathione metabolism	[83]
	Trichoderma harzianum, Trichoderma asperellum, Trichoderma hamatum, Trichoderma atroviride	Chinese cabbage	Irrigation	Increases soil enzyme activity, yield by 37%, and increases the concentration of inorganic nitrogen and phosphorus content of the soil	[84]
	Trichoderma brevicompactum, Trichoderma gamsii, T. harzianum	Tomato	Seedling drenching	Improved growth and yield due to the production of indole-3 acetic acid	[44]
	T. harzianum, *T. asperellum*	Tomato	Seed treatment	Improves phosphorus uptake	[85]
	T. brevicompactm, T. gamsii, T. harzianum	Tomato	Seed drenching	Improves phosphorus solubilization	[44]

Table 1.
Trichoderma *sp. as bio-fertilizers for sustainable crop production.*

3. Conclusion

This review elucidates the ability of *Trichoderma* to be used as an efficient biofertilizer in agriculture. It has shown various mechanisms of providing the crop with biomolecules that can enhance plant growth by increasing nutrient availability, producing plant growth hormones such as indole-3-acetic acid and gibberellin, as well as enzymes including phosphatases and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. Hence, it has various mechanisms to enhance plant growth, giving it an advantage compared to other fungi. Thus, the increased use of *Trichoderma* spp. as commercial biofertilizer offers promising prospects for sustainable and environmentally friendly agriculture. These eco-friendly alternatives can substitute the excessive use of chemical products that can cause long-term problems. These strains have sufficient potential to warrant assessing their practical applications in the real field. However, more studies need to be conducted to elucidate the development of sustainable biotechnological applications of *Trichoderma* species in the soil-plant system. Future research should be conducted on developing new strains that are more effective at promoting plant growth and elucidating the molecular mechanisms through which these effective strains interact with plants. In addition, the use of genetic engineering could provide cultivars more resistant to abiotic and biotic stresses, such as drought and salinity. This could contribute to establishing more sustainable crop varieties that can support changing environmental conditions. Besides, synergetic interactions and the influence of abiotic factors such as extreme pH, soil salinity, drought, and temperature fluctuations should be studied to determine the optimal growth values.

Conflict of interest

The authors declare no competing interests.

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References

[1] Matson PA, Parton WJ, Power AG, Swift MJ. Agricultural intensification and ecosystem properties. Science. 1997;**277**:504-509

[2] de Vries FT, Wallenstein MD. Below-ground connections underlying aboveground food production: A framework for optimising ecological connections in the rhizosphere. Journal of Ecology. 2017;**105**:913-920

[3] Ghorbanpour M, Omidvari M, Abbaszadeh-Dahaji P, Omidvar R, Kariman K. Mechanisms underlying the protective effects of beneficial fungi against plant diseases. Biological Control. 2018;**117**:147-157

[4] Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. Agricultural sustainability and intensive production practices. Nature. 2002;**418**:671-677 [CrossRef]

[5] Alizadeh M, Vasebi Y, Safaie N. Microbial antagonists against plant pathogens in Iran: A review. Open Agriculture. 2020;**5**:404-440

[6] Mueller UG, Sachs JL. Engineering microbiomes to improve plant and animal health. Trends in Microbiology. 2015;**23**:606-617

[7] Bender SF, Wagg C, van der Heijden MGA. An underground revolution: Biodiversity and soil ecological engineering for agricultural sustainability. Trends in Ecology & Evolution. 2016;**31**:440-452

[8] Toju H. Core microbiomes for sustainable agroecosystems. Nature Plants. 2018;**4**:247-257

[9] Gravel V, Antoun H, Tweddell R. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: Possible role of indole acetic acid (IAA). Soil Biology and Biochemistry. 2007;**39**:1968-1977

[10] Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species – Opportunistic, avirulent plant symbionts. Nature Reviews. Microbiology. 2004;**2**:43-56

[11] Shoresh M, Harman GE, Mastouri F. Induced systemic resistance and plant responses to fungal biocontrol agents. Annual Review of Phytopathology. 2010;**48**:21-43

[12] Jaroszuk-Ściseł J, Tyśkiewicz R, Nowak A, Ozimek E, Majewska M, Hanaka A, et al. Phytohormones (auxin, gibberellin) and ACC deaminase in vitro synthesized by the mycoparasitic *Trichoderma* DEMTkZ3A0 strain and changes in the level of auxin and plant resistance markers in wheat seedlings inoculated with this strain conidia. International Journal of Molecular Science. 2019;**20**:19-4923

[13] Karadeniz A, Topcuoğlu ŞF, İnan S. Auxin, gibberellin, cytokinin and abscisic acid production in some bacteria. World Journal of Microbiology and Biotechnology. 2006;**22**(10):1061-1064. DOI: 10.1007/s11274-005-4561-1

[14] Chanclud E, Morel JB. Plant hormones: A fungal point of view. Molecular Plant Pathology. 2016;**17**(8):1289-1297. DOI: 10.1111/mpp.12393

[15] Fayziev V, Jovlieva D, Juraeva U, Shavkiev J, Eshboev F. Effects of PVXN-UZ 915 necrotic isolate of potato

virus X on amount of pigments of *Datura stramonium* leaves. Journal of Critical Review. 2020;7(9):400-403. DOI: 10.31838/jcr.07.09.82

[16] Contreras-Cornejo HA, Macías-Rodríguez L, Alfaro-Cuevas R, López-Bucio J. *Trichoderma* spp. improve growth of Arabidopsis seedlings under salt stress through enhanced root development, osmolite production, and Na^+ elimination through root exudates. Molecular Plant-Microbe Interactions. 2014;**27**:503-514

[17] Qiao C et al. Reshaping the rhizosphere microbiome by bio-organic amendment to enhance crop yield in a maize-cabbage rotation system. Applied Soil Ecology. 2019;**142**:136-146

[18] Howell CR. Mechanisms employed by Trichoderma species in the biological control of plant diseases: The history and evolution of current concepts. Plant Disease. 2003;**87**:1-10

[19] Kamala T, Devi SI, Sharma KC, Kennedy K. Phylogeny and taxonomical investigation of Trichoderma spp. from Indian region of Indo-Burma biodiversity hot spot region with special reference to Manipur. BioMed Research International. 2015;**2015**:21. Article ID: 285261

[20] Rincón AM, Benítez T, Codón AC, Moreno-Mateos MA. Biotechnological aspects of *Trichoderma* spp. In: Rai M, Bridge PD, editors. Applied Mycology. London, UK: CAB International; 2009. pp. 216-223

[21] Yuri. Fusarium Oxysporum. 2012. Retrieved from the Web site: http://thunderhouse4-yuriblogspot.com/2012/06/fusarium-oxysporum.html

[22] Reghmit A, Benzina-tihar F, López Escudero FJ, Halouane-Sahir F, Oukali Z, Bensmail S, et al. Trichoderma spp. isolates from the rhizosphere of healthy olive trees in northern Algeria and their biocontrol potentials against the olive wilt pathogen, *Verticillium dahliae*. Organic Agriculture. 2021;**11**:639-657

[23] Benítez T, Rincón MA, Limón CM, Codón CA. Biocontrol mechanisms of Trichoderma strains. International Microbiology. 2004;7:249-260

[24] Halifu S, Deng X, Song X, Song R. Effects of two Trichoderma strains on plant growth, rhizosphere soil nutrients and fungal community of Pinus sylvestris var. mongolica annual seedlings. Forests. 2019;**10**:758

[25] CainWJ YTT, Wang Q, Cai BD, Feng YQ. A rapid approach to investigate spatiotemporal distribution of phytohormones in rice. Plant Methods. 2016;**12**:47

[26] Gosling P, Hodge A, Goodlass G, Bending GD. Arbuscular mycorrhizal fungi and organic farming. Agriculture, Ecosystems & Environment. 2006;**113**:17-35. DOI: 10.1016/j.agee.2005.09.009

[27] Eisendle R, Oberegger P, Illmer Haas H. Biosynthesis and uptake of siderophores is controlled by the Pae C-mediated ambient-pH regulatory system in *Aspergillus nidulans*. Eukaryotic Cell. 2004;**3**:561-563

[28] Segarra G, Casanova E, Avilés M, Trillas I. *Trichoderma asperellum* strain T34 controls Fusarium wilt disease in tomato plants in soilless culture through competition for Iron. Microbial Ecology. 2010;**59**:141-149

[29] Jaroszuk-Sciseł J, Kurek E, Rodzik B, Winiarczyk K. Interactions between rye (Secale cereale) root border cells (RBCs) and pathogenic and nonpathogenic rhizosphere strains of Fusarium

culmorum. Mycological Research. 2009;**113**:1053-1061

[30] Hawes M, Allen C, Turgeon G, Curlango-Rivera G, Tran TM, Huskey DA, et al. Root border cells and their role in plant defense. Annual Review of Phytopathology. 2016;**54**:143-161

[31] Howell CR. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Diseases. 2003;**87**:4-10

[32] Shoresh M, Yedidia I, Chet I. Involvement of jasmonic acid/ ethylene signaling pathway in the systemic resistance induced incucumber by *Trichoderma asperellum* T203. Phytopathology. 2005;**95**:76-84

[33] Alfiky A, Weisskopf L. Deciphering Trichoderma-plant-pathogen interactions for better development of biological applications. Journal of Fungi. 2021;**7**:61

[34] Viterbo A, Landau U, Kim S, Chernin L, Chet I. Characterization of ACC deaminase from the biocontrol and plant growth promoting agent *Trichoderma asperellum* T203. FEMS Microbiology Letters. 2010;**305**:42-48

[35] Hermosa R, Viterbo A, Chet I, Monte E. Plant-beneficial effects of *Trichoderma* and of its genes. Microbiology. 2012;**158**:17-25

[36] Shi WL, Chen XL, Wang LX, Gong ZT, Li S, Li CL, et al. Cellular and molecular insight into the inhibition of primary root growth of Arabidopsis induced by peptaibols, a class of linear peptide antibiotics mainly produced by Trichoderma spp. Journal of Experimental Botany. 2016;**67**:2191-2205

[37] Ozimek E, Jaroszuk-Scisel J, Bohacz J, Korniłłowicz-Kowalska T, Tyskiewicz R, Słomka A, et al. Synthesis of indoleacetic acid, gibberellic acid and ACC-deaminase by Mortierella strains promote winter wheat seedlings growth under different conditions. International Journal of Molecular Sciences. 2018;**19**:3218

[38] Zhang F, Zhu Z, Yang X, Shen Q. Trichoderma harzianum T-E5 significantly affects cucumber root exudates and fungal community in the cucumber rhizosphere. Applied Soil Ecology. 2013;**72**:41-48

[39] Schmaltz S, Silva MA, Ninaus RG, Guedes JVC, Zabot GL, Tres MV, et al. Biomolecules in modern and sustainable agriculture. 3 Biotech. 2023;**13**(2):70

[40] Nieto-Jacobo MF, Steyaert JM, Salazar-Badillo FB, Nguyen DV, Rostás M, Braithwaite M, et al. Environmental growth conditions of Trichoderma spp. affects indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. Frontiers in Plant Science. 2017;**8**:102

[41] Pallardy S. Plant hormones and other signaling molecules. Physiology Woody Plants. 2008;**2008**:367-377

[42] Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI. Microbial producers of plant growth stimulators and their practical use: A review. Applied Biochemical Microbiology. 2006;**42**(2):117-126. DOI: 10.1134/S0003683806020013

[43] Napitupulu TP, Kanti A, Sudiana IM. Evaluation of the environmental factors modulating indole-3-acetic acid (IAA) production by Trichoderma harzianum InaCC F88. IOP Conference Series: Earth Environmental Science. 2019;**308**:012060

[44] Bader AN, Salerno GL, Covacevich F, Consolo VF. Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum* L.). Journal of King Saud University Science. 2020;**32**:867-873

[45] Saber WIA, Ghoneem KM, Rashad YM, Al-Askar AA. Trichoderma harzianum WKY1: An indole acetic acid producer for growth improvement and anthracnose disease control in sorghum. Biocontrol Science and Technology. 2017;**27**:654-676

[46] Hoyos-Carvajal L, Orduz S, Bissett J. Growth stimulation in bean (*Phaseolus vulgaris* L.) by Trichoderma. Biological Control. 2009;**51**:409-416

[47] Illescas M, Pedrero-Méndez A, Pitorini-Bovolini M, Hermosa R, Monte E. Phytohormone production profiles in Trichoderma species and their relationship to wheat plant responses to water stress. Pathogens. 2021;**10**:991

[48] Hamayun M, Sumera A, Ilyas I, Bashir A, In-Jung L. Isolation of a Gibberellin producing fungus (*Penicillium* sp.MH7) and growth promotion of crown daisy (*Chrysanthemum coronarium*). Journal of Microbiological and Biotechnology. 2010;**20**(1):202-207. DOI: 10.4014/jmb.0905.05040

[49] Jaroszuk-Œcise J, Kurek E, Trytek M. Efficiency of indoleacetic acid, gibberellic acid and ethylene synthesized in vitro by *Fusarium culmorum* strains with different effects on cereal growth. Biologia. 2014;**69**(3):281-292. DOI: 10.2478/s11756-013-0328-6

[50] De Oliveira J, Rodrigues C, Vandenberghe LPS, Câmara MC, Libardi N, Soccol CR. Gibberellic acid production by different fermentation systems using citric pulp as substrate/support. BioMed Research International. 2017;**2017**:5191046

[51] You J, Zhang J, Wu M, Yang L, Chen W, Li G. Multiple criteria-based screening of Trichoderma isolates for biological control of Botrytis cinerea on tomato. Biological Control. 2016;**101**:31-38

[52] Kamalov LS, Turgunov KK, Aripova SF, Abdilalimov O. Gibberillin A-3 from the microscopic fungus Trichoderma harzianum. Chemistry of Natural Compounds. 2018;**54**:421-422

[53] Todorovic B, Glick BR. The interconversion of ACC deaminase and D-cysteine desulfhydrase by directed mutagenesis. Planta. 2008;**229**:193-205

[54] Rauf M, Awais M, Ud-Din A, Ali K, Gul H, Rahman MM, et al. Molecular mechanisms of the 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase producing Trichoderma asperellum MAP1 in enhancing wheat tolerance to waterlogging stress. Frontiers in Plant Science. 2021;**11**:614971

[55] Dubois M, Van den Broeck L, Inzé D. The pivotal role of ethylene in plant growth. Trends in Plant Science. 2018;**23**:1-14 [CrossRef]

[56] Glick BR. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research. 2014;**169**:30-39

[57] Nascimento FX, Rossi MJ, Soares CRFS, McConkey BJ, Glick BR. New insights into 1-aminocyclopropane-1-carboxylate (ACC) deaminase phylogeny, evolution and ecological significance. PLoS One. 2014;**9**:e99168

[58] Saravanakumar K, Mubarak Ali D, Kathiresan K, Wang MH. An evidence of fungal derived 1-aminocyclopropane-1-carboxylate deaminase promoting the growth of mangroves. Beni-Suef University Journal of Applications. 2018;**7**:446-451

[59] Zhang F, Dou K, Liu C, Chen F, Wu W, Yang T, et al. The application potential of Trichoderma T-soybean containing 1-aminocyclopropane-1-carboxylate for maize production. Physiological and Molecular Plant Pathology. 2020;**110**:101475

[60] Zhang S, Xu B, Zhang J, Gan Y. Identification of the antifungal activity of *Trichoderma longibrachiatum* T6 and assessment of bioactive substances in controlling phytopathgens. Pesticide Biochemistry and Physiology. 2018;**147**:59-66

[61] Sood M, Kapoor D, Kumar V, Sheteiwy MS, Ramakrishnan M, Landi M, et al. Trichoderma: The "secrets" of a multitalented biocontrol agent. Plants. 2020;**9**:762

[62] Contreras-Cornejo XA, Macías-Rodríguez L, del Val E, Larsen J. Ecological functions of Trichoderma spp. and their secondary metabolites in the rhizosphere: Interactions with plants. FEMS Microbiology Ecology. 2016;**92**:1-17

[63] Stewart A, Hill R. Applications of Trichoderma in plant growth promotion. In: Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG, editors. Biotechnology and Biology of Trichoderma. Amsterdam, The Netherlands: Elsevier; 2014. pp. 415-428

[64] Rodríguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances. 1999;**17**:319-339

[65] Alori ET, Glick BR, Babalola OO. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontiers in Microbiology. 2017;**8**:971

[66] Khatoon Z, Huang S, Rafique M, Fakhar A, Kamran MA, Santoyo G. Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems. Journal of Environmental Management. 2020;**273**:111118

[67] Saravanakumar K, Shanmuga Arasu V, Kathiresan K. Effect of Trichoderma on soil phosphate solubilization and growth improvement of Avicennia marina. Aquatic Botany. 2013;**104**:101-105

[68] Scervino JM, Mesa MP, Monica ID, Recchi M, Moreno NS, Godeas A. Soil fungal isolates produce different organic acid patterns involved in phosphate salt solubilization. Biology and Fertility of Soils. 2010;**46**:755-763

[69] Lopez AC, Alvarenga AE, Zapata PD, Luna MF, Villalba LL. Trichoderma spp. from Misiones, Argentina: Effective fungi to promote plant growth of the regional crop Ilex paraguariensis St. Hil. Mycology. 2019;**10**(4):210-221

[70] Li YT, Hwang SG, Huang YM, Huang CH. Effects of *Trichoderma asperellum* on nutrient uptake and Fusarium wilt of tomato. Crop Protection. 2018;**110**:275-282

[71] Jain A, Singh S, Kumar Sarma B, Bahadur SH. Microbial consortium–mediated reprogramming of defence network in pea to enhance tolerance against Sclerotinia sclerotiorum. Journal of Applied

Microbiology. 2012;**112**:537-550. DOI: 10.1111/j.1365-2672.2011.05220.x

[72] Singh BN, Singh A, Singh GS, Dwivedi P. Potential role of *Trichoderma asperellum* T42 strain in growth of pea plant for sustainable agriculture. Journal of Pure Applied Microbiology. 2015;**9**:1069-1074

[73] Singh SP, Singh HB, Singh DK, Rakshit A. Trichoderma mediated enhancement of nutrient uptake and reduction in incidence of Rhizoctonia solani in tomato. Egyptian Journal of Biology. 2014;**16**:29-38. DOI: 10.4314/ejb.v16i1.4

[74] Bach E, dos Santos Seger GD, de Carvalho FG, Lisboa BB, Passaglia LMP. Evaluation of biological control and rhizosphere competence of plant growth promoting bacteria. Applied Soil Ecology. 2016;**11**:002. DOI: 10.1016/j.apsoil.2015.11.002

[75] Hemerly A. Genetic controls of biomass increase in sugarcane by association with beneficial nitrogen-fixing bacteria. In: Plant and Animal Genome Conference XXIV. Rio de Janeiro, Brazil: Universidade Federal do Rio de Janeiro; 2016

[76] Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting Rhizobacteria: Current perspective. Journal of King Saudi University Sciences. 2014;**26**(1):1-20. DOI: 10.1016/j.jksus.2013.05.001

[77] Mohiddin FA, Bashir I, Padder SA, Hamid B. Evaluation of different substrates for mass multiplication of *Trichoderma* species. Journal of Pharmacognosy and Phytochemistry. 2017;**6**(6):563-569

[78] Viterbo A, Horwitz BA. Mycoparasitism. In: Borkovich K, Ebbole DJ, editors. Cellular and Molecular Biology of Filamentous Fungi. Washington DC: ASM Press; 2010. pp. 676-693. DOI: 10.1128/9781555816636.ch42

[79] Yu Z, Wang Z, Zhang Y, Wang Y, Liu Z. Biocontrol and growthpromoting effect of *Trichoderma asperellum* TaspHu1 isolate from *Juglans mandshurica* rhizosphere soil. Microbiological Research. 2021;**242**:126596. DOI: 10.1016/j. micres.2020.126596

[80] Zin NA, Badaluddin NA. Biological functions of Trichoderma spp for agriculture applications. Annals of Agricultural Science. 2020;**65**(2):168-178. DOI: 10.1016/j.aoas.2020.09.003

[81] An-le HE, Jia LIU, Xin-hua W, Quanguo Z, Wei S, Jie C. Soil application of *Trichoderma asperellum* GDFS1009 granules promotes growth and resistance to fusarium graminearum in maize. Journal of Integrative Agriculture. 2019;**18**(3):599-606. DOI: 10.1016/S2095-3119(18)62089-1

[82] Rafael L, Valadares-inglis MC, Henrique G, Peixoto S, Eliza B, de Lucas G, et al. Volatile organic compounds emitted by *Trichoderma azevedoi* promote the growth of lettuce plants and delay the symptoms of white mold. Biological Control. 2021;**152**:104447. DOI: 10.1016/j.biocontrol.2020.104447

[83] Juan Z, Ting LIU, Wei-cheng LIU, Dian-peng Z, Dan D, Hui-ling WU, et al. Transcriptomic insights into growth promotion effect of Trichoderma afroharzianum TM2-4 microbial agent on tomato plants. Journal of Integrative Agriculture. 2021;**20**(5):1266-1276. DOI: 10.1016/S2095-3119(20)63415-3

[84] Ji S, Liu Z, Liu B, Wang Y, Wang J. The effect of Trichoderma biofertilizer

on the quality of flowering Chinese cabbage and the soil environment. Scientia Horticulturae. 2020;**262**:109069. DOI: 10.1016/j. scienta. 2019. 109069

[85] Yu Z, Wang Z, Zhang Y, Wang Y, Liu Z. Biocontrol and growth-promoting effect of *Trichoderma asperellum* TaspHu1 isolate from Juglans mandshurica rhizosphere soil. Microbiological Research. 2021;**242**:126596. DOI: 10.1016/j.micres.2020.126596

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