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Malus domestica

New Insights

Edited by Burhanettin İmrak
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Meet the editors

Associate Professor Burhanettin İmrak is an academic staff member at the Çukurova University Faculty of Agriculture in the Department of Horticulture. His research interests include sustainable farming, fruit growing, physiological studies, fruit breeding, physiology of dormancy in temperate climate fruits, and the effects of climate change on fruit growing. He has made short- and long-term visits and training studies in many countries on these issues. Between 1999 and 2000, he attended a plant breeding course organized by CIHEAM in Zaragoza, Spain. Dr. İmrak has written books and book chapters and published around 132 research articles. He has served as a referee for journals. He has participated in many international symposiums, workshops, and national meetings. He has been working on EU Prima and Cost projects. Dr. İmrak is married and has two children.

Professor Nesibe Ebru Yaşa Kafkas graduated with an award from the University of Çukurova Faculty of Agriculture Department of Horticulture in Adana province in Türkiye and is working as a senior lecturer and researcher position at the same university. She is also a member of the Biotechnology Department at the same university. Her Ph.D. was on "Identification of aroma compounds of some strawberry genotypes and relationship between aroma compounds and some fruit quality characteristics". During her Ph.D., she worked as a visiting scientist at the Newe Yaar Research Institute, affiliated with the Israeli Ministry of Agriculture, from 1998 to 2000. She worked on "Flavour and Aroma Formation in Strawberry Fruits (Fragaria × ananassa)" and was involved with the German-Israeli Foundation for Scientific Research and Development (GIF). Lately, she has joined international projects and is the National Coordinator and MC member of the EU COST projects, EU PRIMA Medberry project, and EU HORIZON Breeding Value projects.

Contents

Preface

The edited volume Malus domestica – *New Insights* is a collection of reviewed and relevant research chapters concerning the developments within the book's field of study. The book includes scholarly contributions by valuable authors and is edited by experts pertinent to the horticulture field. Each contribution comes as a separate chapter, complete in itself but directly related to the book's topics and objectives. The book includes chapters covering the following topics: new varieties, rootstock, fertilization, disease and pest control, preservation, transportation, dormancy physiology, irrigation, pruning, harvesting, packaging, fruit storage conditions and chemical composition and implications for processing. The book is addressed to academics and experts in the field.

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Chapter 1

Apple Cider Vinegar in Morocco: Exploring the Nutritional, Phytochemical Profile, and Therapeutic Benefits of This Indigenous Product

Ilham El Arabi and Driss Ousaaid

Abstract

In Morocco, folkloric medicine occupies an important place in the daily practice of people to treat and prevent several life-threatening disorders. Various ethnobotanical studies of Moroccan traditional medicine have been conducted in different regions to archive the traditional knowledge of Moroccan people. Recently, experimental studies have been extensively undertaken to examine the biological properties of common natural remedies against diabetes, inflammation, cancer, infertility, neurodegenerative diseases, and obesity. This chapter reviews the findings obtained from different experimental and ethnobotanical Moroccan studies conducted on apple cider vinegar (ACV) as well as its phytocompound profile, which was determined and identified by several working teams. Finally, different pharmacological activities of apple cider vinegar are discussed.

Keywords: *Malus domestica*, apple by-product, apple cider vinegar, beneficial properties, phytochemistry

1. Introduction

Fruits have long been valued for both their flavor and nutritional value. Recently, there has been a movement in the perspective of fruits as more than just a source of nutrition, as they can also contain pharmacologically active components [1, 2]. The growing interest in understanding the biological qualities of fruits and their by-products led to the development of nutraceuticals, which bridged the gap between food and pharmaceuticals. Recently, there has been a focus on the global usage of apple cider vinegar (ACV) and its potential scope for developing sustainable crop improvement and production. In the last two decades, apple cultivation and the production of apple products have declined in many Western countries. The notable exception to this has been the increase in demand for cider and cider-related products. This has resulted in a shortage of apples for non-cider apple products such as compote and juice. Combined with increasing production costs and the closing of many orchards, cider apple growers and some processing facilities have been in a crisis. It has also sparked debate in traditional apple-growing areas as to the future sustainability of apple production. In contrast to this, there are emerging apple industries in countries or regions with a temperate and Mediterranean climate. Hindered by increased globalization and lower international trade barriers to foreign apples and apple products, these industries are also vulnerable to similar problems within the next two decades. A research and development program aimed at identifying the potential benefits or niche markets for cider apple products is much needed for these regions. The health and medicinal value of ACV could be a possible niche market for product development.

2. From apple-to-apple cider vinegar

2.1 Apple-growing in Morocco

The apple sector in Morocco plays an important socio-economic role. The total area devoted to apple cultivation in Morocco is around 50,590 hectares. The Draa-Tafilalet and Fez-Meknes regions are the main production areas, accounting for 39 and 28% of the national surface area, respectively. The apple tree currently occupies second place among the rosaceous trees grown in Morocco, just after the almond tree. The first commercial orchards were developed in mountainous areas, where climatic conditions are favorable for the development and fruiting of this species.

Socio-economic impact: the apple sector generates income for more than 60,000 Moroccans. It also creates 2.2 million working days each year. In short, the apple sector makes a significant contribution to the economy and employment in Morocco.

In light of the increased globalization of food and agricultural products, sustainable agricultural research is now more important than ever. Numerous studies promoting and comparing global agricultural sustainability have emphasized the importance of region-specific crop systems and small to medium-sized farms. It is clearly evident that there is no one solution for the global improvement of agricultural sustainability. Though a small portion of this has been discussed in relation to apple production in the case of France [3, 4]. Understanding the global usage of apple cider vinegar could provide a potential niche market or health product for small to medium-sized apple farms. Farmers diversifying from current apple products into cider apple production with the intent to value add to their product could also benefit from this.

2.2 Different varieties of apple grown in Morocco

Apples are an important crop in Morocco, and several varieties are grown in different parts of the country:

Golden Delicious: this variety is characterized by its fine flesh and sweet-tart taste. Golden Delicious apples are elongated and yellow in color. They are often eaten fresh or as juice.

Starkimson: the Starkimson is a red to deep red apple variety. The flesh is juicy and the flavor slightly tart. It has a round, conical or elongated shape.

Starking Delicious: this is also a red variety. It has fine flesh and a sweet-tart flavor. Its shape is round-flattened.

These varieties are grown in various regions of Morocco, particularly in the provinces of Midelt and Fez-Meknes. They contribute to the local economy and are appreciated for their flavor and versatility.

2.3 Background of apple cider vinegar

Apple cider vinegar (ACV) has a long history of use. It dates back to 5000 BC, when the Babylonians used it as a preservative. Additionally, its use has been recorded in Egyptian urns as far back as 3000 BC [5]. It is also said to have been used by Pharaoh Cleopatra, who dissolved pearls in apple cider vinegar and then consumed it. Vinegar is also mentioned in the scriptures of the Hindus, and the ancient Greeks produced a sweet beverage very similar to apple cider and subsequently sour wine from honey. Traditional medicine in China uses vinegar to help aid in fighting infections, treating a cold, and to this day apple cider vinegar is used by alternative medicine practitioners to help fight sinus infections and sore throats. It was also used during the time of Hippocrates, known as the father of modern medicine, as a remedy for a variety of ailments. It was also used during the times of the Samurai and in eighteenth-century Europe to fight the threat of poor health and disease, specifically using it to battle food poisoning by consuming fish and chips with vinegar [5].

ACV, derived from the fermentation of apple juice, has been used as a therapeutic and health tonic for centuries. ACV is a type of vinegar which is made from apples. The apples are fermented, therefore, converting the fructose found in the apples to alcohol. The vinegar is made by crushing the apples and squeezing out the juice. Bacteria and yeast are then added to the juice to start the fermentation process. During the fermentation, the sugars are first converted to alcohol. Due to the two-step fermentation process, there are two main types of apple cider vinegar. The first of which contains a high amount of vinegar, and the second is further fermented to form acetic acid. This acetic acid is what gives apple cider vinegar a sour taste and pungent smell [6].

2.4 Importance of studying apple cider vinegar in Morocco

Most Moroccans typically utilize fresh fruits and vegetables, and occasionally meat sourced from the market, to craft their meals, often enjoyed together as a family. Eating patterns are evolving, especially in urban settings. Moroccan dietary practices are shaped by cultural and religious traditions, showcasing Arab, Andalusian, and Jewish influences. Traditionally shared within extended families, meals comprise breakfast as the main course, followed by fruits, nuts, and tea. Regional differences exist in eating habits. In a globalized world, the younger populace witnesses the shift in their dietary routines. A WHO study outlines these transformations in Morocco. An in-depth dietary analysis establishes correlations between eating patterns and illnesses. In low-income communities where a significant portion of Moroccans reside, dietary behaviors and specific diseases demonstrate alterations. Instances of diabetes, cancer, and heart ailments are increasing. The prevalence of deaths connected to these diseases escalated from 14% in 1980 to 28% by 2002. Obesity rates have augmented across generations. Conditions uncommon in traditional Moroccan culture may arise from the adoption of more "Westernized" diets. Apple cider vinegar, a longstanding ingredient in Moroccan cuisine and natural medicine, offers various health advantages, some of the purported medicinal values of ACV have been supported by a number of scientific investigations revealing its properties [7, 8]. The main substance of apple cider vinegar, acetic acid, can kill bad bacteria and prevent it from multiplying. It has also been used to help treat and control weight, cholesterol, and type 2 diabetes [9]. It is notably aiding weight loss. It facilitates reduced calorie consumption, enhanced satiety, lowered blood sugar levels, cardiovascular support, and improved skin health. Culturally significant in Morocco, this vinegar is esteemed for its health and wellness benefits. Its usage extends to a range of skincare and haircare products, emphasizing its importance in traditional Moroccan customs.

2.5 Objectives of the research

- Obtain clear information on the nutritional content of apple cider vinegar.
- Obtain information about the therapeutic benefits of apple cider vinegar in terms of health, prevention, and the healing of diseases that can cause harm to human life.
- Verify the composition and benefits of apple cider vinegar through the results of the conducted research.
- Provide enlightenment and encouragement about the benefits of apple cider vinegar in maintaining health.
- Use the results of this study as a reference for further research.

3. Nutritional profile of apple cider vinegar

Apple cider vinegar's quality and nutritional profile depend highly on the raw matter and the procedure adopted to produce vinegar. To elucidate the nutritional profile of apple cider vinegar, several measurements were performed to characterize different samples regarding their physicochemical properties, vitamin C content, and minerals. For instance, different Moroccan vinegar samples were analyzed in different studies published by Kara et al. [10] and by Ousaaid et al. [11]. In the first study, samples produced using three different methods and different apple varieties were analyzed. The results revealed high variability in the studied parameters. The pH ranged from 3.70 to 5.33 [10]. The electrical conductivity is of prime importance in predicting the mineral composition of sample up to 301 mS/cm [10]. Acidity and alcohol content are the most common quality criteria widely used in Morocco to evaluate the quality of vinegar. Furthermore, the analyzed samples had different total dry matter percentages ranging from 1.52 to 8.51% [10]. In the second study, Ousaaid et al. found that the pH of the studied samples did not exceed 3.83 and the electrical conductivity ranged from 2.11 to 2.90 mS/cm [11]. Different macro and microelements were found in ACV at different amounts, including K, Na, Ca, Mg, P, Fe, and Zn [12]. Importantly, the samples analyzed were exempt from heavy metals and respected the recommendation of the Codex Alimentarius Commission (CODEX) [13]. The same authors found that ACV contains a considerable amount of vitamin C ranging from 13.64 to 15.4 mg/100 mL [13].

4. Phytochemical profile of apple cider vinegar

4.1 Identification and analysis of phytochemicals

Since 1980, there has been a shift in the perception of bioactive phytocompounds, thanks to their safety, efficiency, and lower side effects compared to synthetic compounds [14]. Apple cider vinegar is well-known for its dense chemical composition, which contains organic acids, phenolic acids, flavonoids, melanoidins, minerals, and so on [15]. Several studies have been designed to unravel the mysteries of this apple by-product nutraceutical and its implication for human health. Through a concise exploration, ACV has been shown to serve as rich reservoirs of pharmacologically active compounds [7]. The quality and quantity of vinegar phytochemicals are highly affected by several factors, including manufacturing technique, fruit quality, fruit maturity, and microorganisms implicated in the vinegar-making process [15–17]. The bioactive compounds found in ACV present naturally in raw matter or generated during the vinegar production process confer the unique organoleptic characteristics of vinegar [18]. The delve into phytochemistry of ACV revealed the presence of 44 compounds with different amounts, such as arbutin, trans-ferulic acid, ferulic acid, apigenin, trans-cinnamic acid, and chlorogenic acid [19]. A comparative study conducted to determine the phytochemical content of apple cider vinegars manufactured using two different procedures (artisanal and industrial) indicated a substantial difference in the phytochemical composition [12]. The long fermentation process using the artisanal method led to the formation of newly generated compounds highly implicated in the vinegar quality criteria, such as color, astringency, taste, and flavor [20]. A comparative study between artisanal and industrial vinegar revealed that the artisanal vinegar contains the highest phenolic and flavonoid contents (106.91 mg GAE/100 mL vs. 68.08 mg GAE/100 mL for phenolic content and 11.36 mg QE/100 ml vs. 3.47 mg QE/100 mL for flavonoid content, respectively) [12]. Phytochemical profile exploration showed that artisanal vinegar contained the highest amount of trans-ferulic acid (11.94 vs. 4.32% for industrial vinegar), apigenin (16.48 vs. 2.54%), syringic acid (4.07 vs. 3.22%), sinapic acid (5.73 vs. 2.92%) than industrial vinegar [12]. The dense chemical composition of apple cider vinegar counteracts injurious microbes and could aid multiple physiological functions (**Table 1**).

4.2 Health benefits associated with phytochemicals

Phytochemicals constitute the main objective of a large body of scientific studies designed to examine their unquestionable beneficial properties for body hemostasis. Mounting evidence has proven several beneficial pharmacological properties of bioactive compounds throughout various mechanisms of action, owing to the huge scientific interest [23]. Traditionally, natural products, including medicinal plants, fruits, fruits by-products, and so on, have been used as natural remedies for various life-threatening disorders such as diabetes, obesity, cancer, inflammation, Alzheimer's, and infections [24–27]. Conventional medication is suddenly accompanied by significant adverse effects, which reinforces a surge in interest regarding natural remedies due to their safety, efficiency, and lower side effects. Arubtin is one of the major components of apple cider vinegar considered a biomarker of Moroccan samples [19]. This compound is widely used in the cosmetic industry as an

Country	Manufacturing method	Analysis method	Phytocompounds	References
Morocco	Traditional method	UHPLC/MS	Oleochantal (0.02%), trans-ferulic acid (23.32%), oleuropein (0.02%), trimethoxyflavone (0.10%), arbutin (53.33%), rosmarinic acid (0.05%), apigenin (0.75%), amentoflavone (0.02%), luteolin (0.30%), quercetin-3-O-glucoside (0.008%), quercetin-3-O-glucuronic acid (0.19%), quercetin-3-O.hexose deoxyhexose (0.01%), isorhamnetin-7-O-pentose/luteolin 7-O-glucoside (0.33%), kaempferol-3-O-glucuronic acid (0.07%), kaempferol-3-O-hexose deohyhexose (0.05%), vanillic acid (0.09%), caffeic acid (0.58%), ferulic acid (15.99%), trans-cinnamic acid (2.37%), chlorogenic acid (0.76%), cathechin/epicathechin (0.24%), gallocathechin/epigallocathechin gallate (0.34%), procianidin (0.12%), myricetin (0.19%), kaempeferol (0.36%), rutin (0.09%), and naringin (0.11%)	[19]
Japan	Industrial method	HPLC	5-hydroxymethylfurfural (2.7–4.1%), protocatechuic acid (0.41%), isomer of chlorogenic acid (3.1%), p-hydroxybenzoic acid (0.77%), isomer of p-coumaroylquinic acid (2.5%), chlorogenic acid (0.1–19.6%), caffeic acid (0.76%), isomer of chlorogenic acid (1%), 4-p-coumaroylquinic acid (13.5%), and p-coumaric acid (0.21%)	[21]
India	Industrial method	GC-MS	1,2-Cyclooctanedione, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Bicyclo [2.2.1] Heptane-2-carboxylic acid Isobutyl amide, 1,5-Anhydro-6-Deoxyhexo-2,3-Diulose, 5-Hydroxymethylfurfural, and 3-Deoxy-d-mannoic lactone	[22]

Table 1.
Phenolic profile of apple cider vinegar according to manufacturing and analysis methods.

anti-hyperpigmentation [28]. Furthermore, apple cider vinegar is a natural remedy for tegumentary system disorders widely used in Moroccan regions (Deraa-Tafilalet and Fez-Meknes) [29]. Other compounds, including ferulic acid, trans-ferulic acid, and chlorogenic acid exhibited several pharmacological properties, such as antimicrobial effect, antiseptic effect, antidiabetic effect, anti-inflammatory effect, anticancer effect, and antioxidant effect [30–32].

5. Therapeutic benefits of apple cider vinegar

5.1 Traditional uses of apple cider vinegar in Morocco

Since ancient times, humans have tried to treat and prevent different life-threatening disorders that impact their quality of life with various natural remedies. One of the most natural products widely used in several civilizations' folkloric medicine is apple cider vinegar. The awareness and application of this product back to date 5000 years ago [5, 33]. The Egyptians were most likely the first to discover and utilize vinegar, as they had been manufacturing wine since their civilization's inception [5]. Furthermore, Mesopotamia played a significant role in vinegar-making, as evidenced by archaeological discoveries and written records on cuneiform tablets describing the fermentation process. Hippocrates, the father of modern medicine (460–377 BC) declared vinegar to be the primary natural remedy for a variety of life-threatening disorders, such as cold and cough [34]. Wounds as the oldest surgical problem were treated with wine or vinegar-soaking sponges as prescribed by Hippocrates [35, 36]. After that, Babylonians started manufacturing vinegar from different raw materials [5]. Vinegar is one of the main ingredients of several recipes as documented in De Re Coquinaria. Furthermore, Roman soldiers and the general population have used diluted vinegar in water as a healthy and refreshing drink [5]. It has been used as a treatment against diabetes before the introduction of hypoglycemic pharmaceutical therapy [37]. The oldest prescription of vinegar appeared in the Chinese book Fifty-Two Diseases. The impact of vinegar on fasting blood glucose was confirmed scientifically for the first time by Ebihara and Nakajima [38]. An ethnopharmacological study conducted in Morocco revealed that apple cider vinegar was used traditionally to treat different human-threatening disorders, including digestive system disorders, skin diseases, cardiovascular system disorders, genitourinary system disorders, neuropsychic system disorders, and respiratory system disorders [29].

5.2 Modern application and research findings

Natural products constitute an exhaustible source of biomarkers with health-related properties. The adverse effects and short-term benefits of chemical drugs enhance the shift of the scientific community's interest toward natural therapeutic agents. Historically, apple vinegar has been used for various purposes to treat and prevent numerous diseases. Robust evidence found convincing outcomes proving the beneficial properties of apple vinegar. Metabolic disorders are the main troubles treated with apple vinegar. Numerous studies have found its ability to normalize different metabolic parameters such as LDL, cholesterol, glycemia, insulin level, hepatic enzymes, creatinine, urea, and electrolytes [7, 39–42]. In fact, clinical trials found that the administration of apple cider vinegar significantly reduced total cholesterol, fasting plasma glucose level, and HbA1V concentrations [43]. In a randomized controlled clinical trial, patients diagnosed with diabetes type 2 and dyslipidemia received 20 mL of apple vinegar daily for 8 weeks. The authors found that apple vinegar significantly improved blood glucose levels, HOMA-IR, HOMA-B, and QUICKI [44]. Furthermore, the same authors declared that apple vinegar has no effect on blood pressure and homocysteine after treatment during 8 weeks [44]. Apple vinegar contains a cocktail of bioactive ingredients with different structures and properties that exert their pleiotropic effects on various physiological functions. Acetic acid and other phytochemicals found in apple vinegar regulate blood glucose levels by delaying the gastric emptying process, blocking disaccharidase activity, ameliorating insulin sensitivity of different tissues, and enhancing glucose storage as glycogen [45]. Additionally, the phytochemicals in apple vinegar act their effect through the activation of AMPK pathway, which inhibits multiple genes implicated in the synthesis of fatty acid and promotes oxygenolysis of lipids [16].

Vinegar consumption revealed its ability to ameliorate gut microbiome and metabolome by reducing inflammatory markers, including immunoglobulin, natural killer cells, CD20 expression, and regulating the gut microbiota structure [46]. Administration of vinegar has been shown to up-regulate Verrucomicrobia, *Akkermansia, Hungatella* and *Alistipes*, and down-regulating *Firmicutes, Lachnospiraceae_NK4A136_grou*p and *Oscillibacter* [46]. Pourmozaffar et al. [47] found that the combination of apple cider vinegar at different concentrations (1, 2, and 4%) with propionate acid at a dose of 0.5% significantly reduced *Vibrio spp.*, CFU, R-cells numbers, and cholesterol concentrations. Another study found that the combination of vinegar and *Bacillus coagulans* significantly improves glucose tolerance, lowers overexpression of CD36, IL-1ß, IL-6, LXR and SREBP, and reduces food intake in mice hypercaloric diet-induced metabolic disorders [48]. Gut microbiota is the main target of an effective approach therapy for multiple digestive and inflammatory disorders. Diet fortified with apple cider vinegar ameliorates the probiotic effect, bioavailability of different nutrients, intestinal flora, and regulates the immune system [49].

6. Conclusions

Apple cider vinegar is a functional diet ingredient produced by double fermentation of apple fruit. It contains several added-value substances, including minerals, organic acids, vitamins, phenolic acids, flavonoids, and melanoidins. Apple cider vinegar is highly characterized by its long traditional and pharmacological activities as demonstrated by different in vitro and in vivo experiments. The pleiotropic effects of different compounds found in vinegar could explain its biological properties.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 2

Genotype and Phenotype Diversity in *Malus domestica* Borkh Species – Perusha Variety

Boryana Stefanova, Petko Minkov and Svetoslav Malchev

Abstract

The study was conducted in the period 2020–2022, in the conditions of the Central Balkan Mountain region (600 to 1200 m above sea level), around the town of Troyan and its adjacent villages and hamlets. Some old local apple forms of Perusha variety were discovered and marked, which are threatened with extinction. The genetic diversity of the local specimens of Perusha variety type was evaluated, and the pomological and reproductive properties of some forms were characterized, to select potential cultivars for further cultivation or selection. It was found that all forms have a green fruit skin color, and the fruit flesh in most cases is juicy, slightly sour, with aroma. All the studied forms of the group have different valuable economic qualities and different directions of use; therefore, it can be confidently stated that there is a perspective for their introduction into practice, in creating new orchards for sustainable fruit production.

Keywords: biodiversity, local genotype, physicochemical, pomological properties, resistance

1. Introduction

With the spread of commercial apple cultivars since the second half of the last century, hundreds of different local cultivars quickly disappeared from orchards. The specific quality characteristics of these fruits are still at risk of being lost today. Old apple cultivars (*Malus domestica* Borkh.) are characterized by good morphological and pomological properties, less need for chemicals in their cultivation, and a higher share of biologically active compounds (BACs). Their sensory acceptability is better compared to commercial cultivars and is of interest for biodiversity conservation [1].

The Republic of North Macedonia studied 13 autochthonous apple cultivars and found that some of them deserve to be grown in traditional orchards in typical rural areas, with reduced use of chemicals and in environmental protection programs. However, the local gene pool is of great socio-cultural importance, and the cultivars should be preserved *in situ* [2].

In two-year study in Serbia on the main biological properties of 10 *ex situ* apple genotypes, such as Bihorka, Budimka, Kraljica, Ovčiji Nos, Petrovača-1, Strekinja, Šimun Viparoš Struga, Šumnjaja, Tip 1, and Zejtinka, the best fruit quality among the assessed genotypes was found in Ovčiji Nos according to the fruit's chemical composition. All tested genotypes showed field resistance to fire blight and susceptibility to apple scab and mildew [3].

Vrtodusic & Skendrović [4] evaluated the pomological and physicochemical characteristics of 13 traditional Croatian pear varieties, which were studied in the Karlovac region, Central Croatia, to select potential cultivars for further cultivation or selection. The analyzed characteristics showed that the varieties differed significantly in their fruit weight, height and width, fruit index, length and thickness of stalk, fruit firmness, soluble solids content, acidity, ratio of soluble solids to total acidity, and pH. Varieties, such as Krasanka and Dugačka are prominent for the large fruit size and the harmonious ratio of soluble solids and acids. The Tepka variety stood out for its high content of soluble solids, which makes it a valuable raw material for distillation.

Crop Wild Relative (CWR) species are more or less closely related to the cultivated variety, as they include crop ancestors and are a potential source of characteristics beneficial to our crops. Given their importance for agricultural research and development, their conservation is a high priority, particularly their *in situ* conservation, which allows for the continuous evolution of new adaptive characteristics, as well as maintaining the breadth of genetic diversity present in many CWR species [5].

The main parameters related to quality are sugar and acid content, color, firmness, texture, juiciness, taste, nutritional value, absence of diseases or insects, and general appearance [6]. Old cultivars show good resistance to biotic and abiotic stress factors, as they are characterized by different morphological and pomological characteristics compared to commercial apple cultivars.

Old apple varieties offer great potential for the production of functional products due to their antioxidant activity, which neutralizes free radicals, preventing the formation of cellular damage in the fruit. Dietary fiber found in apples is also a plant substance with many benefits for human health, such as reducing fat and cholesterol absorption, normalizing digestion, maintaining gut health, and helping to control diabetes [7].

Consumers are becoming increasingly aware of the importance of a high intake of antioxidants and beneficial elements in the diet; therefore, attention is paid to foods rich in bioactive compounds that are organically grown, without any chemical substances. The restoration of old apple varieties due to their particular composition, taste, resistance to pathogens, and adaptability to climate and soil can meet this requirement [8].

In the face of global climate change and the invasion of new pests, the local gene pool is a vital step toward sustainable agriculture. Local varieties and forms are generally considered as better adaptable to the conditions of the region of origin than widespread varieties grown elsewhere [9].

Sadova Perusha variety in a private orchard in the area of Cherni Vrah (Troyan region) was found [10]. The tree has a spherical crown with a height of 9.20 m. The fruits are large over 190 g with a height of 63.20 mm, green color, with a pleasant taste with a balanced sugar-acid ratio. The variety is slightly susceptible to apple scab. Large-sized fruit forms and varieties that found suitable soil and climatic conditions for cultivation, appreciated by the local population and well accepted in the studied areas, such as Winter Green, Troyanka, Tsiganka, Reinette du Canada, Lemonki, Perusha, and several other local forms have been reported [11]. They are the basis of the local processing industry for making dried fruits, marmalades, juices, brandy, pectin, vinegar, etc.

Local varieties are described, in several of our studies, as being very well adapted to the agroecological conditions of the Central Balkan Mountain region, with large fruits, very good taste qualities, attractive appearance, suitable for long-term storage, relatively resistant to diseases and pests. Thus, it is required to search, preserve, and study them. They are suitable for nonconventional fruit plantations, such as family farms, as well as for their inclusion in selection programs, to improve some of their qualities.

The objective of the present research is to evaluate the genetic diversity of the local specimens of Perusha variety, to characterize the pomological and reproductive properties of some forms for the agroecological conditions of the Central Balkan Mountain region, and to select potential varieties for further growing and selection.

2. Material and methods

The study was conducted in the period 2020–2022. During the expeditions in the Central Balkan Mountain, old local apple varieties, threatened with extinction, were discovered, marked, and described in the region of Troyan and its adjacent villages and hamlets. The altitude of the studied areas is from 600 to 1200 m. That factor largely determines the period of ripeness. For forms 1 to 6, which were collected in the region of the village of Balkanec (altitude 600-800 m), the period of ripeness was the end of September 25–30. For forms 7 to 14, which were collected in the region of the town of Apriltsi (800-1100 m above sea level), the fruits ripen later, from the beginning to the middle of October.

The studied trees are over 80 years old, with a large trunk circumference (over 1.5 m), a crown height of over 15 m, with strong vigor. They were engrafted at a height of 30–40 cm from the ground and some at 1.20–2.00 m.

The shape of the crowns varies from globose to freely growing conic. The shape and size of the leaf blade are characteristic of Perusha variety. It has approximately the same length and width, which gives it an ellipsoid shape, large size, and dark green color. The leaf blade margin is entire and approximately uniform-integrated.

The trees bear fruit abundantly, but there is a distinct alternateness. Marked trees are single or in abandoned old orchards, along small hamlets, often overgrown with forest vegetation, without the application of any agrotechnical and pomological measures. Drying is often observed on the trunk and crown. Under these conditions, the fruitfulness and yields are of the order of 120–200 kg per tree. No symptoms of economically important diseases were observed.

Some large-fruited forms F10 and F11, with white flesh, were collected, stored and grafted on seed rootstock. Next year, they will be observed in a new field plantation.

The following indexes are taken into account:

- Reproductive – fruit weight (g), fruit size (mm), (height and average diameter) and fruit stalk length (mm)

Fruit - Observations on the fruit should be made on 10 typical fruits taken from a minimum sample of 20 fruits, at the time of ripeness for eating [12].

- Soluble solids (%)

- Sugars (%) (total, invert, and sucrose) – according to Schoorl and Regenbogen method

- Acids (%) – by titration with 0.1n NaOH
- Pectin (%)
- Glucoacidimetric index, ratio total sugars (%), and organic acids (%)
- Density (firmness) of fruit flesh (kgf/cm^2) – determined with a digital penetrometer FHT-15 (3.5 mm), by measuring both sides of 25 randomly selected fruits. The fruit skin of the measured fruits was removed.
- Pomological characteristics:
    - sensory analysis (taste, aroma)
    - color parameters measured with Color meter CM-200S, reported according to the CIELab system of the fruit skin L, a and b;

L – color brightness (L = 0 – black, L = 100 – white); a – the positive values of the indicator display the amount of red, while the negative values signify the intensity of green; b – the positive values are determinant of the yellow hues, while the negative values indicate blue; *H* (hue angle) = b/a. In the evaluation of H, we used the most widely accepted international criterion of assigning the angle. The value of the color tone or the dominant wavelength is represented by the a/b ratio [13].

The biochemical composition and color parameters of the fruits (**Tables 1** and **2**) were analyzed in six forms that are typical because the rest are similar and almost repetitive.

A visual assessment was made of the response of the observed varieties to the economically significant diseases, such as apple scab and powdery mildew on leaves and fruits. Low susceptibility based on single spots or absence of apple scab spots on leaves and fruit was reported and no powdery mildew symptoms were detected.

	Soluble solids (%)	**Total sugars (%)**	**Inverted sugars (%)**	**Sucrose (%)**	**Acids (%)**	**Glucoacidimetric index**	**Vit. C (mg/%)**	**Pectin (mg/%)**
F1	15.5	9.40	5.00	4.18	0.64	14.69	14.08	1.98
F2	13.6	10.20	6.50	3.52	0.64	15.94	17.60	0.83
F3	15.5	7.35	4.50	2.71	0.64	11.48	10.56	1.40
F4	18.1	10.75	8.55	2.09	0.77	13.96	22.88	0.66
F5	14.7	8.05	7.70	0.33	0.63	12.78	17.60	0.72
F6	17.0	18.40	18.4	—	0.34	54.12	12.32	0.64
av	*15.08*	*10.58*	*8.54*	*2.16*	*0.62*		*17.60*	*0.97*
St Dev	*1.74*	*3.22*	*4.02*	*1.21*	*0.11*		*4.69*	*0.44*
CV %	*11.54*	*30.43*	*47.12*	*55.97*	*18.16*		*26.67*	*45.81*

Table 1.
Biochemical composition of fresh fruit.

	L	a	b	*a/b*	H = (b/a)	Density (kgf/cm^2)	Brix (%)
F1	53.30	9.16	68.64	0.14	7.50	5.12	17.60
F2	70.49	−4.71	78.97	−0.06	−16.76	7.18	14.00
F3	66.13	−4.21	66.99	−0.06	−15.91	8.74	15.00
F4	35.13	29.89	25.69	1.34	0.86	8.28	13.00
F5	74.68	1.95	119.22	0.02	61.01	9.39	16.80
F6	74.90	−7.37	54.90	−0.13	−7.45	8.59	16.00

Table 2.
Fruit skin color parameters.

3. Results

The Test Guidelines of UPOV gives detailed practical guidelines for studying and identifying, through appropriate characteristics for researching distinctness, uniformity and stability, (DUS), and the development of harmonized descriptions of new varieties.

According to No 24 in the table of characteristics, the corresponding UPOV scale for fruit size is from 1 to 9. The studied forms of the variety are extremely large-fruited and belong to categories 7 – large (Mutsu); 8 – large to very large (Bramley's Seedling); and 9 – very large (Howgate Wonder) (**Table 3**).

F10 has the largest fruit weight of 220 g, followed by F3–192.63 and F1–191.59 g. In contrast, F6 has the smallest fruit weight with 116.80 g (**Figure 1**).

	Fruit weight (g)	Fruit height (mm)	Fruit diameter average (mm)	Fruit stalk length (mm)
	X ± StDev	X ± StDev	X ± StDev	X ± StDev
F1	191.59 ± *10.73*	64.83 ± *4.15*	*77.73 ± 2.33*	22.21 ± *5.41*
F2	176.75 ± *10.43*	61.43 ± *4.02*	76.29 ± *1.21*	8.87 ± *0.20*
F3	192.63 ± *27.26*	65.33 ± *4.84*	*77.70 ± 2.84*	8.68 ± *1.15*
F4	156.50 ± *27.72*	62.97 ± *1.87*	74.97 ± *2.10*	11.04 ± *1.95*
F5	187.08 ± 37.61	62.83 ± 4.38	80.08 ± 5.70	9.10 ± 1.19
F6	116.80 ± 12.70	49.79 ± 3.54	64.00 ± 4.17	13.54 ± 3.65
F7	132.60 ± 16.43	55.02 ± 1.92	67.50 ± 4.56	10.78 ± 1.55
F8	146.50 ± 5.92	60.67 ± 5.61	75.54 ± 1.65	11.06 ± 1.39
F9	154.60 ± 10.78	55.33 ± 1.32	76.15 ± 2.38	10.15 ± 1.58
F10	202.40 ± 29.80	78.00 ± 2.35	65.50 ± 5.93	11.80 ± 2.28
F11	189.00 ± 38.20	68.60 ± 6.19	81.10 ± 4.15	10.60 ± 1.14
F12	147.60 ± 17.07	54.40 ± 2.07	71.90 ± 5.93	17.40 ± 1.52
F13	127.80 ± *18.95*	58.00 ± *3.39*	67.30 ± *3.08*	19.20 ± *3.35*
F14	119.78 ± *9.26*	54.63 ± *3.14*	67.46 ± *3.43*	8.56 ± *0.88*

Average values indicate the property according to the LDS test with $p \leq 0.05$.

Table 3.
Morphometric indices.

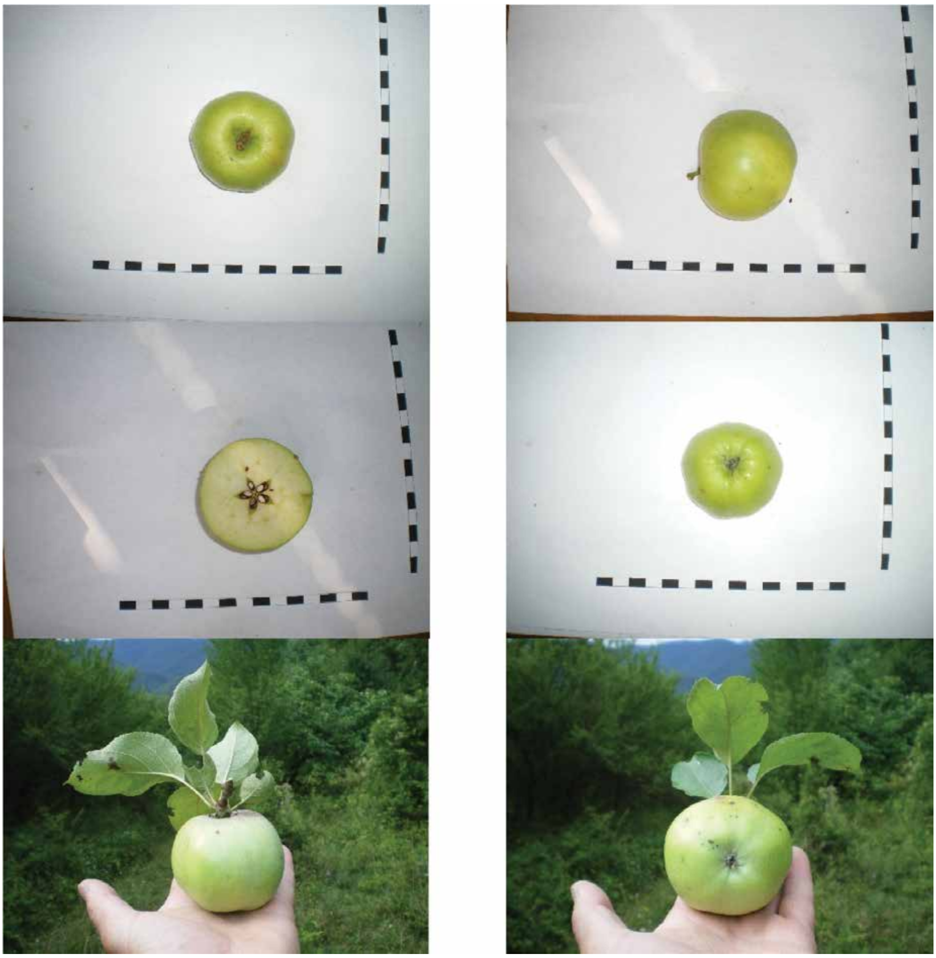

Figure 1.
The apple forms F6.

Fruit shape (No. 28 according to UPOV) is specified as Obloid 7 (symmetrical in diameter, wider than tall). The data on the height and average fruit diameter show that (**Table 3**). The greatest height is registered for F10 (78 mm), whereas the largest diameter is registered for F11 (81.10 mm).

Bozovic, et al. [14] indicate old cultivars that are of interest for production on a larger scale, due to their biological and economic properties. In the apple varieties, they observed ripening occurs from mid-July to mid-October for the Northern Montenegro region. The largest fruit size was found in Ilinjača (167.50 g), Dunjka (170.15 g), and Moračka krstovača krupna (182.34 g). Most cultivars have a round-flat and round-conical shape. The main skin colors of fruits in these varieties are green, greenish-yellow, and yellow, and an additional color (red or pink) is present in varying percentages. This is largely consistent with the present results.

The fruit stalk length of the apple forms (No. 46 according to UPOV) is determined as very short 1 (F2; F3; and F14); short 3 (F5; F7; F9; and F11); very long 9 (F1; F13; and F12).

Table 4 shows the following.

Form Perusha	Coloring of fruit, taste qualities
F1	Fruit skin – green covered with russet and a slight blush, thick, firm. Fruit flesh – whitish, tender, soft, juicy with crystal structure, slightly sour, with aroma
F2	Fruit skin – green with gray spots and a little russet at the stalk area, thick and tough. Fruit flesh – greenish, firm, but not very firm, dense, juicy, slightly sour, with aroma.
F3	Fruit skin – green yellowish with gray spots, a little russet and blush from eye basin, thick, tough, dense, tough. Fruit flesh – whitish, dense, crispy, with aroma.
F4	Fruit skin – green with golden over color at the stalk area, covered with gray spots, thick, greasy, with an abundant wax coating. Fruit flesh – light green, tender, juicy, sweet, with a slight aroma.
F5	Fruit skin – yellowish green, sprinkled with brown spots and russet at the stalk areas. Fruit flesh – white, soft, slightly floury, dry, sweet, without aroma.
F6	Fruit skin – green, thick, covered with an abundant wax coating. Fruit flesh - white, dense with granular structure, sour, crispy with aroma
F7	Fruit skin – yellow to intensively yellow, thick, firm with a wax coating. Fruit flesh – white with floury texture, dry, astringent, without aroma.
F8	Fruit skin – light green with blush on the sunny side, thick, tough, dense. Fruit flesh – white, tender, juicy, sweet, without aroma.
F9	Fruit skin – green with intensive yellow over color, tough, dense, with an abundant wax coating. Fruit flesh – white, very soft, juicy, slightly sour with aroma.
F10	Fruit skin – greenish with a wax coating, thick, dense, tough. Fruit flesh – white, slightly sour to sour, tender, average sour, with a slight aroma.
F11	Fruit skin – light green, smooth, dense, tough with wax coating. Fruit flesh – white, sweet to slightly sour, juicy with aroma.
F12	Fruit skin – green with russet at the stalk areas, thick, dense, tough. Fruit flesh – white, soft, average sour, aroma.
F13	Fruit skin – light yellow with predominant blush, thick, dense, tough, covered with a wax coating. Fruit flesh – yellowish, comparatively tender, sweet, and juicy, without aroma.
F14	Fruit skin – green with yellowish over color and blush, tough, dense. Fruit flesh – white, soft, sweet to slightly sour, with aroma.

Table 4.
Pomological description of the forms of Perusha variety type (2021).

Fruit skin: It is mainly green to yellow-green for the group of PERUSHA variety, often with russet at the stalk area, thick, thin, rough, or tough.

Fruit: ground color (No. 35 according to UPOV).

The color of the fruit skin is a characteristic trait because most forms F1; F2; F3; F4; and F6 (**Figure 1**); F9; F10; and F14 are green (6 green Granny Smith according to UPOV) to light green (5 yellow-green Cox's Orange Pippin According to UPOV) F8; F10; F11.

Forms F5; F7; and F13 are in the yellow range of the color scale (No. 3 is yellow with standard varieties Delorgue, Gala, Transparent de Croncels according to UPOV). The geographical location and the position of the fruits, concerning the sunlight, favoring the formation of the color, probably have an impact.

Fruit: Color of flesh (No. 53 according to UPOV). The fruit flesh varies from soft to firm, but not very firm, in most cases with a juicy, slightly sour, aroma. In some samples, it is dense. The flesh is white to whitish in all forms (F5; 6; 7; 8; 9; 10; 11; 12; and 14), except for F2 and F4, which are greenish. Forms F1: F2; F4; F8; F9; F10; and F11 are distinguished by juicy fruit flesh, whereas F5 and F7 are dry.

Most of the presented forms have aromatic flesh (F1; 2; 3; 6; 9; 11; 12; and 14), and the rest are without aroma. Sweet fruit flesh is found in forms 4; 5; 8; and 13; sweet and sour in 9; 10; 11; and 14; and sour in 1; 2; and 6. According to this indicator, the taste requirements of a large part of consumers are covered – sweet and sour.

In some representatives of the variety, after storage of the fruits, the pulp acquires a floury structure (F5).

The European consumer prefers sour flavors, white fruit flesh, juicy, and aromatic (Forms 1 and 2). In the Balkan countries, the tastes of sweet, sweet-sour, and juicy fruits are preferred (F9; 10; and 11) (**Table 4**).

In our research, the soluble solids in the fruits were from 13.60% (F2) to 18.10% (F4). The coefficient of variation is 11.54%. Higher values of soluble solids are F4, F6, which makes them valuable raw material for processing – drying and distillation (**Table 1**).

Total sugars in these forms are also higher, compared to the whole group, 18.40% and 17.00%, respectively. In general, they vary from 7.35% (F3) to 18.40% (F6), with a coefficient of variation of 30.43%.

Similar results were reported by Kulina et al. [15] for soluble solids content (SSC,%) in apple fruits from 12.93% for the cultivar Bjeličnik to 16.15% for Ljepocvjetka in Bosna and Hercegovina cultivars. According to the soluble solids (14–16%), most of the varieties in their research are classified as having medium-high and high content of SSC. The content of total acids in the studied varieties varied from 0.43% for Petrovača variety to 0.71% for Šampanjka variety.

Bozovic et al. [14] indicated that the SSC in autochthonous varieties in Northern Montenegro ranged from 9.6% to 15.2%, as both authors gave lower levels. Varieties with a high content of soluble solids, such as Aleksandrija (16.0%), Rebrača (15.5%), Jolovača (14.6%), and Dunjka (14.5%) can be recommended as good material for the processing industry. Chemical analyses show that apple varieties differ significantly according to the studied habitats. The fruit chemical composition varies greatly depending on variety, location, and multiple abiotic factors, such as environmental conditions and agrochemical properties of the soil.

The content of soluble solids (17.0%) and total sugars (18.40%) in F6 is high, represented only by inverted sugar in the absence of sucrose, as acids are at least 0.34%. This makes it extremely unbalanced in taste.

The most favorable ratio between sugars and acids is reported at F2 (glucoacidimetric index 15.94) when a harmonious and refreshing taste is felt, which is an important criterion for evaluation and consumption.

Sucrose content is important for the dietary and nutritional properties of fruits. In Form 6, there is no sucrose, which makes it a suitable food for diabetics (**Table 1**).

Vitamin C ranged from 12 to 22 mg/% in the whole group, CV: 26.67%.

Apple pectin improves bowel functions, keeps the feeling of repletion for a long time, prevents obesity, and removes toxins and heavy metals from the body.

Stoyanova et al. [10] reported the highest amount of pectin in Sadova Perusha variety (1.98%), which completely coincides with F1 (1.98 mg/%) from the present study (**Table 1**). The pectin is below 1 mg/% in the variants studied, except for F1 (1.98 mg/%) and F3 (1.40 mg/%).

The color coordinates L – color brightness; +a – red color; −a – green color; +b – yellow color; −b – blue color; the qualitative indicator color tone a/b (dominant wavelength) and hue angle H = (b/a) of six forms were measured (**Table 2**).

The highest value for skin brightness was recorded for F6 (L = 74.90), as F5 had the most dominant yellow color (b = 119.22).

There are also high values for brightness in forms F2 (70.49) and F5 (74.68). Form 4 has the lowest brightness values (L = 35.13), where the red color is most pronounced (a = 29.89).

Minkov [1] studied heirloom local apple varieties threatened with extinction. According to that study, Sadova Perusha has high values for brightness (L = 71.74) and stands out with the largest negative value (−a = 6.09), which defines its green color. The qualitative indicator color tone a/b (dominant wavelength) has the highest value for the fruits of Sadova Perusha (7.92).

The most pronounced are correlation with L to a (r = −0.9399) and L to a/b (r = −0.9128). The highest positive correlation is between (a) and (a/b) r = 0.961941. There is no statistically proven relationship between hue (hue angle (b/a) and color tone (a/b). Color parameters are independent of Density and *Brix* (**Table 5**).

With regression coefficient $R^2 = 1$, the equation $y = -1.0491x + 66.758$ gives us reason to predict the brightness of the color (L), by the value of the parameter (a) (**Figure 2**).

	L	a	b	a/b	H = (b/a)	Density (kgf/cm^2)	Brix (%)
L	1						
a	−0.93,994	1					
b	0.711,146	−0.53,113	1				
a/b	−0.91,286	0.961,941	−0.64,779	1			
H = (b/a)	0.162,686	0.144,813	0.671,304	0.003153	1		
Density (kgf/cm^2)	0.32,754	−0.14,952	0.173,232	0.017086	0.288,632	1	
Brix (%)	0.424,268	−0.37,186	0.571,115	−0.58,478	0.496,633	−0.28,956	1

Table 5.
Matrix of Pearson correlation coefficients for CIELAB values and color indexes considered.

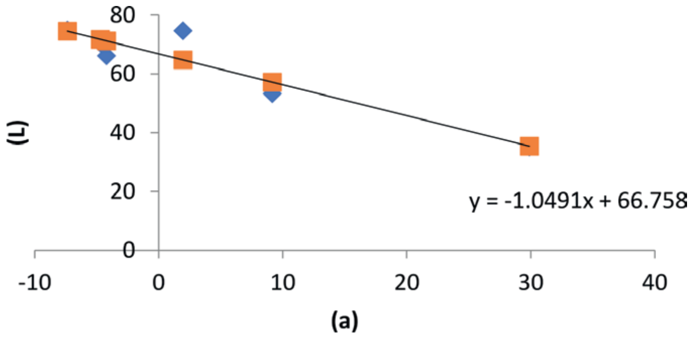

Figure 2.
Regression dependence of (L) against (a), $R^2 = 1$.

4. Conclusions

The study on PERUSHA variety (*Malus Domestica* BORKH) showed the following conclusions:

The fruit skin of all studied Perusha forms is mainly green to yellow-green, dense, thin or rough, firm and tough, giving greater transportability and a long storage period.

The fruit flesh varies from soft to firm, but not very firm, in most cases is juicy, slightly sour, with aroma. These qualities closely resemble standard commercial varieties and satisfy the requirements of all users.

The smallest amounts of sucrose are found in F5 (0.33%) and F4 (2.09%), except for F6 where it is completely absent, which defines them as suitable food for diabetics. The glucoacidimetric index of these forms is, respectively, 17.60 and 22.88, which gives them a pleasant and balanced taste.

All the studied forms of the group have different valuable economic qualities and different directions of use. Therefore, there is a huge perspective for their introduction into practice in the establishment of new orchards for sustainable fruit production.

Considering the diversity of pomological traits, their potential as sources of genetic variation, and their possible tolerance to abiotic and biotic stress factors, it is important to preserve traditional apple varieties as a source of quality fruit for consumption or processing, protecting these valuable genetic resources along with the area's biodiversity.

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Chapter 3

Malus Domestic's Bacterial and Fungal Diseases Impact in Kyrgyzstan Fruit Production

Tinatin Doolotkeldieva

Abstract

Kyrgyzstan is not just a home to wild apple trees, but a global sanctuary for these unique species. The largest concentration of these wild apple trees can be found in the natural walnut forests and the Sary Chelek *reservation.* The *Malus niedzwetzkiana* Dieck—Nedzwiecki apple tree and the *Malus sieversii* are not just rare and endangered species in the Red Book but also global treasures. The preservation of the existing diversity of fruit crops and their wild relatives in Kyrgyzstan is not just critical but of utmost importance at a global level. It provides genetic resources to all user groups now and in the future. Apple orchards are facing significant challenges due to pests and diseases. However, monitoring, scientific study, and identifying these pathogens should be conducted regularly. Only accurate identification of diseases can help determine appropriate protection. It is essential to pay special attention to wild thickets of apple trees mixed in particular forests and foothills, as they serve as genetic resources of cultivated forms and need timely protection from newly introduced species of diseases and pests. To address these needs, this study aimed to identify the economically significant apple tree diseases and develop biological protection against pathogens.

Keywords: *Malus niedzwetzkiana* Dieck, *Malus sieversii*, *Malus domestica*, scab disease, bacterial fire blight

1. Introduction

Kyrgyzstan has the world's largest natural walnut forests, located within a 60,000-hectare area between the Fergana and Chatkal Mountains. These forests are home to 88 plant species. Wild forms of fruit trees such as *Juglans regia, Malus niedzwetzkyana, Malus sieversii, Prunus divaricata, Prunus sogdiana, Crataegus spp., Pyrus korshinsky* dominate the forest's high plant biodiversity [1–4]. The forests are also a significant source of economic and ecological benefits for local communities that provide food, medicine, fuelwood, material, and animal feed.

1.1 Wild relatives of the domesticated apple tree *Malus domestica* and their current status

Kyrgyzstan is home to wild apple trees grown in different locations; the largest concentration of these wild apple trees can be found in the natural walnut forests and the Sary Chelek *reservation.*

1.1.1 Malus niedzwetzkiana *Dieck*

The nedzvetsky apple tree, a rare and endangered species, is a sight to behold. It is listed in the Red Book of Kyrgyzstan and is primarily found in the Chatkal and Fergana ranges within the walnut-fruit forest of Arslanbob. This beautiful tree grows in specific habitats within the forest, adorned with stunning red blooms, and bears fruits with equally striking red skin and flesh. Only 111 specimens of the tree are known to survive in Kyrgyzstan, so in recent years, several activities have been carried out by Fauna and Flora in Kyrgyzstan to plant trees in their natural habitats and increase their numbers [5].

1.1.2 Malus sieversii

Malus sieversii, also known as Sivers' apple, is a rare and endangered species listed in the Red Book. This apple tree is generally found growing alone or in small groups at an altitude of 900 to 2500 meters above sea level (**Figure 1**). It has a weak growth and rarely exceeds a height of 8 meters. The *Malus sieversii* apple has adapted well to harsh climates and high mountain conditions, thanks to its deep root system that makes it resistant to diseases and low temperatures. It has a long lifespan of up to 150 years and starts bearing fruit at 12 years of age. The tree blooms in April and May with large white and pink flowers. The apples produced by this tree are often as delicious as those of garden varieties. These valuable species found growing naturally in forests have been used as rootstocks for apple trees planted in home gardens and garden plots, resulting in the cultivation of various local varieties of apple trees [6, 7].

Figure 1.
Arrays of wild forms of apple growing in Arslanbob walnut forest, 2022.

It has been established through morphological, molecular, and historical data that the primary source of the apple (*Malus domestica)* genome is *Malus sieversii* [8]. Humans have selectively bred *Malus domestica*, resulting in differential selection for fruit flavor and fruit weight, as observed today. The spread of *M. sieversii* and its descendants throughout history occurred via the Silk Road through Asia, into Europe, Russia and then into the America [9, 10]. DNA analysis conducted in 2010 confirmed that *M. sieversii* is indeed the progenitor of the cultivated apple [11].

1.2 Introduced and local varieties of *Malus domestica* in country and their current status

The preservation of the existing diversity of fruit crops and their wild relatives in Kyrgyzstan is critical at a global level. It provides genetic resources to all user groups now and in the future. Some of the ancient varieties of apple trees were brought to the Kyrgyz Republic from Russia, Kazakhstan, and Uzbekistan by Russian and Ukrainian settlers or wealthy landowners in the 19th and 20th centuries. During the Soviet era, the agriculture and horticulture sectors developed rapidly, resulting in the intensive propagation of many varieties of fruit crops, including apples. However, during this period, various environmental factors such as winters with severe frosts, outbreaks of fungal diseases, and the emergence of new diseases caused sharp changes. Many unstable varieties perished during this period, while those that survived and adapted to local conditions continued to produce high yields. This underscores the importance of local adaptation in ensuring fruitful harvests [12].

In the Issyk-Kul region, there are 31 varieties of apple trees that are grown, out of which 24 are local varieties. Five varieties have become widely spread and they are Aport Alexander, Aport blood-red, Gold Ranet, Kyrgyz Winter, and Rashida [12].

Southern Kyrgyzstan is an ideal region for gardening due to its favorable climate zone. The area is characterized by abundant heat, a long frost-free period, and the absence of early autumn and late spring frosts. This creates great opportunities to create modern and intensive gardens. Local varieties such as *Malus kirghisorum* Al. Et An. Theod, which is closely related to *Malus sieversii,* are widely distributed in the gardens of many villages in the region. Other local summer varieties like Zhumagul and Amanbay ripen in early August, while the Kyrgyz Winter and Rashida varieties ripen at the beginning of September. The late-introduced varieties such as Crimean Winter and Rozmarin ripen in October [13, 14].

1.3 Widespread apple tree diseases in Kyrgyzstan

The apple tree is the most commonly grown species in the gardens of the Republic of Kyrgyzstan. During the USSR era, the area of industrial apple orchards in Kyrgyzstan exceeded 52,000 hectares, with each collective farm and state farm owning orchards ranging from 10 to 20 to 100–150 hectares. Small agricultural enterprises typically own orchards that range between 0.5 and 5–10 hectares. According to the National Statistical Committee's data for 2015, 51,175 hectares of land in Kyrgyzstan were dedicated to fruit and berry crops, with a focus on locally adapted species that ensure fruitful harvests.

At present, apple orchards are facing significant challenges due to pests and diseases. The only available treatment for combating these issues involves the use of chemicals. However, the pesticides being used lack environmental safety certificates, and there is no government oversight regarding the chemicals used by gardeners. To address this issue, gardeners require more knowledge and skills to care for their gardens, including information on modern gardening techniques and biological protection of trees from pests. Unfortunately, there is no available station for the production of biological protective agents.

1.3.1 Scab is a common disease that affects apple trees

Different varieties of apples (*Malus domestica*) are extensively cultivated in the orchards of both southern and northern Kyrgyzstan. This economically significant fruit crop is frequently impacted by scab. A survey conducted in 2015 covering 16,189 hectares of orchards revealed that 4643 hectares of plantations were affected by scab.

It is characterized by the appearance of brown spots on the upper part of the leaves, which then leads to yellowing of the entire leaf and falling off. Scabs can cause significant damage to the crops as the spores can infect the ovaries and ripening fruits. This results in the appearance of dark brown or black spots on the apples, making them lose their presentation and deteriorate in taste. It is crucial to take preventive measures to avoid the spread of scab and ensure a healthy crop. The incidence of tree infection ranges from 5.0 to 55.0%, while fruit infection ranges from 1.0 to 56.0% with intensive disease development. In order to reduce the infection rates, chemical fungicides are often used. For example, 1267 hectares of the garden were treated with chemicals [15]. Some authors suggest that the frequency of fungicide treatments can reach up to 20 per vegetation [16–18].

1.3.2 Moniliasis is a disease that affects fruit trees, particularly apple tree seedlings

The disease can also damage young shoots of mature trees. The initial stage of the disease causes leaves, fruit branches, flowers, and ovaries to turn brown and dry out. Additionally, the ripening crop will be damaged again by mid-summer. The fungus responsible for the disease enters the fruit through a hole made by the codling moth, which is an apple tree pest. Apples quickly rot under the influence of the fungus, and the spores are carried by wind and insects to neighboring fruits.

1.3.3 Bacterial fire blight

Fire blight, caused by the bacterium *Erwinia amylovora*, is an important disease affecting most types of Rosaceae plant and represents an enormous threat to fruit cultivation in many parts of the world. The gram-negative bacterium *Erwinia amylovora*, the causative agent of a dangerous and economically significant apple, pear, and quince disease, was first registered in Kyrgyzstan in 2008. In 2009–2013, this disease was noted in several regions of Kyrgyzstan, highlighting the urgent need for control measures. The Phytosanitary Service of the Kyrgyz Republic registered infected trees on 42.1 hectares out of the surveyed 1235 hectares in the Chui region. In one of the country's northern provinces, in Issyk-Kul, symptoms of this disease in orchards were noted in 2012 and 2013, it spread to the eastern regions of this region. Employees of the plant protection station of the Jalal-Abad city registered the first symptoms of fire blight in the forests and orchards of the country's south in 2008–2009. Then, over the following decades, fire blight intensively spread in orchards in the Chui and Issyk-Kul regions. This disease is characterized by the appearance of necrotic spots on the bark, as well as ulcers and cracks on the branches and trunk. Over time, the tree's wood turns brown and eventually dies. The bacteria that cause this disease can easily be spread by wind, insects, or even contaminated tools, underscoring the importance of implementing control measures.

So, bacterial and fungal diseases pose a threat to apple trees in orchards, nurseries, and fruit farms every year. However, proper monitoring, scientific study and identification of these pathogens are not conducted regularly. Only accurate identification of diseases can help determine appropriate protection and the development and application of protective measures. Therefore, it is essential to pay special attention to wild thickets of apple trees mixed in particular forests and foothills, as they serve as genetic resources of cultivated forms and need timely protection from newly introduced species of diseases and pests. To address these needs, the objective of this study was to identify the economically significant apple tree diseases in the republic and develop biological protection against pathogens.

2. Material and methods

2.1 Sampling for the isolation of the *Venturia inaequalis* pathogen

The collection of samples of diseased organs from different apple tree varieties (*Malus domestica* M.) is a crucial step in our research. These samples were collected to isolate a pure culture of the scab pathogen in orchards and fruit farms in the Chui and Issyk-Kul regions (**Table 1**). The collection process was meticulous, with samples taken from different parts of one tree: leaves, shoots, buds, and fruits. Each sample, consisting of five plant parts with signs of disease, was carefully placed in a separate package with a label indicating the name of the crop, variety, age, place, time of collection, and type of damage.

2.2 Isolation of a pure *Venturia inaequalis* culture

The plant material was washed thoroughly under running tap water, ensuring every part was cleansed. The surface was then sterilized with sodium hypochlorite (0.5%) for 2 min, washed twice in sterile distilled water and crushed. To obtain individual colonies, serial dilutions were prepared from the resulting macerate. Then 30 μl of material was applied to the surface of the agar medium. The following nutrient media were used for the primary isolation of scab pathogens:

1. Potato agar (g/distilled water): potatoes—200 g; Agar—15 g; water—1 L; pH —6.2;

2. Apple agar: chopped apple fruit weight—200 g; Agar—15.0; water—1 L; pH —6.5;

3. Czapek's medium; pH —6.2; 4) Wort agar: wort—50 ml; agar—20 g; water—1.0 L; pH —6.5.

A simple, inexpensive, and time-saving unique technique was used to study the morphological characteristics of *Venturia inaequalis*, as suggested in Refs [19, 20] for

Season for collection of samples	The sites of sample collection	The plants	Plant organs
The end of April and May, 2019- and 2020	Botanical garden named after Gareev, the city of Bishkek	Different varieties of apples	Young buds; leaves with symptoms
The end of May, 2020 and 2021	Orchards of Issyk-Kul province	Different varieties of apples	Young buds; leaves with symptoms
September–October, 2022	Orchards of Issyk-Kul and Chy province	Young nursery stock of apple varieties	Affected shoots and leaves

Table 1.
Sample collection from diseased plants.

other fungi. This technique examined the following morphological traits: pathogen colony growth and color, as well as the size and diameter of hyphal bodies and spores.

2.3 Assessing the antibiotic activity of biocontrol *Streptomyces* against scab disease pathogens

The antibiotic activity of biocontrol microorganisms was determined using the perpendicular streak method developed by N.S. Egorov [21]. In this method, the antagonist culture was seeded in a line on the diameter of the Petri dish, and after 4 to 5 days, the test cultures (*Venturia inaequalis*) were added. Additionally, the agar block method was also employed. Here, the culture of the biocontrol bacterium (*Streptomyce*s) was seeded on the surface of the agar medium and allowed to grow for 4 to 5 days to form a continuous growth lawn in the Petri dish. Then, a sterile cork drill (6–8 mm in diameter) was used to cut agar blocks which were then transferred to the surface of the agar medium inoculated with the test organism (*Venturia inaequalis*). The agar blocks, with the lawn facing up, were placed at equal distances from each other and from the edge of the dish, tightly connected to the agar plate.

2.4 In vivo biotests to assess the antibiotic activity of biocontrol agents against scab pathogens on apple seedlings

In order to find potential biocontrol agents that can inhibit the production of *Venturia inaequalis* spores on infected apple leaves, a series of experiments were conducted on a local variety of apple tree called Aichurok, which is highly prone to scab disease. First, young apple trees were deliberately infected by spraying them generously with a solution containing a large number of *Venturia inaequalis* spores (1×10^5 ml^{-1}) until the spores flowed out from the edge of the leaves. The infected trees were then placed in a humid chamber with a plastic tray covered by a transparent lid. After 2 days of being kept at 15°C with diffused light, the lids were opened, and the trees were kept in the chamber for an additional 5 days at 85% relative humidity, 15°C, and 16 hours of light each day. When disease symptoms appeared on the seedlings, they were sprayed with either a suspension containing *Streptomyces* antagonists (with 1×10^7 spores or cells ml^{-1}) or water with 0.01% Tween 80 as a control. Each treatment was done on two seedlings for replication. The treated seedlings were then grown for 9–12 days at 15°C with 16 hours of light per day at a light intensity of 138 $\mu E\ s^{-1}\ m^{-2}$. From each replicate, the five leaves of each seedling were selected and placed in 100 ml Durand bottles filled with 35 ml tap water mixed with 0.01% Tween 80. The bottles were then shaken on a shaker, and the quantity of *V. inaequalis* spores on the leaves was measured using a hemocytometer [21].

2.5 Sampling for the isolation of the *Erwinia amylovora* pathogen

To identify the cause of fire blight, we collected diseased apple tree organs—flowers, leaves, and fruits with necrotic/ulcerative lesions (see **Figure 2**) —for research (refer to **Table 2**).

2.6 Extraction of bacteria from primary materials by shaking in buffer

To extract bacteria from primary materials, the following steps were followed:

1. Samples were placed in a suitable container, such as a disposable 150 ml plastic cup with a lid or a 200 ml Erlenmeyer flask.

2. 30 ml of phosphate buffer or phosphate-buffered saline was added to the container.

Figure 2.
Diseased plant organs analyzed for isolation of Erwinia amylovora.

Season of samples collection	Site of sample collection	The plants	Plant organs
The end of April and May 2019	Botanical Garden named after Gareev, Bishkek city	Different varieties of apple trees	The young buds; blooms with exudate
June, 2019	Arslonbob Ata forestry, Yshelie Sak Mazar, wild growin	*M. sieversii*	The young buds; leaves
The end of May and June 2019	Karakol city, Issyk-Kul province	Different varieties of apple trees	The young buds; blooms with exudate
June, 2019	Fruit farming of Nookat district, Osh province	Different varieties of apple trees	The affected shoots and leaves
June, 2019	Fruit farming of Karasuu	Different varieties of apple trees	The affected shoots and leaves
May, 2020	Fruit farming of Suzak district, Osh province	Different varieties of apples	The affected immature fruits
June, 2020	Nooken District, Aral village, private orchard	Different varieties of apples	The young buds; leaves
June, 2021	Talas province, private orchards	Different varieties of apples	The affected immature fruits
June, 2022	Issyk-Kul province	Different varieties of apples	The affected immature fruits

Table 2.
Collection the samples from diseased plants.

3. The container was placed on a rotary shaker and incubated at 200 rev/min for 1.0 h.

4. For samples with symptoms, an appropriate amount of macerate was selected for polymerase chain reaction (PCR) analysis.

5. For asymptomatic samples, the suspension was concentrated by centrifugation. 50 ml of macerate was carefully poured directly into the centrifuge tube, leaving the pulp in a container or pre-filtered through filter paper. Then, it was centrifuged for 10 minutes at an acceleration of 8000 g at 10°C. The sample was also frozen at −18°C.

6. The supernatant was discarded, and the pellet was resuspended in 1 ml of phosphate buffer and transferred to a sterile microtube.

7. The extract was used immediately for biochemical and PCR analyses.

2.7 Isolation of *E. amylovora* into pure culture

To isolate the bacterial blight pathogen, plant material showing signs of the disease was first surface sterilized using a 0.5% solution of sodium hypochlorite for 2 minutes. It was then rinsed twice with sterile distilled water and crushed. A serial dilution was made from the crushed material, and 30 μl of the diluted mixture was placed on Levan agar [22]. Colonies that exhibited the described characteristics of *E. amylovora* (white, round, slimy, and curved) were then collected and subcultured at 27°C for 72 hours on King B agar (KB) to test for the presence of fluorescent *Pseudomonas syringae* using UV light at 366 nm [22]. Milky-cream colonies with smooth surfaces and complete outlines that were observed on KV agar were selected for further rounds of selection on Levan agar and King B agar until pure cultures of the pathogen were obtained. This rigorous process was essential for accurate identification. Miller-Schroth agar was also utilized to confirm that the colonies belonged to *E. amylovora* [23]. On this medium, the bacterium forms distinct slimy, orange colonies, allowing for clear differentiation from other plant-associated bacteria.

2.8 Biotests are used to assess the virulence of *E. amylovora* isolates

The virulence of *E. amylovora* isolates was determined using a method [24], with slight modifications, on unripe pear fruits. A suspension of *E. amylovora* containing 10^9 cells ml $^{-1}$ in 0.9% saline was prepared. After surface sterilization, unripe pear fruits were inoculated with 10 μl of the pathogenic bacterium suspension using a 0.2 mm syringe needle. The pear fruits were placed in a humid chamber for 5 days at 25°C. Ten unripe fruits were used for each replication, and the assays were repeated three times. The test results were considered positive when symptoms of plant tissue necrosis developed and milky white exudate was released at the inoculation site.

2.9 DNA extraction and sequencing of CRISPR regions in earlier *E. amylovora* isolate

To isolate genomic DNA from the bacterium, the culture was grown overnight at 27°C in a liquid LB medium using the DNeasy Blood and Tissue kit (Qiagen), following the standard protocol provided by the manufacturer.

Samples were kept on ice for immediate subsequent experiments or stored at 4°C for later use. The quality of the extracted DNA was checked by electrophoresis of 5 μl of the resulting eluate on a 1% agarose gel. PCR amplification of the three CRISPR regions were performed with the Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher) in a total volume of 40 μl using 2 μl of a 1:20 dilution in water of overnight bacterial cultures in LB as a template. Primer pairs C1f01/C1r1, C2f01/C2r01, and C4f01/C4r1 (**Table 3**) [25] were used at a final concentration of 0.4 mM for each primer. The PCR program consisted of initial denaturation for 3 min at 98°C, followed by 35 cycles of denaturation for 10 s at 98°C, annealing for 30 s at 60°C, and extension for 90 s min at 72°C. All PCR products were purified using a MultiScreen PCR plate (Millipore, Molsheim, France) and directly sequenced using an ABI Prism Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) using the primer walking strategy previously described. CRISPR spacers and repeats were identified by analyzing the obtained contigs in CRISPR finder [26] and spacers were manually aligned to previously known genotypes following the nomenclature proposed earlier [25].

2.10 Evaluation of antagonistic activity of biocontrol agents in liquid media

To assess the antagonistic activity of biological control agents against the causative agent of fire blight, the antagonist culture and the pathogen culture were co-cultivated in a liquid medium. For this, the *Erwinia amylovora* culture was incubated in 5 ml test tubes in meat-peptone broth for 48 hours. Then, 1 ml of the antagonist culture was added to each test tube: *Streptomyces bambargiensis* SK-6.6; *Streptomyces fumanus* gn-2; *Streptomyces* Pr-3 and *Streptomyces* C1–4. After incubation at 28°C for 24 hours, the contents of the test tubes were analyzed by microscopy and seeding on nutrient media and the activity of biological control agents was assessed.

Name	Target region	Sequence (5′-3′)	Discriminated genotype
G1-F	Chromosomal pEA71 insert	CCT GCA TAA ATC ACC GCT GAC AGC TCA ATG	*E. amylovora*
G2-R	"	GCT ACC ACT GAT CGC TCG AAT CAA ATC GGC	"
C1f01	CRR1, complete region	TGA GTA GCA AAT CCG TGC GTGCT	CRR1 length
C1r01	"	AAT CAG TCC CCC CAT GCT GTGAC	"
C2f01	CRR2, complete region	TCC CGT CTG ACA TGC AAA CCGC	CRR2 length
C2r01	"	GTA ATG TAG CCA GGC TCA GTCC	"
C1f03	CRR1, Spacer 1023	GAG ACT TAA AGA TCG TCT GCT AGT	A/Z
C1r11	CRR1, Spacer 1002	ATG CCC TCA CCG TTG TGT GTG	"
C2f03	CRR2, Spacer 2020	GAT GGT GGC GCT GGT TGC GCT GGC	a/t
C2r02	CRR2, Spacer 2010	CTG AGT CTG GAA TGT ACA CAC TGG	"

Table 3.
Primers used in this work.

2.11 Biotesting biocontrol agents against *E. amylovora in garden*

A biocontrol agent (*Streptomyces fumanus Gn*-2) was tested against a fire blight pathogen in the garden. The first application of the biocontrol agent was carried out on April 1, 2023. One liter of the biological product was added to 15 liters of water. This volume was applied for spraying three old trees (the length of the trees was 8–9 m). The flowers have yet to open. There are branches with symptoms of last year's disease. Air humidity during spraying is 53%, and the temperature is 13°C at 19:00. The trees were monitored for disease symptoms every 3 days. On April 6, the flowers appear on the branches. Air temperature during the day was 12–14°C; in the evening, it was 7–8°C. No symptoms of the disease were observed in the treated trees, demonstrating the effectiveness of the biocontrol agent. On April 20, 2023, the biological preparation was sprayed for the second time. The first disease symptoms were observed in the control trees but none in the treated trees. The weather during the day was 17–19°C, and in the evening, it was 12–14°C. The third spraying was carried out. Every 3 days, the trees were monitored for disease symptoms. On May 23, the disease began to be observed in the control trees. On the trees treated with the bioproduct, the symptoms were noticeable only at the tips of the branches where the aphids settled. In the second control plot, the effectiveness of a commercial chemical product, Roder 80 WP, in treating fire blight symptoms was tested. Five apple trees with apparent symptoms of fire blight after the application of *Erwinia amylovora* were sprayed with Roder 80 WP until runoff. The trees that were sprayed with water were used as a control in a thorough and comprehensive manner, ensuring that all variables were accounted for and the comparison was valid.

2.12 Statistical analysis

The statistical analyses of the obtained data were performed according to the GLIM program of the Royal Society of London [27]. These analyses revealed significant differences between the two means due to different application methods of biological agents or varieties and their interaction at the crop growth stage. The significance of these differences, calculated by comparing their significant levels at $P < 0.05$, underscores the importance of our findings.

3. Results and discussion

3.1 Ecological, cultural, and morphological characteristics of the isolated scab pathogen (*Venturia inaequalis*)

The study of the pathogen's life cycle is the most critical aspect in plant pathology, as our studies have shown that the infection cycle of *Venturia inaequalis* begins in spring when the appropriate temperature and humidity promote the release of ascospores. Their spores are released into the air can be carried to the ground and to the surface of a susceptible tree, where they germinate and form a germ tube. Moreover, with this germ tube, the pathogenic fungus can directly penetrate the wax cuticle of the plant. To isolate natural isolates of *Venturia inaequalis*, we used the method of settling airborne microflora of the crown of fruit trees on the surface of an agar plate on warm days in April. The cultural and physiological characteristics of the selected natural isolates were studied on

Czapek agar, apple agar, and wort-agar medium and incubated at 18–28°C. Apple agar proved to be a more favorable medium, as rapid growth of the pathogen colonies was observed on the first day of sedimentation. Young colonies were fluffy light brown, with radial growth. As the colony matured, its color changed to milky brown, and its texture also changed; it became more uniform and denser, with a cotton-like texture and white color, while mature colonies became darker and smoother. These observations are significant as they provide a deeper understanding of the pathogen's behavior and characteristics, which can be crucial for developing effective control measures. Microscopic examination of colonies of the fungus formed on apple agar revealed thin, pale-yellow mycelia; conidia were transparent, pale yellow, oval-rounded. Conidiophores were intertwined, forming clusters. This picture shows the asexual reproduction stage of the pathogen. When sampling during the growing season, this picture was repeatedly revealed, which means that the generation of conidia continues throughout the summer until the leaves of the trees and fruits fall in early winter. This cycle is called secondary infection (**Figure 3(a)**). When grown on Czapek medium, *Venturia inaequalis* isolates began to proliferate on the second day after inoculation. Young colonies were dense, had a cotton-like texture, were white at the beginning of development, and as the colonies matured, they became darker and smoother. Microscopy of such colonies revealed longitudinally extending colorless conidiophores; conidia were dark in color and oblong-round in shape (**Figure 3(b)**). On wort agar, *Venturia inaequalis* isolates formed soft, fluffy, thick white colonies. However, compared to growth on Czapek and apple agar media, the pathogen showed weak growth on wort medium, although growth did not cease, forming multicellular, thin and colorless mycelium (**Figure 3(c)**).

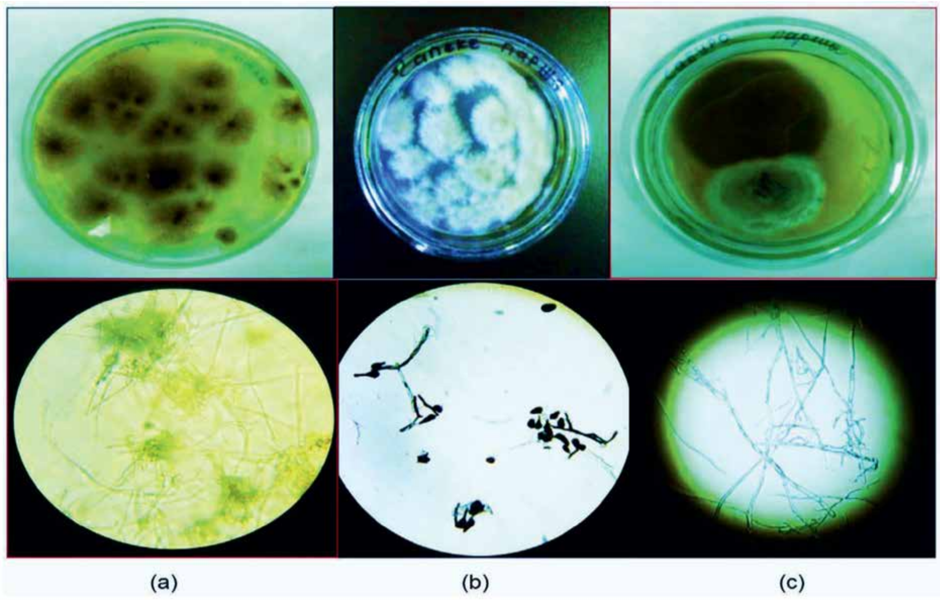

Figure 3.
Cultural and morphological characteristics of Venturia inaequalis, *initially obtained from diseased apple fruits: (a) on apple agar after 24 hours of incubation; (b) on Czapek medium after 48 hours of incubation; (c) on wort medium after 72 hours of incubation.*

3.1.1 Biochemical characteristics of Venturia inaequalis isolates

The biochemical characteristics of *Venturia inaequalis* isolates were studied using special tests to observe the fermentation of carbon substrates such as dispatch-rides, glucose, cellulose, mannitol, and sorbitol. The results indicated that natural isolates of *Venturia inaequalis* have the ability to ferment glucose and cellulose, which turns the liquid medium (with indicator) red, while mannitol and sorbitol turn it yellow.

3.2 Evaluation of the antagonistic activity of *Streptomyces* against *Venturia inaequalis in vitro*

The biotests, a significant step in our research, were conducted using soil actinomycetes against *Venturia inaequalis.* This was done with the aim of developing environmentally friendly local control measures against the scab pathogen of fruit trees. The following actinomycetes strains were used as antagonists: *Streptomyces alfalfae* CI-4, *Streptomyces lividans TR-59*, and *Streptomyces fumanus gn-2.* Antagonistic activity was assessed by measuring the size of the lysis or inhibition zones of the test object in mm (**Figure 4**). The tested *Streptomyces* strains showed varying degrees of antagonistic activity against *Venturia inaequalis.* Notably, the *Streptomyces alfalfae* C1–4 strain demonstrated a solid antifungal effect, with a lysis zone of 10.0 mm after 96 hours (**Table 4**). This high level of effectiveness of the *Streptomyces alfalfae* C1–4 strain underscores its potential as a biocontrol agent in the fight against the scab pathogen of fruit trees, reinforcing the main goal of our research.

After conducting bioassays on a solid medium, two strains of *Streptomyces alfalfa sp*. C1–4 and *Streptomyces fumanus gn-*2 were selected for further tests in a liquid medium due to their potent antagonistic and hyperparasitic properties.

The antagonists were carefully cultivated with *Venturia inaequalis* in a liquid culture for 48 hours. Microscopic analysis of the liquid culture revealed the destruction of plant pathogenic conidia and conidiophores, along with the presence of the branched mycelium and spores of antagonists-Streptomyces. The hyper-parasitic mechanisms were accurately observed in these micro-images (**Figure 5**), demonstrating the precision and reliability of our research methods.

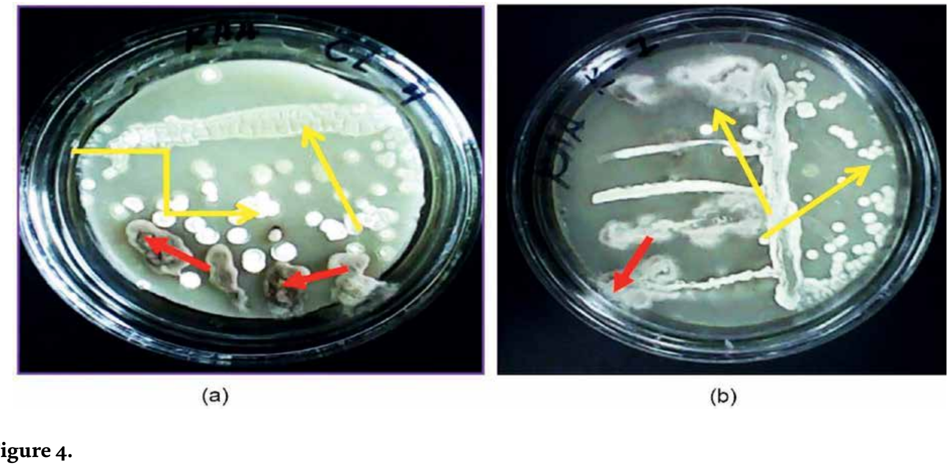

Figure 4.
(a) Inhibition of Venturia inaequalis colony growth under the antagonistic activity of Streptomyces alfalfae *sp. C1–4; (b) Hyperparasitism by* Streptomyces fumanus*, gn-2 toward Venturia inaequalis. Yellow arrows indicate Streptomyces sp. colonies, red arrows indicate Venturia inaequalis colonies.*

The antagonistic strain	Activity, lysis zone, mm			
	24 h	48 h	72 h	96 h
*Streptomyces alfalfae C*1–4	0.3 ± 0.01	0.5 ± 0.02	0.8 ± 0.02	10.0 ± 0.02
Streptomyces fumanus, gn–2	0.1 ± 0.01	0.1 ± 0.01	0.3 ± 0.01	Hyper-parasitism
Streptomyces lividans TR–59	0.1 ± 0.01	3 ± 0.01	The force between two cultures is equal	

Table 4.
Streptomyces *strains' efficiency against the pathogenic fungus* Venturia inaequalis.

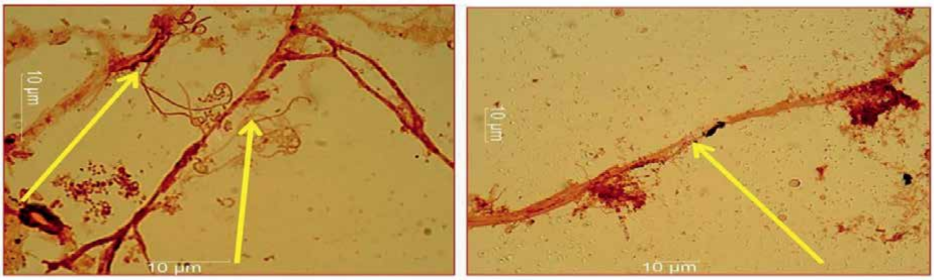

Figure 5.
Lysis of hyphal bodies and mycelium of the pathogen Venturia inaequalis *occurs during co-cultivation with* Streptomyces alfalfae *C1–4 and* Streptomyces fumanus, *gn-2.*

3.3 Evaluation of biological activity of antagonistic bacteria on apple seedlings

The initial signs of scab disease included the appearance of oily spots on the young leaves of vulnerable apple seedlings of the local "Aichurok" variety, which emerged 5 days after deliberate infection. Seven days later, necrotic lesions and leaf death were observed on the edges of the leaves. Once the disease symptoms became apparent, the seedling leaves were treated with a solution containing biocontrol agents. Ten days after the initial treatment, five leaves from each apple seedling were examined under a microscope and then placed on suitable growth media. The number of pathogen colonies on one leaf was counted to calculate the average number of colonies. As illustrated in **Figure 6**, the production of V. inaequalis conidia on apple leaves decreased by 50% 5 days after treatment with *Streptomyces* sp. C1–4. After 10 days, the decrease reached 60% compared to the control trees. Repeat treatment was administered after 15 days. Following the second treatment, at 25 and 35 days, the number of conidia of the pathogen decreased to 35%.

The potential of *Streptomyces sp. C1–4* strains in significantly reducing *V. inaequalis* on leaves has been demonstrated in consistent results from subsequent independent experiments. This promising finding opens up possibilities for future applications. To determine the survival time of antagonist spores *(Streptomyces sp. C1–4*) on the surface of seedling leaves after treatment, samples were taken by tearing off treated leaves from seedlings and transferred to the surface of a starch-ammonia medium from the outside and inside. The appearance of intensive and abundant growth of antagonist colonies on the media around planted leaves indicates their survival in the epiphytic microflora of the apple tree. We believe that the longer they remain on the surface of diseased leaves, the greater the likelihood they will be able to suppress the development of the pathogen. After two applications, *Streptomyces sp. C1–4* spores showed viability on the surface of seedling leaves from 10 to 25 days. In the case of

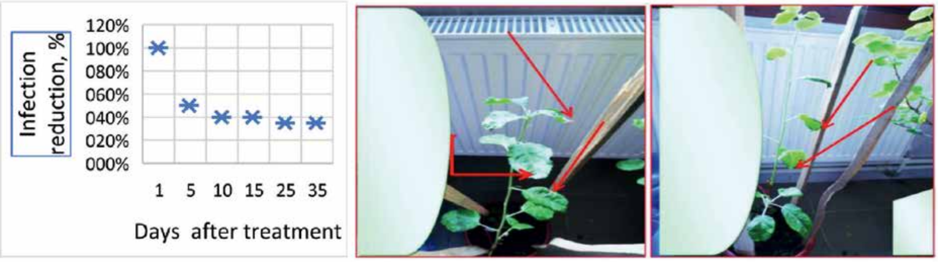

Figure 6.
Scab infection reduction after two treatments by Streptomyces sp. *C1–4 strain.*

using a biopreparation based on *Streptomyces sp. C1–4,* after the second treatment, spores of *Streptomyces sp.* C1–4 remained on the surface of the seedling leaves for 10 to 25 days. During this period, the spores of the antagonist grew slowly, but this contributed to the dense growth of actinomycetes around the leaves planted on the medium (**Figure 7**). In addition, as is known, *Streptomyces sp.* cells also produce the enzyme chitinase and several antibiotic substances that destroy the structure of the pathogen mycelium [28, 29], such an ability to ensure effectiveness against scab in our case.

3.4 Isolation of pure culture of *Erwinia amylovora*

The single colonies with *E. amylovora* characteristics were tested on different media separately for confirmation (**Figure 8**). All isolated and suspected *Erwinia amylovora isolates* gave characteristic colonies on the Levan and Miller-Schroth mediums.

3.5 Analysis and PCR genotyping of *E. amylovora* CRISPR regions

The genetic variability of *E. amylovora* isolates collected from different host plants and geographic locations in 2020–2021 was thoroughly assessed. To do this, we amplified potentially variable parts of their CRR using specially designed primers (see **Table 3**). As a result, we positively amplified 15 different *E. amylovora* isolates collected in 2020–2021 from various regions of Kyrgyzstan. When amplifying with primers C1f03 and C1r11, the following isolates showed PCR results compatible with

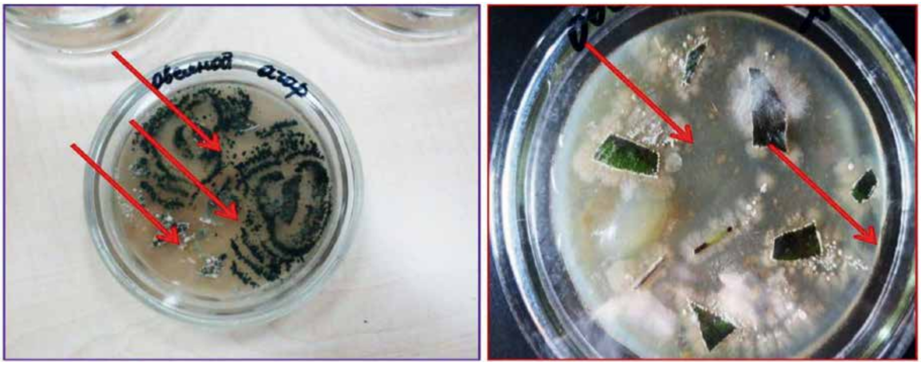

Figure 7.
The leaf pieces taken from seedlings are shown by red arrows, around which the overgrown by colonies Trichoderma virida *on the medium (the left) and by colonies* Streptomyces *sp. C1–4 on the medium (the right).*

Figure 8.
Colonies of E. amylovora *on different solid growth media. On Levan medium (A)* E. amylovora *formed milky or light yellow, circular, domed, smooth, mucoid colonies after 48 h of incubation at 28°C. Under the same growing conditions on KB agar (B) the colonies had a typical white mucoid appearance and were non-fluorescent under UV light at 366 nm. After five days on Miller-Schroth agar (C), the colonies were reddish-orange and approximately 1 mm in diameter and had a round, smooth, and domed appearance with entire margins.*

genotype A: Tal-13, Tal-42, Tal-43, Tal-46, Tal-63 from the Talas region; IS-4, IS-9, IS-19 from areas around Lake Issyk-Kul; and one isolate (Chui-3) from the Chui region (see **Figure 9**).

When by amplifying with primers C1f4/C1r9 in Talas region genotype D was found (**Figure 10**). The monitoring campaign in summer 2021 first time identified D genotyped *E. amylovora* isolates in the Bakai -Ata district of Talas region (N42^{0}30’7; E72°3′23″; 1142 m, Ak Dobo (N42°32′3″ E71°57′7″ 1085m) and Tegirmen Sai (N42^{0}31′12″E71^{0}49′’52″).

In the Talas region, genotypes A, t, and α were found in the studied sites, unlike in other areas. This suggests that there is a distinct geographic distribution of two genotypes, with a predominance of (A, t, α) and (A, a, α) in the northern and southern parts of the country (**Figure 11**).

The maps were made to indicate a location of fire blight distributed sites in regions of country (**Figure 12**).

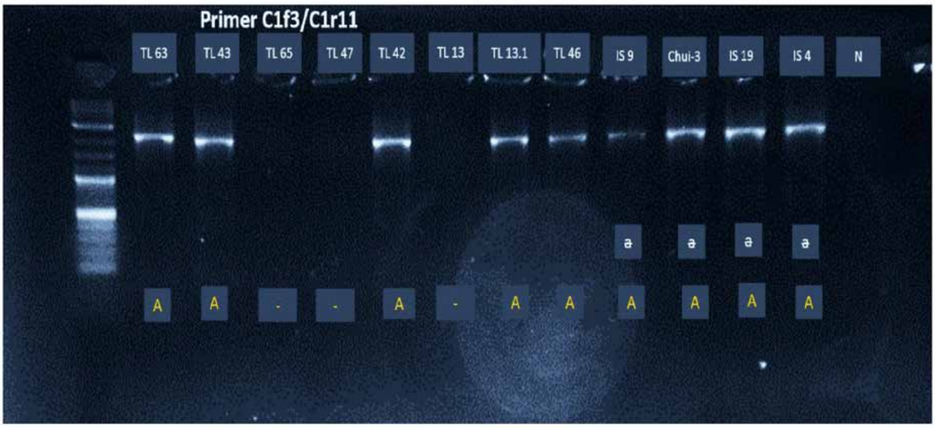

Figure 9.
*Electrophoretic profiles of new E.*amylovora *isolates, obtained in Talas, Ussyk-Kul, and Chui regions in 2020 by amplifying with primers C1f3/C1r11.*

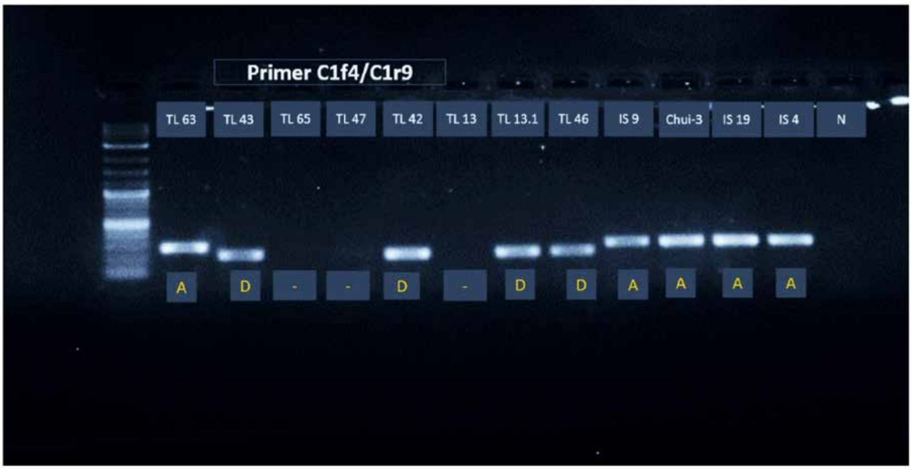

Figure 10.
Electrophoretic profiles of new E. amylovora *isolates, obtained in Talas, Ussyk-Kul and Chui regions in 2022 by amplifying with primers C1f4/C1r9.*

3.6 Virulence tests of *E. amylovora* isolates

The pathogenicity and virulence of the pathogen *E. amylovora* are complex and multifaceted, and continued research could pave the way for significant advancements in the field. The production of the siderophore desferrioxamine, which aids in the acquisition of iron molecules from the host tissue [30, 31], is a significant aspect. Equally important are other virulence factors, such as metalloproteases [32], which play a crucial role in pathogenesis. However, the difference in virulence among different strains of *E. amylovora* is attributed to the production of exopolysaccharides and the function of the type III secretion system (T3SS) along with its associated proteins. Droplets of bacterial exudate first appeared on pear

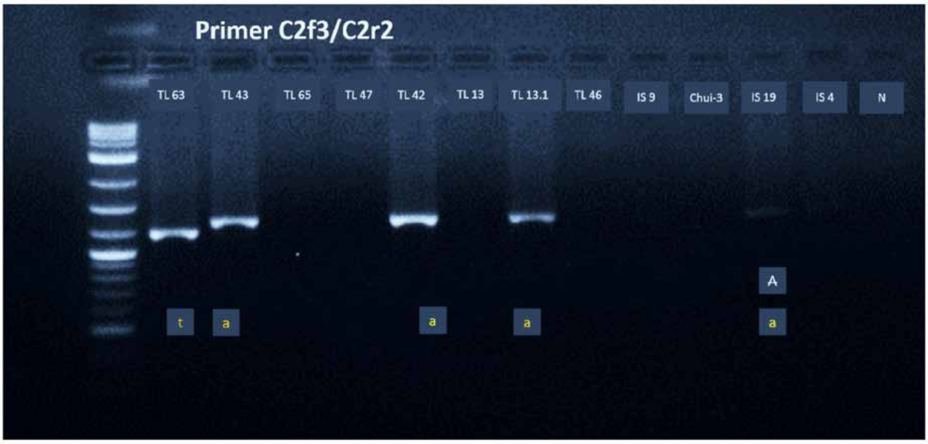

Figure 11.
Electrophoretic profiles of new E. amylovora *isolates, obtained in Talas, Ussyk-Kul and Chui regions in 2020 by amplifying with primers C2f3/C2r2.*

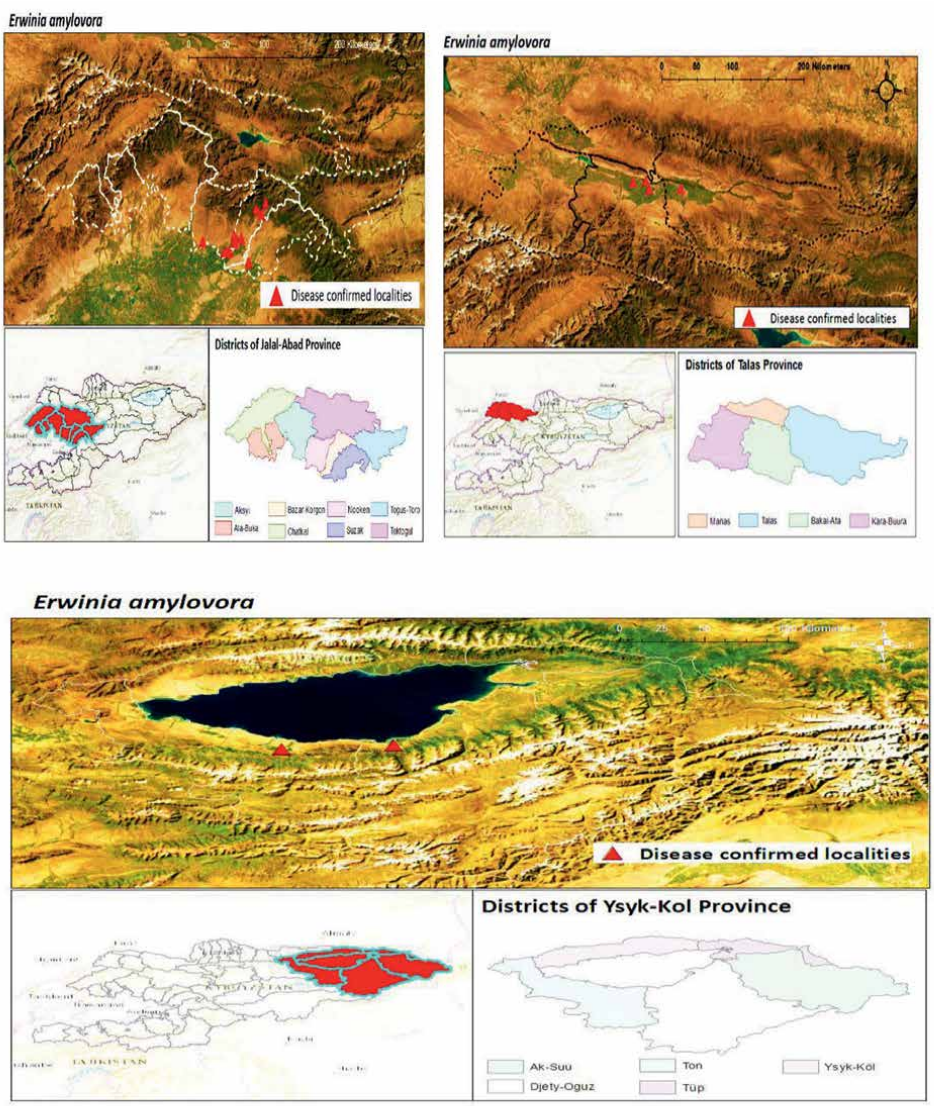

Figure 12.
The maps indicating disease confirmed localities in Jalal -Abad, Talas and Issyk-Kul regions of Kyrgyzstan.

slices 4 to 5 days after they were inoculated with *E. amylovora*. After 7 days, we noticed that inoculation with *E. amylovora culture* isolated from host plant domestic varieties, slice surfaces were completely covered with large amounts of exudate (**Figure 13**).

The bacteria *E. amylovora*, when hosted by apple, pear, and quince, not only thrived but also displayed an intense and aggressive reaction on unripe pear pieces. This thorough research has provided a comprehensive understanding of *E. amylovora's* behavior. However, on a nutrient medium, they either rapidly mutated or perished. All strains from the Talas region, positively identified as *E. amylovora*, were confirmed to be pathogenic in the immature pear fruit assay, producing a visible bacterial ooze 48 h after injection (**Figure 14**).

Figure 13.
Virulence assays on pear slices. Symptoms caused by Erwinia amylovora *isolated from wild and domestic type of host plants.*

3.7 Primary screening of natural *Streptomyces* isolates for antimicrobial activity on sold medium (SCN)

The isolates *Streptomyces alfalfae* C1–4, *Streptomyces fumanus gn*-2, and *Streptomyces* lividans TR-59 have exhibited the highest activity, with their zone of inhibition against the growth of *E. amylvora* colonies ranging from 17.8 to 19.8 ± 0.32 mm (**Figure 15**).

3.7.1 Secondary screening of natural actinomyces isolates for antimicrobial activity in the liquid medium

During the second stage of screening in a liquid medium, both the bioagent and the pathogen were cocultured for 72 hours. Afterward, the contents of each incubated tube were examined under a microscope, followed by inoculation on MPA and SCN medium. A positive and highly active result was determined when the antagonist microorganism completely suppressed the growth of pathogen cells, leaving only the mycelium and the absence of pathogen cells in the incubating tubes—complete inhibition. This comprehensive process ensures the reliability of our results.

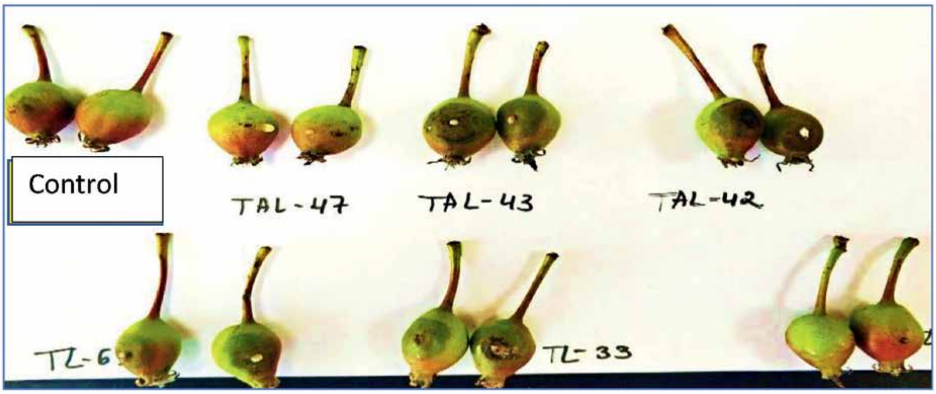

Figure 14.
A visible bacterial ooze in the immature pear fruits 48 h after injection by E.amylovora.

Figure 15.
The photos illustrate the hyperparasitic effect of Streptomyces *isolates, where its mycelium covers and grows on the surface of pathogenic bacteria colonies.*

Additionally, when the contents were inoculated onto nutrient agar media, which is a crucial environment for the growth of microorganisms, the presence of pathogen colonies or biocontrol colonies growth also confirmed the activity of the biocontrol agent. In a liquid medium, *Streptomyces alfalfa* C1–4, *Streptomyces fumanus*, *gn-2* have shown complete inhibition of the *E.amylovora* cells growth during co-cultivation. *Streptomyces lividans TR-59 have* shown moderate activity. **Figure 16** shows microscopic pictures with the solid and weak effects of *Streptomyces* isolates against E. *amylovora* 48 hours after co-cultivation.

3.8 Biotesting biocontrol agents against *E. amylovora* in garden (spring, 2023)

The trees were monitored for disease symptoms every 3 days. On May 23, the disease began to be observed in the control trees. However, on the trees treated with the bioproduct, the symptoms were noticeable only at the tips of the branches where the aphids settled. It is important to note that the disease was not widespread in the orchards (**Figure 17**).

The results of counting tree branches in the control and experimental plots are summarized in **Table 5**.

The number of affected branches in the control group ranged from 15.0 to 22.0 ± 0.91%. In the group of trees treated with a chemical preparation, from 8.0 to 25.0 ± 0.91%. In the group of trees treated with a biological preparation, the damage ranged from 5.0 to 12.0 ± 0.91% (**Figure 18**).

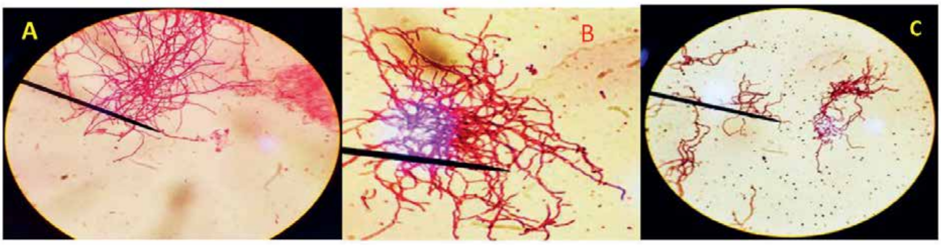

Figure 16.
A- active growth of Streptomyces alfalfa *C1–4 mycelium in the tubes with* E.amylovora *in 48 h co-incubation; B- active growth of* Streptomyces fumanus, gn-2 *mycelium in the tubes with* E.amylovora *in 48 h co-incubation; C- moderate growth of* Streptomyces lividans TR-59 *mycelium in the tubes with* E.amylovora *in 48 h co-incubation.*

Observation for control and experimental trees

Symptoms are start develop in control trees

Symptoms are start develop in experimental trees, at the tips of the branches where the aphids settled.

Figure 17.
The symptoms of fire blight on control trees (left) and on experimental trees (right).

Experiment options	Number of trees	Total number of branches	Number of affected branches
Control, without any treatment	1,2,3	162	32
	2	136	24
	3	210	41
Trees treated with chemicals	1	114	9
	2	200	16
	3	213	55
Trees treated with biological product	1	122	9
	2	209	6
	3	191	7

Table 5.
The number of counted branches affected by the symptoms of fire blight in the control and experimental groups.

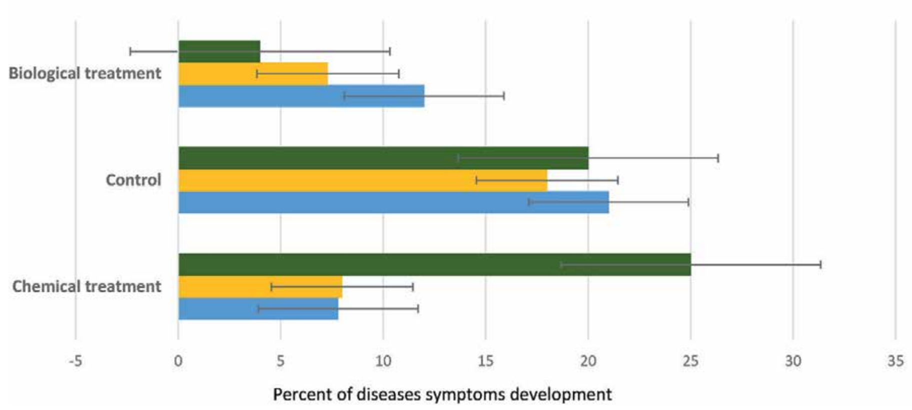

Figure 18.
Percent of fire blight symptoms development in experimental and control plots.

4. Conclusion

Kyrgyzstan is home to wild apple trees and is an excellent region for growing different varieties of this vital fruit tree. In the southern and northern parts of the countries, early, autumn, and late varieties of apple trees adapted to climatic conditions are grown and bred as local varieties and introduced from different countries. Due to the intensive development of fruit nurseries and the export of their products to other countries, public farmers are importing new varieties of apple trees from Europe in order to obtain high yields. Trade in plant material in the form of seedlings facilitates the spread of dangerous diseases and pests from one continent to another and between domesticated and wild forms of the apple tree.

Therefore, modern disease diagnosis is of paramount importance. Without it, we risk losing the biodiversity that is crucial for the economy and genetic resources of fruit plants, particularly apple trees. This underlines the urgent need for developing and implementing protective measures.

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Chapter 4

The Contribution of *Malus sieversii* to the Emergence and Diversity of Domesticated Apple Varieties

Aisha Taskuzhina, Alexandr Pozharskiy and Dilyara Gritsenko

Abstract

The modern apple is a result of hybridization between wild apples and various landraces, with the genetic composition of *Malus domestica* being primarily shaped by the hybridization of wild species *M. sieversii*, *M. baccata*, *M. orientalis*, and *M. sylvestris*. The genetic purity of wild apple populations before and after domestication is a concern. *Malus sieversii* is the primary progenitor of modern apples, with 46% of the *M. domestica* genome originating from this species. Despite facing harsh environmental conditions, the species has continuously adapted, developing genetic resistance to both abiotic and biotic factors. This resilience makes it a valuable source for breeding purposes. The population analysis of *M. sieversii* in Kazakhstan indicates substantial genetic variety; yet there is a notable prevalence of gene flow from cultivated to wild apple populations. This hybridization process is likely intensifying the extinction risk faced by wild progenitors of apples, posing a threat to biodiversity preservation and hindering efforts to improve apple varieties, including enhancing resistance to abiotic stress and optimizing production capabilities.

Keywords: wild apple, *Malus sieversii*, genetic diversity, conservation, apple cultivars

1. Introduction

The wild apple species *Malus sieversii,* which is naturally occurring in Central Asia, is widely recognized as the original ancestor of the domesticated apple *Malus domestica* [1]. *Malus sieversii* is distributed in the mountainous regions of Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan, and China, where it thrives under a wide range of ecological conditions. The genetic diversity of *Malus sieversii* has played a significant role in the development of the several apple cultivars prevalent today. The genetic diversity of this species has served a vital role in contributing features, such as resistance to diseases, large fruit size, desirable flavor, and ability to withstand environmental pressures. These traits are essential for the creation and long-term viability of modern apple cultivars [2].

The Sievers apple tree was named after the scientist and pharmacist, member of the Imperial Academy of Sciences and the Free Economic Society in St. Petersburg, Johann Sievers, who first discovered and described these wild apple trees in the

Urdzhar River valley during his expedition to Altai, Siberia and Central Asia in 1792–1794. In his letters, which were posthumously published by P. S. Pallas in 1796, he wrote: “When I arrived at the foot of the mountain, the goddess Flora delighted me with a forest of the most beautiful dwarf apple trees, which grow wild here at Urdzhar on both banks. I forgot my fatigue, the heat, the rockfall, and everything else and entered the apple trees like a wood sprite and began to feast. Forgive me for this exuberance; you know, I was born in the land of apples. During my 4 years in Siberia, I have not tasted other fruits, except for the wild pears growing beyond Lake Baikal, which are eaten there as a dessert with powdered sugar instead of candies. But those I found now were a good tart fruit, though here, in their wild state, they have shrunk to the size of a bee’s egg and have red and yellow cheeks. In Kyrgyz, they are called ‘alma’. It seems one could present this variety as a new species. The three Siberian peasants who were with me were even more amazed. They had heard from their fathers, who came from Little Russia and Poland, frequent mentions of the abundance of apples there, but they had never seen any themselves” [3]. Baltic German botanist Carl Friedrich von Ledebour conducted an expedition to the Altai and Eastern Kazakhstan (Tarbagatai) and in his book “Flora Altaica” provided a detailed description of *Malus sieversii*, naming it in honor of Johann Sievers [4]. Russian and Soviet agronomist, botanist, and geneticist, who identified the centers of origin of cultivated plants Nikolai Vavilov during his expedition to Kazakhstan noted that the wild apple trees of Central Asia are characterized by relatively large fruits, and some trees bear fruits that are comparable in quality to cultivated forms. This led him to suggest that Central Asian region is the center of origin for apple cultivation [5]. Academician Aimak Dzhangaliev, the follower of Nikolay Vavilov’s research, provided further evidence and expanded on the theory that Central Asia is the primary location where the apple tree originated. He discovered distinct populations of *Malus sieversii* in Kazakhstan, particularly in the mountainous regions of the Dzungarian Alatau, as well as in the Tian Shan. Dzhangaliev conducted research on the genetic variability and resistance of wild apple trees to different diseases, resulting in a substantial contribution to apple breeding programs and the preservation of apple genetic resources. His research significantly advanced the comprehension of the significance of conserving the wild progenitors of cultivated plants to guarantee food security and develop sustainable agriculture in the future [6, 7].

In 1989, 1993, 1995, and 1996, the U.S. Department of Agriculture (USDA)-Agricultural Research Service (ARS)-National Plant Germplasm System (NPGS) organized four expeditions to Central Asian countries. The expedition was mainly conducted in Kazakhstan in collaboration with Aimak Dzhangaliev. A total of 892 *Malus sieversii* samples were collected over four expeditions from 12 regions in Central Asia. Specifically, samples were collected from nine regions in Kazakhstan and one region each in Kyrgyzstan, Uzbekistan, and Tajikistan. The extensive collections have contributed to a deeper understanding of the genetic variation and evolutionary history of *Malus sieversii*. Seedlings have undergone studies in different countries, such as in the United States, Canada, South Africa, New Zealand, Norway, and Germany [8, 9]. The accessions have played a pivotal role in numerous research, encompassing genetic mapping, trait analysis, and breeding programs, with the objective of enhancing disease resistance and environmental adaptability in cultivated apple varieties. The research undertaken using these collections has emphasized the importance of *Malus sieversii* as a crucial genetic resource for apple production worldwide. Advanced scientific methods, including genome sequencing and molecular markers, have allowed us to gain a more profound understanding of the genetic composition and variety of this species. Furthermore, these investigations emphasize the necessity of ongoing conservation efforts to protect these invaluable genetic resources from concerns, such as habitat degradation and climate variability.

This chapter will examine the important role of *Malus sieversii* in the development and variety of domesticated apple species. We will analyze the genetic knowledge acquired from these collections, its influence on modern apple breeding, and the continuous efforts to conserve this crucial species. By comprehending the significance of *Malus sieversii*, we may enhance our grasp of the genetic basis of cultivated apples and the necessity of preserving its wild relatives.

2. Genetic and ecological significance

2.1 Natural habitat and ecological adaptations

Malus sieversii thrives in a wide range of ecological conditions, from the Tian Shan high regions with their prolonged and severe winters to the more temperate foothills and lowlands. They have the ability to withstand a broad variety of temperatures, making them capable of enduring cold winters and hot summers. *Malus sieversii* is distributed in many habitats, such as forests, mountain slopes, and river valleys, characterized by a wide range of soil conditions, ranging from fertile with high organic content to stony and nutrient poor. This apple tree is able to adapt to different levels of water availability, allowing it to efficiently use water from snowmelt and seasonal rainfall in mountainous areas, as well as survive in drier foothills and lowlands. They also serve as crucial food sources for wildlife and provide habitat and shelter for various organisms. By enabling its ability to overcome various environmental conditions, this species is highly important for conservation and breeding efforts that aim to enhance the genetic diversity of cultivated apples [6, 7].

Malus sieversii grows in the mountain systems of the Tian Shan, Dzhungarian Alatau, Altai, and Pamir-Alai in the territory of Central Asia and China at altitudes ranging from 500 to 1900 meters. This wide range of altitudes demonstrates its adaptability to different atmospheric pressures and oxygen levels, which vary significantly with altitude. In China, it is found in the Xinjiang Uygur Autonomous Region in the western Tian Shan Mountains and in Altai (the southern region of the Tarbagatai) [10]. In Kazakhstan, wild apple is distributed mainly in the Zhongar Alatau and the western regions (Karatau and Talas Alatau) and northern regions (Ile-Alatau and Kyrgyz Alatau) of Tian Shan, with a small presence in the Altai (Tarbagatai) mountain systems. In Kyrgyzstan, *Malus sieversii* is primarily distributed in the northern, western, and central parts of the Tian Shan Mountains, specifically within the Fergana Range and the Alai Range, as well as the foothills of the Kyrgyz Alatau. In Tajikistan, the species is found in the Pamir-Alai Mountain system, particularly in the Zarafshan and Hissar Ranges, and in areas around the Fergana Valley. In Uzbekistan, *Malus sieversii* is concentrated in the western part of Tian Shan Mountains, particularly in the Chatkal Range, the Ugam Range, and the western slopes of the Kuramin Range (**Figure 1**) [11].

The wide spread and adaptability of *Malus sieversii* emphasize its importance in both natural ecosystems and agricultural research. Developing an understanding of these particular adaptations of apple cultivars can offer valuable insights into the development of resilient apple varieties that can thrive in a wide range of environmental conditions.

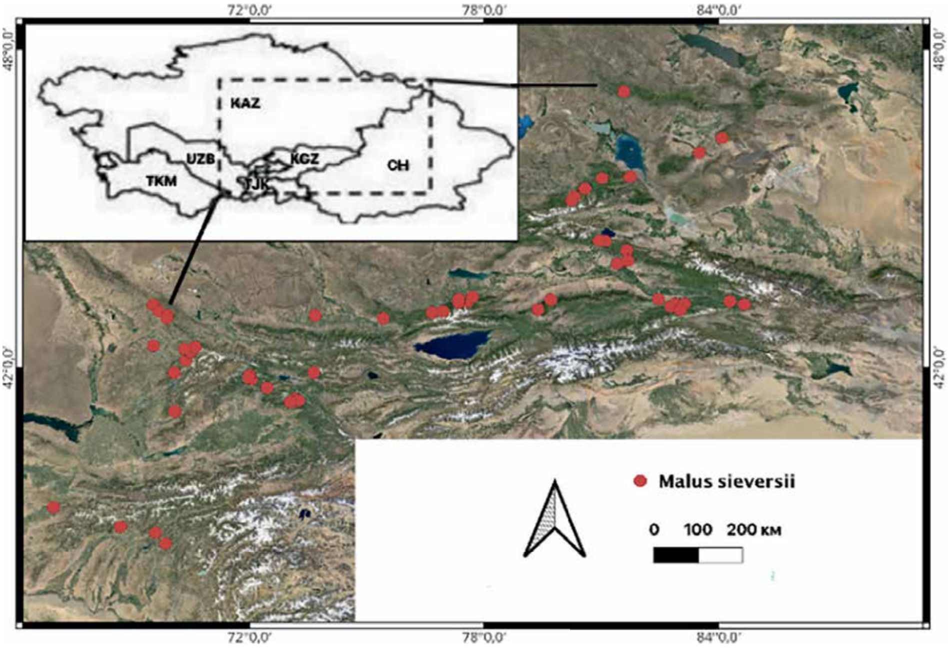

Figure 1.
Distribution of Malus Sieversii *in Central Asia and China.*

2.2 Genetic diversity and contributions to modern apples

In 2010, scientists sequenced the complete apple genome to gain a better understanding of the genesis and evolution of apples. Comparative investigations indicated that *M. domestica* cultivars exhibited a closer genetic relationship with the wild species *M. sieversii*, rather than *Malus sylvestris and Malus orientalis* [1]. The domestication of apples (*Malus domestica*) began 4000 and 10,000 years ago with the selection of *Malus sieversii* from Central Asia, for desirable traits. This process was further enhanced by hybridization with other wild species like *Malus sylvestris*, *Malus orientalis*, and *Malus baccata* introducing new genetic diversity [12–14]. The development of grafting techniques allowed for the propagation of elite cultivars, facilitating the spread of desirable apple varieties. As apple cultivation spread across different regions, local preferences and environmental conditions led to a diverse array of apple cultivars, each adapted to specific climates and tastes. The Silk Road was instrumental in this process, acting as an extensive network of trade routes that connected the East and West, facilitating the exchange of goods, culture, and agricultural practices, thereby enhancing the genetic diversity and adaptability of cultivated apples.

Malus sieversii exhibits an extensive range of morphological characteristics, particularly in its fruits and pollen grains, and possesses significant genetic and phenotypic variation. Morphologically, it closely resembles the cultivated apple tree *(Malus domestica).* The height of the wild apple tree, *Malus sieversii*, varies between 2 and 12 meters, with a few specimens growing reaching as high as 14 meters, with a gray-brown or dark gray trunk and a broad crown. The branches are thick, sometimes with thorns (on young shoots). The one-year-old shoots are short, greenish-brown or brown, pubescent, while the two-year-old shoots are dark gray or gray. The leaves are large, measuring 6–11 cm in length and 3–5.5 cm in width, broadly lanceolate or oblong-elliptical, with a wedge-shaped base, ranging from serrate-dentate to serrate. They are dense, usually glabrous on the upper side, and more or less felted hairy on the underside. The petioles are rather thick and felted hairy. The tree begins to bear fruit at around 12 years of age. The seeds of *Malus sieversii* are wedge-shaped or oval, appearing grayish or brownish-brown when dry; dull and smooth, with a length of 5–6 mm and a width of 3–4 mm. The inflorescence consists of 3–5 flowers. The flowers are large, with a diameter of 3.5–6 cm, and are pale pink or white. The hypanthium is felted pubescent. The fruits of this apple species vary in shape, being distinctly round, flattened-round, rounded conical, elongated round shape, and greater in size compared to other wild apple species. On average, their diameter ranges from 3 to 4 cm, occasionally reaching up to 7 cm. The skin of the fruit can be colored in various shades, including green, yellow, and red, depending on the degree of ripeness and growing conditions. The pulp of the fruit is juicy, with a characteristic sweet and sour, sometimes bitter-sweet taste. The flowering period typically occurs between April and May, while the fruit ripening from August to September. The average age of apple trees is 50 years. Under optimal circumstances, its lifespan can reach a maximum of 150 years [15–17].

In addition to its diverse morphological traits, *Malus sieversii* demonstrates remarkable resistance to various environmental stresses. It exhibits high tolerance to cold, drought, and diseases. Its cold resistance allows it to thrive in harsh climates, while its drought resistance enables survival in arid conditions. Furthermore, *Malus sieversii* has shown resistance to several common apple diseases, including apple scab, fire blight, and powdery mildew. Consequently, *Malus sieversii* has significantly contributed to the genetic makeup and phenotypic traits of *Malus domestica*, particularly in terms of fruit quality, pathogen resistance, and genetic diversity. These contributions have played a crucial role in the domestication and improvement of cultivated apples.

Genetic diversity studies of *Malus sieversii* have been essential for grasping its potential for apple breeding and conservation. The wild apple specimens collected by the USDA-ARS Division of Plant Genetic Resources in Geneva, New York, USA, during expeditions to Central Asia, have been the focus of extensive research on genetic diversity. Researchers have utilized different molecular markers, including SSR (simple sequence repeat) and SNP (single nucleotide polymorphism) markers, to examine the genetic diversity within and among populations of *Malus sieversii*. These studies have uncovered a significant degree of genetic variability, which is crucial for the species' ability to adapt and withstand changes in the environment. Lamboy et al.'s study on the allozyme diversity revealed that *Malus sieversii* has high genetic diversity, surpassing other Malus species, and despite some geographic differentiation, there is significant genetic mixing [18]. Geibel et al. noted significant disease resistance in wild apple trees, with some accessions showing complete absence of apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*). Pre-selected resistant individuals did not show the presence of the scab resistance-associated Vf gene in a study using molecular markers, such as Sequence Characterized Amplified Region (SCAR) and random amplified polymorphic DNA (RAPD), suggesting the potential existence of new resistance genes in these populations [19]. In the study conducted by Forsline et al., seedlings from Kazakhstan (Karatau and Tarbagatai regions) exhibited variations in their resistance to apple scab, rates of natural infection by fire blight in Tarbagatai region, and varied fruit characteristics and highlighted their importance as a valuable resource for apple breeding programs that aim to enhance disease resistance and horticultural traits [20].

The study using microsatellite markers showed high genetic diversity and significant differentiation among populations of *Malus sieversii* in Kazakhstan. Genetic differentiation corresponded with geographical locations, with more admixture observed in the southwestern populations [21]. In the same year, the authors successfully identified a subset of 35 *Malus sieversii* individuals that complements existing core subsets by representing all major genetic lineages and capturing significant genetic and phenotypic diversity, thus aiding in the long-term conservation and utilization of these genetic resources for breeding and research purposes [22]. The research by Volk et al. revealed that the *M. sieversii* collection in the NPGS exhibits diverse genotypic and phenotypic traits, including highly overlapping gene pools within related taxa *Malus pumila*, *Malus pumila* var. *niedzwetzkyana*, *M. sieversii var. kirghisorum*, and *M. sieversii var. turkmenorum* [23]. Recent studies highlighted the prevalent crop-to-wild gene flow of apples in Kazakhstan, posing an accelerated risk of genetic swamping [24, 25].

The study conducted by Tao et al. on the wild apples from different sites of the Ily state in China demonstrated great variation in fruit shape, size, color, and length. The study also found significant genetic variations in mineral elements' content in ripe fruits, suggesting potential for further selection [26]. The study of genetic diversity in Xinjiang, China, using SSR markers revealed the highest genetic diversity and high gene flow between populations, indicating some degree of gene exchange [27]. *Malus sieversii* exhibited resistance to Valsa canker, a disease caused by the fungal fungus *Valsa mali*. Studies have identified particular transcription factors, including MsbHLH41 and MsEIL3, which have significant functions in the plant's defense systems against this pathogen [28].

Genetic diversity studies have continued to underline the critical importance of *Malus sieversii* in the improvement and sustainability of modern apple cultivars. As existing research leads to new discoveries about resistance genes and beneficial traits, conservation of *Malus sieversii* continues to be relevant for making these apple varieties adaptable and resilient in the future.

3. Conservation, challenges, and future prospects

3.1 Current status and threats to wild populations

Despite its historical and genetic importance, *Malus sieversii* faces numerous conservation challenges. The loss of natural habitats, the impact of climate change, and human activities have resulted in a decrease in wild populations, posing a threat to genetic variety [29, 30]. This genetic diversity is essential for future breeding efforts. Conservation actions are crucial for preserving this species and its genetic resources. Diverse strategies, including the establishment of protected areas, *in situ* and *ex situ* conservation projects, and international collaboration, are implemented to save *Malus sieversii*. In 2007, *Malus sieversii* was evaluated for the IUCN Red List of Threatened Species and classified as vulnerable species [31]. It is listed in the Red Books of Kazakhstan, Kyrgyzstan, and China [32]. There are several genetic reserves in Kazakhstan for the conservation of Sievers apple trees. These reserves are mainly located on the territory of the Ile-Alatau and Dzungarian-Alatau national parks and 75% of the wild apple trees of Kazakhstan are distributed in these regions (**Figure 2**). The total area of orchards with wild Sievers apple trees in Kazakhstan, according to 2012 data, was about 14,307 hectares [33].

Figure 2.
Malus sieversii *in the ripening season. A -* Malus sieversii *from Ile Alatau Natural Park, B -* Malus sieversii *from Zhongar Alatau National Park.*

The decline of wild apple habitats in Kazakhstan began in the 1950s, during the Soviet era, when a large-scale process of replacing wild apple trees with cultivated varieties and creating terraced gardens on mountain slopes was carried out [34]. As a result, hundreds of hectares of apple trees were uprooted. In addition, urbanization near the metropolis of Almaty in the southeast of the country, where the Ile-Alatau State National Nature Park is located, also had an impact. This also likely facilitated gene flow from cultivated to wild varieties, as the proximity of cultivated apple trees to wild apple populations increased the chances of cross-pollination. Gene flow has the potential to bring novel genetic material into wild populations, potentially modifying their genetic composition and impacting their capacity to respond to natural environmental fluctuations. This genetic exchange may also have an effect on the total biodiversity of a region, resulting in alterations to the distinctive traits of wild apple populations. Over the past 50 years, the extent of fruit forests in the Ile-Alatau region has declined by 30%, and in certain areas, the decline has been as high as 70%. The apple forest area in the Maly Almaty Gorge was 190 hectares in 1934, but decreased to only 9 hectares in 2004, 70 years later. In the Dzungarian Alatau, the area of apple forests has decreased by 28% since 1960 [35, 36]. The Dzungarian Alatau, due to its isolation, has been less exposed to anthropogenic impact, which has contributed to the preservation of significant genetic diversity of the wild apple. However, the region is still subject to the influence of insect pests, abiotic factors, and natural aging of trees, which threatens the sustainability of populations (**Figure 2B**).

The wild apple trees in Kazakhstan are affected by the spread of various insect pests, such as the apple ermine moth (*Yponomeuta malinellus* Zell), hawthorn leaf roller (*Cnephasia crataegana Hb.),* spongy moth *(Lymantria dispar),* codling moth (*Cydia pomonella*), aphids *(Aphidoidea*), and the San Jose scale (*Quadraspidiotus perniciosus*) [37]. These pests cause damage on the fruits, leaves, shoots, bark, and wood of the trees, leading to a weakening of the plants and a decrease in their productivity.

In 1999–2003, the apple ermine moth (*Yponomeuta malinellus Zell*) caused significant devastation to the apple thickets in the Dzungarian Alatau, resulting in approximately one-third of the trees turning into dead wood. Subsequently, this dangerous pest also appeared in the Ile-Alatau, further threatening the apple populations in that region [34].

Additionally, while some wild apple trees exhibit resistance to various diseases, others remain susceptible to infections, such as apple scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*), fire blight (*Erwinia amylovora*), and phytophthora root rot (*Phytophthora spp.*) [38].

In China, the wild apple is significantly threatened by the wood-boring beetle (*Agrilus mali*), which damages the tree's vascular system, and the fungal pathogen *Valsa ceratosperma* (or *Valsa mali*), which causes cankers on branches and trunks that can lead to tree death [39, 40].

3.2 Conservation efforts and their importance

The conservation of wild apple species is crucial for preserving biodiversity and ensuring the long-term viability and progress of cultivated apple varieties. It is also essential for the sustainability of apple agriculture, particularly for future breeding programs on a worldwide basis. The genetic variety present in populations of *Malus sieversii* is a valuable resource for breeders and researchers, offering a collection of genes that can be utilized to enhance disease resistance, climate adaption, and overall strength of cultivated apple varieties. The genetic reservoir plays a crucial role in the context of climate change, since it provides a valuable resource for developing crop varieties that can withstand new challenges faced by the environment.

However, the main conservation challenges encountering *Malus sieversii* involve substantial habitat degradation resulting from agricultural expansion and urbanization, predicted complete habitat loss due to climate change in regions, such as Zhambyl of Kazakhstan, Tajikistan, and Uzbekistan, and fragmented populations impeding genetic interchange. Insufficient protected areas do not effectively protect appropriate habitats, and land use changes disproportionately impact environmental factors, such as elevation and soil pH. To effectively maintain *Malus sieversii*, conservation planning requires dynamic prioritizing, with a focus on long-term monitoring and adaptive management measures [32].

The process of grafting trees from a natural population with varietal material and cultivating gardens on its lands, which was prevalent in the mid-twentieth century in Kazakhstan, results in alterations to the genetic composition. These changes decrease the ability of a natural population to survive, and the most crucial strategies to protect them involve restoring the original population structure. To begin, it is crucial to prevent the cross-pollination of wild plants with cultivated types and partially cultivated trees. It is important to remove trees exhibiting evidence of varietal DNA and establish buffer zones of two kilometers surrounding natural populations. Within these buffer zones, the growing of both varietal and "semi-cultivated" apple materials should be prohibited. Regular monitoring of the condition of indigenous apple populations is crucial [41].

In situ conservation, which involves protecting *Malus sieversii* in its original habitat, is of the greatest significance. It is crucial to make efforts to save these habitats from deforestation, urbanization, and agricultural encroachment. National parks and genetic reserves are crucial components of this strategy. Academician Aimak Dzhangaliev created several selection and genetic reserves of Sievers apple trees in the territory of Kazakhstan and developed 27 elite varieties-clones of apple trees. Currently, several genetic reserves are experiencing a decrease in vegetation caused by both biotic and abiotic factors, as well as the degradation and aging of the population, this population decline is a significant factor contributing to the extinction of major wild plant species. There are a significant number of old trees in the Zhongar Alatau State National Nature Park (**Figure 3**). To address the degradation of old trees and the overall decline in vegetation, it is essential to implement restoration programs and create new, modern genetic reserves. Restoration involves not only planting new trees but also ensuring that these trees are genetically diverse and resilient.

Ex situ conservation efforts complement *in situ* strategies by preserving genetic material outside of its natural habitat. Botanical gardens, seed banks, and research institutions around the world are actively involved in collecting and maintaining *Malus sieversii* germplasm. These collections serve as genetic libraries that can be accessed for research and breeding purposes. The USDA National Plant Germplasm System (NPGS) and The Main Botanical Garden of Kazakhstan are notable examples of such repositories. These institutions ensure that even if natural populations are lost, the genetic heritage of *Malus sieversii* will be preserved.

Conservation efforts for *Malus sieversii* are vital to maintaining the genetic basis of domesticated apples and ensuring the sustainability of future apple crops. Protecting this species requires a multifaceted approach combining *in situ* and *ex situ* strategies, international cooperation, political support, and community engagement. Continued commitment to the conservation of *Malus sieversii* will not only preserve a key genetic resource but will also contribute to global food security and agricultural sustainability.

Figure 3.
Multispectral imaging of the Malus sieversii *population from a genetic reserve in Zhongar Alatau State National Nature Park.*

4. Conclusion

This chapter explores the historical discovery of *Malus sieversii*, its genetic contributions, and the ecological adaptations that have shaped the physical characteristics of domesticated apples. It also delves into current conservation issues and efforts, emphasizing the importance of preserving *Malus sieversii* for future breeding and research programs. By understanding the role of this wild ancestor, we can better appreciate the diversity and resilience of contemporary apple varieties and work toward sustainable and innovative apple cultivation practices.

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Chapter 5

Traditional versus Commercial Apple Varieties: Chemical Composition and Implications for Processing

Asima Akagić and Amila Oras

Abstract

Apples (*Malus domestica* Borkh.) are a widely consumed fruit recognized for their rich nutritional profile and health benefits. The chemical composition of apples differs significantly between traditional and commercial varieties, influencing their suitability and application in food processing. Both traditional and commercial apples are rich in vitamins, minerals, and dietary fiber. However, traditional apples often have higher concentrations of certain phytonutrients, such as polyphenols, due to less intensive breeding for esthetic qualities. In contrast, commercial varieties are typically bred for uniformity, shelf life, and resistance to pests, which can impact their nutrient profiles. Traditional varieties may offer superior flavor and higher antioxidant activity, while commercial varieties provide standard quality and higher yields. Understanding the distinct chemical compositions of traditional and commercial apples is essential for selecting and optimizing processing techniques to enhance the nutritional value, flavor, and overall quality of apple-derived products, such as juices, jams, 'pekmez', and compotes.

Keywords: nutritive compounds, bioactives, pre- and post-harvest, sustainable production, waste utilization

1. Introduction

Apples, scientifically known as *Malus domestica* Borkh., are widely consumed both in their fresh and processed forms, and they offer a range of health benefits due to their nutritional composition. Almost everyone has heard the expression 'An apple a day keeps the doctor away', reflecting the notion that apples and apple products are nutritious. Its low-calorie content, significant concentrations of vitamins [1], minerals [2], dietary fibers [3], and polyphenols [4, 5] make the apple and its products extremely valuable foods for maintaining health and preventing various diseases [6–9].

Besides the health benefits, apples' chemical makeup makes them a popular fruit for fresh consumption and processing into various products, including juice, vinegar, cider, distilled beverages, dried apples, puree, concentrates, compotes, 'pekmez', and jams (**Figure 1**). Apple juice is the most widely produced apple-based product, representing 65% of total apple product output [10, 11]. During industrial apple processing, 25–30% of the fruit results in pomace by-products [12]. Apple pomace, in particular, is a valuable resource for diverse use in the food, pharmaceutical, and other industries (**Figure 1**).

As the demand for apple products has grown, there has been a shift in the types of apple varieties used for cultivation, from traditional, locally adapted types to commercially dominant varieties bred for low allergenic potential, yield, disease resistance, improved nutritional composition, and uniformity in appearance [13, 14].

Traditional apple varieties are known for their rich and diverse chemical composition. They contain higher levels of fiber, protein, sugars, β-carotene, vitamin E, magnesium, and phenolic compounds than their commercial counterparts [15]. These components contribute to the apple's health-promoting properties, such as supporting digestive health, boosting the immune system, and offering antioxidant benefits. Traditional varieties are also relatively more resistant to pests and climate changes, requiring fewer fertilizers and pesticides [16, 17]. On the other hand, commercial apple varieties, such as 'Red Delicious,' 'Golden Delicious,' and 'Jonathan,' have been bred for traits like disease resistance, esthetic appeal, and consistent yield. While these varieties also provide important nutrients, they tend to have lower concentrations of certain bioactive compounds compared to traditional varieties. This reduction in nutritional density can be attributed to the selective breeding practices focused on commercial viability rather than nutrient content. Despite this, commercially grown apples remain a good source of carbohydrates, dietary fiber, and essential minerals such as potassium and calcium [18].

The chemical composition of apples directly affects how they can be processed and the quality of the resulting products. The quality of apples and their derived products is shaped by a range of factors, both before and after harvest (**Figure 1**)

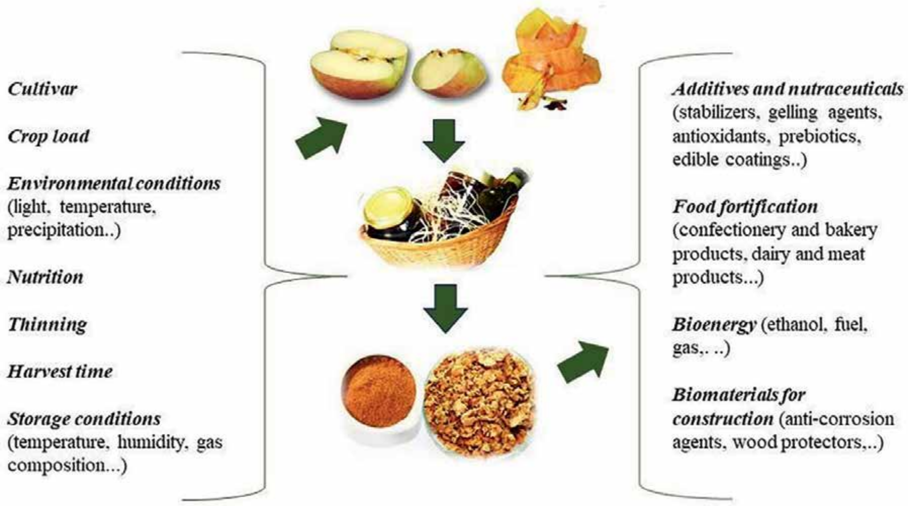

Figure 1.
Factors influencing the quality of apples, their derivatives, and the potential use of apple pomace.

[19, 20]. Pre-harvest influences include environmental conditions, thinning practices, substrate choice, tree yield, apple variety, harvest time, and the stage of maturity [21–25]. Conversely, post-harvest quality is determined by factors such as storage conditions, temperature, ethylene exposure, the composition of packaging gases, and the type of packaging materials used [26, 27].

Apples are processed into a variety of products, both in liquid, semi-solid and solid forms [28–31]. The quality of these products depends on key constituents of the apple, including sugars, acids, pectin, and polyphenolic components [32]. Due to their higher content of phenolic compounds, traditional apple varieties tend to produce apple products that are richer in antioxidants and have better sensory attributes, such as flavor and color stability [33]. These qualities are especially important for products like apple juice and jam, where the balance of sugar, acidity, and bioactive compounds can influence taste and shelf life. Furthermore, traditional varieties, with their natural resistance to pests and diseases, often require fewer chemical interventions during cultivation, which may result in fewer pesticide residues in processed products [34]. Commercial apple varieties, while more uniform in size and appearance, often require more additives during processing to maintain their quality, especially in terms of flavor and preservation. The lower levels of bioactive compounds and the tendency to have a more uniform sugar-to-acid ratio can result in products that are less complex in flavor and have lower health benefits. Additionally, the intensive cultivation of these varieties has led to a reduction in biodiversity, with over 80% of apple cultivars in North America at risk of extinction [35]. A similar trend is present in the European market, where only about 12 apple cultivars are predominantly represented [36], leading not only to a loss of biodiversity but also to a reduced content of important macro- and micronutrients for consumers.

One of the major criticisms of the shift toward commercial apple varieties is the environmental impact of their cultivation. The intensive farming practices required to maintain large monocultures of commercially viable varieties often involve heavy use of fertilizers and pesticides, which can have negative environmental consequences. In contrast, traditional varieties, which are more adaptable to local growing conditions and often more resistant to pests and diseases, require fewer chemical inputs, making them a more sustainable option for apple production [37]. Economically, traditional apple varieties offer advantages for small-scale and organic farmers. These varieties are typically less expensive to grow and maintain, as they do not require the same level of chemical interventions. Moreover, their high concentration of bioactive compounds and excellent sensory attributes make them a desirable option for producing high-quality, artisanal apple products, such as organic apple cider and fruit preserves. These products can fetch a premium price in markets where consumers are willing to pay more for natural, minimally processed foods [15, 38].

Ultimately, regardless of whether apples are traditional or commercial, their chemical composition—particularly content of sugars, acids, pectin, and polyphenols—along with their low cost, excellent storability, versatility in blending with other fruits, and efficient by-product utilization, make them highly sought after by the food industry for processing.

2. Sugars and organic acids in apples

The chemical composition of apples, particularly their sugars and acids, is fundamental to their flavor profile, nutritional value, and suitability for different processing methods. The primary sugars found in apples are fructose, glucose, sucrose, and sorbitol [39]. Among these, fructose is the dominant sugar in mature apples, followed by glucose and sucrose. Fructose is particularly noteworthy because it is sweeter than both glucose and sucrose, contributing significantly to the overall perception of sweetness. The sweetness and flavor of apples are mostly determined by total sugar content and fructose to glucose ratio (F/G) [40]. Sorbitol, a sugar alcohol specific to plants in the rose family (including apples), plays a unique role in determining the perception of sweetness but is often less discussed in comparison to the more prevalent sugars [41]. Sugars in apples are not just responsible for sweetness but also serve as carbon sources for the synthesis of other important compounds such as pigments, vitamins, and aroma compounds [42]. Additionally, the sugars found in apples are crucial for several aspects of product development. They help estimate the fruit's maturity, influence decisions on processing methods, and act as natural preservatives. Sugars play a key role in shaping the final product's flavor, taste, and texture, as well as supporting pectin's gelling properties in jelly products. They also prevent enzymatic browning, reduce the freezing point in frozen products, serve as the foundation for fermentation in alcoholic beverages, and offer mild antioxidant effects [29, 43].

The dominance of fructose in both traditional and commercial apples is crucial for processing, particularly in the production of fruit juices and concentrates. However, fructose is classified as a free sugar by the World Health Organization (WHO), meaning its intake should be limited due to health concerns such as the risk of obesity and type 2 diabetes. While apples themselves have a low glycemic index [36–40], apple juice ranks slightly higher [41, 44, 45], which means that in processing, managing sugar content becomes essential to maintaining the health benefits associated with fresh apples. However, fruit juices are recommended as an integral part of a balanced diet for children and adults [46].

Organic acids, particularly malic acid, are the dominant organic acids in apples, accounting for over 90% of the total acid content [47]. Malic acid is responsible for the tartness in apples, and its concentration varies widely across different apple cultivars. The content of malic acid can significantly affect the flavor balance, especially in apples with higher sugar concentrations, where the acidity provides a counterpoint to the sweetness. Traditional apple varieties often contain higher concentrations of malic acid, which provides a sharper, more pronounced tartness. The role of malic acid extends beyond flavor. As a key organic acid, it contributes to the apple's overall nutritional profile, providing antioxidant benefits and enhancing the fruit's processing characteristics. High acid content can act as a natural preservative in juice production, reducing the need for added preservatives, which is a desirable trait in both traditional and commercial apple processing. Apples also contain other organic acids, including L-ascorbic acid, which, aside from contributing to flavor, acts as a key nutritional factor with antioxidant properties [48].

The composition of sugars and acids in apples is a critical determinant of the fruit's flavor, appeal, and overall quality [29]. While sugars contribute to sweetness, acids impart sourness, and the balance between these two is essential in shaping the sensory experience of consuming an apple. However, the interplay between these components is complex, influenced by factors such as cultivar, maturity, environmental conditions, and harvest timing. The ratio between sugars and acids, often referred to as the sugar/acid ratio, is one of the most critical factors in determining the taste and implications for processing [49]. Optimal sugar/acid ratio for apple juice production used to be 20–35, but nowadays, it ranges from 18–25. According to a study by

Vranac [33], traditional apples from Bosnia and Herzegovina are more suitable for juices production (**Figure 2**) based on their sugars (expressed like total soluble solids content, %) to acids (expressed as titratable acidity, %) ratio.

A higher sugar/acid ratio typically results in a sweeter taste, while a lower ratio is associated with a more tart or sour flavor. The sugar/acid ratio remains a critical factor in consumer preference, with variability across cultivars and growing conditions offering diverse flavor profiles. This complexity in the biochemical composition of apples underscores the importance of ongoing research into apple breeding, aiming to enhance both the taste and nutritional quality of the fruit [50, 51].

The composition and balance of sugars and acids vary widely between different apple cultivars [52]. For instance, some research have showed that traditional cultivars tend to have higher concentrations of sorbitol and organic acids compared to commercial cultivars, which are generally sweeter and less acidic. This genetic variability in sugar and acid content is an area of significant interest for breeders, who aim to develop apple varieties with targeted sweetness and sourness levels [53]. In traditional apple varieties, research indicates that higher concentrations of sorbitol and organic acids contribute to a more complex flavor profile. Traditional varieties often maintain a balance between sweetness and acidity, making them more suitable for certain culinary applications, such as cider production or baking, where a tart-sweet balance is desirable. On the other hand, commercial apple varieties are typically bred for sweetness, with a higher fructose-to-glucose ratio (F/G) aimed at appealing to modern consumer preferences for sweeter flavors. As a result, these varieties tend to have lower acid levels and are generally less tart than traditional cultivars.

The high sugar content in commercial varieties, particularly fructose, poses challenges for juice producers who aim to meet the growing demand for low-sugar or sugar-reduced products. The WHO's recommendation to limit free sugar intake has led to an increased demand for products with reduced sugar content, including fruit juices. Although apple juice is rich in bioactive components such as vitamin C, folate, potassium, and phytochemicals, which offer health benefits such as improved blood

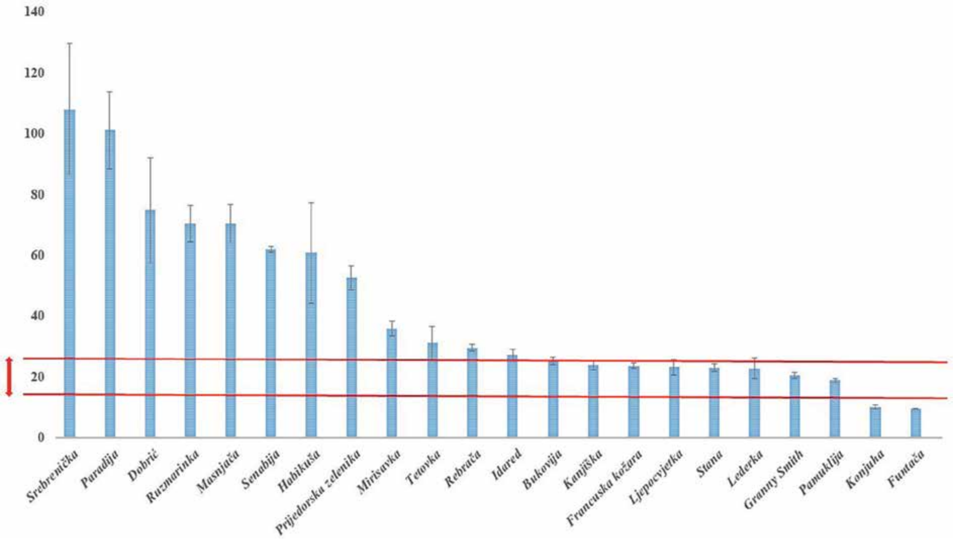

Figure 2.
The average sugars to acids ratio in traditional and commercial apples (± SD) [33].

flow and antioxidant effects, its classification alongside sugar-sweetened beverages underlines the need to manage sugar levels in processed apple products. According to EU Directive 1924/2006, the food industry is trying to develop procedures that will reduce the energy value of juices by at least 30% compared to similar juices [54]. Techniques such as mixing juices from different types of apples [55–59], membrane filtration to separate sugars [60–63], and enzymatic treatments [64–66] have been developed to address this issue. However, these methods often lead to significant changes in the juice's overall composition, raising questions about the integrity and purity of the final product [67]. Traditional apple varieties, with their higher acid content and lower sugar levels, may be more suitable for processing into juices and other products where sugar reduction is desired without compromising on flavor. The higher concentration of malic acid in these varieties can provide a natural counterbalance to the sweetness, offering a more complex and appealing flavor profile. Moreover, the presence of sorbitol in traditional varieties, which contributes to sweetness without adding free sugars, may provide an additional advantage in developing products that cater to health-conscious consumers. By maintaining a higher acidity and lower free sugar content, these varieties can contribute to the development of functional, nutritionally enriched products that align with modern dietary guidelines.

3. Dietary fiber: Pectin

The chemical composition of apples, particularly the pectin content, plays a critical role in determining their processing suitability, influencing juice extraction, texture, and stability of processed products like jams and jellies.

Pectin is a crucial polysaccharide found in the cell walls of apples, along with cellulose, hemicellulose, lignin, and proteins. In apples, cellulose forms the dominant component of the cell wall, comprising 0.70%, while pectin accounts for 0.54% of the composition, hemicellulose 0.34%, and glycoprotein 0.15% [68].

Pectin plays several essential roles in plants and fruit processing, primarily influencing water retention, cell turgor, and fruit texture. During ripening, protopectin, an insoluble precursor of pectin, hydrolyzes into soluble pectin and pectic acid, contributing to the softening of the fruit as the cell walls weaken [69, 70].

Considering that pectin is classified as a soluble dietary fiber, and it has significant health benefits. Numerous studies have demonstrated the positive effects of pectin on human health, including lowering cholesterol levels, regulating blood sugar, and reducing the risk of chronic diseases such as diverticular [71, 72] and cardiovascular disease [73], type 2 diabetes [74, 75], and certain cancers [76]. Pectin's ability to bind water and slow digestion makes it a valuable component of a healthy diet.

While most research has focused on the basic structural characterization of apple pectins, the finer structural differences between cultivars remain largely unexplored [77]. A study comparing traditional and commercial apple varieties in Bosnia and Herzegovina revealed significant differences in pectin content between these groups. Traditional apple varieties, such as 'Prijedorska Zelenika', 'Rebrača', 'Tetovka', 'Paradija', and 'Funtača', generally had higher pectin content than commercial varieties like 'Idared' and 'Granny Smith' [33].

These variations in pectin content can influence the firmness of apples, which is an important quality parameter in both fresh consumption and post-harvest storage [78]. Pectin, combined with calcium-based compounds, forms calcium pectate, a salt that enhances fruit firmness. For example, treating apples with calcium chloride ($CaCl_2$) has been shown to significantly alter proteins and phosphoproteins, impacting the regulation of the cell wall and other physiological processes [79].

Traditional apple varieties, with their higher pectin content, are particularly suited for producing cloudy and pulpy juices, as well as products where viscosity and stability are desired. Pectin acts as a stabilizing agent in these types of juices, preventing the separation of liquid and solid phases [80]. Interestingly, while ripe fruits are often softer, making them seem easier to juice, the increased solubility of pectin actually raises the viscosity of the cellular contents, complicating juice extraction. In this context, the pectin content becomes a vital determinant in the efficiency of juice processing.

The presence of pectin in apples presents both challenges and opportunities in juice production. Pectin increases the viscosity of the fruit mash, reducing juice yield if not properly managed. To address this, the food industry often uses pectinases—enzymes that break down pectin—along with other enzymes, like cellulases and hemicellulases, to improve juice extraction efficiency [81–87]. These enzymes hydrolyze cell wall constituents, reducing viscosity, increasing the extraction yield, galacturonic acid content, reducing sugars, soluble dry matter, and titrable acidity of the products [88, 89]. Studies have shown that increasing the concentration of pectinase in apple pulp can raise juice yield from 72.3 to 83.8%, depending on the amount of enzyme used [90]. Enzyme treatments also improve the extraction of beneficial compounds such as polyphenols [91], which enhance the nutritional quality of the juice, as well as its aromatic profile.

On the other hand, removing the turbidity components from the freshly produced juice is the main objective of the clarification (depectinization + precipitation) during the clear juice production process [92]. The depectinization process of fruit juice through the use of commercial enzymes, most often a mixture of pectinases (e.g., pectinesterase, polygalacturonase, and pectin lyase), has been presented as an efficient alternative to reduce turbidity and to improve clarity [93, 94]. Pectinases degrade pectin, resulting in viscosity reduction and forming pectin-protein complexes [95], which improves separation through centrifugation or filtration. As a result, it is not unexpected that juice has very little pectin—only 0.2%—while apples have a 0.5–1.6% pectin level [96]. However, the removal of colloidal haze particles reduces the nutritional and antioxidant potential of the final juice as well [97].

In addition to juice production, pectin plays a crucial role in the production of jams, jellies, and marmalades. Pectin forms gels when combined with sugar and acid, giving these products their characteristic texture. The degree of esterification (DE) of pectin—whether the pectin is highly esterified or low-esterified—determines its gelling properties and suitability for different products [98]. High-esterified pectins form gels in high-sugar environments (above 50%), such as conventional jams [99], while low-esterified pectins are better suited for low-calorie or reduced-sugar products [100].

The traditional apple varieties, with their higher pectin content, offer an advantage in producing firm, well-gelled products without requiring additional pectin. Commercial varieties, on the other hand, may necessitate the use of pectin additives to achieve the same results. Pectin is a stabilizer and gelling agent widely used in the food industry with GRAS status (generally recognized as safe). As a stabilizing agent, pectin is also added to acidified milk drinks, which are often flavored with fruit juice [101–104].

4. Apple polyphenols

Among the key bioactive compounds found in apples are polyphenols, which have strong antioxidant properties. Polyphenols are a large category of bioactive phytochemicals that plants synthesize in response to environmental stress and developmental processes. Chemically, these compounds contain one or more hydroxyl groups attached to benzene rings. Their antioxidant potential is closely linked to the number and distribution of hydroxyl groups, as well as the pH of the medium in which they are present. In apples, polyphenols are mostly found as esters and glycosides, which require enzymatic breakdown for bioavailability [105].

In apples, over 60 phenolic compounds have been identified, primarily in the form of phenolic acids and flavonoids [106, 107]. The most dominant class of polyphenols in apples are hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols (flavan 3-ols), dihydrochalcones, and anthocyanins (in red-skinned apple varieties). Polyphenols in apples, particularly flavonoids and phenolic acids, contribute to a wide range of health benefits, including antioxidative, anti-inflammatory, and cardiovascular protective effects. Research also shows that apples' polyphenols may have antidiabetic, hypocholesterolemic, and anticancer activities [9, 108].

Beyond their antioxidant activity, polyphenols contribute to the sensory qualities of apples and apple products, including color, bitterness, and astringency [109–111]. Apple polyphenols can serve as valuable markers for monitoring the quality and origin of apple products, providing a means of assuring consumers of the authenticity and nutritional value of what they are purchasing. This is particularly crucial in maintaining and improving food standards [112].

The content of polyphenols and their distribution can vary significantly within the fruit [111]. Apple peel contains higher concentrations of polyphenols in comparison to pulp [5, 113–115]. While the peel, especially in red apple varieties, is rich in flavonols and anthocyanins, the flesh typically contains little to no anthocyanins, except in certain red-fleshed cultivars. Additionally, the flesh has a lower flavonol content compared to the peel, but it holds higher levels of phenolic acids, flavan-3-ols, and dihydrochalcones [116]. Moreover, the polyphenolic profile varies considerably depending on genetic factors, environmental conditions, and cultivation practices [117], making polyphenols useful not only for quality assurance but also for distinguishing between apple varieties [118].

A major distinction between traditional and commercial apple cultivars lies in their polyphenol content. Research indicates that traditional apple varieties tend to have higher concentrations of polyphenols, particularly phenolic acids and flavonoids, compared to modern commercial varieties [119, 120]. Commercial varieties, which have been bred for their appearance and shelf life, often contain lower levels of these beneficial compounds. This has significant implications for processing, particularly in the production of apple juice, where traditional apples may yield a more nutrient-dense product. Traditional apples are valued for their nutraceutical properties, as they have retained much of their phenolic richness through less intensive breeding practices. Conversely, commercial apple breeding over the past two centuries has resulted in a significant decline in polyphenol content [121]. This reduction is often a deliberate outcome of efforts to mitigate enzymatic browning, which is associated with polyphenol oxidation, and to reduce the astringent taste that some phenolic compounds impart [122]. On the other hand, in addition to their high antioxidant potential, superior pomological traits, fruit quality, and resilience to abiotic and biotic stress, the primary goal in preserving traditional apple cultivars is to prevent their possible extinction and safeguard the biological diversity they offer.

Processing methods have a profound effect on the polyphenolic content of apple products. One of the major challenges in apple processing is enzymatic browning, which occurs when polyphenols are oxidized by polyphenol oxidase (PPO) and peroxidase (POD) enzymes, leading to the formation of undesirable brown pigments [123]. Processes such as cutting, grinding, and pressing mechanically disrupt the apple's cellular structure, leading to a loss of polyphenols and browning [124], which not only affects the appearance of apple products but also degrades their nutritional and antioxidant quality. Enzymatic browning, driven by the oxidation of polyphenols, particularly chlorogenic acid, procyanidins, and flavanols [125], is responsible for nearly 50% of fresh fruit loss, as it diminishes both the sensory and nutritional qualities of apples [126]. In addition to the color degradation caused by enzymatic browning of polyphenols in apple products, the interaction of apple polyphenols with the Maillard reaction can further contribute to the browning effect [127]. Variations in chemical composition, particularly phenolic content, across different apple cultivars likely contribute to differences in enzymatic browning. Additionally, genetic relationships between apple cultivars, linked to the genotype-specific activity of polyphenol oxidase (PPO), may explain the variations in PPO activity observed not only between apple varieties but also within the broader Rosaceae family, including apples and pears [128].

Thermal processing methods such as blanching and pasteurization can inhibit enzymatic browning by denaturing the PPO and POD enzymes, although these processes may also reduce the content of heat-sensitive nutrients like vitamins and have a negative impact on fruit texture or aroma [126]. Beyond thermal treatments, several antibrowning agents have been investigated to inhibit enzymatic browning in apple products. In liquid applications, such as juices, these agents can be added directly, while for solid forms like fresh-cut apples, methods such as dipping, coating, or gas exposure with antibrowning solutions are commonly used. Antibrowning agents can function as antioxidants or reducing agents (e.g., L-ascorbic acid, citric acid, kojic acid, quercetin, glutathione, cysteine, and tocopherol), acidulants (e.g., L-ascorbic acid, citric acid, and glutathione), chelating agents (kojic acid, citric acid, and ethylenediaminetetraacetic acid (EDTA)), or halide salts (e.g., NaCl, NaF, and CaCl2). Although antibrowning agents are used to inhibit enzymatic browning, they can also alter the sensory properties of the product [118].

Processing methods used in apple products, particularly juices, have a substantial impact on polyphenol retention [129]. Flavanol monomers, hydroxycinnamic acids, and dihydrochalcones play a key role in the development of oxidation products, which significantly influence the color and flavor of apple juices [130]. Hydroxycinnamic acids, particularly chlorogenic acid, are widely recognized for their significant role in the oxidation process and the resulting undesirable color changes during juice production [131]. Despite this, chlorogenic acid is also noted for its numerous health benefits, including its antioxidant, antimicrobial, anti-inflammatory, and prebiotic properties [132]. Besides the oxidation process, another challenge with apple polyphenols is that a great amount of these compounds and dietary fiber components are not transferred into the liquid phase during the juicing process but remain in the pomace after pressing. Hydroxycinnamic acids and dihydrochalcones have the highest extraction rates, at 65 and 80%, respectively, while procyanidins have the lowest at just 32% [133]. Apple phenolics, particularly procyanidins, tend to bind to cell wall material, which reduces polyphenol levels in the juice [134]. In the conventional apple juice production process, pressing is the main stage where polyphenol losses occur due to the binding of flavonols to the pressing cake [135]. However, different processing techniques yield varying outcomes in terms of polyphenol preservation [136]. In traditional pressing methods, only 43 to 85% of polyphenols are recovered in the juice, with the remainder lost to the pomace. Cold-pressing frozen apples, for instance, recovers almost 100% of the polyphenols present in the fruit, compared to traditional methods, which retain far less. This highlights the importance of both the apple matrix and the processing method in determining the final polyphenol content of apple products [137].

It is not just the juicing process that impacts the bioavailability of phytochemicals. Clarification, a crucial step in producing clear apple juice, also plays a significant role in altering the phenolic composition of the final product [138]. Clear apple juice, which undergoes extensive clarification to remove pectins and solids, retains significantly fewer polyphenols than cloudy juice [9]. In particular, procyanidins and flavonoids bind to the cell walls during pressing and are partially lost during clarification [139]. Consequently, clear juice exhibits reduced antioxidant activity and diminished health benefits compared to cloudy juice, which contains more suspended solids and preserves more of the fruit's original polyphenol content.

5. Apple pomace as valuable by-product

It is estimated that of the total amount of apples produced, about 70–75% is used for fresh consumption, while 25–35% is processed into nutritionally valuable products [140–142]. Depending on the method of processing and pre-harvest factors, processing results in about 25–30% waste, such as peels and mesocarp (95%), seeds (2–4%), and stems (1%), which make up apple pomace [143]. Globally, the amount of apple pomace ranges from 3,000,000 to 4,200,000 tons annually [144].

Due to its high nutrient and water content, plant-based food waste quickly undergoes microbiological decomposition, causing health problems [145]. On the other hand, the resulting organic waste, due to its high biological and chemical oxygen demand, variable composition, and pH value, poses a serious environmental problem if, as is often the case, it is directly disposed of in landfills [12, 146]. According to Monspart-Sényi [147], the biological oxygen demand for waste generated after apple juice production is 2 kg/unit of product. Although it is a common practice, apple pomace is not recommended for livestock feed due to its low protein content [148], nor for composting due to pollution caused by greenhouse gas emissions [149].

Nowadays, available synthetic corrosion inhibitors used in construction are being replaced with eco-friendly inhibitors due to environmental pollution concerns. These inhibitors are primarily non-toxic, biodegradable, readily available, and inexpensive. Therefore, apple pomace, which is rich in polyphenolic components and has a high antioxidant capacity, has been analyzed as a potential corrosion inhibitor [150]. According to Honarvar Nazari et al. [151], apple pomace prepared in a 3.5% NaCl solution has proven effective, with its mechanism of action based on blocking active anodic sites on the steel surface and transforming Fe_3O_4 into a more corrosion-resistant form, Fe_2O_3. Additionally, to protect wood from decay, traditional toxic biocides are being replaced with alternative biodegradable agents like apple pomace, thanks to the antimicrobial properties of polyphenols [152, 153].

Apple pomace can be used as a substrate for biogas production; however, it is considered that its utilization for other purposes, such as the production of additives, supplements, and nutraceuticals, is approximately 3.5 times more profitable compared to biofuel production [154]. Given that apple pomace is a valuable source of dietary fiber, pectin, minerals, and polyphenolic components [155], its use in the fortification of various food products and as a medium for the production of additives and nutraceuticals is unquestionable [156].

The pomace from traditional apple varieties is richer in phenolic components compared to commercial ones. This is supported by the research of Akagić et al. [157], which found that the total polyphenol content in the peel of the traditional apple variety 'Prijedorska Zelenika' was significantly higher (nearly up to three times) in comparison with the commercial varieties 'Granny Smith' and 'Jonagold'.

Confectionery and bakery products are often enriched with apple pomace to increase antioxidant activity (polyphenolic components), improve aroma and appearance, substitute fat with pectin, and enhance nutritional value [158–162]. As more people exhibit some form of gluten intolerance, the food industry is striving to expand the range of gluten-free products enriched with nutritionally valuable components. To this end, gluten-free cakes [163] and crackers [164] have been produced with up to 10% apple pomace, resulting in acceptable sensory attributes and enrichment with fibers, minerals, and polyphenolic components, as well as a reduced glycemic index [165].

Apple pomace is also used in yogurt production, reducing acid and fat content while increasing fiber content, stabilizing the emulsion, and improving sensory characteristics such as color, aroma, and texture [166–168].

There is a growing trend of reduced meat and meat product consumption due to negative impacts on the environment and human health, primarily because of high-fat content. To reduce fat content and increase yield, antioxidant activity, fiber content, and sensory properties, chicken and beef are being supplemented with apple pomace [169].

Since apple pomace is a rich source of pectin and polyphenolic components, it can serve as a medium for producing additives that are widely used in the food industry as stabilizers, gelling agents, and antioxidants [170]. However, the extraction method of these valuable components from apple pomace is a limiting factor because conventional methods use expensive solvents, result in extracts of unsatisfactory purity, and cause degradation of thermo-sensitive components [171–173]. Therefore, recent research has focused on alternative, environmentally friendly extraction methods that achieve high yield and quality extracts in a shorter time [174]. The most promising innovative methods for pectin extraction include enzyme application [175], ultrasound [176], microwave heating [177], high hydrostatic pressure [178], and supercritical fluid extraction [179]. The part of the apple pomace that remains after the extraction of antioxidants and pectin could be used in bone and cartilage tissue replacement therapy, adhering to the 'zero waste' philosophy [180]. Additionally, pectin as an additive is often used for producing films and coatings, promoting sustainable and environmentally friendly food packaging solutions [174, 181].

Since intensive apple cultivation involves the use of growth bioregulators and pesticides, their residues are expected in apple pomace. According to research [182, 183], the detected values of pesticide residues in pomace from different apple varieties were negligible and posed a low toxicity risk, as did the residues of bioregulators. Furthermore, apple seeds remaining in the pomace after juice extraction present a health risk due to their cyanidin content. However, studies indicate that the level of amygdalin, which degrades into cyanidin, in apple seeds is safe for human consumption [184]. Additional safety concerns with apple pomace include the potential accumulation of patulin, a toxin produced by fungal contamination. Regulatory bodies such as the European Food Safety Authority (EFSA) and the U.S. Food and Drug Administration (FDA) have set maximum allowable patulin levels at 50 μg/kg for adults and 10 μg/kg for infants and young children to ensure product safety [105]. As new growth stimulators and protective agents are continuously introduced to the market, and as there is an expected increase in food waste utilization to reduce negative environmental impact, it is necessary to conduct continuous and detailed analysis of these residues to protect consumer health.

6. Conclusion

Modern apple orchards are being designed to suit the constantly increasing demand for food, emphasizing broadly adaptable genotypes that have a detrimental effect on biodiversity. However, these cultivars are frequently of lower quality, less content of bioactive components that are beneficial to human health, and have less noticeable sensory qualities than traditional ones.

Despite traditional cultivars have numerous advantages, their more intensive cultivation is limited by alternative bearing and their variable external appearance. As a result, they are primarily suggested for adding value by enhancing the sensory qualities and nutritional makeup of products derived from commercial cultivars.

In the age of global warming, all parties involved in the food supply chain, both domestically and internationally, should be primarily interested in protecting biodiversity by building plantations of diverse assortments including traditional apple genotypes.

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The edited volume *Malus domestica - New Insights* is a collection of reviewed and relevant research chapters, offering a comprehensive overview of recent developments in the field of horticulture. The book comprises single chapters authored by various researchers and edited by an expert active in the horticulture research area. All chapters are complete in themselves but united under a common research study topic. This publication aims to provide a thorough overview of the latest research efforts by international authors on *Malus domestica* and open new possible research paths for further novel developments.

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