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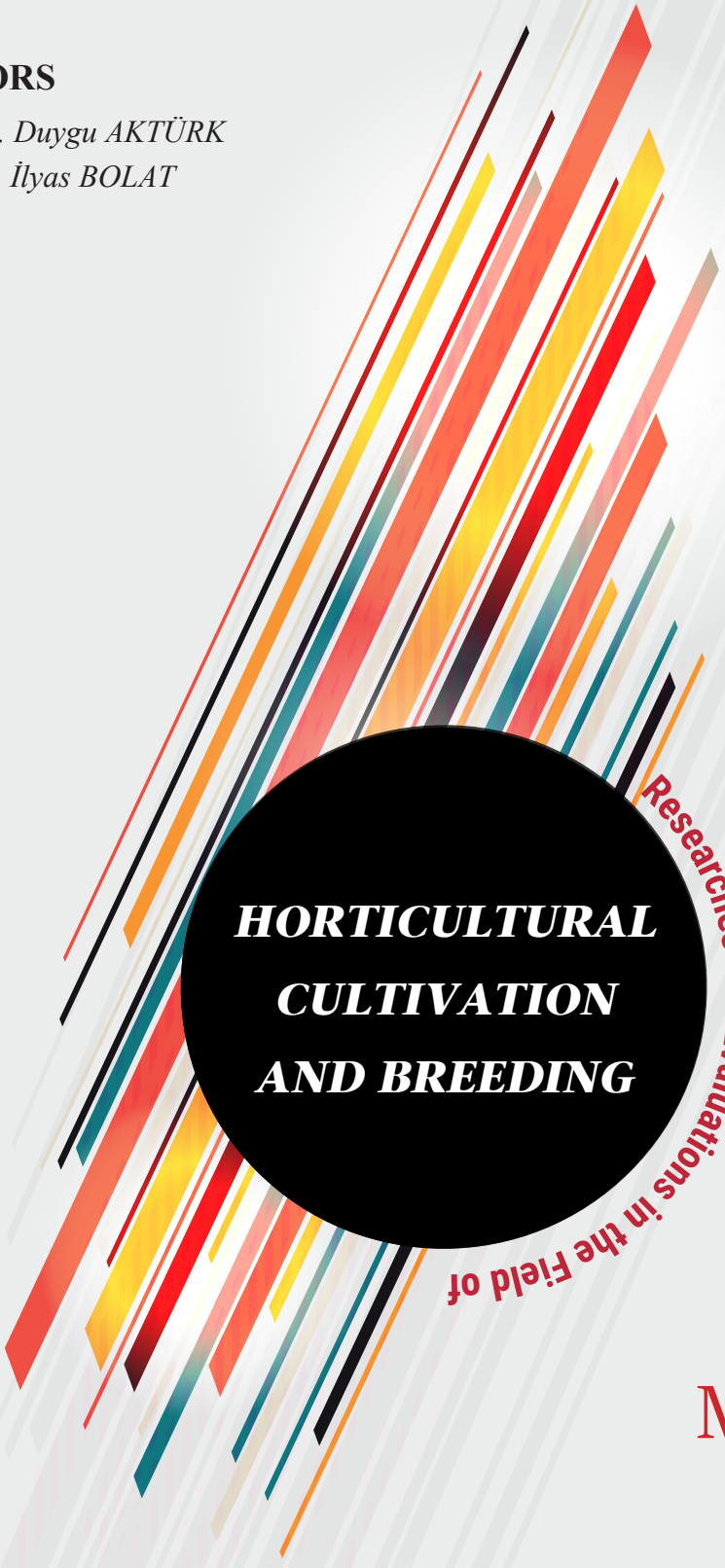

HORTICULTURAL CULTIVATION AND BREEDING

Researches and Evaluations in the Field of

March 2025

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Research And Evaluations In The Field Of Horticultural Cultivation And Breeding

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İÇİNDEKİLER

CHAPTER 1

PRESERVATION OF SEED VIABILITY UNDER STRESS CONDITIONS

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1. Introduction

Seeds play a crucial role in ensuring the perpetuation of plant species, sustaining agricultural production, and preserving the biodiversity of ecosystems. Seed viability is directly associated with the metabolic activity of the embryo, genetic stability, and resistance to environmental stress factors. However, climate change, increasing environmental degradation, and pathogenic threats pose significant challenges to seed viability and germination capacity (Bewley et al., 2013; Rajjou & Debeaujon, 2008). In this context, maintaining seed viability has become a focal point of scientific research, particularly in relation to food security and sustainable agriculture.

Abiotic stress factors (such as drought, salinity, extreme temperatures, and heavy metals) and biotic stress factors (such as fungal pathogens and insect pests) can compromise the cellular integrity of seeds, leading to irreversible damage, including lipid peroxidation, protein denaturation, and DNA damage (Bailly, 2004; Munns & Tester, 2008). For instance, drought stress limits water uptake, triggering the accumulation of reactive oxygen species (ROS), whereas salinity disrupts osmotic balance and induces ionic toxicity (Farooq et al., 2017). Similarly, mycotoxins produced by fungal pathogens can result in severe consequences, including embryo mortality (Agrios, 2005).

To mitigate these challenges, a range of strategies, both conventional and innovative, have been developed. Physical and chemical protection methods (such as seed coating, priming, and chemical treatments) have proven effective in enhancing seed resistance to environmental stresses (Ashraf & Foolad, 2007; Khan et al., 2020). Meanwhile, biotechnological and nanotechnological approaches (including CRISPR-Cas9 and nanoemulsions) offer targeted solutions (Zhang et al., 2022; Kah et al., 2018). Additionally, biological agents such as arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria (PGPR) naturally improve nutrient uptake and stress tolerance in seeds (Smith & Read, 2008; Glick, 2012).

This book chapter aims to provide an in-depth analysis of the stress factors that threaten seed viability, examine protection strategies based on scientific foundations, and discuss future research directions from the perspective of sustainable agriculture.

1.1. Seed Viability and Its Importance

Seeds play a crucial role in plant propagation and the preservation of genetic diversity. As one of the fundamental components ensuring the continuity of agricultural productivity, seeds must retain their viability even

under long-term storage conditions (Bewley et al., 2013). Seed viability is influenced by genetic composition, physiological status, and environmental factors. Properly stored seeds can maintain their optimal germination capacity, enabling the emergence of new plants (Rajjou & Debeaujon, 2008).

The viability of a seed depends on the integrity of its embryo, energy reserves, and protective layers. From a physiological and biochemical perspective, seeds sustain their metabolic activities by utilizing stored carbohydrates, proteins, and lipids (Nonogaki, 2014). The preservation of seed viability is of particular importance for ensuring agricultural productivity, especially under prolonged storage and stressful growing conditions (Kaur et al., 2016).

1.2. Effects of Stress Conditions on Seeds

When exposed to environmental stress conditions, seeds may experience metabolic imbalances, leading to reduced germination rates and impaired seedling development (Finch-Savage & Bassel, 2016). Stress factors are generally classified into two categories: abiotic (such as drought, salinity, extreme temperature, and oxidative stress) and biotic (such as diseases and pests) (Mittler, 2006).

Drought stress adversely affects seed metabolism, potentially delaying or completely inhibiting germination (Farooq et al., 2009). Similarly, salt stress disrupts the osmotic balance within seeds, restricting water uptake and ultimately reducing germination rates (Munns & Tester, 2008). Extreme temperatures and oxidative stress can damage the seed coat and DNA structure, leading to harm in embryonic tissues (Bailly, 2004).

Biotic stress factors originate from pathogens and harmful organisms. Fungal and bacterial infections can negatively impact seeds by decreasing germination percentages and preventing the healthy growth of seedlings (Neergaard, 1977). Seed-borne diseases not only contribute to yield losses in agricultural production but also facilitate the spread of pathogens that can persist in the soil for extended periods (Maude, 1996).

1.3. The Importance of Strategies for Preserving Seed Viability

Various physical, chemical, biological, and biotechnological methods are employed to maintain seed viability. One of the critical factors in preserving seed health is optimizing storage conditions (Walters et al., 2005). Seeds stored under appropriate moisture and temperature conditions can retain their viability for extended periods.

Seed coating and priming techniques are widely used to enhance seed resilience against stress conditions. Priming treatments expose seeds to specific substances, improving metabolic activity and thereby enhancing germination capacity (Ashraf & Foolad, 2005). Biostimulants such as salicylic acid, proline, and seaweed extracts are also utilized to increase stress tolerance (Korkmaz et al., 2010).

Advancements in biotechnological methods have made it possible to develop genetically stress-tolerant seeds. Gene-editing techniques such as CRISPR/Cas9 contribute to the improvement of seed performance, potentially leading to the development of more resilient plant varieties in the future (Gao, 2021).

Preserving seed viability supports sustainable agricultural practices and contributes to global food security. Therefore, the development and protection of stress-resistant seeds remain a vital issue for the agricultural sector.

2. Stress Factors Affecting Seed Viability

Seed viability is of critical importance for the perpetuation of plant species and the continuity of agricultural production. However, environmental and biotic stress factors can negatively impact seed germination capacity, metabolic activity, and genetic stability. The following sections provide a detailed overview of abiotic and biotic stress factors affecting seed viability.

2.1. Abiotic Stress Factors

Abiotic stress factors refer to non-biological environmental conditions that influence seed viability.

2.1.1. Temperature Stress

Extreme temperatures (>35°C) disrupt the structural integrity of the seed coat, leading to protein denaturation and reduced enzymatic activity. Lipid peroxidation causes irreversible damage to cell membranes, adversely affecting the germination process (Bewley et al., 2013). Conversely, low temperatures (<5°C) induce intracellular ice crystal formation and organelle damage, hindering seedling development (Hasanuzzaman et al., 2020).

2.1.2. Drought Stress

Water deficiency limits seed water uptake, ultimately disrupting metabolic processes such as respiration and enzymatic activity. Drought conditions promote the accumulation of reactive oxygen species (ROS), leading to damage in DNA and protein structures (Farooq et al., 2017).

2.1.3. Salinity Stress

High salt concentrations lower the osmotic potential in the seed environment, restricting water absorption. Sodium (Na^+) and chloride (Cl^-) ions interfere with cellular ionic balance, leading to enzyme inhibition and metabolic dysfunction (Munns & Tester, 2008).

2.1.4. Oxidative Stress

Excessive ROS production overwhelms the antioxidant defense system, causing oxidative damage to lipids, proteins, and DNA. This results in reduced germination rates and delayed seedling growth (Bailly, 2004).

2.1.5. Heavy Metal Stress

Heavy metals such as cadmium (Cd), lead (Pb), and arsenic (As) inhibit enzymatic functions and induce mitochondrial dysfunction in seeds. Additionally, they hinder cell division, thereby suppressing germination (Rajjou et al., 2012).

2.2. Biotic Stress Factors

Biotic stress factors involve the negative effects of living organisms on seed viability.

2.2.1. Fungal, Bacterial, and Viral Diseases

Fungal pathogens such as Fusarium and Aspergillus produce mycotoxins that can lead to embryo mortality. For instance, aflatoxin production disrupts the biochemical composition of seeds (Agrios, 2005). Bacterial pathogens like Pseudomonas and Xanthomonas secrete exopolysaccharides that hinder water absorption, negatively affecting germination (Maude, 1996). Seed-borne viruses, such as the Tobacco Mosaic Virus (TMV), replicate within embryo cells, disrupting gene expression (Mandahar, 1990).

2.2.2. Insect and Nematode Pests

torage pests (e.g., Sitophilus spp.) cause physical damage to the seed endosperm, leading to nutrient loss and creating favorable conditions for microbial infestations (Dhaliwal et al., 2015). Similarly, nematodes of the Meloidogyne genus form galls in the root zone, obstructing water and nutrient uptake, thereby suppressing seedling growth (Jones et al., 2013).

Seeds can employ various adaptive mechanisms to protect themselves against stress conditions. These mechanisms include externally applied priming techniques, mycorrhizal symbiosis, and gene expression regulation. Priming treatments regulate seed hydration-dehydration cycles, enhancing antioxidant capacity. Mycorrhizal fungi establish a symbiotic relationship with plants, mitigating the adverse effects of heavy metal and salt stress. Additionally, heat shock proteins synthesized under stress conditions help prevent cellular damage, thereby preserving seed viability and germination potential (Bewley et al., 2013; Paparella et al., 2015; Smith & Read, 2008).

3. Strategies for Preserving Seed Viability

3.1. Physical and Chemical Protection Methods

3.1.1. Seed Coating Technologies

Seed coating is a technique aimed at enhancing resistance to stress factors encountered before and after germination by applying a protective layer to the seed surface. Currently, biopolymers (such as chitosan) and microencapsulation techniques are widely used. Chitosan coatings exhibit antifungal properties, helping to protect seeds against soil-borne pathogens (Farooq et al., 2020). Additionally, hydrogel layers can enhance water retention capacity, thereby mitigating the effects of drought stress (Sánchez-González et al., 2022).

3.1.2. Seed Priming Techniques

Seed priming is a pre-treatment technique designed to accelerate germination and enhance resistance to stress conditions by controlling water uptake or applying specific chemical agents. These methods activate the metabolic processes of seeds, ensuring faster and more uniform germination. Some commonly used seed priming techniques include:

Hydropriming

Hydropriming involves exposing seeds to water or a controlled humidity environment for a specific period, followed by drying. This method

initiates metabolic processes while regulating early water uptake, providing protection against excessive water stress or oxidative damage. Through controlled hydration-dehydration cycles, seeds strengthen their antioxidant defense systems to cope with stress before germination.

Benefits:

- Reduces oxidative stress by enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Paparella et al., 2015).
- Increases germination speed and improves germination rate.
- Simple to apply and cost-effective.

Limitations:

The effectiveness of hydropriming may be limited in some seed species, and prolonged exposure can lead to excessive water uptake, accelerating seed aging.

Osmopriming

Osmopriming involves pre-treating seeds in osmotic solutions with low water potential for a specific period. Commonly used osmotic agents include polyethylene glycol (PEG), mannitol, and salt solutions such as KNO_3 and $CaCl_2$. This method regulates water uptake in a controlled manner, initiating embryo development while preventing excessive water influx. As a result, osmopriming minimizes the negative effects of osmotic stress during germination.

Benefits:

- Enhances resistance to salt and drought stress. For instance, osmopriming with PEG can increase germination rates by up to 30% in seeds exposed to salt stress (Hussain et al., 2021).
- Stimulates metabolic activity, supporting embryo development.
- Promotes uniform germination and accelerates seedling growth.

Limitations:

The substances used in osmopriming solutions may leave residues on the seed coat, and improper dosage or treatment duration can result in toxic effects.

Hormonal Priming

Hormonal priming is a pre-treatment technique that involves the use of plant growth regulators (phytohormones). Commonly used hormones in this method include salicylic acid (SA), gibberellic acid (GA_3), abscisic acid (ABA), and jasmonic acid (JA). These plant hormones enhance stress resistance and promote seed development by activating cellular signaling pathways during germination (Rajjou et al., 2019).

Benefits:

- **Salicylic acid (SA):** Reduces oxidative stress by increasing antioxidant enzyme levels, thus improving seed germination under stress conditions.
- **Gibberellic acid (GA_3):** Breaks dormancy and accelerates embryo development, promoting faster germination.
- **Abscisic acid (ABA):** Enhances stress tolerance by providing protection against extreme drought and salt stress.
- **Jasmonic acid (JA):** Activates defense-related genes, improving seed resilience to pathogens and environmental stressors.

Limitations:

If not applied at appropriate doses, hormonal priming may inhibit germination. Additionally, the cost and precise application requirements of hormones make their widespread use impractical for all seed types.

These priming techniques can effectively enhance seed germination capacity under different stress conditions. The application method and duration should be carefully determined based on the seed species and the targeted stress factor.

3.1.3. Enhancing Seed Viability with Chemical Treatments

Various chemical compounds are used to maintain seed viability and improve germination rates. These compounds act through different mechanisms, such as reducing oxidative stress, regulating seed metabolism, and

providing protection against pathogens. Chemical treatments are widely preferred, especially for optimizing seed quality during long-term storage or under stressful growing conditions.

Gibberellic Acid (GA_3): Gibberellic acid (GA_3) is a crucial phytohormone that regulates plant growth and development. It plays a critical role in seed germination by breaking dormancy, stimulating enzyme production, and accelerating embryo development. GA_3 is widely used to initiate or enhance germination, particularly in seeds with dormancy. The application typically involves soaking seeds in GA_3 solutions for a specified period. For seeds with low dormancy, 25-50 ppm GA_3 is generally effective. For species with deep dormancy, 100-500 ppm GA_3 can significantly improve germination rates.

Salicylic Acid (SA): Salicylic acid (SA) mitigates oxidative stress by scavenging reactive oxygen species (ROS) and maintaining the stability of cell membranes, thereby enhancing seed tolerance to salinity, drought, and temperature stress (Khan et al., 2020). SA can be applied as a priming treatment at low concentrations (0.5–1 mM) in solution form.

Proline: Proline contributes to osmotic balance, thereby enhancing resistance to water stress. Additionally, it supports intracellular protein stabilization, improving seed tolerance to drought and salinity stress (Ashraf & Foolad, 2007). Proline treatment involves soaking seeds in solutions containing 25–200 mg/L proline for a specific duration.

Glycine Betaine: Glycine betaine plays a crucial role in cellular water retention mechanisms, offering protection against drought and salinity stress (Chen & Murata, 2011). Seed priming with glycine betaine is typically performed using solutions with concentrations ranging from 10 to 50 mM.

3.1.4. Antioxidant Applications

Antioxidants play a critical role in preventing oxidative damage caused by free radicals, thereby delaying seed aging and enhancing germination rates.

Ascorbic Acid (Vitamin C): Ascorbic acid (AA) is essential in seed metabolism, preventing lipid peroxidation and mitigating the detrimental effects of oxidative stress. A study conducted by Paparella et al. (2015) demonstrated that seeds treated with ascorbic acid exhibited enhanced resistance to oxidative stress and improved germination rates. It can be applied through seed coating or priming, particularly for seeds exposed to salinity and drought stress.

Tocopherol (Vitamin E): Tocopherol inhibits lipid peroxidation, thereby protecting cell membranes from oxidative stress. Research on seed aging has shown that seeds treated with tocopherol maintain higher germination rates (Rajjou & Debeaujon, 2008). This application typically involves soaking seeds in tocopherol-enriched solutions before storage.

Glutathione (GSH): Glutathione neutralizes reactive oxygen species (ROS) and supports the cellular detoxification system. Studies have indicated that glutathione treatments contribute to the rejuvenation of aged seeds. It can be applied through pre-treatment with glutathione solutions or by incorporating it into seed coatings.

3.1.5. Seed Storage Conditions

Proper seed storage conditions are crucial for maintaining germination potential and ensuring seed viability over time. Environmental factors such as temperature, humidity, oxygen levels, and light directly influence seed biological activity, ultimately affecting germination rate, seedling emergence, and longevity.

a- **Temperature** **:** Influences seed aging and metabolic activity.
b- **Humidity Levels** **:** Regulates oxidative stress and microbial growth.
c- **Oxygen Levels :** Determines metabolic activity and seed aging.
d- **Light Conditions** **:** Can affect dormancy through photoreceptors.

Controlling these factors is essential for preserving seed viability and germination capacity.

For long-term viability, seeds should be stored under low humidity conditions (5–8%) and at cool temperatures (4–10°C). Controlled atmosphere storage (O_2 <1%, CO_2 <5%) can prevent lipid peroxidation (Walters et al., 2020). Additionally, vacuum packaging and the use of silica gel can regulate moisture levels, extending seed shelf life (Ellis & Hong, 2021).

3.2. Biotechnological and Nanotechnological Applications

3.2.1. Nanoemulsions and Nanofertilizers

Nanoemulsions: Lipid-based nanoparticles facilitate the controlled release of antifungal and antibacterial agents, thereby enhancing seed health. For instance, nano-chitosan applications have been reported to suppress Fusarium infections by up to 70% (Kah et al., 2018).

Nanofertilizers: Iron oxide (Fe_3O_4) and zinc oxide (ZnO) nanoparticles contribute to seedling development by enhancing the stability of essential micronutrients in the soil (Rai et al., 2021).

3.2.2. Genetic Modification and Stress Tolerance

Genetic engineering techniques enable the modification of seed traits to improve resistance against drought, salinity, and temperature stress.

CRISPR-Cas9 Technology: Modifications in Late Embryogenesis Abundant (LEA) genes enhance seed resistance to water loss, thereby improving agricultural productivity (Gao, 2021; Zhang et al., 2022).

Transgenic Approaches: The expression of Heat Shock Protein 70 (HSP70) genes helps maintain protein stability in seeds exposed to high-temperature stress (Wang et al., 2020).

Microbial Gene Transfer: The introduction of Bacillus subtilis-derived genes can stimulate the synthesis of osmolytes, thereby increasing seed tolerance to salinity (Vílchez et al., 2023).

The preservation of seed viability is of strategic importance in the context of climate change and rising global food demand. While physical and chemical treatments offer practical solutions, biotechnological and nanotechnological approaches provide targeted interventions to enhance efficiency. However, concerns regarding the environmental impact of nanomaterials and the ethical and regulatory aspects of genetic modification necessitate further research in these areas.

4. Biological Approaches to Enhancing Stress Tolerance

Plants exposed to environmental stress factors such as drought, salinity, and extreme temperatures often experience substantial losses. Consequently, biological approaches to improving plant stress tolerance are gaining increasing attention.

4.1. Microbial Applications

4.1.1. Effects of Arbuscular Mycorrhizal Fungi (AMF) on Seed Viability

Arbuscular mycorrhizal fungi (AMF) establish symbiotic associations with plant roots, enhancing nutrient and water uptake. This interaction strengthens plant resilience against stress conditions such as drought and salinity. AMF fungi have been particularly noted for their ability to enhan-

ce phosphorus and nitrogen absorption, promote plant growth, and improve root system development, thereby facilitating water uptake (Smith & Read, 2008; Gianinazzi et al., 2010).

4.1.2. Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria (PGPR) reside in the root zone and contribute to plant stress tolerance by producing phytohormones, enhancing nutrient availability, and protecting plants against pathogens. PGPR have been shown to improve plant resilience under drought, salinity, and heavy metal toxicity conditions (Glick, 2012; Vessey, 2003).

4.2. Plant Extracts and Biostimulants

Biostimulants and plant-derived extracts are increasingly explored as eco-friendly solutions to improve plant stress tolerance. These substances enhance nutrient absorption, boost antioxidant defenses, and regulate plant physiological responses under adverse environmental conditions.

4.2.1. Seaweed Extracts

Seaweed extracts contain plant growth-promoting hormones, amino acids, and essential minerals. Research has demonstrated that seaweed extracts enhance plant tolerance to environmental stresses such as drought and salinity (Craigie, 2011; Khan et al., 2009).

4.2.2. Humic and Fulvic Acids

Humic and fulvic acids, which are formed through the decomposition of organic matter, improve nutrient uptake and stimulate root development, thereby enhancing plant stress tolerance. Studies have indicated that these compounds provide significant protection against drought and heavy metal toxicity (Stevenson, 1994; Calderón & Clapp, 2001).

4.2.3. Plant-Based Antioxidant Applications

Reactive oxygen species (ROS) accumulate in plants under stress conditions, leading to cellular damage. Antioxidants help neutralize ROS, thereby improving plant stress tolerance (Mittler, 2002; Foyer & Noctor, 2005).

5. Scientific Studies on Seed Viability and Their Findings

Seed viability is a critical factor in agricultural production and the conservation of natural ecosystems. Understanding the factors that influence seed viability and the strategies to maintain it is essential for sustainable agriculture and biodiversity conservation.

5.1. Studies on Seed Viability Under Different Stress Conditions

Drought Stress: Drought is one of the most significant stress factors affecting seed germination and seedling development. Studies have shown that seeds from drought-tolerant plant species exhibit higher viability rates under drought stress conditions. Additionally, some research has reported that seeds exposed to drought stress exhibit increased antioxidant enzyme activity, which reduces cellular damage (Osakabe et al., 2014).

Salinity Stress: Salinity is another major stress factor threatening seed viability, particularly in arid and semi-arid regions. Saline soils hinder water uptake by seeds, leading to reduced germination rates. Studies have indicated that seeds from salt-tolerant plants are more resistant to salinity stress due to their ability to maintain ion homeostasis and employ osmotic adaptation mechanisms (Flowers & Colmer, 2015; Munns & Tester, 2008).

Temperature Stress: High temperatures can adversely affect seed viability by disrupting protein structures and increasing membrane permeability. Research suggests that seeds from heat-tolerant plant species are protected against high-temperature stress through the production of heat shock proteins and increased antioxidant capacity (Hasanuzzaman et al., 2013; Wahid et al., 2007).

5.2. Application of Seed Preservation Strategies in Various Plant Species

Seed Coating and Pre-Treatments: Seed coating involves covering seeds with protective materials to enhance their resistance to stress conditions. Pre-treatments refer to specific processes applied to seeds before germination to improve their viability. For instance, coating seeds with arbuscular mycorrhizal fungi (AMF) or plant growth-promoting rhizobacteria (PGPR) can enhance nutrient uptake and increase stress tolerance (Taylor et al., 1998).

Conservation of Genetic Diversity: Preserving seeds with diverse genetic traits enhances plant adaptation to stressful environmental conditions. Seed banks play a crucial role in maintaining genetic diversity by pre-

serving rare and indigenous plant species, thereby contributing to the long-term sustainability of agricultural and ecological systems (FAO, 2010).

5.3. Seed Viability in Sustainable Agriculture and Future Perspectives

Ensuring seed viability is a crucial factor for the long-term sustainability of agricultural systems. Climate change, biodiversity loss, and technological advancements are dynamic factors that must be considered in seed storage, germination capacity, and productivity.

Adaptation to Climate Change: Climate change introduces multiple challenges, including rising temperatures, irregular precipitation patterns, drought, salinization, and the spread of plant diseases. Maintaining seed germination capacity and viability under these changing conditions is critical for sustainable agriculture. Strategies for preserving seed viability should support plant adaptation to the adverse effects of climate change (Altieri, 2009; Tester & Langridge, 2010).

Conservation of Biodiversity: Sustainable agriculture is not solely dependent on increasing productivity but also on preserving local and traditional seed varieties. The decline in biodiversity poses a significant threat to global food security. Protecting indigenous and heirloom seed varieties plays a fundamental role in maintaining sustainable agricultural systems and ensuring genetic diversity.

Technological Advancements: Technological innovations offer significant opportunities to enhance seed viability and strengthen plant resilience to environmental stresses. Genome Editing Technologies: Techniques such as CRISPR-Cas9 enable the development of seed varieties with improved resistance to drought, salinity, and diseases, thereby contributing to more sustainable agricultural production. Nanotechnology: Nano-fertilizers and nano-coating techniques optimize nutrient uptake, increase germination rates, and accelerate seedling growth. Microbial Applications: Beneficial microorganisms, such as Trichoderma and Bacillus species, play a crucial role in biological control by protecting seeds from pathogens and enhancing plant health. Artificial Intelligence and Sensor-Based Technologies: AI-driven systems and sensor technology optimize seed storage conditions, ensuring long-term seed viability by regulating temperature, humidity, and other critical environmental factors. These innovative approaches hold significant potential for ensuring global food security and promoting sustainable agricultural practices.

6. Conclusion

This study clearly demonstrates that seed viability holds vital importance for the future of our planet. Seeds not only form the foundation of agricultural production but also play a crucial role in preserving natural ecosystems, maintaining biodiversity, and ensuring food security. However, in today's world, seed viability is under serious threat due to various abiotic and biotic stress factors.

The increasing impacts of climate change, such as heat stress, drought, salinity, and other environmental challenges, negatively affect seed germination ability and overall viability. In addition, biotic stress factors, including fungal, bacterial, and viral diseases, as well as insect and nematode pests, hinder the healthy development of seeds. To overcome these challenges, it is essential to develop and implement strategies for preserving seed viability. Traditional approaches, such as physical and chemical protection methods, seed coating technologies, priming techniques, and chemical treatments to enhance seed viability, remain significant. Moreover, biotechnological and nanotechnological applications offer promising solutions for improving seed health.

Innovative approaches, including genetic modification, nanoemulsions, and nanofertilizers, have significant potential to enhance seed stress tolerance and productivity. Additionally, biological methods, such as microbial applications and plant extracts, strengthen the natural defense mechanisms of seeds, increasing their resistance to stress conditions.

Scientific research provides valuable insights into seed viability under different stress conditions, guiding the development and implementation of seed protection strategies. Sustainable agricultural practices play a key role in maintaining seed viability and preserving biodiversity.

Future research should focus on developing new approaches to protect seed viability. Advancements in fields such as molecular biology, genetic engineering, nanotechnology, and artificial intelligence hold great potential for improving seed health and increasing stress tolerance.

In conclusion, preserving seed viability is an indispensable priority for the future of humanity. The integration of science, technology, and sustainable agricultural practices will enable the effective protection of seeds, ensuring food security, safeguarding biodiversity, and leaving a more livable world for future generations.

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CHAPTER 2

EFFECT OF THIDIAZURON (TDZ), GIBBERELLIC ACID (GA_3) AND I-NAPHTHALENEACETIC ACID (NAA) ON CLONAL SEED (SYTHETIC SEED) PRODUCTION VIA SOMATIC EMBRYOGENESIS FROM LEAF EXPLANTS IN SOME BLUEBERRY VARIETIES

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Introduction

Blueberries (Vaccinium corymbosum L., V. angustifolium Ait., V. ashei Reade; Ericaceae) are perennial, dicotyledonous angiosperms that belong to the genus Vaccinium. These plants exhibit a wide range of growth habits, from small, dwarf forms to medium and tall shrub-like structures (Vander Kloet, 1988; Kepenek, 2020). The Vaccinium genus includes approximately 400 species, with at least one species being native to every continent except Antarctica and Australia, as well as various remote islands (Vander Kloet, 1988). These plants thrive in acidic, sandy, peaty, or organic soils and are well adapted to environments characterized by an average annual temperature of 16°C, with the warmest periods occurring in March and April. They also prefer regions receiving 500–600 mm of annual rainfall and situated at altitudes of around 800 meters.

There are five primary categories of blueberries, each adapted to specific climatic conditions: the large bush type (Vaccinium corymbosum L.) found in northern regions, the tall bush type (a hybrid of Vaccinium corymbosum and Vaccinium darrowii Camp) suited to southern climates, the dwarf bush (Vaccinium angustifolium Ait.), the semi-dwarf type (Vaccinium corymbosum × Vaccinium angustifolium), and the rabbiteye blueberry (Vaccinium ashei Reade) (Vander Kloet, 1988). The semi-dwarf varieties arise from a hybridization between large bush and dwarf bush blueberries, offering a combination of adaptability and high fruit quality (Debnath, 2016). These berries have gained widespread popularity due to their superior nutritional and sensory properties, making them a preferred choice among consumers.

The commercial production of blueberries largely depends on wild populations, primarily located in North America, Asia, and Northern Europe. However, on a global scale, large-scale agricultural blueberry production remains in its early stages (Ballington, 2001). Despite this, blueberries have emerged as a high-value crop, with substantial cultivation in Latvia, Poland, Austria, Germany, and Russia, while the United States and Canada dominate global production (Debnath, 2016; Kepenek, 2020).

The large-scale cultivation of blueberries dates back to the early 19th century, and since then, the fruit has gained prominence in both fresh and processed markets. Blueberries are widely consumed in their natural form, as well as in frozen and processed varieties, including canned products, yogurts, juices, jams, and jellies. Furthermore, their extracts have become a valuable component of functional foods and dietary supplements due to their rich phenolic content and strong antioxidant properties (Prior et al., 1998; Howell et al., 2001). The bioactive compounds present in blueberries have been extensively studied for their potential role in mitigating chronic

diseases such as cancer, cardiovascular ailments, and neurodegenerative disorders (Ames et al., 1993; Adams et al., 2010). These health-promoting effects are primarily linked to their capacity to counteract free radicals, which contribute to oxidative stress and related diseases (Macheix Mazur et al., 1991; Wang et al., 1996; Mazur et al., 2000; Ehlenfeldt and Prior, 2001).Blueberry plants exhibit genetic heterozygosity, making vegetative propagation the preferred method for preserving desirable genetic traits and ensuring rapid fruit production. While cuttings from softwood and hardwood remain a common propagation technique, this method is labor-intensive, slow, and dependent on various factors, such as genotype, plant age, and seasonal conditions (Gajdošova et al., 2006). Moreover, conventional propagation methods are often limited in their ability to produce pathogen-free planting material in large quantities. These challenges have driven the adoption of in vitro propagation techniques, which enhance propagation efficiency and improve overall plant quality. Studies have shown that blueberry plants propagated via tissue culture exhibit greater plant crown volume (habitus) and higher fruit yields compared to those propagated through traditional cutting methods (El-Shiekh et al., 1996; Kepenek, 2020).

In recent decades, notable advancements have been achieved in the micropropagation of blueberries, particularly through axillary shoot proliferation. Studies have extensively investigated the influence of cytokinins and various growth media on shoot development efficiency (Lyrene, 1980; Jiang et al., 2009; Zhou et al., 2023). Additionally, research has demonstrated successful adventitious shoot regeneration from leaf explants in multiple blueberry cultivars, highlighting the potential of this technique for large-scale propagation (Gajdošova et al., 2006; Cao & Hammerschlag, 2002; Meiners et al., 2007; Zhou et al., 2023). Moreover, the role of auxins in promoting both axillary and adventitious shoot growth has been explored, with findings indicating their significant contribution to shoot induction and elongation (Gonzalez et al., 2000; Meiners et al., 2007; Litwinczuk & Wadas-Boron, 2009; Kepenek, 2020; Zhou et al., 2023).Technological advancements in bioreactor-based micropropagation have further enhanced efficiency and cost-effectiveness in blueberry propagation. Although micropropagation in liquid bioreactors presents opportunities for large-scale production, its success is dependent on a thorough understanding of the plant's physiological and biochemical responses to the culture environment (Debnath, 2011; Kepenek & Karoğlu, 2016). While bioreactors hold great promise for commercial-scale propagation, further optimization is needed to balance cost efficiency and plant quality.

Despite the success of stem cuttings as a propagation method, they are often time-consuming and require intensive labor. In contrast, in vitro

micropropagation techniques provide an efficient, rapid, and scalable alternative that enables year-round production of high-quality plants (Debnath, 2018). These methods can be categorized into axillary bud proliferation, direct somatic embryogenesis, and adventitious shoot formation, depending on the desired propagation approach (Steward et al., 1970; Zimmerman, 1993).

Recent studies have also focused on genetic transformation and somaclonal variation, which provide new avenues for breeding improved blueberry cultivars (Debnath & McRae, 2001a, b). Methods involving Thidiazuron (TDZ)-induced somatic embryogenesis have demonstrated potential in promoting both shoot proliferation and adventitious shoot formation (Debnath, 2017). Furthermore, bioreactor-based propagation methods, particularly those involving liquid culture systems, continue to be explored for their potential to automate and optimize large-scale production (Levin & Vasil, 1989; Kepenek & Karoğlu, 2016; Zhou et al., 2023).

Blueberries continue to gain global recognition due to their nutritional value, health benefits, and increasing commercial demand. However, efficient propagation techniques remain essential for sustaining and expanding commercial blueberry cultivation. While traditional propagation via stem cuttings remains widely used, micropropagation techniques offer significant advantages in terms of efficiency, scalability, and disease-free plant production. Future research should focus on further refining bioreactor-based propagation methods, optimizing plant growth regulators, and exploring genetic improvements to enhance blueberry production worldwide.

Materials and Methods

Plant material

Mature plants of ‘Chandler’, ‘Patriot’, ‘Bluecrop’ and ‘Duke’ grown in pots containing Peat soil + Farmyard manure + Garden soil mixture mortar in the open greenhouse located in the field of Isparta Applied Sciences University, Faculty of Agriculture, Isparta were used as explant source. Each plant was planted in acidic peat bed with 10 liters of peat volume per plant and pH 3.8 (Kepenek, 2020).

Establishment of aseptic cultures

The four semi-dwarf blueberry cultivars examined in this study—Chandler, Patriot, Bluecrop, and Duke—were cultivated and managed under greenhouse conditions. The blueberry cultivars were grown in plastic

pots (12.5 × 12.5 × 15.5 cm) containing peat:1 perlite (v/v) (Prior et al., 1988; Kepenek, 2020; Zhou et al., 2023) under a natural light source with a maximum light intensity of 90 µmol m^{-2} s^{-1}, a temperature of 20 ± 2 °C, a relative humidity of 85% and an automatic irrigation system (Kepenek and Kolağası, 2016; Kepenek, 2019; Kepenek, 2020).

Surface sterilization of explants

Young shoot-shaped branches were collected from actively growing plants in the greenhouse and cut with their leaves. The leafy shoot tips taken from the plants were washed with detergent and rinsed under running tap water for 30 minutes. For sterilization, the samples were first washed under running water for 1 hour, followed by immersion in 70% ethanol for 2 minutes. They were then treated with a 0.1% mercury chloride (HCl_2) solution containing three drops of Tween-20 for 6 minutes. Finally, the sterilization process was completed by rinsing them three times with sterile water for a total of 15 minutes (Kepenek, 2020).

Preparation of nutrient medium

The basic nutrient medium originally developed by Debnath and McRae (2001) was later modified by Kepenek (2020) to optimize nutrient composition for blueberry somatic embryo and clonal seed production. In this modified version, certain micro- and macronutrients were used at half-strength, ensuring a balanced nutrient supply for in vitro propagation (Kepenek, 2020) (Table 1). The KK-2023 nutrient medium, specifically formulated as a shoot initiation and production medium, was employed for the efficient generation of somatic embryos and clonal seeds (Kepenek, 2020).

For this study, which focused on clonal seed production from somatic embryos, both petri dish cultures and bioreactor systems were utilized. The KK-2023 nutrient medium was supplemented with 2 mg/L CPPU, 10 g sucrose, 1.25 g Gelrite™, and various plant hormones, including gibberellic acid (GA3) at 1.5, 3.0, 4.0, and 6.0 µM, 1-naphthaleneacetic acid (NAA) at 3.0, 5.0, and 7.0 µM, and thidiazuron (TDZ) at 3.0, 5.0, 7.0, 10.0, or 12.0 µM. To achieve the final medium composition, double-distilled water was added to the solution, which was then heated until completely dissolved.

The pH of the medium was adjusted to 5.0 using 1N HCl and KOH prior to autoclaving. The sterilization process was conducted at 121 °C under 1 kg-1.cm-2 (15 psi) pressure for 20 minutes to ensure a sterile environment suitable for plant tissue culture. Since certain components, such as

vitamins, GA3, and TDZ, are heat-labile, they were filter-sterilized separately before being incorporated into the medium after autoclaving (Nissen et al., 1990). In contrast, NAA was directly added to the medium prior to autoclaving, as its stability allowed for this process.

The concentrations of GA3, NAA, and TDZ used in the KK-2023 medium remained consistent across both petri dish cultures and bioreactor systems, ensuring standardized growth conditions for the development of somatic embryos and clonal seeds (Kepenek & Kolağası, 2016; Kepenek, 2020).

Table 1. Micro and macro salts and their amounts (mg/L) in the KK-2023 nutrient medium modified by **Kepenek (2020)** *of the basic nutrient medium of Debnath and McRae (***Debnath and McRae, 2001***) for the in vitro somatic embryo (SE) induction and somatic embryo production (clonal seed production) of some blueberry cultivars (Chandler, Patriot, Bluecrop and Duke).*

Components		KK-2023 composition of the nutrient medium (mg/L)	Bileşenler		KK-2023 composition of the nutrient medium (mg/L)
Macronutrients	NH_4NO_3	412	Micronutrients	$MnSO_4.4H_2O$	11
	$CaCl_2.2H_2O$	110		$ZnSO_4.7H_2O$	4.3
	$Ca(NO_3)_2.4H_2O$	205		Boric acid H_3BO_3	3
	KNO_3	475		Potassium iodide KI	0.4
	$KH_2.PO_4$	164		$CuSO_4.5H_20$	0.012
	K_2SO_4	70		$Na_2MoO_4.2H_2O$	0.12
	$MgSO_4.7H_2O$	185		$CoCl_2.6H_2O$	0.012
	$NaH_2.PO_4.H_2O$	50		Na_2-EDTA	19
	$(NH_4)_2.SO_4$	25	Vitamins	Myo -İnositol	100
Other additives	Glycine	1		Thiamine-HCl	0.6
	Casein Hydolysate	40		Nicotic Acid	0.4
	Adenine sulfate dihydrate	15		Pyridoxine-HCl	0.4
	Calcium gluconate	1300			

Effect of plant hormone (GA_3, NAA and TDZ) types and concentrations on maturation and formation of somatic embryos

For the formation, maturation, and subsequent growth of plants derived from somatic embryos, 10-week-old embryos that had developed

in nutrient medium within petri dishes containing 10.0 μM TDZ were transferred to 500 mL bioreactors. These bioreactors contained 80 mL of KK-2023 nutrient medium, supplemented with varying concentrations of gibberellic acid (GA3) (1.5, 3.0, 4.0, and 6.0 μM), naphthaleneacetic acid (NAA) (3.0, 5.0, and 7.0 μM), and thidiazuron (TDZ) (3.0, 5.0, 7.0, 10.0, and 12.0 μM).

To maintain hormonal stability during preparation, GA3 and TDZ, which are heat-sensitive, were filter-sterilized and added to the cooled nutrient medium after autoclaving, whereas NAA, being more heat-resistant, was incorporated before autoclaving. Each treatment condition was tested using four bioreactors, with ten explants placed in each bioreactor, ensuring adequate replication for statistical analysis. The experiment followed a completely randomized factorial design, encompassing four different blueberry cultivars and 12 hormone concentration treatments, alongside hormone-free controls (Kepenek & Kolağası, 2016; Kepenek, 2020).

Following transfer, the cultures were maintained under controlled climatic conditions to support shoot and root elongation. The bioreactors were placed in a climate chamber, where the plantlets were exposed to a 16-hour photoperiod under 60 μmol m^{-2} s^{-1} PPFD light intensity. To assess developmental progress, data on embryo maturation and plantlet elongation were collected eight weeks post-transfer. These observations provided insights into the effects of various hormone combinations on blueberry somatic embryo development and subsequent plant growth.).

Influence of TDZ Concentration on Somatic Embryogenesis in Blueberry Cultivars

For the induction of somatic embryo formation, leaves from 2–3-week-old actively growing blueberry plants cultivated in a greenhouse were collected, subjected to surface sterilization, and then cultured in a modified KK-2023 medium, which was adapted from the methodology of Debnath and McRae (2001) (Kepenek, 2020). Thidiazuron (TDZ) was incorporated into the nutrient medium prior to autoclaving to optimize conditions for embryogenic development.

Leaf explants were placed in 100 × 25 mm petri dishes with transparent lids, each containing 25 mL of KK-2023 medium supplemented with TDZ at concentrations of 0 (control), 3.0, 5.0, 7.0, 10.0, and 12.0 μM. Explants were positioned with their abaxial (lower) surfaces in direct contact with the medium to facilitate nutrient uptake. To preserve sterility and prevent moisture loss, the petri dishes were wrapped with two layers of Parafilm™. The medium's pH was set to 5.0 prior to sterilization, and the

solution was subsequently autoclaved at 121°C for 20 minutes to ensure sterility.

Each treatment condition was tested using four petri dishes, with five leaf explants placed in each dish, ensuring adequate replication for statistical analysis. Initially, the cultures were kept in complete darkness at 23 ± 2°C for two weeks to stimulate somatic embryo induction. Following this dark incubation, they were transferred to a cultivation chamber, where they were maintained under 60 µmol m^{-2} s^{-1} PPFD light intensity, provided by LED lamps, with a 16-hour photoperiod to support further development.

To assess somatic embryo (SE) formation, data were recorded at two-week intervals over an eight-week cultivation period. A 4 × 5 randomized factorial design was implemented to evaluate the effects of five TDZ concentrations across four semi-dwarf blueberry cultivars, allowing for direct comparisons of treatment outcomes (Kepenek & Kolağası, 2016; Kepenek, 2020). In addition to petri dish cultures, this experiment was concurrently replicated in submerged bioreactors, following the same experimental design, to evaluate the efficiency of liquid-based systems for blueberry somatic embryogenesis..

In vitro growth conditions and subculture duration

The cultures were kept under regulated environmental conditions at 23–25°C ± 1°C, with a 16-hour light cycle and an illumination intensity of 60 µmol m^{-2} s^{-1}, supplied by white LED fluorescent lamps. These conditions were optimized based on the specific requirements of different blueberry varieties to support their development.

After eight weeks of cultivation, five plantlets measuring 4–5 cm in length and bearing 8–10 leaves were removed from the bioreactors from all TDZ-treated conditions. The selected plantlets were washed with sterilized water to eliminate any residual medium and subsequently transplanted into plastic pots (25 × 18 × 6 cm) filled with a peat-to-perlite mixture (3:1 v/v). However, plantlets grown on media containing NAA or GA3 did not undergo acclimation due to their poor development and inability to transition effectively to external conditions.

For acclimation, the pots were placed in a humidification room maintained at 23 ± 2°C, equipped with a cold steam generator, ensuring an initial relative humidity of 95%. The plantlets remained in this environment under a 16-hour photoperiod at 60 µmol m^{-2} s^{-1} PPFD for 2–3 weeks. As part of the acclimation process, humidity was gradually reduced to 85%, which was found to be an effective strategy for adapting tissue culture-de-

rived plantlets to external conditions in the growth chamber (Debnath, 2017; Kepenek, 2020).

Due to the underdeveloped cuticle, epicuticle, and cuticle wax layers, as well as delayed stomatal functionality, in vitro-propagated plantlets exhibit high transpiration rates through both stomata and cuticular surfaces during the acclimation phase. This excessive water loss poses a significant challenge to successful transition. To mitigate this issue, micropropagated plantlets must be gradually shifted from a high-humidity to a lower-humidity environment, allowing for progressive adaptation (Chandra et al., 2010).

To evaluate the effectiveness of the acclimation process, the survival rate of blueberry plantlets was recorded six weeks after their transition to ex vitro conditions. Following successful acclimatization, the plantlets were transferred to 6 cm plastic pots filled with a peat-to-perlite mixture (3:1 v/v) and cultivated under controlled greenhouse conditions. The greenhouse environment was maintained at a stable temperature of 23 ± 2°C, with a 16-hour photoperiod and a maximum photosynthetic photon flux density (PPFD) of 90 μmol m^{-2} s^{-1}. Additionally, relative humidity levels were regulated at approximately 85% to support optimal plant adaptation and growth (Debnath, 2017; Kepenek, 2020). These carefully maintained conditions facilitated the establishment of tissue culture-derived blueberry plantlets, promoting their continued development.

Statistical data analysis.

To ensure statistical reliability and reduce variability, all experimental treatments were arranged in a randomized design with four replications. The collected data were subjected to a two-way analysis of variance (ANOVA) using a general linear model to evaluate the primary effects. Statistical computations were carried out using Statistica version 10 (Statsoft Wipro, East Brunswick, NJ, USA), a well-established tool commonly utilized in biological research for data evaluation.

During the analysis, the hormone-free control medium was excluded, as it did not induce somatic embryo formation in any of the experimental conditions. Additionally, the 3.0 μM NAA treatment was determined to be ineffective for somatic embryo maturation across all four blueberry cultivars; therefore, the associated data were also removed from the final statistical evaluation.

For the comparison of treatment means, Tukey's Test was applied at a significance level of $P \leq 0.05$, allowing for the identification of statistically significant differences among treatments. The results of the analysis are

presented as means ± standard error, providing clarity on variability and treatment effectiveness.

Results

Effects of plant hormone type and concentration on maturation (formation) of somatic embryos

Somatic embryos with elongated shoots and well-developed apical root meristems, initially formed in KK2023 medium containing 10.0 µM TDZ, were transferred to the same medium supplemented with varying concentrations of GA_3, NAA, or TDZ to promote further maturation. The findings demonstrated that both the hormone-free medium and the 3.0 µM NAA treatment were ineffective in supporting somatic embryo maturation and development in all four blueberry cultivars. The detailed outcomes of the different hormone treatments are summarized in Table 2.

Statistical ANOVA analysis indicated a significant influence of hormone type and concentration ($P \leq 0.05$) on embryo maturation rates. Notably, GA_3 at 4.0 and 6.0 µM was particularly effective in enhancing embryo maturation, especially in the Duke and Patriot cultivars. In contrast, all other hormonal combinations contributed to improved embryo maturation across all tested cultivars, with the highest percentage recorded at 3.0 µM TDZ, ranging from 31.40% to 39.67%.

Conversely, the maturation rate of somatic embryos in KK2023 medium containing GA_3 and NAA was markedly lower, with values ranging from 1.22% to 8.29% for GA_3 and 4.91% to 16.46% for NAA. The seedlings that developed following maturation in GA_3- and NAA-containing KK2023 medium exhibited poor growth, and ultimately, failed to survive beyond two months of cultivation.

The elongation of root apical meristems originating from somatic embryos became evident seven weeks after culture initiation at 3.0 µM TDZ, with seedlings exhibiting vigorous root development after an additional month of culture (Figure 1). Following one month of growth in the maturation medium, seedlings reached heights of 4–7 cm, rendering them suitable for transfer into a peat-perlite medium to initiate acclimatization to external conditions.

Table 2. Effect of different hormone concentrations in starter cultures on somatic embryo formation and root development of four semi-dwarf blueberry cultivars.

Hormone concentration (µM)	Somatic embryo formation-maturation percentage			
	Chandler	Bluecrop	Patriot	Duke
GA_3				
0.0 (Control)	0.01 ± 0,00	0.00 ± 0,00	0.03± 0,00	0.05± 0,01
1.5	1,22 ± 0,39 st	1,43 ± 0,08 ^{s§}	1,30 ± 0,10 st	1,50 ± 0,31 st
3.0	2,45 ± 0,51 ^{s§}	2,67 ± 0,62 ^{s§}	2,92 ± 1,53 ^{s§}	2,72 ± 0,83 ^{s§}
4.0	5,71 ± 0,53 ^{rs}	6,91 ± 1,14 ^{pr}	4,55 ± 0,30 ^{rs}	6,63 ± 1,49 ^{pr}
6.0	6,83 ± 1,09 ^{pr}	5,49 ± 1,03 ^{rs}	8,17 ± 1,35 ^{op}	8,29 ± 1,24 ^{op}
NAA				
5.0	5,42 ± 1,12 ^{rs}	6,52 ± 1,55 ^{pr}	5,22 ± 0,99 ^{rs}	4,91 ± 1,05 ^{rs}
7.0	10,70 ± 1,39 ^{no}	13,18 ± 1,65 ^{mn}	12,98 ± 1,66 ^{mn}	11,51 ± 1,86 ^{no}
10.0	14,25 ± 1,73 ^{lm}	16,46 ± 0,91 ^{kl}	16,31 ± 1,70 ^{kl}	14,88 ± 1,03 ^{lm}
TDZ				
3.0	14,81 ± 1.80 ^{lm}	13,21 ± 1.73 ^{mn}	16,16 ± 1.11 ^{kl}	13,45 ± 1.95 ^{mn}
5.0	16,77 ± 0,91 ^{kl}	15,85 ± 0,47 ^{lm}	20,97 ± 0,83 ^{ij}	18,73 ± 1,49 ^{jk}
7.0	18,90 ± 1,26 ^{jk}	16,58 ± 0,95 ^{kl}	22,59 ± 0,96 ^{ii}	19,90 ± 1,16 ^{ij}
10.0	39,67 ± 1,36 ^{a}	31,40 ± 1,60 ^{ef}	37,74 ± 1,56 ^{ab}	35,61 ± 1,19 ^{bc}
12.0	22,53 ± 1,67 ^{ii}	20,69 ± 0,81 ^{ij}	18,43 ± 1,12 ^{jk}	21,86 ± 1,30 ^{ij}

Each treatment was planned with four replications. Somatic embryo formation data of the varieties were evaluated after four months of cultivation. Standard error associated with different letters indicates significant differences according to Tukey test at $P \leq 0.05$.

Effects of TDZ concentrations on somatic embryo induction

At all TDZ concentrations, distinct rectangular protrusions began forming along the leaf edges within the first 2–3 weeks of incubation in dark conditions. However, no embryo induction was observed in mediums lacking TDZ. Over time, these protrusions developed into spherical embryos within 4–5 weeks under light conditions. The transition to heart-shaped or torpedo-shaped embryos typically required five weeks. Notably, embryo formation predominantly occurred along the leaf margins, rather than in the central regions. By the eighth week, epicotyl development was evident in the formed embryos.

The most pronounced callus formation was observed 7–10 days after culture initiation in leaves grown in medium containing 10 µM TDZ. These calli were generally compact, spherical, and exhibited a light yellow color with minimal granular structures. The percentage of embryos formed within these calluses ranged from 73.34% in Chandler to 85.33% in Bluecrop, while the average number of somatic embryos per explant varied between 27.89% in Chandler and 32.28% in Duke (Figure 1; Table 3). By the fourth week, shoot and root initiation from embryo meristems became evident. The rooting rate of somatic embryos derived from leaf explants was consistently high, ranging from 83% to 93%. Additionally, bud formation following root initiation was vigorous, supporting rapid growth. However, mild stress symptoms were observed in some rooted embryos.

When analyzing the impact of TDZ concentrations on somatic embryo formation and maturation, the highest embryogenesis percentage was recorded at 10 µM TDZ for all cultivars:

- Chandler: 39.67%
- Patriot: 37.74%
- Duke: 35.61%
- Bluecrop: 31.40%

These results were statistically significant ($P \leq 0.05$). In contrast, 3.0 µM TDZ exhibited the lowest embryo formation percentages, with values ranging from 16.16% (Patriot) to 14.81% (Chandler). A clear trend was observed, where higher TDZ concentrations corresponded with increased somatic embryo formation, varying between 13.21% and 39.67% across all four cultivars (Table 3).

Statistical analysis demonstrated a significant interaction ($P \leq 0.05$) between cultivar type and TDZ concentration regarding embryo formation percentage in somatic embryogenesis (SE). Cultivar differences were also statistically significant ($P \leq 0.05$), with Duke exhibiting the highest SE rate (117.45%), followed by Patriot (115.72%), Bluecrop (112.56%), and Chandler (110.18%) (Table 3). The highest number of somatic embryos per explant was found in:

- Chandler (49.13% at 10 µM TDZ)
- Duke (48.96%)
- Patriot (47.84%)
- Bluecrop (46.64%)

These values differed significantly ($P \leq 0.05$). At 3.0 µM TDZ, embryo formation percentages remained low across all cultivars, ranging between 1.97% (Chandler) and 11.89% (Patriot) (Table 3).

Variance analysis (ANOVA) indicated that TDZ concentration was the only variable with a statistically significant influence on somatic embryo formation ($P \leq 0.05$). A pattern similar to the embryo formation percentage was observed, where the number of embryos per explant consistently increased with rising TDZ concentrations in all four blueberry cultivars (Table 3).

The lowest number of embryos per explant was recorded at 3.0 µM TDZ, with an average of 1.97 embryos per explant, while the highest count was observed at 10.0 µM TDZ, reaching 49.13 embryos per explant. Across all TDZ concentrations, embryo numbers per explant varied between 27.89 and 32.28, whereas at 10.0 µM TDZ, values ranged from 46.64 to 49.13 across the four cultivars (Table 3). These results highlight the dose-dependent influence of TDZ on somatic embryogenesis, reinforcing its role in promoting embryo induction and development.

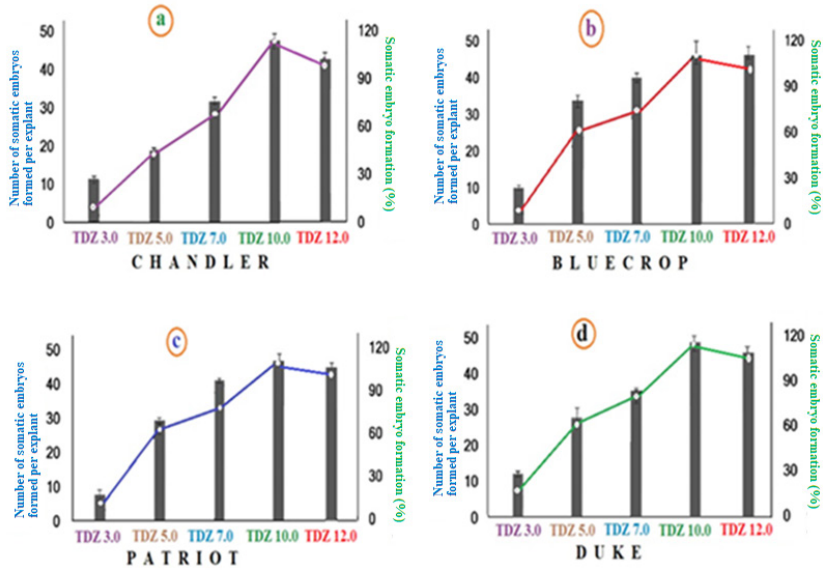

Figure 1. The impact of varying TDZ concentrations (µM) on somatic embryo formation in semi-dwarf blueberry cultivars after 10 weeks of culture. The primary axis (line graph) represents the average number of somatic embryos per explant, while the secondary axis (column graph) shows the percentage of explants forming embryos. Data are presented for the following cultivars: a) Chandler, b) Bluecrop, c) Patriot, and d) Duke.

Each treatment was replicated four times, and statistical significance was assessed using Tukey's test at $P \leq 0.05$.

Table 3. Effect of TDZ concentrations on the number of somatic embryos per plant part (explant) and embryo formation percentage in blueberry cultivars.

Variety	TDZ Dose (μM)	Number of somatic embryos per explant	Embryo formation percentage
Chandler	3.0	**1.97 ± 0.84 şt**	**29.46 ± 0.51 pr**
	5.0	**19,18 ± 0.25 lm**	**46.11 ± 0.69 op**
	7.0	**28.39 ± 0.63 ıi**	**75.63 ± 0.48 l**
	10.0	**49.13 ± 0.92 a**	**110.18 ± 1.17 de**
	12.0	**40, 80 ± 1.06 de**	**105.34 ± 1.56 gh**
	Average	**27,89**	**73,34**
Bluecrop	3.0	**4.63 ± 0.14 sş**	**25.60 ± 0.49 sş**
	5.0	**27.10 ± 0.39 ij**	**82.79 ± 0.22 kl**
	7.0	**33.72 ± 0.31 gh**	**97.39 ± 0.80 ıi**
	10.0	**46.64 ± 0.69 bc**	**112.56 ± 1.09 cd**
	12.0	**43.89 ± 0.99 cd**	**108.32 ± 1.82 fg**
	Average	**31,19**	**85,33**
Patriot	3.0	**11.89 ± 0.39 op**	**29.15 ± 0.71 rs**
	5.0	**19.79 ± 0.45 kl**	**54.81 ± 0.63 no**
	7.0	**29.53 ± 0.71 hı**	**83.69 ± 0.76 jk**
	10.0	**47.84 ± 0.67 ab**	**115.72 ± 0.85 bc**
	12.0	**42.90 ± 1.04 cd**	**99.89 ± 1.19 hı**
	Average	**30,39**	**76,65**
Duke	3.0	**8.33 ± 0.37 pr**	**30.94 ± 0.63 öp**
	5.0	**23.80 ± 0.70 jk**	**66.93 ± 0.50 mn**
	7.0	**34.56 ± 0.67 gh**	**87.12 ± 0.43 ij**
	10.0	**48.96 ± 0.89 ab**	**117.45 ± 0.78 a**
	12.0	**45.79 ± 0.92 bc**	**109.48 ± 1.01 ef**
	Average	**32,28**	**82,38**

Each treatment was planned with four replications. Somatic embryo formation data of the varieties were evaluated after four months of cultivation. Standard error associated with different letters indicates significant differences according to Tukey test at $P \leq 0.05$.

Acclimatization of seedlings to external conditions

Rooted plants measuring 4–6 cm, which were propagated in vitro, were transplanted into a substrate mortar mixture composed of 80% peat, 15% Agro-perlite, and 5% vermiculite. These plants were then acclimatized to in vivo conditions under greenhouse settings, where they were

regularly watered without requiring any special light treatment. The acclimatization process involved gradually reducing humidity levels through ventilation over a 2–4 week period. After this initial phase, the plants were removed from viola pots and transferred into larger pots (12 cm in diameter, 8–10 cm in height). By the end of two months, the acclimated seedlings were successfully transitioned to open-air conditions.

The new regenerant seedlings, obtained from somatic embryos that developed in semi-solid and liquid media, were derived from leaf explants cultivated under in vitro conditions in both bioreactors and petri dishes. These seedlings were successfully transferred from in vitro to ex vitro conditions and acclimated to the external environment. Notably, 80–90% of seedlings cultured in KK2023 medium supplemented with 3.0 μM TDZ survived upon transfer to outdoor conditions.

Additionally, seedlings obtained through direct rooting of micro-cuttings—derived from somatic embryos developed under in vitro conditions—were successfully transitioned into in vivo environments within misted greenhouse benches. These micro-cuttings, originating from shoot explants cultured in KK2023 medium containing 3.0 μM TDZ, bypassed separate rooting and acclimatization stages, enabling a rapid and efficient mass propagation process for blueberry plants. A comparative evaluation between these directly rooted seedlings and conventionally acclimated ones indicated high success rates, demonstrating the effectiveness of somatic embryogenesis for large-scale propagation.

Once transferred into plastic pots filled with a growing medium (80% peat, 15% Agro-perlite, 5% vermiculite) within greenhouse conditions, the seedlings exhibited strong adaptability to external conditions. Importantly, all plants survived, and those acclimated to outdoor environments displayed normal growth without any morphological variations, confirming the stability and viability of the micropropagated blueberry seedlings.

Discussion

This study aimed to establish an in vitro micropropagation system for blueberry seedlings derived from clonal embryos (synthetic seeds) obtained through somatic embryogenesis (SE) in selected semi-dwarf blueberry cultivars. The results demonstrated that TDZ played a crucial role in inducing somatic embryogenesis from leaf explants in a semi-solid medium. While earlier research by Cui et al. (2008) explored some preliminary aspects of somatic embryogenesis in blueberries, it lacked detailed findings on SE formation, highlighting the need for further investigation.

While thidiazuron (TDZ) is classified as a cytokinin, its effects in plant tissue culture extend beyond conventional cytokinin functions, influencing various aspects of in vitro development. Studies indicate that TDZ can exhibit both auxin- and cytokinin-like properties, playing a role in cellular differentiation and morphogenesis (Mok & Mok, 1985; Murthy et al., 1995, 1998). Despite its widespread use, the exact mechanism by which TDZ operates remains uncertain. Some researchers suggest that it may contribute to the synthesis or accumulation of endogenous plant growth regulators, thereby enhancing morphogenetic responses in cultured tissues (Mok & Mok, 1985; Radhakrishnan et al., 2009). Experimental evidence shows that the physiological effects of TDZ are concentration-dependent—higher concentrations encourage callus formation, shoot regeneration, and somatic embryo development, while lower concentrations primarily stimulate axillary bud proliferation. This response pattern is comparable to that of other N6-substituted cytokinins, including N-N-diphenylurea and N-(2-chloro-4-pyridyl)-N′-phenylurea (Mok & Mok, 1985; Huetteman & Preece, 1993).

In this study, the highest number and percentage of somatic embryos were observed at 10.0 μM TDZ following two months of in vitro culture. These results align with previous reports where higher TDZ concentrations (10–20 μM) were linked to direct somatic embryogenesis, as opposed to adventitious shoot regeneration, as demonstrated in pigeonpea (Singh et al., 2003). Similar patterns have been documented in grapes (Dhekney et al., 2016) and raspberry-blackberry hybrids (Rubus) (Fiola et al., 1990), where TDZ concentrations between 0.5–10 μM successfully induced somatic embryogenesis. Further supporting this trend, geranium (Pelargonium × hortorum) hypocotyl explants cultured in TDZ-enriched media (0.2–1 μM) exhibited somatic embryo formation within two weeks (Visser et al., 1992). Additionally, research on African violet (Saintpaulia ionantha) revealed that TDZ at 5–10 μM was more effective in promoting somatic embryogenesis compared to traditional cytokinins such as benzyladenine (BA) and N-(2-chloro-4-pyridyl)-N′-phenylurea (CPPU) (Mithila et al., 2003). These findings reinforce the dose-dependent influence of TDZ on somatic embryo induction, demonstrating its potential for optimizing micropropagation protocols in a variety of plant species. The study also revealed that somatic embryo formation rates varied among cultivars but generally increased with higher TDZ concentrations. This observation is consistent with earlier research on pigeonpea, where somatic embryo induction was genotype-dependent and required higher TDZ concentrations (10–20 μM) (Singh et al., 2003). In our study, maturation of somatic embryos was successfully achieved in KK-2023 medium supplemented with NAA and GA3. However, plantlets derived from these somatic embryos

exhibited poor growth and survival rates, a limitation also noted in geranium, where exogenous GA3 application negatively impacted somatic embryo formation by inhibiting key developmental stages (Hutchinson et al., 1997).

Auxin-type plant growth regulators, such as NAA and IBA, are commonly used to stimulate root induction in woody plants propagated in vitro or from cuttings (James, 1983). However, IBA is known to degrade under light exposure and undergoes photooxidation, leading to reduced effectiveness during prolonged tissue culture conditions (Nissen et al., 1990; Drew et al., 1991). Research indicates that when IBA-containing media are incubated in darkness for 28–30 days, the IBA concentration declines by 10–38%, potentially impacting its role in root development (Nissen et al., 1990; Drew et al., 1991). Furthermore, studies on Orchid Oncidium (Oncidium 'Gower Ramsey') revealed that IBA completely inhibited somatic embryo formation when incorporated into the nutrient medium (Chen & Chang, 2001).

Our findings indicate that TDZ and NAA effectively influenced both somatic embryo formation and maturation in blueberries. Lower TDZ concentrations were conducive to root formation, while higher TDZ levels primarily promoted shoot proliferation, hindering rooting ability—an effect previously reported by Gaspar & Coumans (1987). Although no direct studies have explored the impact of Zeatin or TDZ on multi-step embryogenesis, evidence suggests that these hormones influence root formation in various plant species. For example, Zeatin has been shown to enhance in vitro rooting in Bounty strawberry (Fragaria ananassa Duch.), particularly when applied at 1–2 μM concentrations (Debnath, 2006). Additionally, rooting without pretreatment has been observed in strawberry (Debnath, 2005b), cranberry (Qu et al., 2000), and blueberry under both in vitro and ex vitro conditions. Similarly, in soybean, the highest root formation rates were recorded in B5 nutrient medium supplemented with 3.5–4.6 μM TDZ (Radhakrishnan et al., 2009).

In this study, plantlets derived from somatic embryo regeneration were successfully transferred to the greenhouse, where full survival was achieved following acclimation to external conditions. These results are in agreement with Debnath (2017), who reported 80–90% survival rates in semi-dwarf blueberry plants propagated in vitro. Furthermore, our study and similar research confirm that somaclonal variation risk is significantly reduced when plants are generated through direct somatic embryogenesis, bypassing the callus phase (Singh et al., 2003).

This study provides comprehensive insights into the somatic embryogenesis process in semi-dwarf blueberry cultivars, emphasizing the role

of TDZ in embryo formation, maturation, and plantlet regeneration. The findings suggest that in vitro micropropagation using bioreactors offers a significant time advantage in seedling production and represents a valuable tool for large-scale commercial propagation of blueberries via somatic embryos..

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CHAPTER 3

REGULATION OF SEEDLING GROWTH AND MORPHOLOGICAL TRAITS IN TOMATO, POTATO, AND BANANA THROUGH PACLOBUTRAZOL APPLICATON

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Introduction

In Turkey, the cultivation of tomatoes, potatoes, and bananas has evolved considerably, particularly in response to advancements in seedling production techniques. Two key factors have influenced this transformation: the spread of pests and diseases into previously unaffected areas and the expansion of large-scale orchards for export markets. The adoption of micro-propagated seedlings, in vitro propagation methods, and growth regulators such as paclobutrazol has enabled the efficient production of seedlings with high phytosanitary standards in a relatively short period.

One of the fundamental challenges in seedling production is controlling plant size. During early development, plants allocate a substantial amount of their energy reserves to vegetative growth, which can deplete resources necessary for reproductive development. Redirecting these reserves toward reproductive growth while limiting excessive vegetative expansion—through the application of growth regulators—has been shown to enhance productivity and reduce variability in fruit-bearing cycles. Among these regulators, triazole-type plant growth retardants, such as paclobutrazol, have demonstrated effectiveness across various species (Jung et al., 1986).

Paclobutrazol acts as a potent inhibitor of gibberellic acid (GA3) biosynthesis, restricting GA3 synthesis in leaves and fruits (Aloni and Paskkar, 1987). This inhibition leads to reduced vegetative vigor, as evidenced in several plant species (Tukey, 1986; Li et al., 1989). Due to its slow metabolism in plants (Sterrett, 1985) and acropetal transport via the xylem, paclobutrazol accumulates in plant tissues, extending its effects over time (Lever, 1986).

The primary physiological responses to paclobutrazol application include reduced plant height and foliar area, shorter shoot length, increased chlorophyll concentration, and intensified green coloration. Unlike many other growth regulators, paclobutrazol remains effective whether applied as a foliar spray, soil drench, or incorporated into potting media. In horticultural crops, its application has successfully controlled excessive vegetative growth without compromising yield. A significant reduction in stem elongation has been reported, resulting in more compact plant architecture. Paclobutrazol's growth-inhibiting effects have been documented in species such as Lolium perenne (Hebblethwaite et al., 1982), tulips (Menhenett and Hanks, 1983), chrysanthemums (McDaniel, 1983), and carrots (Thomas et al., 1982). Similar effects have been observed in tomato (Brigard et al., 2006; Magnitskiy, 2004; Magnitskiy et al., 2006), banana (El-Otmani et al., 1992; Maia et al., 2009), and potato (Tekalign et al., 2005). Additionally, paclobutrazol has been reported to enhance flowering and

fruiting while limiting excessive vegetative growth in apple orchards (Greene, 1986). Studies have also shown that paclobutrazol reduces leaf area in apples (Steffens et al., 1986) and cranberries (McArthur and Eaton, 1989), but does not delay flowering or harvesting time.

Moreover, research indicates that paclobutrazol can increase overall fruit yield, as observed in apple production (Lever, 1986), and has no detrimental impact on fruit quality in strawberries, grapes, or citrus (Atkinson and Crisp, 1986; Lolaei et al., 2012; Intrieri et al., 1986; Monselise, 1986). However, a significant increase in fruit starch content has been noted when paclobutrazol is applied as a foliar treatment. Findings suggest that paclobutrazol does not negatively affect fruit yield in apples (Elfving and Proctor, 1986) or influence fruit size (Erez, 1986).

Increasing paclobutrazol concentration has also been shown to elevate total chlorophyll content, particularly chlorophyll a and b. For instance, Wang (1985) observed that soil-applied paclobutrazol inhibited plant growth while increasing chlorophyll content in cucumber and zucchini squash. Similar results were reported in potatoes, where Tekalign et al. (2005) found that treated leaves displayed darker green pigmentation, attributed to higher chlorophyll concentrations and the stimulation of cytokinin synthesis.

Regulatory bodies impose specific residual limits for paclobutrazol, such as 0.01 mg/kg in stone fruits, 0.5 mg/kg in dry beans, and 0.5 mg/kg in apples and small berries (Singh and Ram, 2000). However, studies by Magnitskiy et al. (2006) detected no residual accumulation in cucumbers and tomatoes when seeds were treated with 1000–4000 ppm paclobutrazol for 180 minutes. These findings suggest that paclobutrazol does not accumulate in fruit tissues.

Assuming that growth retardants like paclobutrazol can effectively regulate plant size without significantly reducing per-plant yield, increasing planting density per hectare could potentially lead to a higher total yield.

This study investigates the growth-regulating effects of paclobutrazol on tomato, banana, and potato seedlings. The primary aim is to compare its influence on key developmental traits using in vitro micro-propagation techniques under controlled greenhouse conditions.

Materials and Methods

For This experiment was conducted at the Plant Tissue Culture Laboratory, Department of Agricultural Biotechnology, Faculty of Agriculture, Süleyman Demirel University, where seedlings of tomato, potato, and banana were propagated using in vitro micropropagation techniques. Tomato

seedlings were generated from seeds of Lycopersicon esculentum Mill., cv. T83-48F1, while potato seedlings were derived from meristem cultures of Solanum tuberosum, cv. Granola. Banana seedlings were propagated using tissue culture techniques applied to Musa acuminata, cv. Dwarf Cavendish.

Tomato seeds were sown in trays containing 250 cells, each lined with a fiber sheet and placed in a ¼ MS aerated liquid solution. The trays, holding approximately 1000 seeds, were transferred to a controlled growth chamber (21±3°C, 80% relative humidity, dark conditions) in a completely randomized design. Tomato seedlings were maintained in liquid ¼ MS medium for 25 days, after which they were acclimatized and transplanted into plug trays (45-cell trays filled with a mixture of vermiculite, polystyrene granules, and peat in equal volumes) under greenhouse conditions.

Following acclimatization, both paclobutrazol-treated and untreated seedlings were transplanted into plug-mix compost in 45-cell transplant trays, where they were left to establish for one week. Paclobutrazol was applied as a foliar spray at concentrations of 0, 100, 250, 500, 750, and 1000 ppm. Each seedling received 4 mL of solution using a hand sprayer, while control plants were sprayed with distilled water. The seedlings were then transferred to a greenhouse environment maintained at 21±3°C, 50-60% relative humidity, and a light intensity of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Similarly, potato and banana seedlings propagated in vitro were acclimatized under greenhouse conditions and transplanted into plug trays containing plug-mix compost after one week. Paclobutrazol treatments were applied using the same procedure as for tomato seedlings, with foliar sprays administered one week post-transplantation in greenhouse conditions.

Seedlings of uniform size were transplanted into plug flats containing the same plug-mix compost and placed on capillary mats for subirrigation. After 15 days, the trays were removed from the capillary mat and subjected to overhead irrigation. Banana, tomato, and potato seedlings were fertilized with a water-soluble fertilizer (20N-8P-16K) at a concentration of 2.5 g/L every fourth irrigation over a 21-day cultivation period.

To ensure uniform growth conditions, seedlings of similar size were transplanted into plug flats filled with the same plug-mix compost and placed on capillary mats for subirrigation. This method facilitated consistent moisture distribution across all treatments. After 15 days, the trays were removed from the capillary mat system and switched to overhead irrigation to maintain appropriate water availability. Throughout the 21-day cultivation period, banana, tomato, and potato seedlings were fertilized regularly using a water-soluble fertilizer (20N-8P-16K) at a concentration of

2.5 g/L, applied every fourth irrigation to support balanced growth and nutrient uptake.

The experimental setup was structured as a completely randomized factorial design, ensuring unbiased treatment allocation and statistical robustness in evaluating the effects of paclobutrazol on seedling development. Each treatment condition was replicated four times, with 25 seedlings per replication, leading to a total of 100 treated seedlings per paclobutrazol dose. This approach provided a reliable dataset, allowing for thorough comparisons across treatments while minimizing the influence of random variability.

After 21 days of cultivation, a detailed evaluation of seedling survival and usability was conducted to determine both the viability of treated plants and the overall effectiveness of paclobutrazol application. In addition to survival rates, the assessment focused on key morphological and physiological traits, ensuring that any changes resulting from paclobutrazol treatment were comprehensively documented.

To further quantify the impact of paclobutrazol on seedling growth and development, five seedlings per replication were selected for in-depth analysis, measuring the following parameters:

- Total seedling height – to assess the degree of growth regulation and suppression.
- Internodal length – to determine changes in plant architecture and compactness.
- Leaf area – to evaluate the impact on photosynthetic capacity and canopy expansion.
- Leaf thickness – to examine changes in mesophyll structure and chlorophyll density.
- Chlorophyll content (chlorophyll a and b) – to assess potential alterations in pigment accumulation and photosynthetic efficiency.
- Stem diameter – to analyze the influence of paclobutrazol on shoot strength and mechanical stability.
- Root length – to investigate possible shifts in below-ground biomass allocation and nutrient uptake potential.

This structured methodology provided a comprehensive understanding of how paclobutrazol influenced seedling morphology and physiology, allowing for data-driven insights into its application in controlled plant production systems.Chlorophyll concentration was analyzed in both paclobutrazol-treated and untreated seedlings. Ten leaves per treatment

were collected, along with ten control samples. The chlorophyll content was extracted using 80% acetone (v/v) over 24 hours, following the method described by Arnon (1949). Absorbance was measured at 645 nm and 663 nm using a spectrophotometer (Uvikon 922 Kotron), and total chlorophyll content (µg·mg^{-1} fresh weight) was calculated using the formula:

$$Chlorophyll\ Content = [(20.29 \times A_{645}) + (8.02 \times A_{663})] \times \left[\frac{Volume\ of\ acetone\ (ml)}{Fresh\ weight\ (mg)}\right]$$

where A_{645} and A_{663} represent the absorbance at 645 and 663 nm, respectively.

Paclobutrazol Residue Analysis

Following foliar paclobutrazol application, five seedlings per treatment were destructively harvested to examine residual effects. The samples were surface-dried, placed in paper bags, and dried at 30°C for three days. Subsequently, the dried samples were stored at -18°C for future paclobutrazol residue analysis.

The experiment was conducted following a fully randomized plot design with three replications of 15 plants per treatment. Each replication consisted of ten seedlings per treatment, totaling 45 seedlings per dose. One-way analysis of variance (ANOVA) was performed to evaluate treatment effects. The Tukey-Kramer test (JMP, Version 5) was applied to determine significant differences among paclobutrazol doses. Mean separation was conducted using LSD at a 5% significance level.

Results and Discussion

Paclobutrazol application at all tested doses resulted in a reduction in seedling growth, including height, root length, leaf area, and internodal length, while leaf thickness increased. Seedlings in the concentration trial exhibited greater sensitivity to paclobutrazol compared to those in the formulation test, as reflected by the final reduction in growth. The most significant growth suppression was observed at 500–750 ppm, whereas the least reduction occurred with the 100 ppm foliar application.

The survival rate of seedlings was influenced by paclobutrazol concentration after transplantation. The results indicate that paclobutrazol treatment had a positive effect on seedling growth and improved adaptation to greenhouse conditions (Table 1, 2, 3). A strong correlation was observed between paclobutrazol concentration and the reduction in seedling height. Final measurements of total seedling height in banana, tomato, and potato plants revealed statistically significant differences (Table 1, 2, 3). While all

paclobutrazol treatments (100–750 ppm) caused a substantial decrease in seedling height compared to the control group, the difference between 750 and 1000 ppm was not statistically significant.

Paclobutrazol application resulted in shorter seedlings (total height = plant height + internode length) and reduced stem diameter relative to untreated seedlings (Table 1, 2, 3). Internodal length was significantly restricted across all treatments, with the greatest reduction observed between 100–1000 ppm. Notably, the 750 ppm treatment exhibited a highly inhibitory effect, nearly halting internodal growth. In tomato seedlings, the mean height decreased from 138.9 cm (control) to 40.7 cm (750 ppm) following paclobutrazol application, while stem diameter increased from 7.9 mm (control) to 15.1 mm (750 ppm) (Table 1).

Table 1

The effect of foliar application of Paclobutrazol doses applied twentyone days after adaptation on total seedling height, internode length, survival rate, leaf area, leaf thickness, leaf chlorophyll content, usable plugs, stem diameter and root lenght on potted tomato at the propogated seedlings in vitro conditions

Paclobutrazol doses (ppm)	Survival rate number of the seedling (%)	Usable plugs (%)	Total seedling height (cm)	Stem diameter (mm)	Lenght of internode (cm)	Total leaf area (cm^2)	Total leaf thickness (µm)	Leaf chlorophyll content (µg mg^{-1} fresh weight)		Root lenght (cm)
								a	*b*	
0.0	98.6±1.2	91.7^{a}±1.5	138.9^{a}±1.0	7.9^{i}± 3.4	6.1^{a}±0.5	97.9^{a}±1.3	201.12^{i}± 0.3	0.58^{h}± 0.07	0.34± 0.04	28.0^{a}±0.9
100.0	98.4^{a}±1.6	84.4^{b}±2.0	96.4^{g}±1.2	10.3^{h}± 3.6	4.7^{e}±0.3	37.7^{h}±1.1	269.88^{g}± 0.6	0.75^{d}± 0.04	0.46± 0.06	27.9^{a}±1.2
250.0	98.2^{a}±1.9	87.6^{b}±1.8	81.6^{j}±1.6	12.8^{g}± 4.5	3.3^{i}±0.5	24.8^{n}±1.5	275.75^{g}± 0.4	0.80^{c}± 0.08	0.53± 0.02	26.1^{c}±0.7
500.0	97.1^{a}±1.4	83.9^{b}±1.5	47.3^{k}±1.3	13.4^{d}± 3.9	2.9^{j}±0.7	20.5^{o}±1.5	283.16^{f}± 0.6	0.84^{b}± 0.06	0,57± 0.05	24.4^{e}±1.4
750.0	99.2^{a}±1.5	90.5^{a}±1.4	40.7^{l}±1.7	15.1^{b}± 4.1	2.4^{j}±0.4	17.3^{p}±1.6	304.33^{e}± 0.4	0.92^{a}± 0.04	0.59± 0.04	25.8^{b}±0.8
1000.0	97.8^{a}±1.7	85.3^{b}±1.6	41.9^{l}±1.8	16.4^{a}± 3.7	1.8^{l}±0.8	18.4^{p}±1.3	395.91^{a}± 0.7	0.87^{b}± 0.05	0.55± 0.07	26.3^{c}±0.6

Significant differences between treatment effects were analyzed by regression analysis.

Values ± mean standard deviation

Means followed by the same letters do not differ significantly at 5% level of significance

In banana seedlings, paclobutrazol application resulted in a significant reduction in pseudostem height, decreasing from 38.4 cm in the control to 10.1 cm at 750 ppm. At the same time, pseudostem diameter nearly doubled, increasing from 9.1 mm (control) to 17.6 mm (750 ppm) (Table 2). This inverse relationship between height suppression and stem thickening suggests that paclobutrazol redirects growth energy from elongation toward structural reinforcement, leading to shorter, sturdier plants.

A similar pattern was observed in potato seedlings, where mean height was reduced from 123.4 cm (control) to 50.5 cm (750 ppm), whereas stem diameter increased from 5.7 mm (control) to 13.6 mm (750 ppm) (Table 3). This change in morphology is indicative of gibberellin inhibition, which reduces cell elongation while promoting compact, thicker growth patterns. The resulting thicker stems and shorter internodes contribute to stronger plant structures, which may enhance resistance to mechanical stress, lodging, and environmental fluctuations in field conditions.

Paclobutrazol's growth-inhibitory effects became more pronounced as application rates increased, indicating a dose-dependent suppression of stem elongation. This trend is consistent with findings from previous research on various plant species, reinforcing paclobutrazol's role as an effective growth regulator. Studies conducted by Brigard et al. (2006), Magnitskiy (2004), and Magnitskiy et al. (2006) on tomato, along with Tekalign et al. (2005) on potato, reported similar reductions in plant height following paclobutrazol treatment. These results indicate that the inhibitory effect of paclobutrazol is consistent across different species, demonstrating its broad applicability in plant height management.

Additionally, research by Passian and Bennet (2001), Pill and Gunter (2001), and Still and Pill (2003) suggests that higher paclobutrazol concentrations may be required for sustained long-term height control, especially in species prone to excessive vegetative growth. However, the extent of height suppression varies depending on both species-specific responses and environmental conditions, which can influence the efficiency and persistence of paclobutrazol's effects over time. Factors such as light availability, temperature, and soil composition may also play a role in determining the optimal dose for maximum effectiveness.

These findings highlight paclobutrazol's potential as a valuable tool for managing plant growth, particularly in nursery production and intensive cropping systems where excessive stem elongation can compromise plant stability, disrupt spacing efficiency, and reduce overall uniformity. However, further studies are needed to identify species-specific optimal concentrations, as well as to assess the potential trade-offs between growth regulation and yield performance. Understanding long-term physiological effects and interactions with other plant growth regulators will be essential for maximizing paclobutrazol's benefits without negatively impacting plant productivity.

Table 2

The effect of foliar application of Paclobutrazol doses applied twenty-one days after adaptation on total seedling height, internode length, survival rate, leaf area, leaf thickness, leaf chlorophyll content, usable plugs, seedling (*pseudostem*) height, stem diameter, and root length on potted banana at the propogated seedlings *in vitro* conditions.

Paclobutrazol doses (ppm)	**Survival rate number of at the seedling (%)**	**Usable plugs (%)**	**Total seedling (pseudo stem) height (cm)**	**Stem diameter (pseudo stem) (mm)**	**Lenght of internode (cm)**	**Leaf area (cm^2)**	**Total leaf thickness (µm)**	**Leaf chlorophyll content (µg mg^{-1} fresh weight)**		**R o o t lenght (cm)**
								a	*b*	
0.0	98.9^{a}±1.2	97.8^{a}±1.3	38.4^{a}±1.9	9.1^{k}± 3.7	14.9^{a}±0.9	59.9^{a}±1.3	356.4^{fg}± 0.2	0.40^{ij}± 0.06	0.19± 0.04	43.7^{ab}±1.1
100.0	98.6^{a}±1.4	94.3^{a}±1.4	25.6^{de}±1.6	12.5^{fg}± 4.5	10.8^{fg}±1.3	28.3^{ij}±0.9	384.7^{ef}± 0.2	0.52^{gh}± 0.03	0.28± 0.06	41.5^{ab}±1.3
250.0	96.2^{a}±1.2	96.7^{a}±1.1	22.7^{fg}±1.9	14.9^{de}± 3.9	8.1^{hi}±0.8	25.6^{jk}±1.1	403.9^{de}± 0.4	057^{fg}± 0.05	0.35± 0.08	40.2^{bc}±0.7
500.0	95.8^{a}±1.3	94.9^{a}±1.2	16.5^{hi}±1.5	15.7^{cd}± 4.4	7.4^{ij}±0.9	20.4^{m}±0.9	427.1^{cd}± 0.1	0.64^{de}± 0.07	0,39± 0.05	39.8^{bc}±0.8
750.0	97.9^{a}±1.5	95.2^{a}±1.2	10.1^{ij}±1.4	17.6^{bc}± 3.8	6.6^{kl}±1.2	16.9^{gh}±1.0	451.8^{bc}± 0.3	0.77^{ab}± 0.04	0.41± 0.08	37.6^{cd}±1.1
1000.0	97.7^{a}±1.2	95.6^{a}±0.9	7.8^{jk}±1.5	18.0^{ab}± 4.2	5.9^{kl}±1.3	14.5^{kl}±1.2	507.5^{a}± 0.2	0.79^{ab}± 0.06	0.47± 0.04	36.4^{cd}±0.9

Values ± mean standard deviation. Means followed by the same letters do not differ significantly at 5% level of significance.

High concentrations of paclobutrazol applied via foliar spraying had a significant impact on leaf area, leading to a dose-dependent reduction, while simultaneously increasing leaf thickness. The observed reduction in leaf area was closely related to paclobutrazol's role in inhibiting gibberellin biosynthesis, which results in reduced cell elongation and expansion. This effect is particularly relevant in crops where leaf size plays a crucial role in photosynthetic efficiency and transpiration regulation. Analysis of variance indicated highly significant differences across all treatments (Table 1, 2, 3), confirming that leaf area decreased proportionally with increasing paclobutrazol doses.

In tomato, the largest leaf area was recorded in the control (97.9 cm^2), with a noticeable decline at 100 ppm (37.7 cm^2) and a further reduction at 1000 ppm (18.4 cm^2) (Table 1). Similarly, in banana, the highest leaf area was measured in the control group (59.9 cm^2), but it was significantly lower in the 100 ppm (28.3 cm^2) and 1000 ppm (14.5 cm^2) treatments (Table 2). In potato, leaf area decreased from 73.8 cm^2 (control) to 22.2 cm^2 (100 ppm) and 13.9 cm^2 (1000 ppm) (Table 3). These results are consistent with previous studies, including those by Ramina and Tunutti (1985), Assem (1986), and Maia et al. (2009), which emphasize species-specific differences in response to paclobutrazol treatment.

These findings are consistent with those of Atkinson et al. (1985), further reinforcing the role of paclobutrazol in regulating leaf area across various plant species. The observed increase in leaf thickness following paclobutrazol treatment suggests that the compound induces structural changes within the epidermal and mesophyll layers, likely due to modifications in hormonal balance.

At a concentration of 750 ppm, leaf thickness increased considerably compared to the control, with values rising from 201.12 µm to 395.91 µm in tomato (Table 1), from 356.4 µm to 507.5 µm in banana at 1000 ppm (Table 2), and from 188.75 µm to 279.14 µm in potato (Table 3). This increase in thickness can be linked to higher chlorophyll accumulation, a denser mesophyll structure, and reduced intercellular spaces, all of which contribute to darker green pigmentation and potentially greater photosynthetic capacity. Similar findings have been reported in tomato (Brigard et al., 2006; Magnitskiy, 2004; Magnitskiy et al., 2006) and potato (Tekalign et al., 2005), where paclobutrazol treatment not only suppressed plant height but also enhanced leaf thickness, indicating a consistent trend across multiple species.

Beyond its impact on leaf area and thickness, paclobutrazol also led to a reduction in internodal length, directly affecting overall plant morphology. The regulation of internodal elongation is a critical factor in plant architecture, as it determines light interception, nutrient distribution, and mechanical stability. Measurements from this study (Table 1, 2, 3) revealed a statistically significant decrease in internodal length across all treatments, with higher paclobutrazol concentrations resulting in greater reductions.

In tomato, the longest internodal length was recorded in the control group (6.1 cm), whereas it decreased sharply at 1000 ppm (1.8 cm) and 750 ppm (2.4 cm) (Table 1). In banana, internodal length was reduced from 14.9 cm in the control to 5.9 cm at 1000 ppm and 6.6 cm at 750 ppm (Table 2). Similarly, in potato, internodal length declined from 4.2 cm in the control to 2.2 cm at 750 ppm and 2.3 cm at 1000 ppm (Table 3). The statistical significance of these differences confirms that paclobutrazol effectively suppresses excessive elongation while promoting more compact plant growth.

These results highlight the potential of paclobutrazol as a tool for managing plant architecture, particularly in crops where compact growth, increased structural integrity, and controlled leaf expansion are desirable traits. While moderate doses may improve plant stability, excessive concentrations could negatively impact biomass accumulation, emphasizing the need for careful dose optimization based on the crop species and growing conditions. Further research should explore the long-term physiological effects of paclobutrazol, its interactions with other plant growth regulators, and the potential trade-offs between controlled growth and overall yield efficiency.

Table 3

The effect of foliar application of Paclobutrazol doses applied twentyone days after adaptation on total seedling height, internode length, survival rate, leaf area, leaf thickness, leaf chlorophyll content, usable plugs, stem diameter and root lenght on potted potato at the propogated seedlings in vitro conditions.

Paclobutrazol doses (ppm)	**Survival rate number of at the seedling (%)**	**Usable plugs (%)**	**Total seedling height (cm)**	**Stem diameter (mm)**	**Lenght of internode (cm)**	**Total leaf area (cm^2)**	**Total leaf thickness (µm)**	**Chlorophyll content (µg mg^{-1} fresh weight)**		**Root lenght (cm)**
								a	*b*	
0.0	92.9±1.3	87.5±2.0	123.4±1.7	5.7^{h}± 3.9	4.2^{a}±0.8	29.8^{a}±1.6	188.75^{g}±0.9	0.46± 0.06	0.25± 0.07	20.4±1.1
100.0	90.3^{a}±1.1	86.9^{a}±1.8	98.9^{e}±1.4	8.9^{g}± 4.4	3.2^{f}±0.8	22.2^{f}±2.2	242.67^{c}± 1.1	0.64^{a}± 0.08	0.34± 0.09	18.7^{b}±1.2
250.0	91.7^{a}±1.5	87.3^{a}±1.7	83.3^{g}±1.5	10.7^{e}±3.8	3.0^{g}±0.9	17.9^{h}±1.9	255.63^{b}± 0.9	0.68^{b}± 0.09	0.42± 0.06	17.9^{cd}±0.9
500.0	89.8^{a}±1.3	86.1^{a}±1.7	59.6^{i}±1.9	11.1^{d}±4.3	2.9^{gf}±1.0	14.3^{j}±1.5	268.12^{a}± 1.0	0.73^{b}± 0.03	0,45± 0.07	18.5^{b}±1.3
750.0	91.5^{a}±1.7	89.8^{a}±1.6	50.5^{j}±1.4	13.6^{b}±4.1	2.2^{fg}±1.2	10.5^{k}±1.7	281.45^{a}± 0.7	0.81^{a}± 0.09	0.48± 0.09	17.2^{cd}±1.4
1000.0	90.2^{a}±1.5	86.5^{a}±1.4	52.8^{kl}±1.8	12.8^{b}±4.0	2.3^{fg}±1.2	13.9^{j}±1.9	279.14^{a}± 0.8	0.76^{b}± 0.05	0.46± 0.05	19.6^{ab}±1.2

Values ± mean standard deviation.

Paclobutrazol application during the seedling growth stage resulted in a notable increase in total leaf chlorophyll content, with higher concentrations leading to greater accumulation. This increase in chlorophyll content contributed to the darker green coloration of leaves, which is commonly associated with enhanced photosynthetic efficiency and improved plant vigor. At 750 ppm, treated leaves exhibited significantly higher chlorophyll a and b levels compared to control plants, suggesting a strong dose-response relationship. Specifically, in tomato, chlorophyll a reached 0.92 μg mg^{-1} fresh weight and chlorophyll b 0.59 μg mg^{-1} fresh weight (Table 1). In banana, chlorophyll a was 0.77 μg mg^{-1} and chlorophyll b 0.41 μg mg^{-1} (Table 2), whereas in potato, chlorophyll a measured 0.81 μg mg^{-1} and chlorophyll b 0.48 μg mg^{-1} (Table 3). In contrast, control plants exhibited significantly lower chlorophyll levels, measuring 0.58 and 0.34 μg mg^{-1} in tomato, 0.40 and 0.19 μg mg^{-1} in banana, and 0.46 and 0.25 μg mg^{-1} in potato, for chlorophyll a and b, respectively. These findings are in line with previous research, including studies by Senoo and Isoda (2003), Elfving and Proctor (1986), and Steffens and Wang (1986), which reported similar enhancements in chlorophyll content following growth regulator treatments.

The increase in chlorophyll content is likely related to paclobutrazol's influence on endogenous cytokinin levels, as suggested by Fletcher et al. (2000). Cytokinins are known to play a key role in chloroplast development, enhancing chlorophyll biosynthesis while inhibiting chlorophyll degradation, ultimately leading to extended leaf longevity and delayed senescence. Given that paclobutrazol acts as a gibberellin biosynthesis inhibitor, its secondary effect on cytokinin levels may contribute to enhanced photosynthetic activity and prolonged functional leaf area, which are critical for maintaining plant productivity. The dark green leaf pigmentation observed in paclobutrazol-treated seedlings further supports this hypothesis, as increased chlorophyll density is associated with higher light absorption efficiency, potentially improving the plant's ability to adapt to variable environmental conditions.

A statistically significant reduction in root length was also observed among paclobutrazol-treated seedlings, which may be attributed to its influence on gibberellin suppression and root-shoot energy allocation dynamics. Analysis of average root length (Table 1, 2, 3) showed that in tomato, root length at 750 ppm was 25.8 cm, which was slightly shorter than the control (28.0 cm) (Table 1). Similarly, in potato, root length decreased from 20.4 cm (control) to 17.2 cm (750 ppm) (Table 3). Root growth inhibition

by paclobutrazol has been reported in various species and is often linked to a shift in assimilate distribution, favoring shoot thickening and compact growth rather than root elongation. However, the extent of inhibition is dose-dependent, as mild concentrations tend to strengthen root architecture, whereas higher doses may suppress root expansion, potentially affecting water and nutrient uptake efficiency.

In banana seedlings, paclobutrazol application resulted in an increase in lateral shoot formation, though higher concentrations led to a subsequent decline. This response suggests that while moderate doses may promote axillary bud development, excessive inhibition of gibberellin biosynthesis at higher concentrations can negatively impact overall shoot proliferation. These findings are consistent with reports by Brigard et al. (2006) and Magnitskiy (2004), who observed similar effects in other species where paclobutrazol induced compact, bushier growth but at high doses suppressed normal shoot elongation.

These findings underscore the potential role of paclobutrazol in seedling growth regulation. By controlling excessive elongation, increasing chlorophyll retention, and modulating shoot-root energy allocation, paclobutrazol presents an effective means of managing plant structure, particularly in greenhouse and commercial nursery settings. However, its long-term effects on root functionality, nutrient uptake, and overall plant metabolism require further investigation.

While the results indicate that 750 ppm paclobutrazol (applied via foliar spraying) effectively regulated the growth of tomato, potato, and banana seedlings, further experimental validation under varying environmental conditions is necessary. This approach may benefit commercial nurseries by preventing excessive elongation, particularly in crops susceptible to height-related lodging issues. Although paclobutrazol has been widely studied in vegetable and ornamental crops, its potential for regulating plant architecture in other species remains an area for future research.

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CHAPTER 4

ARTIFICIAL NEURAL NETWORKS AND THEIR APPLICATIONS IN HORTICULTURE

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1. What is the Artificial Neural Network

Artificial Neural Networks, which resemble biological neural networks, are very good at predicting nonlinear systems. Thanks to this feature, it is widely used in solving complex and nonlinear problems (Pacci et al., 2022; Odabaş et al., 2016).

Artificial Neural Networks are systems that perform the learning process based on the human brain. It realizes the learning process thanks to the examples obtained beforehand. There are artificial neural cells in which each sample is connected to each other. Each connection has a different weight value. Artificial Neural Networks keep these values hidden and spread them to the networks. The calculation method of Artificial Neural Networks works differently from other calculation methods. It is a calculation method that can work with incomplete information, can make decisions in uncertainties in the environment, and can tolerate the errors encountered (Öztemel, 2003).

There are different definitions of Artificial Neural Networks in the literature. According to one definition, Artificial Neural Networks are systems that work in parallel among themselves. The operation of this network structure is realized by the functioning of neurons and their weights (Darpa, 1988). In another definition, Artificial Neural Networks are information processing elements and consist of many artificial neural cell connections (Simpson, 1996). In other words, artificial neural networks are parallel distributed processors assembled from simple units (Haykın, 1999). Artificial neural networks are self-learning programs by imitating biological neural networks. This system was developed by imitating the human brain. In addition to learning, it also has the ability to establish relationships between events and memories (Elmas, 2007).

Artificial Neural Networks provide computer learning in general. After the learning process, they make decisions about similar events. They process information differently than traditional methods. In order to realize the learning process in Artificial Neural Networks, appropriate examples must be selected and given to the artificial neural network. Otherwise, artificial neural networks cannot be trained. Artificial Neural Networks use the examples given to them to generate information on the examples they have not seen. Artificial Neural Networks are also used in classification processes. The data given to the network is clustered and divided into classes. Then, the next examples coming to the network determine which class they will fall into. Artificial Neural Networks can detect missing information in the data that contains incomplete information. Artificial Neural Networks could learn new events with the data given to them. Artificial Neural Networks can continue to work with missing data after the learning

process and develop results. The ability of Artificial Neural Networks to produce results even with incomplete data increases their tolerance to errors. Artificial Neural Networks continue to work even if some cells become inoperable. In this case, there is a decrease in the efficacy of Artificial Neural Networks. Artificial Neural Networks could use uncertain data. After the learning process, they can produce results by establishing relationships on uncertain events. Artificial Neural Networks only operate with numerical data. Symbolic statements must be transformed into numerical format (Öztemel, 2016).

Artificial Neural Networks have many advantages. They can produce reliable solutions in a wide range of applications. They do not make assumptions. Artificial Neural Networks can produce solutions within categorized data. They can produce results on data they have not seen before. The computer programs required for the use of artificial neural networks are varied and their use is constantly becoming easier. It is more flexible than other modeling techniques (Çakır, 2020):

In addition to these advantages, Artificial Neural Networks also have some disadvantages. In order for the reliability of the results produced by Artificial Neural Networks to be high, the number of data to be used while training the network should be high. The relationships within Artificial Neural Network models remain unknown and how it obtains solutions is still a subject of research. It can only use numerical data, not symbolic and verbal data. There are no specific rules in the creation of the network, it depends entirely on the user's experience. Artificial Neural Networks may not always produce the best performance results. There is no certainty in the parameters to be used when training the network. These parameters are changed by the user through trial and error until the best result is obtained. The data to be given to Artificial Neural Networks should be defined in the zero-one range (Tu, 1996; Berry and Linoff, 2000; Hu, 2002; Şen, 2004; Öztemel, 2016).

The capacity to learn, past experience, perception, and thought that resemble human intelligence is known as artificial intelligence. This ability enables devices, computers, or systems to make judgments in the presence of predictable or unpredictable new situations and to take the requisite action. Variables are instructed during this decision-making process, and formulas are generated through the use of computer simulations that can generate interpretations that are comparable to how humans think in order to guarantee the accuracy of the decision. Consequently, a computer software-based paradigm of choices and reasoning is developed based on human thought processes (Özgüven, 2019).

Artificial Intelligence is a comprehensive discipline that encompasses a diverse array of devices that are designed to allow machines to perform tasks that typically necessitate intellectual ability, including figuring out solutions, deductive thinking, and understanding. Within the field of artificial intelligence, artificial neural networks are computational models that are derived from the anatomy and neuroscience of the human brain. Such networks are composed of connected components or "neurons" that are organized in layers. They analyze input data to identify patterns and make decisions. The design and functionality of artificial neural networks have been impacted by our comprehension of neural networks in humans, which are characterized by neuronal connections that transmit signals to process information. Neural networks that emulate this design have been instrumental in the advancement of artificial intelligence capabilities, facilitating the development of applications such as autonomous systems, the processing of natural language, and picture and speech identification (Gerven and Bohte, 2017).

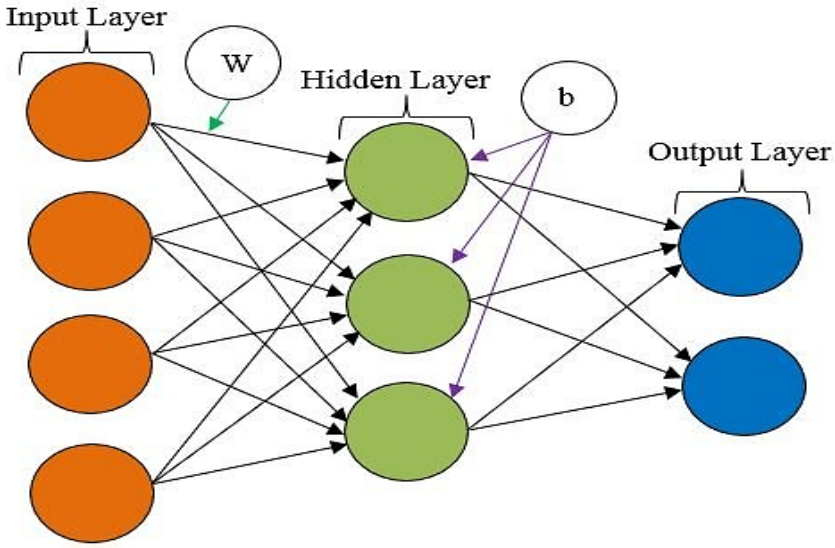

Figure 1. The basic structure of artificial neural networks (Rahman et al., 2019)

The fundamental structure of neural networks is illustrated in Figure 1. The neural network system's simplest working element is the neuron. The sigmoid activation function operates on the inputs and the weight to determine the output. Biases (b) are the association of weight values with individual nodes. The continual process for learning information determines the weight values throughout the network. The coefficient values are validated during the training phase, during which the network identifies a particular cluster by analyzing the characteristics of typical input data.

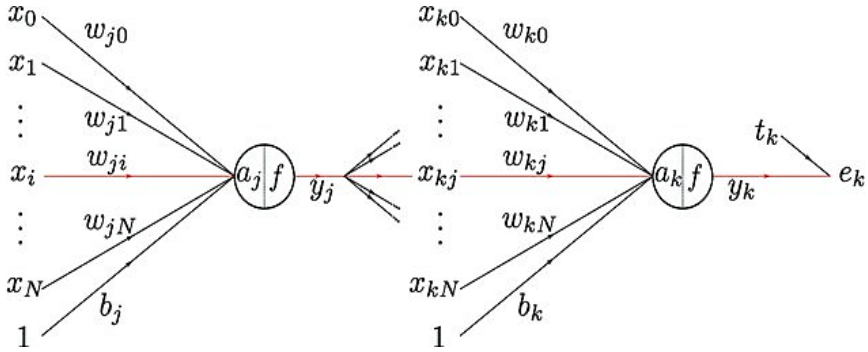

Figure 2. *A neural network with one layer that is concealed is illustrated in detail.*

Figure 2 is a detailed representation of a neural network that only has a single concealed layer. A group of inputs, denoted by the letters x1, x2,..., and x3, make up the input layer. A bias b i, net neuron activity a j defined as Σ i w ji, and an activation function f are all components that are present in the buried layer. Weights w i0,..., w iN u are also present. The output section, which is represented by the notation output y k, is equipped with weights tw k0,..., w jN u, as well as a bias b j. The target label t k is used in order to ascertain the mistake that arises for the output y k, e k. As you can see, the expression x n kj is the same as y n j.

1.1. Structure and basic components of Artificial Neural Networks

Biological neuron cell

The biological nervous system is described as a three-layered system with the brain at its center, which continuously receives and interprets information and produces an appropriate decision. These layers are listed as the receptor nerves that transform the inputs from the environment into electrical signals and transmit them to the brain, the response nerves that transform the electrical signals produced by the brain into appropriate responses as output, and the central nervous network that produces appropriate responses by feeding forward and feedback between the receptor and response nerves (Baş, 2006).

The brain has millions of closely linked neurons. An average neuron is interconnected with over ten thousand other neurons inside the neurological system (Eğrioğlu et al., 2020). The axon of a neuron is separated and linked to the dendrites of other neurons via a junction known as a synapse. The transmission at the junctions is chemical, with the signal's intensity

being transmitted as well as received via dendrites, contingent upon the quantity of chemicals produced by the axon. The size of synapses reflects the modifications occurring in the brain throughout the learning process. This synapse integrates with the neuron's computation of data, grounded in the fundamental memory mechanism of the brain (Duman, 2006).

Artificial neuron cell

The artificial neural cell is the result of an attempt to mimic the human nerve cell. Figure 3 illustrates the correlation among an artificial neural cell and a biological neural cell (Ghorbani and Soleimani, 2023). In overall, five essential elements comprise an artificial neural cell.

- Inputs
- Weights
- Aggregation function
- Activation function
- Output

Once the inputs enter the neuron, they are multiplied by the respective connection weights, then combined with a combining function (usually an addition function) to obtain the net input of the neuron. The net input is processed by an activation function. The output of the activation function determines the net output of the neuron (Hamzaçebi, 2011).

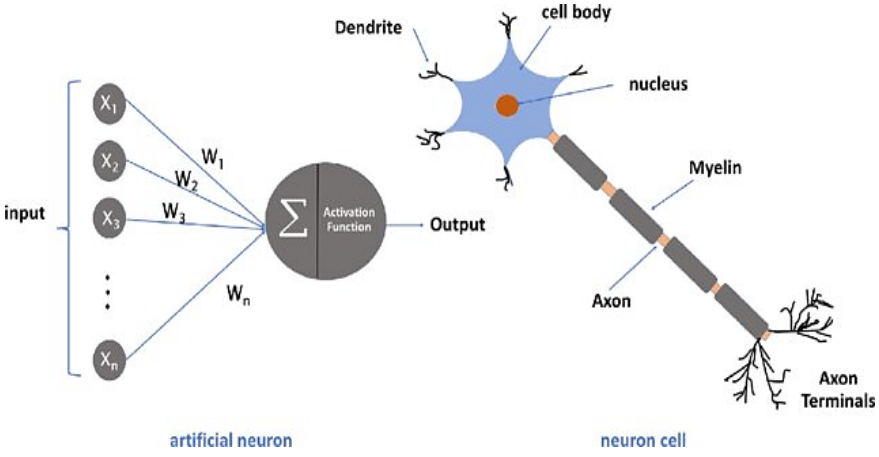

Figure 3. Biological and Artificial Neuron Cell (Ghorbani and Soleimani, 2023)

Inputs

An artificial neural cell receives information from the outside world. These are determined by the examples that the network is asked to learn. The artificial neural cell can receive information from the outside world as well as from other cells or from itself (Öztemel, 2016).

Weights

The impact of external stimuli on the cell is defined by these parameters, which are called weights. By means of the weights connected to relationships, data is introduced into the cell. The parameters provided to the artificial neural network application are given proportional strength (a numerical factor) by the weights. In an artificial neural network, different weights are given to each connection. The transmission of inputs between cells is carried out via these linkages (Elmas, 2007).

Summation function

Calculating the net input to a cell is the purpose of this procedure. For that reason, a variety of functions are used. Finding the weighted average is the first and most usual method. Each incoming input value is multiplied by its weight, and then the total of those values is calculated. It is thus possible to determine the net input to the network (Öztemel, 2016).

Activation function

This function processes the net input to the cell and determines the output that the cell will produce in response to this input. Various types of activation functions are used. The most appropriate function for a problem is also a situation that the designer can determine as a result of experiments. A formula showing the appropriate function has not been found (Öztemel, 2016).

Output of the cell

The value obtained from the activation function is the output value of the neuron. This value can either be given to the outside world as the output of the artificial neural network or can be used again in the network. Although a neuron has an output, this output can depend on any number of neurons (Kubat, 2016).

1.2. Learning, adaptive learning and testing in artificial neural networks

In artificial neural networks, training the network is the determination of the weight values of the process elements. These values are initially determined randomly. As samples are shown to the neural network, new weight values are determined. The important thing is to determine the weight values that will produce the correct results. The most appropriate weight values are tried to be found by showing the examples to the neural network many times. In this way, the neural network can generalize about the event. This process is called network learning. Changing weight values depends on the learning rule. There are different learning rules used in applications (Öztemel, 2016).

Learning is performed in two stages in artificial neural networks. Determining the output that the network should produce, for example shown to the network constitutes the first stage. In the second stage, the weight values in the network connections are changed according to the accuracy of this output value. These processes may differ depending on the learning rule (Öztemel, 2016).

2. The Use of Artificial Neural Networks in Horticulture

2.1. Applications for Artificial Neural Networks of Vegetables

Research in California utilized multispectral photographs of leaf reflectance in the visible and infrared bands, ranging from 384 to 810 nm, to differentiate between lettuce and weeds. A mean classification accuracy of 90.3% for crops and weeds was attained. Machine vision was employed to delineate leaf borders, hence enhancing classification accuracy (Slaughter et al., 2008).

For their 2017 study, Rad et al. used an artificial neural network to analyze how several agricultural and phenological factors affected the weight of eggplant fruits. Several factors were taken into account in the study, including plant height, fruit length and breadth, quantity per plant, ratio of fruit length to width, total yield, days to blooming, days to first harvest, temperature of the canopy, chlorophyll content, and relative water content. Parameters such as R2 = 93%, mean prediction error (MPE) = 2.01, and mean square deviation = 2.35 demonstrate that the 7-4-1 ANN model achieved a high degree of quality in the research. The study found that flowering time, the proportion of fruit length to width, and the amount of days before first harvest were the most important factors in determining the weight of individual fruits. The fruit weight and days to flowering correlation

coefficient was 0.99**, while the individual fruit weight and total yield correlation coefficient was 0.88**. The largest standard deviations were seen in total yield (308.8 and 67.5, respectively) and each fruit weight.

Demir et al. (2017) highlighted that artificial neural networks are innovative computational tools that offer a rapid and precise approach for forecasting the physical characteristics of agricultural materials, demonstrating superior performance relative to conventional approaches. Various physical parameters of pumpkin seeds, such as linear dimensions, volume, surface area, projected area, geometric mean diameter, and sphericity, were determined under laboratory settings. Neural networks were used to estimate these parameters. Experimental inquiry and simulation analysis of neural networks make up the two main parts of the study. To predict the physical properties of pumpkin seeds, radial basis neural networks and back propagation neural networks were used. The Back Propagation Neural Network designs were found to have RMSE values of 0.0025 and 0.6875, respectively. We found that the Radial Basis Neural Network structure is more effective at making predictions, therefore it might be a good substitute for the old ways of doing things.

Zaborowicz et al. (2017) carried out studies using computer image analysis and artificial neural modeling approaches to evaluate the quality of Cappricia cultivar greenhouse tomatoes, utilizing samples ranging from 40 mm to 67 mm in size. The image depicts the empirical data collection conducted during the growing period, which lasts from the first harvest in mid-May to the last harvest in November.. The RBF 37: 37-39-1: 1 and RBF 22: 22-20-2: 2 models were utilized in order to get the quality features that were desired. With regard to the RBF 37: 37-39-1: 1 network, the output variable that represents tomato color was able to attain a training quality of 0.930827, a validation quality of 0.911982, and a test quality of 0.979390. For the training set, the root mean square error (RMSE) training rates are 0.075986, while for the set to be validated, they are 0.072194, and for the test set, they are 0.061714. Hue and softness were the factors that were used for the 22-20-2:2 network network. A training quality of 0.985038, a validation quality of 0.990694, and a test quality of 0.985130 are all displayed by the network. For training purposes, the RMSE values for the artificial neural network were 0.065667, while for validation purposes, they were 0.066187, and for testing purposes, they were 0.073868. According to the findings of a study, accurate classification requires the collection of two digital images of the tomatoes that are being evaluated. One of these photographs should show the roots, while the other should show the entrance of the tomato. Moreover, training sets must be created, consisting of the mean values of the acquired features.

Banjac et al. (2018) investigated the impact of post-harvest circumstances on the profile of bioactive chemicals and antioxidant activity in lettuce by artificial neural network modeling. To elucidate the impact of post-harvest circumstances on lettuce plants, all samples underwent compositional analysis of bioactive components and assessment of antioxidant activity. Experimental data were utilized for simulating antioxidant activity via the artificial neural network methodology. The created artificial neural network model has been determined to predict antioxidant activity.

Gholipoor and Nadali (2019) applied an Artificial Neural Network to assess the impact of various factors on pepper fruit yield, including the duration from sowing to emergence, the interval from sowing to flowering onset, the period from sowing to 50% flowering, plant height, canopy width, fruit count per plant, fruit water content, and the duration of the reproductive stage. Seeds from 692 indigenous chili pepper (*Capsicum annuum* L.) genotypes were seeded to gather data for the development and evaluation of the ANN. The findings indicated that the ANN with an 8:10:1 configuration attained the maximum accuracy (R2 = 0.97). Sensitivity analysis was conducted to ascertain the relative impact of plant traits on fruit output. They were graded from high to low based on their respective contributions in the following parameters: number of fruits per plant, days from sowing to 50% blooming, days from sowing to flowering beginning, duration of the reproductive stage, fruit water content, canopy width, days from sowing to growth, and plant height.

Zhang et al. (2020) used a three-layer neural network to forecast the differentiation rate of melon under four cultivation conditions: agar concentration, light duration, culture temperature, and humidity. Ten-fold the cross-validation indicated that the ideal reverse propagation neural network was configured with traingdx as the training function and a final architecture of 4-3-1 (four neurons in the input layer, three neurons in the hidden layer, and one neuron in the output layer). This configuration achieved a high coefficient of correlation ($R^2 = 0.9637$) between actual and predicted results, along with a root-mean-square error (RMSE) of 0.0108, indicating effective performance of the artificial neural network. Tissue culture studies were conducted based on the ideal culture parameters established by a genetic algorithm. The findings indicated that the actual differentiation rate of melon attained 90.53%, which is just 1.59% below the expected figure generated by the genetic algorithm's output. The results of the optimization surpassed that achieved by response surface methods, with a projected induced diversification rate of 86.04% and an actual value of 83.62%, resulting in a discrepancy of 2.89% below the expected value. The integration of artificial neural networks and genetic algorithms may effectively

improve plant tissue culture conditions with excellent predictive accuracy, indicating promising applications in other biological studies.

The research by Koyama et al. (2021) evaluated the effectiveness of a nondestructive computational imaging approach for assessing spinach quality. Images of spinach leaves were taken using a smartphone camera after different storage periods. 12 sensory panels categorized spinach freshness into one of four classifications using these photos. The turned mean of all twelve panel assessments was assigned as the actual label. The spinach image was removed from the background and then converted to grayscale, CIE-Lab color space (L*a*b*), and Hue, Saturation, and Value (HSV). The mean, minimum, and standard deviation of each color component in spinach leaves were obtained as color attributes. Local characteristics were extracted using the bag-of-words methodology from significant data points obtained by Oriented FAST (Features from Accelerated Segment Test) and Rotated BRIEF (Binary Robust Independent Elementary Features). The attribute pairs obtained from the spinach images were employed to train machine learning models for the identification of freshness levels. The correlation analysis of the retrieved characteristics and the sensory assessment score indicated a positive correlation ($0.5 < r < 0.6$) for four color characteristics, and a negative correlation ($0.6 < r < –0.5$) for six clusters within the local features. The support vector machine classifier and artificial neural network technique proficiently classified spinach samples, achieving an overall accuracy of 70% in four classes, 77% in three classes, and 84% in two classes, which is equivalent to each person's panel evaluations.

Eşidir and Metin (2021) sought to forecast tomato exports utilizing the ANN methodology in their research. The agricultural price of tomatoes, the market price of tomatoes, the production quantity of tomatoes, the monthly exchange rate of the US Dollar, and the amount of tomato exports from Turkey were identified as the variables influencing tomato exports for the ANN model. The SPSS 25 software program was utilized to estimate the export value using ANN. The program employs a feedforward backpropagation neural network model, which is frequently utilized for such predictions. The constructed ANN model is multilayered, utilizing the hyperbolic tangent function as the activation function. The input layer has six variables. The artificial neural network has two hidden layers, with 10 units in the first layer and 7 units in the second layer. In the study, around 74.6% of the dataset was designated for the training set, whilst 25.4% was designated for the test set. The regression result was 0.96, and the correlation result was 0.98. The market price of tomatoes has impacted the outcome by 60.6% among the independent factors. The results indicate that the ANN technique yielded valid and trustworthy predictions for tomato exports.

Belouz et al. (2022) endeavored to forecast greenhouse tomato productivity and ascertain the inputs that significantly influence tomato production by employing an artificial neural network in conjunction with sensitivity analysis. Models were developed from data randomly gathered by face-to-face questionnaires from 25 greenhouse tomato farms. The quantity of neurons in the hidden layer was adjusted from 1 to 40 to evaluate various ANN models. The optimal structure identified based on statistical criteria is 12–34–1. This artificial neural network model has been employed to forecast tomato production based on energy inputs. The total energy inputs were 94,748.2 MJ ha-1, with an energy ratio of 1.055, indicating rather low energy efficiency. Subsequently, the outcomes of the ANN model were juxtaposed with those derived using the multiple linear regression (MLR) method. They asserted that the ANN yielded more precise predictions than the MLR method. Sensitivity analysis indicates that the critical inputs in greenhouse tomato cultivation are pesticide, farm manure, potassium, nitrogen, energy, and fungicide.

2.2. Applications of Artificial Neural Networks in Fruits

Jahromi et al. (2007) forecasted the mass and surface area of bergamot fruit utilizing several physical parameters in linear models. The study employed single and multiple variable regressions to analyze bergamot dimensional properties, projection areas, mass and surface area based on measured volume and assumed shapes, as well as surface area on a mass basis. In the initial classification of mass modeling, the lowest determination coefficients were $R^2 = 0.87$ for length and $R^2 = 0.88$ for width, however all other determination coefficients exceeded $R^2 = 0.90$. In all mass models, the maximum determination coefficient based on real volume was achieved at $R^2 = 0.99$. The findings indicate that all determination coefficients for the surface area modeling exceeded $R^2 = 0.92$. In all models, the maximum determination coefficient achieved was R2 = 1.00 for certain combinations of projection areas. The results indicate that all mass and surface area models are adequately fitting models.

Ebrahimi and Mollazade (2010) asserted in their study that the categorization of agricultural products constitutes a pivotal research domain within post-harvest engineering, and they introduced an algorithmic system for classifying four distinct almond varieties: Yalda, France, Shokofeh, and Shahrood 15. This system utilized acoustic inputs, chosen characteristics, decision tree-derived rules, and a fuzzy inference system. Two distinct plates, plywood and stainless steel, were utilized, and two varying drop heights (14 cm and 24 cm) were established as test circumstances. It was discovered that an ideal fuzzy system could be achieved with a stainless steel impact plate at a drop height of 24 cm, resulting in a classification

accuracy of 84.16% for almond types using fuzzy inference performance. The findings also demonstrated the influence of almond drop height and impact plate type on the precision of the fuzzy inference system.

Fathi et al. (2011) employed image analysis and artificial neural networks in their research to forecast mass transfer kinetics and color alterations in osmotically dried kiwi slices. The investigation involved the dehydration of kiwi fruits using four distinct sucrose concentrations, three processing temperatures, and four osmotic durations. A multilayer, feedforward neural network utilizing three inputs and a single hidden layer with 16 neurons has been determined to be the optimal model for prediction. They have shown that picture analysis and the utilization of artificial neural networks may provide online status prediction, along with automated, objective, and quick inspection.

By integrating acoustic emission analysis, principal component analysis (PCA), and an adaptive neuro-fuzzy inference system (ANFIS) classifier, Khalifa and Komarizadeh (2012) created and evaluated a powerful walnut recognition system. During the preprocessing step, when impact signals were generated, the study made use of 281 sample data for data collecting and system efficiency assessment, as well as a shiny steel plate slanted at 60° to produce sound signals. To begin with, we extracted features for classification based on the statistical properties of a subset of time-domain impact events. Then, we used principal component analysis (PCA) to reduce the features. During the identification step, the ANFIS classifier was fed the chosen statistical characteristics. It has been found that the suggested PCA-ANFIS intelligent system has a classification accuracy of 100%.

Lorestani et al. (2012) proposed models for predicting the mass and volume of limes based on their geometric properties. In the study, the models were classified into three categories: regression of single and multivariable lime dimensions (1st classification), regression of single and multivariable predicted areas (2nd classification), and estimation of lime shape; ellipsoidal or spherical based on volume (3rd classification). The research results showed that the modeling based on the volume of the lime had a higher determination coefficient than mass modeling.

Soares et al. (2013) asserted that the pyrotechnical characters observed in field experiments are phenotypic in nature, and their assessment is frequently founded solely on the observer's knowledge. The correlations among factors anticipate shifts in a single personality based on modifications in other traits. They anticipated the impact of agronomic characteristics on the cluster weight of banana plants in their research. The yield-related characteristics that were calculated during harvest included

group weight, the quantity of fruits, fruit weight, fruit width, and fruit length while the vegetative characters evaluated during flowering included plant height, pseudostem circumference, number of branches, and number of live leaves. The artificial neural networks method has been employed as a modeling instrument to establish a system that forecasts banana productivity. Both the accuracy and efficacy of artificial neural networks in predicting bundle weight in banana plants have been illustrated by R2 = 91%, MPE = 1.40, and MSD = 2.29.

In their work, Torabi et al. (2013) employed nonlinear regression analysis to simulate the volumes of Red Delicious, Golden Delicious, and Granny Smith apple cultivars, utilizing parameters such as dimensions, surface area, mass, and sphericity. The findings indicated that the optimal models for volume estimation, based on the designated physical parameters, were third-degree, exponential, and second-degree equations for size, mass, and sphericity, respectively, with the most effective model for volume estimation in apple varieties being contingent upon the mass of the fruits. The determination coefficients for the Red Delicious, Golden Delicious, and Granny Smith cultivars were 0.984, 0.955, and 0.961, respectively. Sphericity exhibits the lowest coefficient of determination among all kinds in the modeling of apple volume.

Shahbazi (2015), in his investigation of the relationships between various physical properties of walnut fruits and their masses, determined that all correlations of the examined properties were significant at the 0.01 level and asserted that the optimal model for estimating walnut fruit mass based on size properties was a quadratic equation utilizing the fruit's width (W).

Vivek Venkatesh et al. (2015) employed an image processing approach in their research to forecast the correlation between the volume and mass of axially symmetric fruits, including apples, lemons, and oranges. This technology employed a single camera to record five distinct photos of the fruit, allowing for volume estimation based on their morphology. The fruits were classified into spherical, ellipsoidal, and paraboloidal forms, and a suitable analytical model was employed to compute the volume for each group. Consequently, the mass of the fruits has been determined by a straightforward method of estimating volume using a camera. The proposed procedures were executed utilizing the C++ programming language and an open-source computer vision library, with observations indicating that the findings were sufficiently accurate.

Balcı et al. (2016), in their paper "Classification of Napoleon cherries using image processing techniques," sought to ascertain the dimensions of Napoleon cherries by image processing methods. The work involved capturing photos of cherries and developing a programming language within

the MATLAB environment. The study revealed a color prediction accuracy of 99% and a species prediction accuracy of 95.52%. Future research should examine other elements, including the combined use of artificial neural networks and artificial intelligence technologies, images of completely ripe cherries, a better resolution camera, and a more professional setting.

In their 2016 paper "Automatic Yield Estimation System for Apricots," Varjovi and Talu sought to automatically ascertain the yearly output of apricots in orchards. The study utilized apricot orchards located in Malatya as the subject matter. The Gaussian mixture model was employed as the methodology. Consequently, they reported a success rate of R2 0.77 using the proposed technique. Future research indicated that improved outcomes would be achieved by utilizing factors associated with the ratio of fruit density to tree bulk.

Javadikia et al. (2017) employed image processing methodologies to categorize the Bam, Khooni, and Thompson orange types. Furthermore, data on fourteen physical attributes acquired via image processing in three variations were utilized in the ANFIS model to forecast orange mass. In the ANFIS model, 70% of the data was designated for the training set and 30% for the test set. Bam, Khooni, and Thompson established that the optimal model for the orange varieties had determination coefficients (R^2) of 0.948, 0.99, and 0.98, respectively. The prediction accuracy of the optimal model for the Bam, Khooni, and Thompson orange types was determined to be ± 3.7 g, ± 1.28 g, and ± 3.2 g, respectively. The findings collected demonstrate that ANFIS is adequate for determining the mass of oranges.

Solak and Altınışık (2018), in their study titled "Detection and Classification of Hazelnut Fruit Using Image Processing Techniques and Clustering Methods," aimed to classify hazelnuts using image processing techniques. They classified the hazelnuts as significant (K3), medium (K2), and small (K1). For the analysis, they used mean-based classification and K-means clustering methods. As a result of the study, they achieved a 100% success rate in detecting hazelnut fruits using image processing techniques. Thus, they concluded that the proposed software would be successful in classification processes using different objects.

Ahmad et al. (2019), in their paper "Mango Shape Ripeness Classification Using Image Processing," sought to ascertain the maturity degree of Harumanis Mango using shape analysis utilizing image processing techniques. They asserted that the primary physical attributes for assessing fruit maturity are exterior qualities including color, shape, size, and texture; however, this is not the case for Harumanis mangoes. The study classified mangoes based on form, as it is a crucial determinant of ripeness and

quality. The study employed a computer vision approach (CVM) encompassing picture capture, preprocessing, segmentation, feature extraction, and classification, asserting that the CVM classification aligns with human shape perception.

In their 2019 study, "Mango Classification System Using Image Processing Technology and Artificial Intelligence," Thong et al. aimed to create a system that could classify mangoes in Vietnam based on their color, volume, size, shape, and fruit density. A framework that integrates artificial intelligence, such as CCD cameras, C programming, computer vision, and artificial neural networks, has been established by the system for classification that employs imagery processing. The method employed graphics to represent the mango fruit's surface shortcomings, bulk, and volume. The study determined that an automated mango category system should be implemented to evaluate and regulate the quality of mangoes prior to packaging and exportation, incorporating data processing technique.

In their 2019 study, "An Example of the Use of Image Processing Technologies in Apple Orchards," Kaymak et al. sought to identify and quantify red apples on trees via image processing methodologies. They created software within a computing environment to accomplish this objective. The program created indicated and recognized the center points of apple objects, hence facilitating the counting of apples. They highlighted that the created application software can capture pictures via real-time snapshots, photographs, or live videos. The program successfully identified the red apples on the tree with an accuracy of 78.47% regarding color. They asserted that this scenario may be deemed effective for apple detection regarding color using image processing techniques.

Zhang et al. (2022), in their study "Automatic Flower Cluster Prediction in Apple Orchards Using Airborne and Ground-Based Point Clouds," sought to elucidate pruning and thinning operations through the utilization of unmanned aerial vehicles and ground-based artificial vision systems to assess flowering intensity in apple orchards. Two linear regression models were employed utilizing unsupervised machine learning techniques. Consequently, they said that the suggested techniques offer an innovative approach for directing floral thinning through the utilization of basic location-based artificial vision system imagery and location data.

In a study titled "Design and Testing of Apricot Kernel Shell Breaking Machine," Zhaoshuai et al. (2022) created a device to fracture apricot kernels and used it to study the mechanical properties of the fruit. We engineered the main parts of the shell breaking machine and tested it to see how changing the shell breaking gap, feeding speed, and differential speed ratio of the rollers affected the machine's performance. We also measured how

much damage the almonds sustained. This led to an impressive 99.04% shell breaking rate and 2.28% almond damage rate, proving the machine's excellent shell breaking efficacy and operating efficiency, with a feeding speed of 350 kg/hour, a differential speed ratio of 1.75, and a shell breaking gap of 8.5 mm.

Ramezanpour and Farajpour (2022) utilized artificial neural networks and genetic algorithms to forecast and enhance banana fruit output in greenhouses. To ascertain the optimal concentrations of three macronutrients—nitrogen (N) (0, 100, and 200 g), potassium (K) (0, 100, 200, and 300 g), and magnesium (Mg) (0, 50, and 100 g)—on fruit yield (FY), fruit length (FL), and variance analysis (ANOVA), the perspective row number (NRPS) of greenhouse bananas was employed, followed by a post hoc LSD test and two established neural networks: multilayer perceptron and generalized regression neural network (GRNN). The ANOVA findings indicate that the optimal average value of FY was achieved with 200 g of nitrogen, 300 g of potassium, and 50 g of magnesium. This study indicates that both ANN models had great prediction accuracy, with R2 values ranging from 0.66 to 0.99 for training and test data for FY, FL, and NRPS. Reports indicate that the GRNN model surpassed the MLP model in modeling and forecasting the three attributes of greenhouse bananas. A genetic algorithm (GA) was utilized on the GRNN model to determine the best quantities of N, K, and Mg for achieving elevated levels of FY, FL, and NRPS. The GRNN-GA hybrid model has demonstrated that significant yields may be attained by decreasing nitrogen, potassium, and magnesium-based chemical fertilizers by 65%, 44%, and 62%, respectively, in comparison to conventional methods.

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CHAPTER 5

EFFECTS OF CLIMATE CHANGE ON FRUIT GROWING: CHALLENGES AND ADAPTATION STRATEGIES

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1. Introduction

Climate change stands as one of the most pressing global challenges of the 21st century, profoundly transforming ecosystems, economies, and livelihoods worldwide (IPCC, 2021; Bilgili et al., 2024). This phenomenon is primarily characterized by rising global temperatures, increasingly erratic weather patterns, and a heightened frequency of extreme climatic events, including prolonged droughts and severe storms, with far-reaching consequences across multiple sectors. Agriculture, being intrinsically dependent on stable climatic conditions, emerges as one of the most vulnerable domains (Malhi et al., 2021). Among agricultural systems, fruit cultivation is particularly susceptible due to the precise environmental requirements necessary for each stage of development, including vegetative growth, flowering, fruit set, and maturation (Bhattacharjee et al., 2022; Bacelar et al., 2024).

The urgency of studying the effects of climate change on fruit production extends beyond the agricultural sector, as fruits play a critical role in global food security, serving as primary sources of essential nutrients while also making significant economic contributions (Ameen et al., 2023). Many tropical and subtropical regions rely heavily on fruit production not only to meet domestic consumption demands but also as a vital source of export revenue (Nath et al., 2018). Moreover, as high-value commodities, fruits are fundamental to the economic stability of millions of smallholder farmers worldwide, particularly in developing countries where agriculture constitutes the backbone of rural economies (Asfaw et al., 2019).

The increasing unpredictability of weather patterns—manifesting as unseasonal frosts, prolonged heatwaves, and erratic precipitation—poses severe disruptions to established farming cycles, adversely affecting fruit quality and overall yield (Lamichhane, 2021; Türkoğlu et al., 2021). Simultaneously, shifts in temperature and precipitation regimes are modifying pest and disease dynamics, further complicating fruit production and exacerbating existing challenges (Caselli & Petacchi, 2021; Subedi et al., 2023). These issues are particularly acute in developing economies, where financial constraints and limited access to advanced agricultural technologies hinder adaptive capacity (Reyes-García et al., 2019). A comprehensive understanding of these interrelated challenges is essential for developing effective mitigation and adaptation strategies that safeguard global food security and ensure economic stability in agriculturally dependent regions (Piñeiro et al., 2020).

This study aims to provide a systematic analysis of the multifaceted impacts of climate change on fruit cultivation. By examining shifts in phenological cycles, variations in yield and quality, and the increasing pre-

valence of pests and diseases, this paper seeks to offer a comprehensive perspective on this critical issue. Furthermore, it evaluates a range of adaptation strategies, including advanced agricultural practices, innovations in genetic resources, and policy-driven interventions. The ultimate objective is to present actionable recommendations that mitigate the adverse effects of climate change on fruit cultivation, fostering resilience and long-term sustainability in this vital agricultural sector.

1.2. Literature Review

A growing body of scholarly research highlights the significant vulnerability of fruit cultivation to the widespread impacts of climate change (IPCC, 2021; Osorio-Marín et al., 2024). One of the most consistently documented consequences is the alteration of phenological patterns, particularly the advancement of flowering and fruiting periods (Campoy et al., 2011; Legave et al., 2013). Empirical studies indicate that rising temperatures in temperate zones have led to earlier flowering in apple and cherry orchards, inadvertently synchronizing critical developmental stages with periods of heightened frost risk (Fujisawa & Kobayashi, 2010). Similarly, modifications in chill hour accumulation—a crucial factor for dormancy release—have been observed in stone fruits such as peaches and apricots (Luedeling et al., 2011; Fadón et al., 2020).

Beyond these phenological disruptions, climate change has profound and multifaceted effects on fruit yield and quality (Bacelar et al., 2024). The increasing frequency and intensity of drought stress, heatwaves, and irregular rainfall patterns directly contribute to lower fruit yields and deterioration in key quality attributes. These attributes include fruit size, organoleptic properties such as flavor, and essential nutritional content (Mattos et al., 2014; Wolfe et al., 2018). For instance, extensive research on grapevines has demonstrated that elevated temperatures accelerate sugar accumulation within berries, disrupting the delicate balance between sugar and acidity—a critical factor in premium wine production (Van Leeuwen & Darriet, 2016; Rogiers et al., 2022).

Another pressing issue is the increasing prevalence of agricultural pests and diseases. The combined effects of rising temperatures and increased humidity create more favorable environmental conditions for invasive species and pathogenic organisms (Subedi et al., 2023). Consequently, crop losses are intensifying, necessitating more rigorous management strategies. Research has documented the geographic expansion of pest populations, such as fruit flies, into regions that were previously unsuitable for their survival and proliferation (Caselli & Petacchi, 2021).

Despite significant advancements in understanding the effects of climate change on fruit cultivation, notable knowledge gaps remain. For example, the regional and species-specific consequences of climate change are still insufficiently studied, particularly in developing countries where limitations in data collection infrastructure and research capacity hinder comprehensive assessments (Asfaw et al., 2019). Additionally, while some studies have explored adaptation strategies, a more extensive body of evidence is necessary to rigorously evaluate their effectiveness across diverse environmental, economic, and social contexts (Zhang et al., 2018; Piñeiro et al., 2020). Addressing these gaps requires sustained research efforts and enhanced collaboration among scientists, policymakers, and fruit-growing communities to develop comprehensive, evidence-based strategies for mitigating climate-related risks.

2. Climate Change Impacts on Fruit Cultivation

Climate change imposes significant and multifaceted pressures on fruit cultivation, primarily through disruptions in phenological cycles, yield stability, and fruit quality. These challenges, driven by rising global temperatures and increasingly unpredictable precipitation patterns, pose substantial threats to the sustainability and productivity of fruit agriculture worldwide (Malhi et al., 2021; Bilgili et al., 2024).

2.1. Disruptions in Phenological Timing

Phenological changes are among the most evident and consequential effects of climate change in fruit cultivation systems (Legave et al., 2013; Türkoğlu et al., 2021). The steady increase in global mean temperatures has accelerated the timing of anthesis across a wide range of fruit-bearing species, including *Malus domestica* (apple), *Prunus persica* (peach), and *Prunus avium* (cherry). This advancement in flowering phenology can result in asynchronies between peak pollinator activity and critical reproductive stages, leading to measurable declines in fruit set efficiency (Fujisawa & Kobayashi, 2010). Additionally, earlier fruit ripening shifts traditional harvest periods, placing strain on labor availability and creating logistical challenges for commercial fruit producers (Moriondo et al., 2011).

The timing of both flowering and fruit ripening in many fruit species is highly sensitive to fluctuations in temperature and seasonal climatic variations. As global temperatures continue to rise, many fruit-growing regions are experiencing earlier flowering and ripening trends, with significant implications for fruit production systems. Elevated temperatures accelerate the accumulation of thermal units required for phenological development, often leading to premature flowering and ripening in economically

significant crops such as apples (*Malus domestica*), grapes (*Vitis vinifera*), and citrus fruits (*Citrus* spp.) (Baldocchi & Wong, 2022).

For example, commercially important apple cultivars in temperate regions have been observed to flower up to two weeks earlier than historical averages due to increased spring temperatures (Guo et al., 2020). This shift disrupts synchronization with pollinators, particularly bees, leading to lower fruit set rates and reduced overall yield. Similarly, premature fruit ripening can misalign with traditional harvesting schedules, complicating labor management and market distribution strategies (Moriondo et al., 2011).

Many fruit crops require specific temperature ranges to achieve optimal vegetative growth and reproductive development. Rising temperatures have led to earlier flowering and fruiting in various species, increasing their susceptibility to late-season frost events. For example, *Malus domestica* and *Prunus avium* trees cultivated in temperate regions have exhibited premature floral anthesis, heightening the risk of frost-induced damage to sensitive floral tissues and causing significant reductions in fruit set (Lamichhane, 2021).

The impact of climate-induced phenological shifts is particularly evident in apple cultivation across Europe. Studies indicate that apple orchards in major fruit-producing countries such as France and Germany now exhibit flowering times 10 to 15 days earlier than those recorded in the 1980s (Legave et al., 2013). This shift has increased vulnerability to late spring frosts, leading to significant yield losses in years with unfavorable climatic conditions.

2.1.1. Case Study: Impacts on Viticulture

Within viticulture (*Vitis vinifera* cultivation), climate-driven phenological shifts are influencing key aspects of grape quality, particularly sugar accumulation, acidity balance, and secondary metabolite composition (Van Leeuwen & Darriet, 2016). Elevated temperatures accelerate sugar accumulation within grape berries, disrupting the equilibrium between sugar and acidity, which is crucial for wine production (Rogiers et al., 2022). Research has shown that prolonged heatwaves during critical grape development stages induce physiological heat stress, leading to berry desiccation and a subsequent decline in fruit quality parameters.

While certain cooler regions may benefit from extended growing seasons due to warming trends, the broader consequences of phenological shifts are largely detrimental. Premature flowering increases the risk of exposure to late frosts, which can cause substantial crop losses. For

example, in *Prunus avium* (cherry), late-season frosts can result in cryogenic damage to floral structures, often leading to total crop failure (Atkinson et al., 2013). Furthermore, changing phenological patterns may drive shifts in optimal cultivation zones, forcing traditional fruit-growing regions to adapt or risk losing their suitability for specific crops due to evolving temperature constraints.

2.1.2. Chill Hour Requirements and Low Temperature Effects

Many fruit crops native to temperate regions require a specific cumulative period of chill hours to break dormancy and initiate uniform budburst in the spring. Chill hours are defined as the total duration of exposure to low temperatures, typically ranging between 0°C and 7.2°C, which are essential for overcoming endodormancy and ensuring synchronized flowering (Luedeling et al., 2011; Fadón et al., 2020). However, as winter temperatures continue to rise due to climate change, these critical chilling requirements are increasingly unmet in various fruit-growing regions. This deficit in accumulated chill hours leads to irregular and asynchronous flowering, ultimately resulting in reduced fruit set and lower commercial yields (Campoy et al., 2011). Conversely, the increasing occurrence of unseasonal frost events in early spring can cause severe cryogenic damage to flower buds, significantly affecting fruit production. Such frost-induced losses have been well-documented in economically significant species such as *Prunus armeniaca* (apricots) and *Prunus domestica* (plums) (Atkinson et al., 2013).

Temperate fruit species, including *Prunus avium* (cherries), *Prunus persica* (peaches), and *Prunus armeniaca* (apricots), rely on an adequate accumulation of chilling hours to successfully transition from dormancy to active growth. The ongoing decline in winter chilling, a direct consequence of global warming, poses a major challenge to the sustainable cultivation of these crops. Studies indicate that in key fruit-growing regions such as California, where *Prunus* species are extensively cultivated, the number of annual chill hours has steadily declined over the past century. As a result, fruit orchards are experiencing irregular and unsynchronized flowering, leading to lower fruit set percentages and reduced overall yields (Baldocchi & Wong, 2022). Similarly, projections suggest that regions in southern Europe and the Mediterranean basin—longstanding centers of fruit production—could lose up to 50% of their available chill hours by 2050, jeopardizing the economic viability of fruit cultivation in these historically significant agricultural zones (Luedeling et al., 2011).

The reduction in chill hours can cause “dormancy disruption,” a physiological condition in which developing flower buds fail to progress uni-

formly. This results in asynchronous and prolonged flowering, irregular fruit development, and uneven maturation, all of which negatively impact harvest efficiency and fruit quality. Adding to these challenges, late-season frosts—expected to become more frequent and unpredictable due to climate change—pose a severe risk to fruit crops. When frost occurs during the early flowering period, it can cause irreversible damage to floral tissues, particularly in *Malus domestica* (apples) and *Prunus domestica* (plums), where frost-induced injury frequently leads to significant commercial losses (Atkinson et al., 2013).

In response to these climatic changes, researchers and plant breeders are actively working on developing low-chill or no-chill cultivars that can maintain productivity under warmer winter conditions. These advanced cultivars are selectively bred to require fewer chilling hours while still producing viable yields (Gogorcena et al., 2020). However, large-scale commercial adoption of such climate-resilient cultivars remains in its early stages, and widespread implementation may take several decades. Until then, the continued decline in winter chilling remains a critical challenge for global fruit production, particularly in regions already experiencing marginal chilling conditions and limited adaptive capacity.

2.2. Effects on Yield and Quality

2.2.1. Role of Temperature and Precipitation Changes on Yield

The productivity of fruit crops is intricately linked to climatic conditions, with fluctuations in temperature and precipitation being key determinants of yield. Climate change has led to increased variability in these factors, resulting in a rise in extreme weather events, including heatwaves, unseasonal frosts, prolonged droughts, and excessive rainfall. These climatic disruptions pose significant challenges to fruit production at regional, national, and global scales (IPCC, 2021).

2.2.1.1.Temperature Extremes and Heat Stress

Extreme heat events, particularly during critical phenological stages such as flowering, fruit set, and early fruit development, can severely impact fruit yields. Excessive temperatures reduce pollen viability, impair pollination success, and increase floral and fruit drop. For instance, *Mangifera indica* (mango) production in India has suffered significant yield reductions due to heat-induced flower abortion and reduced fruit set (Shah et al., 2017). Similarly, high temperatures during fruit development in *Citrus* and *Prunus* species can cause sunburn on fruit surfaces, reducing marketable yields (Wolfe et al., 2018). Additionally, thermal stress disrupts essential

physiological processes such as photosynthesis, leading to reduced fruit size and lower sugar content, as observed in *Vitis vinifera* (grape) cultivars used in winemaking (Van Leeuwen & Darriet, 2016). Furthermore, heat stress increases plant susceptibility to pests and diseases, compounding yield losses (Subedi et al., 2023).

2.2.1.2. Drought and Water Scarcity

Water availability is another crucial factor influencing fruit yields. Extended drought periods and declining water resources, exacerbated by climate change, have severely affected fruit production across different regions. Drought stress during flowering and fruit development leads to smaller fruit, increased fruit drop, and overall yield reductions. In California, *Prunus dulcis* (almond) and *Vitis vinifera* vineyards have suffered significant yield declines due to recurrent droughts and restricted irrigation supplies (Medellín-Azuara et al., 2015). Water shortages also impair nutrient uptake, further compromising fruit quality. In regions such as Sub-Saharan Africa, where *Musa* spp. (bananas) and *Musa paradisiaca* (plantains) are staple crops, declining rainfall has threatened food security and rural livelihoods (Nelson et al., 2010).

2.2.1.3. Excessive Rainfall and Flooding

While drought is a major concern, excessive rainfall and flooding present equally severe challenges. Saturated soils limit oxygen availability to roots, leading to root anoxia and reduced yields. *Fragaria* × *ananassa* (strawberries) and *Citrus* species are particularly vulnerable to waterlogged conditions, which also promote fungal diseases such as *Phytophthora* and *Botrytis* (Wolfe et al., 2018). In Southeast Asia, prolonged monsoonal rains have disrupted *Durio zibethinus* (durian) and *Nephelium lappaceum* (rambutan) production by negatively affecting flowering and fruit development. Similarly, in South America, excessive rainfall during *Coffea* spp. (coffee) flowering has caused irregular fruit set, reducing overall yields (daMatta et al., 2007).

2.2.1.4. Case Studies of Climate-Induced Yield Declines

In Southern Europe, *Olea europaea* (olive) production has been significantly affected by rising temperatures and drought stress, with projections indicating potential yield reductions of 25–30% by 2050 in traditional growing regions (IPCC, 2021). Similarly, *Malus domestica* (apple) production in China's Loess Plateau has been disrupted by earlier flowering and insufficient rainfall, increasing annual yield variability (Guo et al., 2020).

2.2.2. Changes in Fruit Quality

In addition to yield reductions, climate change also affects fruit quality, which is a key factor influencing market value and consumer preference. Alterations in temperature, precipitation, and atmospheric CO_2 levels impact fruit sugar content, acidity, texture, coloration, and nutritional composition (Bacelar et al., 2024).

2.2.2.1. Sugar Content and Acidity

Higher temperatures during fruit ripening accelerate sugar accumulation while simultaneously reducing acidity. This imbalance affects the organoleptic properties of fruits, diminishing consumer appeal. *Vitis vinifera* grapes used for winemaking are particularly sensitive to these changes, as lower acidity levels negatively affect wine quality and aging potential (Van Leeuwen & Darriet, 2016; Rogiers et al., 2022). Similarly, *Malus domestica* apples grown under elevated temperatures exhibit higher sugar content but reduced acidity, altering their traditional regional flavor profiles (Guo et al., 2020).

2.2.2.2. Color and Shelf Life

Temperature fluctuations and heat stress significantly affect fruit pigmentation. The intensity and uniformity of fruit color are important quality parameters that influence consumer perception of ripeness and freshness. Heat stress can reduce fruit firmness and shorten shelf life, as seen in *Fragaria* × *ananassa* (strawberries), where increased temperatures lead to softer fruit that is more susceptible to mechanical damage and post-harvest spoilage (Wolfe et al., 2018).

2.2.2.3. Nutritional Composition

Climate change also alters the nutritional content of fruits, particularly their micronutrient and antioxidant profiles. Elevated atmospheric CO_2 levels have been shown to increase carbohydrate concentrations in fruit tissues while simultaneously reducing protein, vitamin, and mineral levels. This so-called "nutrient dilution effect" has been observed in *Citrus* species and other fruit crops, raising concerns about potential declines in fruit nutritional value (Myers et al., 2014; Loladze, 2014). For instance, under elevated CO_2 conditions, *Citrus sinensis* (oranges) and *Musa* spp. (bananas) have exhibited increased sugar content but reduced concentrations of essential minerals such as calcium and magnesium. Furthermore, heat stress can disrupt the biosynthesis of secondary metabolites, including anthocyanins and carotenoids, which are responsible for fruit pigmentati-

on and antioxidant properties. *Vaccinium corymbosum* (blueberries), for example, produce lower anthocyanin levels under high-temperature stress, reducing their antioxidant capacity and commercial value (Zhao et al., 2021).

2.2.2.4. Impact on Specific Crops

- **Apples (*Malus domestica*)**: Higher temperatures can advance flowering, increasing susceptibility to frost damage. Reduced winter chill accumulation can also affect fruit set and quality attributes (Legave et al., 2013).
- **Grapes (*Vitis vinifera*)**: Elevated temperatures accelerate ripening, necessitating earlier harvests and potentially affecting wine quality. Water stress further reduces yield and quality (Rogiers et al., 2022).
- **Citrus Fruits (*Citrus* spp.)**: Temperature and precipitation changes impact fruit size, sweetness, and acidity. Higher CO_2 levels may increase fruit size but reduce vitamin C content (Wolfe et al., 2018). Warmer conditions may also exacerbate pest and disease pressure (Caselli & Petacchi, 2021).
- **Berries (e.g., *Fragaria* × *ananassa*, *Vaccinium corymbosum*)**: These crops are highly sensitive to temperature and water availability. Warmer temperatures accelerate ripening, alter sugar-acid balance, and reduce post-harvest shelf life (Johnson et al., 2023). Excess rainfall increases fungal infection risks during harvest (Wolfe et al., 2018).

2.2.2.5. Regional Variability in Quality Changes

In geographically circumscribed tropical regions, fruit commodities such as *Musa* spp. (bananas) and *Mangifera indica* (mangoes) have shown significant post-harvest physiological changes in direct response to environmental stress mediated by climate change. For example, *Mangifera indica* fruits exposed to prolonged periods of elevated temperatures during post-harvest ripening exhibit uneven exocarp coloration patterns and suboptimal texture, which negatively impacts consumer acceptance and reduces export value (Shah et al., 2017). This highlights the necessity of adapting harvesting and storage practices to cope with changing environmental conditions.

2.3. Role of CO_2 and Nutrient Content

While elevated atmospheric CO_2 concentrations may increase photosynthetic carbon assimilation rates and enhance biomass production in certain fruit crops, they also lead to a dilution of proteinaceous compounds and micronutrients in the fruits. This phenomenon, known as the "nutrient dilution effect," poses significant concerns about the future nutritional quality of fruit crops grown under progressively elevated atmospheric CO_2 concentrations (Loladze, 2014; Myers et al., 2014). The decrease in micronutrient content under high CO_2 conditions could have long-term implications for human nutrition, particularly in regions heavily reliant on fruits as a primary food source.

2.5. Increased Pest and Disease Pressure

As ambient temperatures continue to rise, they create favorable conditions for a wide range of agricultural pests and pathogens that pose significant threats to fruit production. This shift in environmental suitability for pests and pathogens, driven by climate change, increases the dependency on synthetic pesticides in global fruit production systems (Deutsch et al., 2018). Additionally, milder winter temperatures in many fruit-growing regions facilitate the survival of pests, such as the *Drosophila suzukii*, which can increase infestation pressures and result in substantial economic losses during subsequent growing seasons (Caselli & Petacchi, 2021).

2.6. Invasive Species and Pest Migration

Climate change, particularly global warming, facilitates the migration of agricultural pests into previously unaffected regions. This includes species such as the spotted wing drosophila (Drosophila suzukii), which has rapidly expanded its range due to warmer temperatures. This invasive species is causing significant damage to berry and stone fruit crops in areas where it was not previously a concern, posing a major threat to fruit production worldwide (Walsh et al., 2011). Understanding and forecasting the migration patterns of these pests will be critical in developing effective pest management strategies (Sharma, 2014; Caselli & Petacchi, 2021).

2.7. Water Scarcity and Drought Effects

The increasing frequency and intensity of droughts, marked by prolonged periods of below-average precipitation and reduced soil moisture, are significantly impacting fruit production in many regions. Grapevines and other fruit crops, which require consistent soil moisture, are particularly vulnerable to the adverse effects of drought, including reduced yields

and decreased fruit quality (Chaves et al., 2007). This trend emphasizes the importance of adopting water-efficient irrigation technologies and sustainable water management practices to mitigate the impacts of water scarcity on fruit production (Trenberth et al., 2014; Rey et al., 2017; El Jaouhari et al., 2018).

2.8. Drought Resilience Techniques

In response to escalating drought risks, fruit growers are increasingly adopting water-conserving irrigation strategies. One such approach is deficit irrigation, which involves strategically applying water stress during specific growth stages to enhance desirable fruit attributes, such as increased sugar concentration and improved fruit coloration, while conserving water resources (Medrano et al., 2015; Ali et al., 2020). These adaptive strategies are essential for maintaining fruit production in regions facing increasing water scarcity and will be crucial for ensuring the sustainability of global fruit agriculture under future climate scenarios.

2.9. Shifts in Geographic Distribution of Fruit Crops

Optimal geographical regions for the economically viable and commercially profitable cultivation of numerous fruit crops are undergoing discernible shifts at regional, continental, and global scales. These shifts are primarily driven by the rising global average temperatures and the accompanying changes in climatic suitability for traditional fruit-growing regions (Hannah et al., 2013; Fraga et al., 2016). Regions once considered optimal for certain fruit crops may become less suitable, while previously marginal areas may become increasingly viable due to changing climatic conditions. This redistribution not only alters global fruit production dynamics but also necessitates significant investments in agricultural infrastructure and post-harvest supply chains in emerging regions. Furthermore, these changes have the potential to disrupt long-standing global fruit trade networks, potentially transforming international trade patterns and market demands (Davis et al., 2019).

2.9.1. Example: Grapevine Shifts in the Wine Industry

Long-established wine-producing regions, particularly in California and Southern Europe, are witnessing a decline in the economic viability of viticulture due to rising temperatures and other climate change impacts (Hannah et al., 2013). As a result, wine production is shifting to previously cooler regions, such as Canada and Northern Europe, where these areas are now becoming more suitable for viticulture. The transformation of these

marginal regions into viable wine-producing areas presents new economic opportunities but also introduces challenges in adapting to new climatic conditions and potential market shifts (Jones et al., 2005).

2.10. Impact of Climate Change on Pest and Disease Populations

Climate change is significantly altering the geographic distribution, life cycle, and severity of pest and disease populations affecting fruit cultivation globally. Rising temperatures create more favorable conditions for pests such as *Bactrocera* spp. (tephritid fruit flies), *Aphis* spp. (aphids), and *Thripidae* (thrips), which are expanding their ranges into previously temperate regions. Additionally, warmer winter temperatures contribute to higher survival rates and larger pest populations in subsequent growing seasons, exacerbating the risk of crop damage. Changes in precipitation patterns also play a role by creating environments conducive to the proliferation of fungal pathogens like *Podosphaera leucotricha*, *Plasmopara viticola*, and *Colletotrichum* spp. These diseases thrive in elevated moisture conditions and have been linked to substantial crop losses in fruits such as *Vitis vinifera* (grapevine), *Fragaria* × *ananassa* (strawberry), and *Persea americana* (avocado) (Thomson et al., 2010; Jones & Barbetti, 2012).

2.10.1. Ecological Imbalances

Climate change is disrupting the ecological balance between pests, their natural predators, and host plants. For example, increased temperatures accelerate the development of pests, leading to more frequent reproductive cycles. At the same time, elevated temperatures reduce the efficacy of natural control agents such as parasitoid wasps and entomophagous insects. This imbalance increases the vulnerability of fruit crops to pest infestations and disease outbreaks, highlighting the need for more adaptive pest management strategies (Gutierrez et al., 2008).

2.10.2. Control Strategies

In response to the growing threats from pests and diseases, fruit producers are increasingly adopting Integrated Pest Management (IPM) strategies that combine biological, cultural, and chemical methods to minimize damage while reducing reliance on broad-spectrum pesticides. The use of pheromone traps and the introduction of natural predators, such as parasitoid wasps, are gaining traction as effective, environmentally sustainable solutions for managing fruit fly populations (Brewer & Goodell, 2012; Zhou et al., 2024). Concurrently, organic farming practices, including the use of biopesticides and disease-resistant crop varieties, are becoming

more widely implemented as alternatives to conventional chemical-intensive pest control methods.

Recent advances in precision agriculture technologies, such as remote sensing and unmanned aerial vehicles (UAVs), also enable more precise monitoring of pest populations and disease outbreaks. This real-time data allows for targeted interventions, optimizing pest and disease management while minimizing environmental impacts and improving long-term sustainability in fruit production (Zhou et al., 2024).

3. Breeding Climate-Resilient Varieties

One of the most effective and sustainable strategies to mitigate the diverse impacts of climate change on commercial fruit production is the development and deployment of climate-resilient fruit varieties. By combining traditional plant breeding methods with modern biotechnological tools, such as targeted genetic engineering and CRISPR-Cas9 genome editing, there are significant opportunities to enhance the tolerance of fruit crops to abiotic stresses (e.g., heat and drought) and biotic stresses (e.g., insect pests and plant diseases) (Luedeling et al., 2011; Gogorcena et al., 2020).

For example, ongoing research focuses on developing *Prunus persica* (peach) and *Prunus armeniaca* (apricot) cultivars that require fewer chilling hours, which helps them thrive in regions with milder winter temperatures. In a similar vein, breeding efforts are focused on improving *Vitis vinifera* (grapevine) cultivars to better withstand high temperatures and resist economically significant fungal diseases, such as Podosphaera leucotricha, which are exacerbated by climate change (Lobos et al., 2012).

4. Sustainable Water Management

Given the documented increase in the frequency and severity of prolonged droughts and escalating water scarcity in many fruit-growing regions worldwide, implementing efficient and environmentally sound water management practices is crucial for the long-term sustainability and economic viability of commercial fruit production. Advanced irrigation techniques, such as micro-irrigation systems (e.g., drip irrigation) and fertigation (the combined application of water and soluble nutrients), allow for the precise and targeted delivery of water and nutrients directly to the root zone. This approach significantly reduces water losses compared to traditional flood or furrow irrigation, optimizing both water usage and crop productivity (Zhang et al., 2018; Ali et al., 2020).

In addition, rainwater harvesting systems and the reuse of recycled water for irrigation are becoming increasingly important, especially in regions facing chronic water shortages, like California and Australia (Fentabil, 2016).

5. Soil Management

Healthy, biologically robust soil is the foundation of climate-resilient and ecologically sustainable agricultural systems. Implementing soil conservation practices, such as reduced or no-tillage farming, incorporating cover crops in crop rotations, and applying organic surface mulches, can improve soil structure, enhance water retention, and reduce the risk of soil erosion during extreme weather events, such as heavy rainfall and high-velocity winds (Lal, 2013; Mangalassery et al., 2015).

6. Mulching and Shade Nets

Fruit producers are increasingly using both organic and synthetic mulching materials to retain soil moisture, regulate temperature fluctuations, and suppress weed growth in orchards and vineyards. The use of shade netting systems made from specialized photoselective textiles is also becoming more common. These nets provide targeted protection from excessive solar radiation and extreme temperatures, reducing heat stress on fruit-bearing plants and preventing solar radiation-induced fruit damage (Kalcsits et al., 2017; Mupambi et al., 2018).

7. Sunburn Protection

Advanced agricultural technologies, such as specialized shade netting systems and the application of reflective exocarpic materials (e.g., kaolin clay films), can effectively reduce the incidence and severity of sunburn caused by solar radiation in economically important fruit crops, such as *Malus domestica* and *Citrus* spp. (Kalcsits et al., 2017; Mupambi et al., 2018).

8. Policies and Education

8.1. Development of Agricultural Policies

Governments play a critical role in addressing the multifaceted challenges posed by anthropogenic climate change to the agricultural sector, particularly in fruit cultivation systems. The development and proactive implementation of comprehensive agricultural policies designed to enhan-

ce system-wide resilience and adaptive capacity are essential for sustaining robust fruit production amid increasing climate-related stressors. Key policy initiatives include providing targeted financial subsidies to promote the adoption of climate-resilient crop varieties and implementing incentive programs that encourage ecologically sustainable farming practices (Wolfe et al., 2018; IPCC, 2021).

Targeted financial support—such as direct subsidies for purchasing certified seeds or grafted saplings of drought-tolerant, heat-resistant, or pest-resistant fruit cultivars—can alleviate the economic burden on farmers and enhance crop establishment and long-term productivity under adverse environmental conditions (IPCC, 2021). Additionally, policy frameworks can incentivize sustainable practices like Integrated Pest Management (IPM), precision agriculture, and certified organic farming through tax benefits and direct financial assistance, thereby mitigating the impacts of climate change while reducing the environmental footprint of conventional agriculture (Zhou et al., 2024). Moreover, public policies promoting water-efficient irrigation systems, such as micro-irrigation (drip systems) and rainwater harvesting infrastructure, are vital, especially in regions suffering from severe water scarcity and recurrent droughts (Zhang et al., 2018; Ali et al., 2020).

Furthermore, governments at various levels must invest strategically in critical agricultural infrastructure and deploy effective early-warning systems to enable fruit producers to prepare for and mitigate extreme weather events. The establishment of robust agricultural insurance schemes that provide fair financial compensation for climate-induced crop losses can further reduce economic risks, encouraging proactive adaptive measures (Mârza et al., 2015). Finally, sustained collaboration among government agencies, agricultural research institutions, and fruit producer communities is crucial to ensure that policies are grounded in practical realities and effectively address the needs of stakeholders most impacted by climate change (Reyes-García et al., 2019).

8.2. Farmer Education and Awareness Programs

Farmer education and outreach initiatives are fundamental to any effective, scalable climate change adaptation strategy in agriculture. Empowering fruit producers with the necessary knowledge, technical skills, and operational competencies to adopt climate-adaptive practices is essential for building resilience at local, regional, and national levels. Targeted training programs, accessible agricultural extension services, and widespread public awareness campaigns constitute a set of critical, mutually reinfor-

cing tools that promote farmer education and facilitate practice change (Pretty et al., 2011; Altieri & Nicholls, 2017).

Agricultural extension services play an instrumental role in bridging the gap between cutting-edge scientific research and practical on-farm operations. By disseminating actionable, research-based information on climate-resilient techniques—including crop diversification, optimized planting schedules, and sustainable soil management—extension services enable fruit producers to make informed management decisions. For example, extension programs that highlight the benefits of agroforestry or polyculture systems have effectively encouraged the integration of ecologically sustainable practices into fruit production (Altieri & Nicholls, 2017).

Public awareness campaigns that emphasize the escalating risks posed by climate change and the benefits of adaptive strategies can further motivate fruit producers to adopt climate-adaptive practices. Initiatives such as farmer field schools and community-based experiential learning have proven particularly successful in regions like Sub-Saharan Africa and South Asia, where localized knowledge exchange fosters context-specific adaptation strategies (Pretty et al., 2011).

Moreover, the growing accessibility of digital information and communication technologies offers novel, scalable opportunities for farmer education. Mobile applications providing localized weather forecasts, real-time pest and disease alerts, and decision-support systems for optimized irrigation scheduling have emerged as valuable tools for fruit producers. These digital resources empower farmers to make timely, data-driven management decisions, thereby enhancing both on-farm productivity and overall agroecosystem resilience (Zhai et al., 2020). Collaborative efforts among governments, NGOs, and private sector technology providers are essential to ensure equitable access to these digital tools, especially for smallholder and economically marginalized fruit farmers who are most vulnerable to the adverse impacts of climate change (Reyes-García et al., 2019).

9. Conclusion and Recommendations

9.1. General Evaluation

Research shows that human-induced climate change has wide-ranging and complex effects on global fruit cultivation. These effects include major changes in key growth phases, increased year-to-year variability in yields, a decline in fruit quality, and a greater vulnerability to both common and emerging pests and diseases. Rising temperatures, unpredictable rain-

fall, and more frequent extreme weather events are disrupting traditional growing seasons and reducing the suitability of many established fruit-producing regions. Although scientific understanding of these impacts has advanced, important knowledge gaps remain. In particular, more research is needed on species-specific responses and regional differences in how fruit crops respond to various climate-related stressors (Luedeling et al., 2011; Fraga et al., 2016).

For example, many studies have clearly shown that reduced winter chilling hours negatively affect temperate fruit production. However, there is limited research on the physiological and growth-related mechanisms that help tropical and subtropical fruit crops adapt to severe heat stress. Similarly, although the negative effects of climate change on yield and quality have been well documented, the combined impacts of heat, drought, and pest or disease outbreaks are not fully understood and require further study (Jones & Barbetti, 2012; Sharma, 2014).

Current adaptation strategies, including the development of climate-resilient fruit varieties, the adoption of sustainable farming practices, and the use of precision agriculture technologies, show significant promise in improving overall system resilience. Nevertheless, these approaches need further refinement, wider on-farm adoption, and faster implementation to meet the scale of the challenge. Transitioning to more ecologically resilient and economically sustainable fruit production also depends on stronger public policies and enhanced collaboration among fruit producers, researchers, policymakers, and international agricultural organizations (IPCC, 2021; Zhou et al., 2024).

9.2. Future Strategies and Conclusion

9.2.1. Research and Innovation Imperatives

Future agricultural research must focus on quickly developing robust, widely adapted, climate-resilient fruit varieties that can endure extreme temperatures, limited water supplies, and increased pest and disease pressures. Advances in functional genomics, gene editing techniques such as CRISPR-Cas9, and improved traditional breeding methods provide promising pathways for developing these resilient cultivars (Luedeling et al., 2011; Gogorcena et al., 2020). Moreover, research should investigate how multiple environmental stressors interact to affect fruit crop physiology. This knowledge will support the creation of integrated and effective adaptation strategies (Fraga et al., 2016).

9.2.2. Policy and Governance Imperatives

Policymakers at local, regional, national, and international levels need to develop and continually update comprehensive agricultural policies that support climate adaptation and strengthen resilience in the fruit production sector. This should include targeted financial subsidies and economic incentives for adopting climate-resilient practices, sustained public funding for agricultural research and infrastructure, and investments in essential systems like water storage and early-warning networks for extreme weather and pest or disease outbreaks (Wolfe et al., 2018; IPCC, 2021). International collaboration is vital to ensure the fair sharing of adaptation knowledge, technological innovations, and necessary resources (Reyes-García et al., 2019).

9.2.3. Farm-Level Adaptation Actions

Fruit producers, as the primary managers of agricultural landscapes, can adopt a range of practical and cost-effective strategies to reduce climate-related risks. These include adjusting planting dates and crop development schedules to match shifting growing seasons, implementing water-saving irrigation and improved soil management practices, and diversifying cropping systems to spread risk (Lal, 2013; Ali et al., 2020). In addition, community-based extension programs and farmer-to-farmer knowledge-sharing networks can greatly enhance local adaptive capacity and speed up the adoption of new practices (Pretty et al., 2011).

9.2.3.4. Collaboration and Capacity Building Imperatives

The success of climate adaptation efforts in the fruit sector depends on strong collaboration among all stakeholders, including fruit producers, researchers, policymakers, private technology providers, and international organizations. Capacity-building initiatives, such as technical training programs, farmer workshops, and accessible extension services, are essential to equip producers with the skills and knowledge needed for effective adaptation (Reyes-García et al., 2019; Zhai et al., 2020). Such collaboration ensures that adaptation strategies are both tailored to local conditions and economically viable.

9.3. Concluding Synthesis

In conclusion, overcoming the challenges posed by climate change in global fruit cultivation requires a comprehensive approach that combines continuous scientific innovation, supportive public policies, and proactive on-farm management. By strengthening resilience at every level—from re-

search institutions to individual farms—the fruit production sector can not only withstand the impacts of climate change but also thrive, contributing to global food security, supporting rural economies, and ensuring long-term sustainability (IPCC, 2021; Zhou et al., 2024).

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