THEORY, CONTEXT, AND PRACTICE IN AGRICULTURAL, FORESTRY, AND AQUATIC SCIENCES

Editor: Prof. Dr. Gökhan AYDIN

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Editor

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Use Of Tissue Culture Techniques In Plant Breeding Studies

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1. INTRODUCTION

Throughout history, efforts to improve the use of plants for human and animal nutrition have played a significant role in the development of agriculture. There have been two major phases where these efforts were intensified. The first is known as the Green Revolution, and the second is the Gene Revolution. The Green Revolution, a cornerstone of modern agriculture, occurred in the mid-20th century. It is characterized by the advancement of traditional plant breeding methods, the widespread use of commercial fertilizers, and improvements in agricultural practices such as irrigation (Baydar, 2020).

This approach led to a significant increase in the production of staple crops, particularly wheat, maize, and rice (Llewellyn, 2018). As a result, the rapidly growing global population's food demands were addressed more effectively (Algül et. al., 2017). On the other hand, beginning in the late 20th century, the Gene Revolution revolutionized agriculture by establishing the foundation of plant biotechnology. This was made possible by the discovery of DNA's structure, the advancement of genetic engineering techniques, and the integration of plant tissue culture applications. During this era, new plant varieties with desired traits began to be developed by altering their genetic makeup (Arı, 2001).

Nowadays, plant breeders are utilizing biotechnological methods to complement, support, and accelerate traditional plant breeding efforts. This helps them obtain new plant varieties that are high-yielding, of good quality, and resistant to various stress conditions (Baydar, 2020). When it comes to plant products, several important issues stand out. As the global population rapidly increases, the amount of arable land has reached its potential limits. Approximately two-thirds of the world's population suffers from inadequate or

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unbalanced nutrition. In response, innovations are emerging in industrial and processing technologies, while advancements are being made in preservation and transportation methods. Plant breeding is the science and art of altering or improving the genetic makeup of plants to meet human needs (Oğlakçı and Tiryaki, 2014).

The foundation of this discipline is built upon genes, the units of inheritance, genetic manipulation methods, and the principles of genetic behaviour that allow for the accurate prediction of outcomes. The main goal of a plant breeder is to obtain a new variety or rootstock with one or more improved traits and to develop suitable parents for the production of hybrid varieties (Cloutier and Landry, 1994). The primary objectives in general breeding programs are to increase product yield, enhance quality, develop species resistant to adverse environmental conditions, and create varieties that are durable against diseases and pests.

Considering that it's difficult or even impossible to expand agricultural land today, obtaining more products from the same area, reducing costs, and increasing quality are of vital importance for human nutrition. This is where biotechnological applications offer significant advantages. Biotechnology is used in many different fields besides plant breeding, such as human and animal health, environmental protection, mining, the development of alternative energy sources, and the chemical and food industries. One of the biotechnological methods used in plant breeding is plant tissue culture (Ergül et. al. 2001).

2. PLANT TISSUE CULTURE

Plant tissue culture technology is a method frequently used in large-scale plant production today. In addition to its use in research, plant tissue culture techniques have recently gained significant industrial value in areas such as plant propagation, disease elimination, plant breeding, and secondary metabolite production (Baydar, 2020).

Through the plant tissue culture method, it is possible to obtain hundreds or even thousands of new plants from a small piece of plant called an explant (Kumar and Loh, 2012). Plant tissue culture is the process of in vitro proliferation of sterile plant parts, known as explants, taken from plant organs such as roots, stems, and leaves, or from meristematic tissues and apical meristems, in special nutrient-rich media under laboratory conditions (Vuran and Turker, 2021). This method enables plant production under controlled conditions. The primary objectives of this method are to produce plants free from microbial diseases, to generate biochemical compounds, to conserve plant genetic resources, to solve problems that cannot be addressed with conventional agricultural methods, and

ultimately, to achieve high-quality and economic plant production (Özcan et al., 2001). Furthermore, in the field of genetic engineering, tissue culture techniques play a significant role in creating plants with specific genetic structures, obtaining rapidly developing individuals, and identifying plants that are resistant to abiotic and biotic stress factors (Vuran and Türker, 2021).

The first attempt at in vitro plant tissue culture was conducted by Haberlandt in 1902 (Algül et. al., 2017). Subsequent tissue culture research, until 1975, was generally based on the principles of callus culture and plant regeneration (Özcan et. al. 2021).

Thanks to plant tissue culture, it is possible to produce plants without being affected by environmental factors, increase the amount of beneficial chemical components in plants, create a suitable environment for gene transfer, and conserve endangered plant species (Amente and Chimdessa, 2021). The applications of plant tissue culture include genetic engineering, artificial seed production, asexual propagation, the production of an unlimited number of genetically identical plants, the provision of raw materials for the pharmaceutical and food industries, and the production of biochemical products (Özcan et. al. 2021).

3. AIMS OF PLANT TISSUE CULTURE APPLICATIONS

3.1. Healthy Plant Production

Meristem tissue culture is one of the most well-known methods for obtaining a large number of healthy and genetically identical plants from a diseased plant in a short period (Amente and Chimdessa, 2021) Numerous studies since 1952 have shown that the meristem tissue culture technique can be used to produce virus-free individuals in various plant species. For example, research conducted in 1965 successfully produced virus-free freesia plants using meristem culture (Arı, 2001).

3.2. Rapid Propagation of Plants

In addition to producing a large number of healthy copies from a single plant in a short amount of time, the micropropagation method offers advantages such as year-round production, minimal space requirements for the propagation material, and reduced labor needs (Thorpe and Stasolla, 2001).

3.3. Production of Transgenic Plants

Genetic transformation, a modern application of plant cell and tissue culture, offers possibilities for transferring genes with desired traits to target plants and for obtaining genetically modified, or transgenic, plants (Kumar and Loh, 2012).

The process of producing transgenic plants aims to modify the genetics of selected plant cells, tissues, and the entire plants that are regenerated from them. In a tissue culture environment, the probability of plant regeneration is increased by meticulously controlling environmental conditions and the growth medium (Özcan et. al. 2021).

This technique holds significant potential for improving the genetic makeup of various plant species by integrating it into plant biotechnology and breeding programs. It plays a promising role in conferring agriculturally valuable traits such as increased yield, improved quality, and enhanced resistance to pests and diseases (Kumar and Loh, 2012).

3.4. Conservation of Genetic Resources

The conservation of plant genetic resources is vital for global food security and agricultural biodiversity, ensuring the continued existence of a vast genetic wealth. Genetic diversity offers various opportunities for selecting and developing innovative and higher-yielding crops that are resistant to biological and environmental challenges (Ergül et. al., 2001). Advances in in vitro culture techniques and molecular biology, in particular, allow for more effective preservation of plant genetic resources. In vitro culture stands out as a viable alternative for conserving the genetic material of plant species for which seed banking is not suitable.

3.5. Production of Secondary Metabolites

Secondary metabolites are compounds with antimicrobial effects in humans and animals, and they protect the plants in which they are found from diseases. Additionally, some secondary metabolites absorb UV rays, protecting plant leaves from the harmful effects of the sun (Vuran and Türker, 2021). These bioactive compounds can also have pharmacological or toxicological effects on humans and animals. However, obtaining these secondary metabolites from plants on a commercial scale is quite difficult, as these valuable substances often constitute less than 1% of the plant's weight. Therefore, plant tissue culture technology is considered a promising alternative for the production of these valuable secondary metabolites. Recent research supports the idea that plant tissue culture applications can be an alternative method for obtaining these valuable molecules instead of growing the entire plant (Vuran and Türker, 2021).

3.6. Somatic Hybridization

Somatic hybridization is a key method for creating intra- and inter-specific hybrids through protoplast fusion. This process involves the fusion of protoplasts

with different genomes, followed by the selection of desirable somatic hybrid cells, and finally, the regeneration of the hybrid plant (Vuran and Türker, 2021). The somatic hybridization technique is frequently used to obtain new hybrids in various horticultural crops to increase yield and improve disease resistance. Additionally, this method has been applied to enhance salt tolerance, improve quality, transfer cytoplasmic male sterility, produce seedless triploid plants, and improve rootstocks (Özcan et. al., 2001). Somatic hybridization via protoplast fusion has enabled the resolution of various problems in citrus cultivation and the creation of new genetic structures. In citrus, somatic hybridization applications have not only increased the resistance of rootstocks to various biotic and abiotic stress factors and enhanced productivity but also led to improvements in fruit quality (Vuran and Türker, 2021).

3.7. Organogenesis

Organogenesis is based on the principle of organ formation from a piece of plant, either directly or through callus culture. There are three different methods of plant regeneration via organogenesis. The first two methods rely on adventitious organ formation, developing either from a callus culture or directly from an explant. A third alternative is the method of axillary bud formation and growth, which is also used to regenerate the whole plant from certain types of tissue cultures. The process of organogenesis relies on the inherent plasticity of plant tissues and is controlled by altering the composition of the growth medium. Specifically, the developmental pathway followed by the regenerated tissue depends on the ratio of auxin to cytokinin hormones in the medium. (Amente and Chimdessa, 2021).

4. TISSUE CULTURE PRODUCTION METHODS

Plant tissue culture is, in essence, a production technique. This approach, which differs from traditional production methods, is based on the principle of cultivating a small tissue sample (explant) taken from different parts of a plant in a sterile, nutrient-rich medium (in vitro) under controlled environmental conditions (light, humidity, and temperature), after it has been sterilized (Amente and Chimdessa, 2021).

In vitro techniques offer various possibilities, such as the rapid multiplication of elite genotypes, the early-stage determination of resistance traits in a laboratory environment, rejuvenation for conventional vegetative propagation, the production of disease-free plants, the generation of haploid plants, the creation and selection of mutant plants, the conservation of genetic diversity (through cryopreservation), the formation of somatic embryos and production of artificial

seeds, in vitro somatic hybridization via protoplast culture, and gene transfer through DNA technology (Kumar and Loh, 2012).

Furthermore, production via the tissue culture method offers advantages such as not being dependent on the growing season, enabling the unlimited production of genetically identical plants, obtaining polyploid individuals, and establishing gene banks. It also plays a significant role in the easy propagation of certain plant species that are difficult to propagate by cuttings, and in the production of tree species where years with abundant seeds are rare, and seeds cannot be stored for long periods, or their storage is difficult.

Geneticists use the tissue culture technique as an effective tool for evaluating carefully selected genetic structures (genotypes), identifying rapidly developing individuals, and selecting plants resistant to cold, drought, diseases, salinity, and herbicides. These in vitro techniques, which offer a relatively easy and fast production possibility, provide significant advantages that can be utilized in subsequent breeding stages. Some of the commonly used nutrient media in tissue culture can be listed as follows: WS (Wolter and Skoog medium), WPM (Woody Plant Medium), MS (Murashige and Skoog medium), DKW (Driver and Kuruyuki medium), SH (Shenk and Hildebrand medium), GD (Gresshoff and Doy medium), DL (Durzan and Lopushansk medium), and LS (Linsmaier and Skoog medium).

4.1. Callus Culture

Callus culture is the sterile production of unspecialized cell masses, known as callus, from plant parts in a suitable nutrient medium. To initiate callus culture, parts of the plant that contain cells with the ability to divide can be used. Examples include endosperm, pollen, embryo, petiole, root sections, and internodes of the stem. The hormone 2,4-D is typically added to the culture medium to promote callus formation.

Callus culture is one of the fundamental applications of plant tissue culture and plays a critical role in plant biotechnology. Callus is an amorphous (shapeless) mass of disorganized and undifferentiated cells formed on an explant (a piece of plant tissue like a leaf, root, or stem) (Kumar and Loh, 2012). The formation of this tissue is induced in an in vitro environment through the use of balanced and specific concentrations of plant growth regulators such as auxins and cytokinins. These hormones alter the genetic programming of the explant cells, causing specialized cells that would normally form a specific tissue or organ to begin dividing uncontrollably.

Callus formation is a testament to the concept of cellular plasticity in plants. Callus cells are considered totipotent; that is, they have the potential to regenerate

an entire plant when provided with the right conditions. Callus culture utilizes this totipotency for various purposes in plant breeding and genetic engineering studies, such as regeneration, genetic transformation, and secondary metabolite production (Kumar and Loh, 2012).

Thanks to the opportunities it provides for accelerating plant breeding, developing new varieties, and in the field of industrial biotechnology, callus culture has become an indispensable tool for modern agriculture and science. However, challenges like genetic instability (somaclonal variation) that can sometimes arise in callus culture require careful management of the technique.

4.2. Organ Culture

Organ culture is a specialized sub-branch of in vitro plant tissue culture that aims to support the growth and development of a specific organ (e.g., a bud, root, leaf, or flower) under controlled laboratory conditions. Unlike cell and callus culture, this technique focuses on maintaining the structure and function of the existing organ as it continues to develop, rather than forming a mass of undifferentiated cells. Organ culture is used for various scientific and commercial purposes, such as understanding plant developmental biology, genetic engineering, and metabolite production.

The success of organ culture depends on the physiological age and genotype of the chosen explant, as well as the balance of the culture medium. The nutrient medium must contain essential macro and microelements, vitamins, and carbon sources, in addition to the correct proportions of appropriate plant growth regulators (auxins and cytokinins). For instance, shoot tip meristem culture is widely used to produce healthy, virus-free plants. In this method, the apical meristem, the youngest and virus-free part of the plant, is cultured in vitro to regenerate a new plant. Similarly, root culture is used to study the mechanisms of root system development or to produce "hairy roots" that generate medically valuable secondary metabolites.

Organ culture is a crucial tool in plant breeding because it enables the production of genetically stable clones and directly facilitates applications like genetic transformation. It also provides an effective method for the conservation of rare and endangered plant species (germplasm conservation).

In production using tissue culture techniques, organ culture is the most frequently preferred method for its ease of application and its ability to maintain genetic stability. For shoot development and plant regeneration in organ culture, various parts of the plant are used as source material, such as embryo parts (leaves, cotyledons, and hypocotyl), shoots and shoot tips, and axillary and terminal buds.

4.3. Embryo Culture

Embryo culture is the process of developing or preserving isolated mature or immature embryos in a laboratory environment (in vitro). Carbohydrates in the culture medium are of great importance for the embryo's survival and growth. Sucrose, the most commonly used carbohydrate, not only serves as an energy source but also acts as an osmotic stabilizer. The embryo culture technique is widely used to overcome germination problems frequently encountered in forest tree species.

Embryo culture is a specialized application of plant tissue culture that involves cultivating isolated embryos in a sterile nutrient medium under in vitro (laboratory) conditions. This technique is used to rescue and develop into a complete plant those embryos that are difficult or impossible to germinate from seed, or those that have lost viability or stopped developing. Embryo culture is a vital tool in plant breeding and in efforts to conserve genetic diversity.

Embryo culture has two main application areas:

1. *Mature Embryo Culture*: This approach is applied to seeds that are difficult to germinate due to dormancy (e.g., some forest trees or orchid species). The seed coat may contain substances that inhibit embryo germination. By excising mature embryos from the seed and placing them in a sterile nutrient medium, these barriers are removed, accelerating germination and plant development. This method shortens the long period required for seed germination and increases efficiency in nursery operations.

2. *Immature Embryo Culture:* This application is used to rescue embryos that result from wide hybridization between distantly related species and would normally have low viability or fail to develop at all. When such crosses occur between plants with different chromosome numbers or genetic incompatibility, "hybrid inviability" can occur in the embryo. In this case, the young embryo is separated from the mother plant and kept alive by being nourished in a special nutrient medium. This technique makes it possible to transfer desirable genes, such as disease resistance, drought tolerance, or high nutritional value from wild species to cultivated plant varieties, thereby expanding the gene pool.

Embryo culture serves as a bridge in plant breeding programs, both shortening the time required and overcoming the physiological barriers that traditional cross-pollination methods face. It also offers an important method for the conservation of germplasm (genetic material) of rare and endangered plant species. In this way, the diversity and future of plant genetic resources are secured.

4.4. Cell Culture

In cell culture, cells can be used individually or in aggregates. Cell culture is fundamentally carried out using two different techniques: the filter paper technique and the Petri dish technique. In the filter paper technique, a single cell is taken from an active callus tissue using a micropipette and allowed to divide, first forming a shoot and then a root. These newly formed plantlets are then transferred to different culture media (Arı, 2001). In the Petri dish technique, cell aggregates mixed with a sterilized nutrient medium are subjected to a special sterile procedure, transferred to containers of various sizes, and finally cultured in Petri dishes. Rooted shoots are then transplanted into new growing media.

Cell culture is a fundamental application of plant tissue culture that involves the proliferation of isolated single cells or cell groups under sterile in vitro (laboratory) conditions. This technique leverages the totipotency (the potential to differentiate into a whole plant) of cells, providing a unique model system for research into plant physiology, genetics, and biochemistry. Unlike callus or organ culture, cell culture aims to produce a homogeneous cell population by growing cells in an amorphous suspension or on a plate.

The success of cell culture depends on the meticulous control of environmental conditions, starting with the selection of the explant and the preparation of the appropriate nutrient medium. The nutrient medium contains not only macro and microelements but also plant growth regulators (auxins and cytokinins) that stimulate cell division and proliferation. The most common form of cell culture is cell suspension culture. In this method, cells obtained by mechanically or enzymatically breaking down callus tissue are placed in a liquid nutrient medium and continuously agitated on magnetic stirrers or shakers. This promotes rapid cell proliferation by providing better access to oxygen and nutrients.

Cell culture has found broad applications, particularly in the following areas:

- *Secondary Metabolite Production*: The controlled production of valuable compounds that plants naturally produce and that are used in the pharmaceutical, cosmetic, or food industries (e.g., vincristine, shikonin).
- *Genetic Transformation*: Cell suspensions are ideal targets for gene transfer (genetic transformation). Foreign genes can be easily introduced into these cells using methods like Agrobacterium tumefaciens or particle bombardment, followed by the regeneration of genetically modified plants.
- *Mutagenesis Studies*: New genetic variants can be created using chemical mutagens or radiation, and these variants can be selected to develop plant lines with novel traits.

Cell culture is a powerful tool that accelerates plant breeding processes, facilitates genetic manipulations, and optimizes the production of valuable plant compounds. However, genetic instabilities, such as somaclonal variation that can occur in cell lines, are an important consideration that requires careful management of this technique.

4.5. Protoplast Culture

Protoplasts are obtained by removing the cell wall through chemical treatment. Two protoplasts, derived from two different plants, are brought together in the same culture medium to fuse, resulting in a new, hybrid plant. Protoplast isolation is performed using two different methods: mechanical isolation and enzymatic isolation. Through asexual (vegetative) reproduction via protoplasts, it is possible to obtain a large number of new plants in this way (Amente and Chimdessa, 2021).

Protoplast culture is one of the most advanced techniques in plant biotechnology and a specialized application of plant tissue culture. Protoplasts are living plant cells enclosed by a cell membrane, with their cell walls completely removed using enzymes (typically cellulases and pectinases). The absence of a cell wall makes protoplasts an ideal model for manipulations such as genetic transformation and somatic hybridization. This technique offers unique opportunities to develop new plant varieties by overcoming the physiological and genetic barriers that traditional plant breeding methods cannot.

The process of obtaining protoplasts begins with the selection of fresh and healthy plant tissue (usually leaves or a cell suspension). This tissue is immersed in a mixture of enzymes that digest the cell wall in a sterile environment. Under the effect of the enzymes, the cell walls dissolve, and the cells are released as protoplasts. The resulting protoplast suspension is purified and transferred to a special nutrient medium to minimize osmotic stress.

Culturing protoplasts requires a high degree of care and precision. Because they lack a cell wall, protoplasts are highly sensitive to osmotic shock. Therefore, the osmotic pressure of the nutrient medium is adjusted with osmotic agents like mannitol or sorbitol. Under suitable growth regulators and environmental conditions, the cultured protoplasts begin to form a new cell wall, divide, and form a callus tissue. A complete plant is then regenerated from this callus tissue using regeneration techniques (Arı, 2001).

Protoplast culture has revolutionized plant breeding, but it is a field that must be managed carefully due to certain technical challenges, such as a high risk of contamination, regeneration difficulties, and somaclonal variation. In the future,

as these challenges are overcome, protoplast culture will play an even more significant role in food production and biomaterials.

5. PLANT PRODUCTION STAGES WITH TISSUE CULTURE METHODS

5.1. Preparation Stage

This stage involves taking a sample from a plant and placing it into Petri dishes or test tubes containing a nutrient medium. The disinfected plant material is carefully placed into the prepared sterile medium. The containers are then transferred to climate-controlled cabinets with fixed temperature and light levels, where they are incubated to allow for growth, development, and proliferation.

The preparation stage is the starting point of the plant tissue culture (in vitro) process and is critically important for its success. This phase involves the meticulous preparation of three main components: the nutrient medium (culture medium), the plant material to be used (explant), and the sterile environment. The slightest error in any of these components can lead to the contamination of the entire culture and, consequently, the failure of the project.

The nutrient medium is a gel or liquid mixture containing all the macro and micro nutrients, vitamins, carbohydrates (usually sucrose), and plant growth regulators (hormones) necessary for the in vitro growth and development of plant cells, tissues, or organs. The most commonly used media are formulations like Murashige & Skoog (MS) medium or Woody Plant Medium (WPM). These media are customized according to the needs of a specific plant species and the desired developmental stage (callus formation, shoot, or rooting). During preparation, it is mandatory to precisely weigh and mix all chemicals, adjust the pH (typically between 5.6-5.8), and then sterilize the mixture using an autoclave.

The explant is the plant part to be cultured (e.g., a leaf, stem segment, bud, or seed). The type and health of the explant directly affect the success of the culture. Explants from young, healthy, and disease-free plants are generally preferred.

The explant undergoes a series of sterilization procedures to eliminate microorganisms (bacteria and fungi) on its surface. This process typically involves washing with detergent water, followed by disinfection with chemical agents like sodium hypochlorite (bleach) or ethanol, and finally rinsing with sterile water. Surface sterilization requires a delicate balance to ensure complete disinfection without harming the explant's viability.

All preparation and culturing procedures are carried out within a laminar flow hood to minimize airborne microorganisms. These cabinets use hepa filters to provide a continuous flow of sterile air to the work area. Constant disinfection of

the work surface, tools, and hands is a fundamental rule to eliminate the risk of contamination.

The meticulous and coordinated management of these three components in the preparation stage maximizes the chances of success for in vitro plant tissue culture. This phase establishes a solid foundation for subsequent stages and prevents a significant portion of potential problems (contamination, explant loss) from the outset.

5.2. Shoot Stage

The Shoot Stage is one of the most fundamental and critical phases of the plant tissue culture (in vitro) micropropagation process. The main goal of this stage is to ensure the formation of numerous, healthy, and genetically uniform shoots from a selected plant part (explant) under sterile conditions. This phase is a crucial step for the rapid and mass production of new plant varieties.

The success of the shoot development stage is strictly dependent on the composition of the nutrient medium and the control of environmental conditions. Hormones known as plant growth regulators play a vital role in this phase. Specifically, cytokinins promote cell division and the formation of shoot buds, thereby increasing proliferation. Cytokinins like Benzylaminopurine (BAP), kinetin, and zeatin are used at concentrations optimized for different plant species. Maintaining a high cytokinin-to-auxin (shoot-to-root) ratio in the nutrient medium supports shoot formation while inhibiting root development.

Once the shoots reach a certain size, they can either be transferred back to this stage for further proliferation or moved to the next phase, the rooting stage. The efficiency of the shoot development stage is one of the key factors that determines the commercial viability of plant tissue culture. The healthy and vigorous shoots obtained during this phase directly affect the quality of the final product.

After the plant material is cultured, varying numbers of new shoots emerge, depending on the proliferation capacity of the plant species and variety used. These shoots are carefully separated and transferred to new culture media in larger containers (subculture). This subculturing process is repeated until the rooting stage is reached.

5.3. Rooting Stage

The rooting stage is one of the most crucial and final phases of the plant tissue culture (in vitro) micropropagation process. The main purpose of this stage is to ensure that the shoots produced under in vitro conditions develop healthy root systems and to prepare the plantlets for transfer to ex vitro (outside the laboratory) conditions. Successful rooting is vital for the economic and practical success of

the entire process, as it directly affects the plantlets' chances of survival and growth in soil.

The success of the rooting stage depends on the plant's genetic makeup, the type and concentration of plant growth regulators used, environmental factors like light and temperature, and the genotype's response to these factors. A healthy and strong root system obtained at this stage is a fundamental prerequisite for the plantlets' survival during the acclimatization process and their eventual development into a marketable product.

The shoots are transferred to a special nutrient medium with an adjusted growth regulator content, where they are left to root. Depending on the plant material used, the nutrient concentration of the medium may also be reduced by specific ratios.

5.4. Acclimatization

Acclimatization is a critical process that ensures the successful adaptation of plantlets produced under in vitro (laboratory) conditions to natural environmental conditions. Tissue culture laboratories have artificially controlled conditions such as high humidity, low light intensity, sterile air, and constant temperature. Plantlets grown in this environment develop different morphological, anatomical, and physiological characteristics compared to their counterparts in nature. For example, their cuticles are thinner, stomatal function is limited, and photosynthetic activity is low. Additionally, their leaves largely lose the ability to control moisture loss.

Successful acclimatization directly affects the survival rate and commercial success of plants produced through tissue culture. Failure in this process can lead to the plantlets drying out, succumbing to diseases, and ultimately resulting in crop loss. Therefore, acclimatization is not just one step of plant tissue culture, but one of the most critical phases that determines the economic feasibility of the entire process.

After the rooted plantlets are planted in soil, they are kept in a climate-controlled cabinet for a specific period to minimize moisture loss before being transferred to a greenhouse environment.

6. CONCLUSION

Plant tissue culture and biotechnology have become the cornerstones of modern plant breeding. These approaches have significantly accelerated and enhanced the effectiveness of the slow and labor-intensive processes of conventional breeding methods. Traditional breeding relies on natural cross-pollination and selection to achieve genetic diversity; however, these processes

can take years of work to bring together desired traits (such as disease resistance and high yield) in a single plant. Tissue culture is the cultivation of plant cells, tissues, or organs in a controlled nutrient medium under sterile laboratory conditions. This technique offers many advantages in plant breeding, including micropropagation, haploid production, embryo rescue, and disease elimination. Plant biotechnology involves understanding and manipulating the genetic structure of plants at a molecular level. The fundamental tools used in this field make breeding processes more precise and predictable. The integration of these modern techniques creates tangible benefits for both producers and consumers. Plant varieties that are more resistant to diseases and pests contribute to food security by increasing agricultural productivity. At the same time, the reduction in pesticide and herbicide use supports environmental sustainability. Consumers gain access to higher-quality, nutritionally enhanced products. The shortened breeding time allows farmers to adapt more quickly to new challenges, like climate change, and respond faster to market demands. In conclusion, plant tissue culture and biotechnology are critical tools that make the fundamental processes of conventional breeding—cross-pollination and selection—faster, more efficient, and more predictable. These techniques will continue to shape the future of plant breeding and make significant contributions to sustainability in food production.

Tissue culture techniques are a critical tool that supports and accelerates the fundamental processes of conventional breeding, namely selection and hybridization. When used in conjunction with modern biotechnological approaches like genetic engineering and marker-assisted selection, these techniques maximize the efficiency of breeding programs. In the face of global challenges such as climate change and a growing world population, the need for faster and more effective plant breeding is increasing. Tissue culture will continue to play a fundamental role in developing new plant varieties with superior traits that can respond to these challenges.

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Chapter 2

Determination of Seafood Meat Quality by Histological Methods

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Abstract

Seafood constitutes a pivotal component of the human diet, owing to its high protein and fatty acid content. In addition to the consumption of seafood in its fresh state, a variety of processing methods are employed to transform it into other products suitable for consumption. Following the processing methods applied to seafood, a series of physical, chemical, microbiological and sensory changes are undergone by the product. Recent technological advancements have facilitated the observation of alterations in muscle structure through histological analysis, complementing the existing methods. The application of histological methods facilitates the observation of tissue deformations. Histological examination is the most effective method of analysis for water, protein, fat, connective tissues and all the breakdowns in the tissues. A comprehensive review of the extant literature revealed that the application of histological methods is a pivotal factor in the assessment of the quality of fish meat. It is evident that a review study was conducted for the following reasons: to emphasise the importance of histological methods in determining meat quality. Consequently, it was observed that, particularly following freezing and thawing, the proteins in the inner and outer cells, fibres and content of fish muscle tissues underwent denaturation.

Key words Histology, Seafood, Quality, Fish muscle.

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1. Introduction

Considering dietary habits, animal-derived proteins such as red meat, poultry, and fish constitute essential sources of protein. In addition to being a major protein source, meat also contains water, minerals, vitamins, and other nutrients, making it highly important for human health and nutrition (Budak and Kayaardı, 2006; Özlü and Ercoşkun, 2021). However, among these food sources, seafood is known for its highly perishable nature (Mol and Ceylan, 2011). Freezing is one of the most crucial methods used to preserve foods and extend their shelf life. Particularly for seafood, freezing is a widely applied method to ensure long-term storage and extend shelf life. Nevertheless, it is known that the freezing process leads to structural changes in muscle tissue and induces chemical modifications, thereby affecting the organoleptic quality of fish products (Zhu et al., 2004; Venugopal, 2006; Burgaard, 2010; Uddin, 2010; Gökoğlu and Yerlikaya, 2015). Structural (mechanical) damage, oxidation, dehydration, and protein denaturation that occur during the storage of frozen products are considered the main causes of quality loss in fish (Benjakul and Bauer, 2001). It has been reported that the freezing rate and storage conditions significantly influence muscle structure (Shahidi and Botta, 2012). Ice crystals formed between the fibers of frozen food products cause significant damage to muscle fibers and lead to their contraction (Gambuteanu et al., 2013). Although the microbial flora is partially inactivated during the freezing process, it may proliferate during and after thawing due to the release of tissue fluids, which provide a favorable environment for microbial growth (Pan and Chow, 2004; Kolbe and Kramer, 2007).

High-quality frozen seafood products may suffer from quality losses due to interruptions in the cold chain during transportation (Gormley et al., 2002), or as a result of non-industrial and improper freezing methods used to slow down tissue degradation in unsold fresh seafood (Bozzetta et al., 2012). Fluctuations in storage temperature can lead to the accumulation of ice crystals within the product. The distribution and size of ice crystals on the surface negatively affect various quality parameters of frozen products and influence tissue integrity and water-holding capacity (Petzold and Aguilera, 2009). Additionally, it is known that consumers have difficulty distinguishing between fresh fish and fish that has been frozen and thawed based on organoleptic parameters (Karoui et al., 2006). Consequently, the intentional sale of frozen/thawed fish products in place of fresh fish has been documented (Uddin et al., 2005; Fasolato et al., 2008; Upton, 2015).

2. Histological Parameters of Muscle Tissue Quality

Histology is one of the oldest methods used for detecting the composition of animal tissues in meat and meat products (Tremlová and Štarha, 2003). Histological examinations in meat and meat products were first employed in the early 1910s. A researcher named Jaeger was the first to propose that tissue types comprising a meat product could potentially be identified under a microscope by staining sections taken from meat products and examining the prepared slides. In 1921, another researcher, Braunert, emphasized the importance of histological examinations of cooked meat products such as salami, sausages, and fermented sausages (Güçer and Gövercin, 2010).

Through examinations performed on stained histological slides, it has been demonstrated that tissues and organs with low nutritional value — such as skin, smooth muscle, connective tissue, skeletal muscle, lymphoid tissues, glands, intestines, hair fragments, blood vessels, and similar components — as well as edible tissues, can be identified in meat products, especially in cases where the inclusion of certain organs and tissues is not permitted (Kaymaz et al., 1989; Yıldız et al., 2004; Torun, 2005).

The staining technique (Hematoxylin & Eosin), used to verify the compliance of meat products and to protect consumer rights, is considered reliable for the identification of all animal tissues added to meat products. This staining method allows for the detection of a variety of tissue types — including bone, cartilage, adipose tissue, blood vessels, and nerve tissues — through microscopic examination (Ghisleni et al., 2010).

An alternative method to differentiate between fresh and frozen–thawed meat products is based on identifying histological structural changes (such as empty vacuolar spaces) induced by freezing (Love, 1958; Simeonidou, 1997; Sigurgisladóttir et al., 2000; Alizadeh et al., 2007; Alizadeh et al., 2009).

In seafood, histological analyses were first applied to common carp (*Cyprinus carpio*) (Pavlov et al., 2008). In recent years, these analyses have increasingly been used to determine muscle composition and lipid content in various fish species, including *Oncorhynchus mykiss, Engraulis encrasicolus, Sparus aurata, Mullus barbatus, Sarda sarda, Salmo salar, Thunnus alalunga, Psetta maxima, Euthynnus alletteratus*, and *Xiphias gladius* (Bozzetta et al., 2012; Richelmi et al., 2013; Popelka et al., 2014; Meistro et al., 2016).

The melting of ice crystals is defined as the thawing process. Thawing generally takes a longer time compared to freezing and causes various chemical and physical changes in frozen food products. These changes can significantly affect the quality of the food (Wu et al., 2017). As the ice crystals melt, protein degradation occurs, leading to the formation of voids within the tissue (Hassoun

et al., 2020). During the freezing process, the water in tissues forms ice crystals that exert intense pressure on unfrozen cellular components. Upon thawing, these conditions may affect microstructural recovery (Fukuda, 1996; Herrero et al., 2005; Miyawaki, 2018). Ice crystals formed both intracellularly and extracellularly during freezing cause separation and degradation of muscle fiber bundles. Due to the formation of ice crystals in muscles and the rupture of cell membranes during freezing, thawing results in the release of water-soluble components from muscle tissues (Dawson et al., 2018). The voids formed following ice crystal melting and protein denaturation contribute to water loss, resulting in softer texture, structural gaps, and alterations in flavor and taste (Leygonie et al., 2012; Nakazawa and Okazaki, 2020). Changes in quality during thawing are influenced by multiple factors, including storage temperature, freezing rate, thawing duration, and thawing temperature (Leygonie et al., 2012).

When comparing large ice crystals formed during slow freezing to smaller ones from rapid freezing, it is evident that slower freezing causes greater tissue damage. The mechanical effects of existing ice crystals are considered responsible for quality deterioration in meat products (Kaale and Eikevik, 2013; Dalvi-Isfahan et al., 2019; Jiang et al., 2019c). Ice crystals formed within the meat can deform muscle fibers and influence not only the structural integrity of fiber fragments but also the distribution and characteristics of the cellular area. The osmotic pressure changes and contracted fibers induced by extracellular ice crystals during freezing also contribute to these structural alterations (Shi et al., 2020). Deformation and damage in muscle tissues can be detected using morphological methods (Latorre et al., 2015).

Pastırma, a meat product, is prepared through a series of processing steps. The categorisation of the meat is based on its processing stage, with the meat being classified into four distinct groups: salted-washed, dried-pressed, dried with fenugreek paste (çemen), and plain meat. Histological analyses revealed no structural changes in any of the samples; however, it was observed that the pressing process altered the orientation of muscle fibres. As a consequence of the particular production process, biochemical analyses demonstrated a decline in pH and water activity, whilst increases were evident in protein, moisture, ash, fat, and salt content (Yakışık et al., 1992).

3. Studies on Determining Quality Parameters of Seafood Using Histological Method

In a study investigating the changes in quality characteristics of salted and unsalted tuna meat subjected to different freezing and thawing processes, histological and biochemical analyses revealed that salting improved water-

holding capacity and textural properties. In unsalted meat products, degradation of myofibers and the presence of extracellular ice crystals were observed after three or more thawing cycles. Additionally, increases in protein concentration, color deterioration, and lipid oxidation were reported in salted tuna following freezing and thawing treatments (Jiang et al., 2019a). Bahuaud et al. (2008) reported that ice crystals formed during freezing disrupt tissue concentrations, leading to dehydration, protein denaturation, and membrane damage. Damage occurring in muscle tissues during freezing is considered significant and often results in irreversible changes (Lu et al., 2020).

The use of slow freezing processes can trigger osmotic phenomena that result in morphological changes such as dehydration and shrinkage (Pham, 2008; Kiani and Sun, 2011). Moreover, the rate of temperature decline has been reported to increase the formation of extracellular ice crystals (Kiani and Sun, 2011). Slow freezing leads to the formation of large ice crystals between muscle fibers, and the pressure exerted by these crystals can cause structural damage to the muscle tissue. As a result, protein denaturation, tearing of the endomysium, fiber shrinkage, and the formation of large gaps between muscle fibers may occur (Ozogul, 2019; Dalvi-Isfahan et al., 2019).

Popelka et al. (2014) investigated post-freezing myofiber damage and tissue alterations in rainbow trout using histological analyses. In another study examining histological findings after single and double freezing/thawing cycles at different temperatures (−10, −18, and −27°C), it was reported that a single freeze–thaw cycle did not cause significant changes in muscle tissue, whereas double freezing led to muscle cell shrinkage, fiber fragmentation, and increased extracellular spaces (Strateva and Penchev, 2019). Similarly, another study found that muscle cells shrank and assumed irregular shapes, with increased extracellular spaces and vacuole formation within intracellular areas, ultimately leading to fiber disintegration (Shi et al., 2020).

The more open microstructure observed in thawed meat after freezing may contribute to mass transfer and the loss of cellular components during the salting process (Lauritzsen et al., 2004; Aursand et al., 2009). Brine salting has been shown to induce modifications in soluble myofibrillar proteins and the fine tissue structure, both of which are critical for the qc)ality parameters of meat (Offer and Trinick, 1983; Xiong, 2005; Jiang et al., 2019b. The red color of meat is known to depend on the concentration of myoglobin and its derivatives (Viriyarattanasak et al., 2011). The structural integrity of muscle tissue is essential for the quality of muscle-based foods, which rely on a fine cellular organization (Offer et al., 1989; Ertbjerg and Puolanne, 2017).

In tuna subjected to salting for different durations, redox changes in myoglobin and pigment losses have been observed (Jiang et al., 2019b), and similar pigment

losses were identified as the cause of color changes in Atlantic salmon subjected to different salting levels (Hughes et al., 2014). As demonstrated in these studies, intensive salting—either by immersion in high-salt brine or through dry salting—generally results in low yield due to muscle fiber shrinkage, protein denaturation or aggregation, particularly in myofibers (Thorarinsdottir et al., 2011).

Histology has been identified as an appropriate method for distinguishing between fresh and frozen–thawed fish in terms of quality assessment (Tinacci et al., 2018). In a study examining muscle tissue changes in three different fish species (*Sparus auratus, Mullus barbatus, Xiphias gladius*), histological alterations in muscle structure were observed as a result of freezing, confirming the significance of histological analysis as a parameter in quality determination (Bozzetta et al., 2012). In another study, vacuum-packed gilthead seabream (*Sparus auratus*) fillets stored at −22°C for 340 days exhibited muscle structure deterioration and protein denaturation within the muscle tissue (Makri, 2009).

In studies conducted by Tinacci et al. (2018, 2020), specific histological criteria were defined to differentiate between fresh and frozen fish and octopus meat. These criteria focus on the general structure of the muscle tissue, the presence of voids in different regions, and the existence of proteinaceous material in the intercellular spaces.

Furthermore, it has been emphasized that tissue alterations during shelf life are associated with spoilage processes resulting from the combined effects of microbial growth and enzymatic oxidation (Ghaly et al., 2010; George et al., 2016).

Conclusion and Recommendations

A review of the current literature reveals that freezing and thawing processes have negative effects on the meat quality of fish. This review has demonstrated that these processes disrupt muscle fiber integrity, lead to an increase in intra- and extracellular spaces, and cause protein denaturation in fish muscle tissue. These structural alterations can adversely impact the physical and sensory quality of the product.

It is therefore recommended that histological analyses be routinely implemented as a complementary method for the objective evaluation of product quality. Such an approach would enhance the assurance of product quality offered to consumers and support the optimization of processing technologies. Furthermore, expanding histological assessments across different species and processing methods in future studies will contribute scientifically to the seafood industry.

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Chapter 3

Epigenetic Mechanisms and Their Relationship with Nutrition and Health in Farm Animals

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Introduction

The aim of animal production is to increase the health and welfare of animals as well as to increase productivity for the benefit of humans (Ibeagha-Awemu & Zhou, 2015). In addition to the ever-increasing human population in the world, which naturally increases the demand for animal food, the challenges brought by global climate change are very important for the sustainable development of animal food production and reveal the fact that high-quality animal proteins need to be produced more with the current environmental impacts. For these reasons, the number of studies has increased in recent years to develop different approaches to reduce production costs and develop environmentally friendly livestock production systems in order to increase efficiency in animal production (Capper & Bauman, 2013; Scott, 2018; Wang & Ibeagha-Awemu, 2021).

Sequencing studies conducted on the genomes of farm animals have revealed coding and non-coding genome sequences associated with productivity and disease resistance in animals, which has accelerated genetic progress (Do, Dudemaine, et al., 2017; Kamath et al., 2016; Wara et al., 2019). Nevertheless, notwithstanding the plethora of research conducted, the current findings remain inadequate in elucidating the ideal degree of variability necessary for the attainment of sustained advancements in animal health and productivity. Because less research has been done in the field of epigenetics, which includes clues about genetic and environmental conditions. Thanks to epigenetics, additional variation levels can be determined that can be used to improve productivity and disease resistance traits in livestock farming (Wang & Ibeagha-Awemu, 2021).

The principles of Mendelian genetics have significantly influenced numerous

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contemporary research endeavors. For an extended period, it was presumed that genes responsible for specific phenotypes were exclusively derived from the DNA sequence. Notwithstanding various studies, models of non-Mendelian inheritance contest these established theories and propose that an alternative mechanism may exist to elucidate certain inheritance patterns. Historically, the designation "epigenetics" was first articulated by Conrad Waddington during the 1940s (Waddington, 1942). Huxley subsequently refined this definition by acknowledging that variability in the determination of cellular phenotype was not intrinsically linked to the gene sequence. Since that time, the notion and characterization of epigenetics have progressively diverged from the initial definition conceptualized by Waddington (Triantaphyllopoulos et al., 2016). Epigenetics involves molecular mechanisms that offer insights into phenomena not explained by classical Mendelian genetics. This field investigates heritable and reversible modifications that influence genome function and gene expression without altering the underlying DNA sequence (Feeney et al., 2014; Skinner et al., 2010).

Epigenetics refers to the study of heritable molecular modifications that regulate genomic activity and gene expression without altering the underlying DNA sequence, thereby contributing to phenotypic variation (Wang & Ibeagha- Awemu, 2021). The regulation of gene expression through epigenetic mechanisms primarily occurs via four pathways: DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs (Feeney et al., 2014). The epigenome, encompassing these regulatory processes, remains dynamic across the lifespan and is closely influenced by interactions between genetic regulation and environmental factors (Monk et al., 2019). Evidence from both human and animal studies highlights the critical roles of epigenetic mechanisms in fundamental biological functions, including growth, development, metabolism, and overall health (Paiva et al., 2019). Moreover, a deeper understanding and application of epigenetic principles offer promising opportunities for elucidating complex quantitative traits and advancing genetic improvement in livestock, particularly in enhancing productivity and disease resistance. In light of recent research developments, epigenetics has garnered increasing attention within the scientific community (Wang & Ibeagha-Awemu, 2021).

2. Epigenetic Mechanisms

2.1. Epigenetic Transcriptional Regulation At The DNA Level

Epigenetic transcriptional regulation at the DNA level encompasses mechanisms that influence gene expression through direct modifications of DNA nucleotides or histone proteins, which organize chromatin into functionally distinct regions (Bártová et al., 2008). Histone proteins facilitate the packaging of eukaryotic genomes into euchromatin (transcriptionally active) and heterochromatin (transcriptionally repressed) states, thereby governing gene accessibility. In contrast, DNA methylation involves the addition of methyl groups to cytosine residues, typically within promoter regions, resulting in gene silencing by inhibiting the transcriptional machinery (Navarro-Martín et al., 2020). Various classes of enzymes, such as DNA methyltransferases and histone acetyltransferases, mediate the addition or removal of functional groups on DNA or histones, thus modulating gene expression. These epigenetic processes underpin key biological functions, including development, reproduction, metabolism, and behavioral phenotypes. Notably, epigenetic modifications can be heritable, enabling offspring to adapt more effectively to challenging or unpredictable environmental conditions. Nevertheless, it is crucial to recognize that these heritable epigenetic marks are susceptible to reprogramming in response to environmental stressors both anthropogenic (e.g., exposure to chemicals) and abiotic (e.g., temperature fluctuations, hypoxia) (Dorts et al., 2016). Given their central role in physiological adaptation and evolutionary potential, further research employing comparative animal models is essential to deepen our understanding of epigenetic transcriptional regulation at the DNA level.

2.1.1. DNA methylation

Among the diverse configurations of DNA methylation identified in various animal species, the modification of 5'-cytosine is recognized as the most thoroughly investigated (Kumar et al., 2018). This epigenetic phenomenon encompasses the covalent incorporation of a methyl group (CH_3) at the 5' position of the cytosine moiety within the nucleotide sequence of DNA. The catalytic action of this process is mediated by DNA methyltransferase enzymes, predominantly including DNMT1, DNMT3a, and DNMT3b, which utilize S- adenosyl-methionine as the methyl group donor (Ibeagha-Awemu & Zhao, 2015). DNMT3a and DNMT3b are primarily involved in the establishment of de novo methylation patterns during embryonic development or as a response to environmental cues, whereas DNMT1 plays a crucial role in the preservation of these methylation marks

throughout the processes of DNA replication and cellular division (Schmitz et al., 2019).

DNA methylation is of paramount importance for genomic integrity and is instrumental in the regulation of cellular differentiation within mammalian systems. Predominantly, DNA methylation is observed at cytosine-phosphate-guanosine (CpG) dinucleotides (commonly referred to as CpG islands) and is comparatively infrequent at CpA, CpT, or CpC dinucleotides (Wang & Ibeagha- Awemu, 2021; Ziller et al., 2011). The occurrence of hypermethylation within gene promoter regions is associated with transcriptional repression, whereas hypomethylation has been correlated with an enhancement in transcriptional activity (Ibeagha-Awemu & Zhao, 2015). DNA methylation can impede gene expression through direct inhibition of transcription factor binding to specific loci or indirectly via methyl-binding domain proteins, which facilitate alterations in chromatin architecture induced by methylated DNA (Greenberg & Bourc'his, 2019). Consequently, although all cells within an organism share identical genetic information, distinct DNA methylation profiles arise during development and are preserved following cellular differentiation. These profiles are crucial for ensuring accurate DNA replication and enabling tissue- or cell-specific gene expression (Ibeagha-Awemu & Zhao, 2015).

DNA methylation plays a crucial role in the normal growth and development of organisms, including their response to pathogenic agents. Its patterns are highly cell- and tissue-specific, making DNA methylation a valuable biomarker for biological processes. Alterations in DNA methylation levels during early life can contribute to the onset of various diseases, such as cancer, diabetes, cardiovascular disorders, and respiratory illnesses. These epigenetic modifications also influence gene expression across different organs and environmental contexts. Moreover, epigenetic mechanisms provide insight into the variability in productivity observed among animals of the same breed when exposed to distinct environmental conditions (Satheesha et al., 2020)

2.1.2. Histone modification

Histone modification constitutes a pivotal epigenetic mechanism that profoundly affects chromatin architecture and the modulation of transcriptional activity (Wang & Ibeagha-Awemu, 2021). Within eukaryotic cells, DNA is condensed into a meticulously organized structure through its winding around histone proteins to create nucleosomes, which serve as the fundamental constituents of chromatin. Nucleosomes furnish the necessary

packaging to systematically accommodate the cellular genome within the nucleus. The organization of nucleosome compaction and packaging is achieved by the assembly of two copies of each of the four histone proteins, namely H2A, H2B, H3, and H4, which coalesce to form an octameric configuration, with the nucleosome core arising from the wrapping of approximately 147 base pairs of DNA around this octameric structure. Owing to these histone proteins, the accessibility of proteins responsible for DNA repair functions, in addition to the mechanisms of nucleosome transcription and replication, is meticulously regulated (Zentner & Henikoff, 2013). Each nucleosome that is formed is interconnected to adjacent nucleosomes by linker DNA, while the H1 protein finalizes the packaging process by binding the termini of the approximately 147 base pairs of DNA together.

Histones are protein molecules that assemble into dimeric structures through various configurations, facilitating the organized and efficient packaging of DNA within the cell. Beyond their N-terminal and C-terminal domains, histones possess “tails” that undergo structural alterations through modifications such as phosphorylation, sumoylation, ubiquitylation, methylation, and acetylation, alongside other mechanisms that remain incompletely characterized. Among the enzymes mediating these histone modifications, the two principal categories are histone acetyltransferases (HATs), which add acetyl groups, and histone deacetylases (HDACs), which remove them (Navarro-Martín et al., 2020). Histone acetyltransferases provide histone acetylation, which is responsible for the relaxation of compacted chromatin (histone-DNA interaction) and increased transcription activity. Conversely, histone deacetylases are involved in the suppression of gene expression by causing deacetylation (Schmauss, 2017). In addition to these, histone methylation, known as monomethylation of arginine and lysine, is regulated by histone methyltransferases and demethyltransferases and plays an effective role in gene expression (Wang & Ibeagha-Awemu, 2021).

2.1.3. Chromatin Remodeling

The dynamic architecture of chromatin serves as a fundamental genetic reservoir, providing the essential information required for cellular differentiation and lineage specification throughout organismal development (Wang & Ibeagha- Awemu, 2021). Beyond covalent modifications of histones and DNA, nucleosome remodeling constitutes a critical determinant of chromatin structure, contributing significantly to the regulation of gene accessibility and expression. Nucleosomes are important in the packaging of genomic DNA and formation of chromosomes, as well as having internal

mechanisms that contain reorganization complexes for the rearrangement of chromatin in order for the DNA in the chromatin structure to function actively (Kobayashi & Kurumizaka, 2019). Chromatin remodeling encompasses processes such as repositioning and restructuring chromatin to either enable or restrict access to specific DNA regions. These processes are primarily driven by ATP-dependent chromatin remodeling complexes, which function by shifting, ejecting, or altering the configuration of nucleosomes associated with DNA (Ho & Crabtree, 2010). Notably, the first ATP-dependent chromatin remodeling protein, SWI2/SNF2, was identified through genetic screening in yeast (Nayan et al., 2014).

It has been reported that adenosine triphosphate (ATP)-dependent chromatin remodeling complexes generally play a regulatory role in the access of transcription factors (TFs) to DNA to make changes in nucleosome structure through the energy released from ATP hydrolysis (Hota & Bruneau, 2016). Another ATP-dependent complex, known as BRM/BRG1 (BAF)-related factor, has been revealed to be involved in the activation of disease-resistance-related genes during development in mammals (Hota et al., 2019). In addition, it has been reported that the BAF complex has 15 different subunits that undertake different tasks during the developmental stages of systems such as embryo development, immune cells, skeletal muscle and cardiovascular system (Wang & Ibeagha- Awemu, 2021). However, chromatin organization factors are not completely the same during the developmental stage of living things and differ (Kress et al., 2016). Another important point is that some serious diseases such as cancer are associated with abnormal chromatin remodeling (Mirabella et al., 2016). DNA methylation, covalent modifications of histones, and ATP-dependent chromatin remodeling are tightly interconnected processes that play essential roles in regulating fertility and various other physiological functions (Nayan et al., 2014). Emerging research has identified numerous new chromatin remodeling complexes, elucidating their involvement in transcriptional regulation and their effects on gene expression across different developmental stages and disease contexts in vivo (Kim & Kaang, 2017; Stachowiak et al., 2020).

2.2. Post-transcriptional control

2.2.1 Non-coding RNA Regulation

In addition to the aforementioned epigenetic mechanisms DNA methylation, histone modification, and chromatin remodeling non-coding RNAs (ncRNAs) also play a pivotal role in regulating gene expression and modulating chromatin structure, thereby influencing both productivity and

health outcomes in animal husbandry (Benmoussa et al., 2020). ncRNAs are RNA molecules that function without being translated into proteins, meaning they do not encode amino acid sequences. Instead, they predominantly regulate gene expression at both the transcriptional and post-transcriptional levels (Wang & Ibeagha-Awemu, 2021). In intergenerational genetic inheritance, maternally formed messenger RNAs (mRNAs), long non-coding RNAs (lncRNAs), RNAs affecting the transcription and translation stages and mRNA stabilization in the gene expression mechanism (siRNAs), piwi RNAs (piRNAs), micro RNAs (miRNAs), etc., various small different RNA types play a role (Heard & Martienssen, 2014). Among these, miRNA is the most researched mechanism and has been revealed to be effective in disease resistance, physiological processes, fertility and developmental stages of animals. MicroRNAs are approximately 20-24 nucleotides in size and are operated by an endoribonuclease enzyme called Dicer, which belongs to the RNase III family (Brosnan & Voinnet, 2009).

MicroRNAs suppress gene expression through the RNAi mechanism. Studies have shown that maternal microRNAs are important for the development of the zygote in mice. In addition, studies have shown that miRNAs suppress oocyte development during the reprogramming phase of gene expression (Nayan et al., 2014). A study conducted on dairy cattle demonstrated that miRNAs regulated by the DNMT1 enzyme significantly influence mammary gland development and are pivotal for the lactation process in these animals (Do, Li, et al., 2017). The interaction between miRNAs and their target mRNAs leads to the suppression of transcription and can also contribute to DNA methylation processes (Lamouille et al., 2014 Moreover, non-coding RNAs (ncRNAs) have been identified as participants in epigenetic regulation at both the histone modification and DNA methylation levels (Sabin et al., 2013 It has also been shown that ncRNAs can modulate the expression of the gene encoding the relevant protein or adjacent genes, particularly in the presence of mRNAs containing CpG islands (Mercer et al., 2009). A specific example is the ncRNA known as AIR, which originates from a CpG island within the second intron of the IGF2R gene and plays a role in the silencing of the paternal allele (Sleutels et al., 2002).

2.2.2 RNA methylation

In animals, RNA methylation has been investigated mostly at the level of coding mRNAs, but it has been reported that RNA methylation also plays an effective role in the regulation of ncRNAs (Navarro-Martín et al., 2020). Although there are many forms of RNA methylation, the best-explained one is

m6A methylation (Chen & Witte, 2019). Since methylation occurs at the sixth N of RNA adenylate, it is called m6A modification. m6A is the most common type of methylation in eukaryotic mRNAs and can affect every process of the RNA cycle (Roundtree et al., 2017).

For adaptation to environmental conditions, mRNA levels in the cell must change as quickly as possible. Studies have revealed that m6A on mRNA changes depending on environmental stress and that external stress has various effects on transcriptional, post-transcriptional and translational processes. For example, it has been observed that certain adenosines within the 5′ UTR region of newly transcribed mRNAs in embryonic fibroblasts of mice are preferentially methylated due to heat stress (Zhou et al., 2015). In a study conducted on lambs, it was observed that the addition of essential amino acids (EAA) to the diets of lambs fed with low protein levels regulated fat accumulation and increased protein synthesis. In addition, it was revealed that methionine (Met) and lysine (Lys) in EAA increased protein synthesis and regulated lipid metabolism. In the group fed with a diet with a deficient or sufficient level of Met, lipid transformation was affected by affecting the expression level of genes related to fat metabolism in the liver and other tissues. In addition, it has been reported that increasing the lysis level in the diet reduces lipid accumulation and increases protein synthesis by regulating essential genes involved in various mechanisms (Dannenberger et al., 2014). It has been reported that Met and betaine serve as donors of methyl groups involved in DNA and m6 A RNA methylation processes (Gebeyew et al., 2022). In another study, Met was supplemented to diets and it was revealed that it had an effect on DNA methylation process by changing SAM concentration (Miousse et al., 2017). Similarly, when betaine was supplemented, it was observed that it was effective on m6 A RNA methylation (Chen et al., 2015). Considering the results of these studies, it is revealed that consumption of foods containing methyl groups plays an important regulatory role in the epigenetic modification process.

3. Relationship of Epigenetic Processes with Nutrition and Animal Health in Animals

3.1 Nutrition and Epigenetics Relationship in Farm Animals

Feeding in farm animals is one of the most important expenses affecting profitability. Post-pregnancy feeding is the most important environmental factor that interacts with the genomes of animals and affects the growth, development and other phenotypic characteristics of the organism. Data emerging as a result of studies have revealed that the amount and types of

feeding can be transferred to the offspring by changes in the genomes of the embryos or germline of animals, and this has led to the formation of the concept of intergenerational epigenetic inheritance (Ibeagha-Awemu & Yu, 2021).

In animal species that can be consumed as food, it has been reported that unfavorable conditions in the uterus during pregnancy affect the health, development rate, fat storage rate, decrease in muscle mass and meat quality of the offspring after birth (Wu et al., 2006). Even in the first studies conducted in the 1960s, it was revealed that insufficient or excessively intensive nutrition in the early period of pregnancy affected the viability of the embryos (Bellows et al., 1963). Thanks to current studies, it has been proven that the level of nutrition of the mother is related to the body composition and glucose metabolism of the offspring after birth and that the feeding program and duration during pregnancy change these parameters. For example, it has been reported that feeding in the late period of pregnancy affects the body condition, growth level and insulin sensitivity after birth in ruminants (Tollefsbol, 2017). In addition, significant evidence has emerged showing that prenatal nutrition affects milk production and calf immune globulin levels (Tollefsbol, 2017), alters gene expression in offspring, and affects epigenetic mechanisms in pigs (Altmann et al., 2012). A study by Martin et al. (2007) demonstrated that daughters born to cattle supplemented with dietary protein during the final trimester of gestation exhibited earlier onset of puberty and higher pregnancy rates during their first breeding season. These findings suggest that the maternal nutritional status, particularly fattening performance in late gestation, exerts a significant influence on the subsequent reproductive performance of female offspring. In another study, it was determined that the antral follicle count decreased at the age of 2 in heifers born to mothers fed high-protein diets in the second trimester of pregnancy (Sullivan et al., 2009).

Vitamin B-12, methionine, betaine, folate, and choline represent essential nutrients that have the capacity to influence DNA and histone methylation. The function of folate, which is categorized as a water-soluble B vitamin, in the modulation of DNA methylation has been extensively investigated in scholarly literature. In a study involving cattle, Juchem et al. (2012) explored the implications of dietary supplementation with a rumen-protected B-vitamin complex that included folate on DNA methylation and reproductive outcomes. The results indicated that the group receiving supplementation demonstrated a significantly elevated pregnancy rate, thereby suggesting a potential association between folate-induced methylation mechanisms and enhanced fertility. In a separate investigation conducted on cattle, dietary methionine

was introduced during the peripartum phase; although a reduction in the overall DNA methylation rate in the liver was observed, there was a notable increase in methylation within the promoter region of the PPARA gene, alongside an upregulation of PPARA gene expression in cattle that received methionine supplementation compared to their non-supplemented counterparts (Osorio et al., 2016). The activation or upregulation of the hepatic PPARA gene signifies an improvement in lipid metabolism and immune functionality. Furthermore, the incorporation of methionine into the diet during the latter stages of gestation was found to enhance calf productivity by sustaining methionine homeostasis, facilitating DNA methylation, and optimizing energy metabolism (Alharthi et al., 2019). In a study conducted in pigs, omega-3 fatty acid supplementation to pre- and post-natal diets affected the DNA methylation mechanism and caused changes in the development and resistance of newborn piglets to inflammation (Boddicker et al., 2016). In addition to this information, it was determined that supplementation of methyl-containing nutrients to the diet during pregnancy increased the level of digestion and absorption in the intestines and the development of piglets, and these were found to be compatible with DNA methylation levels in certain genes (C. Jin et al., 2018).

High-quality roughage is a critical component of ruminant nutrition, with alfalfa hay and silage being among the most commonly utilized sources (Tasdelen et al., 2024). Mammary gland development in ruminants is a complex process that occurs across multiple life stages, including fetal development, puberty, pregnancy, and lactation. Notably, research has shown that nutritional excess during the peripubertal period exerts a particularly pronounced influence on mammary tissue development, and that the composition of the diet plays a significant role in modulating this growth. For example, it has been found that commonly used corn-based rations cause a decrease in cellular DNA of mammary tissue compared to an alfalfa-based ration (Gotoh, 2014). It has also been observed that methylation levels of certain genes responsible for fat and protein production in mammary tissue cells were affected in dairy cows fed a ration containing high concentrations of corn straw (Dong et al., 2014). In another study, the addition of substances rich in unsaturated fatty acids to the diet of dairy cows significantly affected the expression of histone acetyltransferase (HAT1 and KAT2) (Ibeagha-Awemu & Zhao, 2015). All these studies have shown that the nutritional effect of milk fat and protein content can be regulated through epigenetic mechanisms.

3.2 Relationship Between Health and Epigenetics in Farm Animals

Infectious diseases attributable to a diverse array of pathogens, including bacteria, viruses, parasites, and fungi, constitute a substantial risk to the productivity of global livestock and rank among the principal contributors to production deficits. Notwithstanding the comprehensive investigations into the pathogenesis and management approaches pertaining to these pathogenic entities, therapeutic interventions remain complex, and complete elimination has not yet been realized. In light of these challenges, investigating the function of epigenetic biomarkers in the mechanisms underlying diseases presents a promising trajectory for enhancing our comprehension of pathogenesis and refining management strategies for diseases affecting farm animals (Ibeagha-Awemu & Zhao, 2015).

Biomarkers are defined as measurable factors, features, or characteristics that serve as indicators of normal biological processes or pathological conditions. According to the Food and Agriculture Organization (FAO), a biomarker encompasses any substance, structure, or biological process that can be quantified and that influences or predicts the occurrence or outcomes of diseases (Organization, 2001). Molecular biomarkers are increasingly important in modern animal husbandry because they help monitor large animal populations and provide objective confidence in the data they provide (Myers et al., 2017). Therefore, epigenetic biomarkers have become promising in farm animal production due to their integration of multifaceted information. The use of methylation in farm animals has enabled the development of complex DNA methylation biomarkers (Clarke et al., 2021).

In a study investigating epigenetic influences on mastitis a prevalent and economically significant disease in dairy farming Escherichia coli was found to induce hypermethylation in a previously hypomethylated segment of the upstream promoter region of the αS1-casein gene, thereby inhibiting its expression. Remethylation associated with mastitis caused chromatin remodeling in this region and showed the importance of the acute regulatory role of the promoter on CpG methylation by regulating high prolactin levels in the circulation (Vanselow et al., 2006). It was revealed that the presence of mastitis- associated bacteria in Chinese Holstein dairy cows with clinical mastitis caused abnormal promoter methylation in the cluster of differentiation 4 (CD4) gene in blood cells (Wang et al., 2013).

Environmental factors such as heat stress, pathogens, ration changes, etc. experienced by farm animals cause a certain level of stress in animals and significantly affect their health and productivity levels individually. Studies conducted on different animal species have revealed that environmental stress

sources cause epigenetic changes in animals (David et al., 2019; Hao et al., 2016; Littlejohn et al., 2018; Lu et al., 2019). Unfortunately, it is predicted that heat stress experienced by farm animals will continue to cause problems in terms of animal health and animal production due to increasing global temperatures. It has been revealed that heat stress in animals plays an important role on the DNA and histone methylation mechanism of heatshock proteins, which are important for adaptation to heat (Wu et al., 2020). Furthermore, thermal stress has been documented to profoundly influence embryonic development and reproductive efficacy in bovines through its impact on epigenetic alterations, including DNA methylation, DNA hydroxymethylation, and modifications of histones (Sun et al., 2019; Wang & Ibeagha-Awemu, 2021).

Hypoxic stress constitutes another critical determinant affecting both the well- being of animals and their epigenetic modifications. Empirical studies demonstrate that hypoxia significantly impacts the physiological growth of swine raised in elevated altitude conditions, revealing genome-wide alterations in DNA methylation profiles across diverse tissues (L. Jin et al., 2018; Zhang et al., 2019). Additionally, variations in methylation patterns have been documented in genes responsive to hypoxia. In particular, heightened methylation levels in HIF-1α, HIF-3α, and EPO, combined with diminished methylation in HIF-1, have been observed in various organs such as the heart, liver, lungs, kidneys, muscles, and brain of caprines and ovines raised in plateau areas (Y. Wang et al., 2017).

4. Conclusion

This chapter reviews recent epigenetic advancements within the domain of animal husbandry and explores the potential applications of epigenetic processes in enhancing livestock productivity and health management. The growing body of data on epigenetic modifications in livestock is expected to significantly contribute to improvements in animal health and performance. A deeper insight into epigenetic alterations, particularly DNA methylation, will enrich existing knowledge of molecular, cellular, biological, and immune system processes associated with the genome, offering a more integrated perspective. Furthermore, it is anticipated that the detailed elucidation of interactions among epigenetic mechanisms that influence phenotypic traits will advance in the coming years.

Advancements in DNA sequencing technologies have greatly accelerated the genomic assessment of variability in productivity and disease resistance traits among animals. However, it has become clear that DNA sequencing and

sequence variations alone are insufficient to fully account for phenotypic diversity. Consequently, various epigenetic mechanisms, including mRNAs, DNA methylation, and histone modifications, have become integral to accurately deciphering gene expression regulation. Additionally, the realization that these mechanisms can elicit profound biological responses to environmental changes during gestation, and that such responses can be heritable, has positioned epigenetics as a highly discussed area in contemporary research. Consequently, there is a pressing need to advance studies examining the impacts of epigenetic markers on complex diseases and production-related traits in animals under varying conditions. Integrating these findings into livestock production systems is crucial for enhancing productivity and achieving sustainability in the industry.

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Chapter 4

Negative Effects Of Climate Change on Pollen Germination in Fruit Trees

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In recent years, the biggest negative factor in agriculture and food security is climate change. The main issue caused by climate change is droughts, which result from irregular rainfall and water resource depletion. Intergovernmental Panel on Climate Change (IPCC) reports indicate that Mediterranean countries, including Turkey, will be among the most affected by climate change. The report states that as global temperatures rise, predicted to reach 1.5°C by 2100, an increase to 2.13 times the expected 3.2°C will lead to more natural disasters, decreased plant diversity, and a food crisis (Lee et al., 2023). Recent frequent disasters like floods, forest fires, and droughts are primarily caused by climate change in Turkey. The agriculture and food sectors will be the most impacted by climate change. Along with global warming, temperature fluctuations, pollution of clean water sources, erosion, high greenhouse gas emissions, and drought are key factors that harm agriculture. It has become essential to focus research on the sustainability of agriculture amid the adverse effects caused by climate change (Venkateswarlu and Shanker, 2012). Like other agricultural products, climate change and drought are expected to be the major factors limiting fruit cultivation. Climate change can increase winter air temperatures, and it has been reported that these projected temperature changes could impact regions that produce various fruits, grapevines, and nuts (Şahin et al., 2015).

Pollination is an ongoing process in fruit production that includes fertilization and fruit growth. The first necessary components for successful pollination and fertilization are the amount of pollen on the pistilthe ovarium's viability during this process, as well as its capacity to germinate and create a pollen tube (Tosun and Koyuncu, 2007; Güçlü et al., 2015). Optimal pollen germination levels can vary depending on the species and cultivars, environmental nutrients, temperature, pressure, pH, and ecology (Eti, 1991; Voyiatzis and Paraskevopoulou-Paroussi, 2002; Koyuncu, 2006). The healthy development, viability, and high germination capacity of pollen—the male sex cells of plants—

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are essential for successful fertilization (Engin and Ünal, 2002; Özcan, 2020). In addition to these qualities, which are considered pollen quality criteria, a high quantity of flower pollen is also desirable (Sütyemez and Eti, 1999). Pollination and fertilization are key factors influencing fruit set. Therefore, understanding the properties of pollen and other characteristics of species and cultivars is important for growers and breeders. Pollen properties (such as quantity and germination rate) significantly affect fertilization success and high fruit set in fruit species. Heat waves are predicted to become more common in a number of areas as a result of global warming, which poses a serious risk to agricultural security. Temperature rises, whether short-term or long-term, reduce crop production. These circumstances change the morphology, physiology, and biochemistry of plants, which has an adverse effect on their growth (Begcy and Dresselhaus, 2018).

It has been reported that the adverse effects of high temperatures on reproductive organs are greater than those on vegetative organs. Besides the harmful effects of high temperatures on bee activity—which is crucial for pollination and fertilization—they also adversely affect fruit growth by reducing pollen viability, morphological uniformity, pollen performances. Pollen and pollen tube growth serve as reliable indicators for how plant species, different cultivars within the same species, and even various genotypes respond to stress (Çetinbaş-Genç et al., 2019). Gametophyte development, the progamic phase, and embryo and seed development are the three phases of angiosperm reproduction. The male gametophyte (pollen), which produces male sperm cells and transfers them to the female gametophyte for double fertilization, is essential to plant reproduction and crop output (Carrizo Garcia et al., 2017). Because male organs are more susceptible to heat stress than female organs, temperature stress has distinct effects on male and female gametophytes (Zinn et al., 2010; Hedhly, 2011). Changes in temperature influence pollen quantity, shape, cell wall structure, and pollen metabolism (Hedhly, 2011). A thick callose membrane holds the four haploid microspores that are produced by meiosis in diploid pollen mother cells (microsporocytes) in anthers together in a tetrad. ndividual microspores are subsequently released when the callase enzyme, which is produced by the tapetum, breaks down the callose wall. These microspores grow, discharge their nuclei into the environment, and form vacuoles. A tiny generative cell and a big vegetative cell are produced as a result of the polarized microspores' following substantial asymmetric mitotic division. Consequently, the microspore generates cells with two distinct potentialities and serves as the male germline's pluripotent source (Berger and Twell, 2011). The two sperm cells required for double fertilization are created by another cycle of mitotic division of the germ

cell. Whether the second mitotic division takes place during pollen germination or prior to pollen maturation determines whether mature pollen originates from the anthers in a two-celled or three-celled form. Successful and coordinated pollination and fertilization require the simultaneous development of microspores within an anther. Multiple checkpoints regulate this process, and when it malfunctions (for example, as a result of stress), developmental asynchrony causes physiological and metabolic variations in microspores (Giorno et al., 2013). Then, during development, competition for water increases for stigma rehydration and development of pollen tubes. Microspore formation at the onset of meiosis is the most susceptible to environmental stress in the majority of plants (De Storme and Geelen, 2014; Muller and Rieu, 2016; Rieu et al., 2017; Begcy et al., 2019). Climate change is expected to have a major influence on pollen formation and pollen tube enlargement, the most influenced by temperature stages of plant growth, which would lower agricultural crop (Scaven and Rafferty 2013; Bisbis et al., 2018; Rutley et al., 2021). Pollen metabolism, cell wall structure, and pollen quantity and quality are all impacted by abrupt temperature changes (Hedhly et al., 2005). Because it lowers pollen germination, reduces pollen viability, causes pollen to stay in anthers, and causes mononuclear microspore abortion, this has been connected to aberrant tapetum formation (Harsant et al., 2013). Temperature rises have been observed to cause faster pollen tube growth and quicker ovule degeneration (Hedhly, et al., 2005). Furthermore, heat stress causes premature deterioration of the tapetum, a layer of nutrient-rich cells that nourish developing pollen. The tapetum contains more mitochondria than vegetative tissues (Lee et al., 2023; Selinski and Scheibe, 2014). As a consequence of aerobic metabolism, ROS generation rises dramatically at high temperatures due to the large mitochondrial concentration (Mittler, 2017). ROS induce oxidative damage and necrosis when they build up in significant quantities as a result of stress (Sharma et al., 2012). Angiosperm sexual reproduction depends on pollen germination and tube development, two critical phases for fruit set and crop output. Pollen release and germination are necessary for successful fertilization after seed and fruitset (Mondal and Ghanta, 2012). In some cases, failure of plants to flower and pollen to germinate can greatly impact seed production and reduce crop yields. Pollen growth, viability, germination, and overall quality are all impacted by a variety of factors, including fertilization capacity and pollen tube development. These elements include high or extremely low temperatures, air pollution, chemical fertilizers, and pesticides (Padilla et al. 2017; Pers-Kamczyc et al. 2020). Environmental stress can also modify nutrient availability and phytohormone levels, which may impact plant reproduction (Cho et al. 2017; Pacini and Dolferus 2019; Dong et al. 2021). It has

recently been observed that severe temperature swings are occurring in numerous areas (King and Harrington 2018; Kew et al. 2019; Perkins-Kirkpatrick and Lewis 2020). An increase or decrease of just a few degrees above or below the optimal growth limit can cause significant crop yield losses (Peng et al. 2004). Temperature changes can harm the pollination period, affecting the final fruit set and crop yield (Sanzol and Herrero, 2001). Additionally, research indicates that the environment during anther development influences bioactive pollen metabolites. Unfavorable ecological conditions during pollen development lead to notable changes in pollen performance and quality, potentially causing pollen sterility and resulting crop losses (Powell et al., 2012). All phases of pollen formation are impacted by abiotic stress, which commonly results in morphological, structural, and metabolic abnormalities that can cause male sterility, premature spore abortion, and dysfunctional pollen (Prasad and Djanaguiraman, 2011; Firon et al., 2012; Carrizo-Garcia et al., 2017). It can also prevent pollen from being released from the anthers and cause clustering on the stigma (Jagadish et al., 2010; Parish et al., 2013). Due to the tapetum's extreme susceptibility, any interference with its growth results in nonviable pollen, which drastically lowers dicotyledon grain yield. For example, early microspore stage temperature stress results in premature tapetum deterioration (Ku et al., 2003). Climate change could restrict the cultivation of certain crops in various regions. The effects of high temperatures on fruit tree pollen germination were investigated in a number of research. In a research on pollen from many Rosecea species, Beltrán et al. (2019) assessed pollen germination rates, average pollen tube length, and maximum pollen tube length at different temperatures. They found that pollen germination was highest between 15°C and 30°C across all species, with 30–52% germination occurring between 15°C and 20°C for quince, apple, cherry, plum, peach, pear. Germination declined in all species once the temperature reached 30°C. The ideal temperature for blackberry pollen germination is 20°C, according to a study on the effects of various temperatures and incubation periods on the process. High temperatures have a detrimental effect on pollen germination and tube development (Güçlü et al., 2021). According to a study on almond species, pollen germination and tube development are adversely affected by both high and low temperatures (Sorkheh et al., 2018). A study on mango found that the cardinal temperature ranges for pollen germination (Tmin, Topt, and Tmax) were 20.3-22.8 °C, 26.7-30.6 °C, and 30.4-34.3 °C, respectively. Similarly, the cardinal temperatures for pollen tube growth (Tmin, Topt, and Tmax) were 20.3-21.2 °C, 27.9-32.1 °C, and 30.2-34.4 °C, respectively (Liu et al., 2023). In a Korean research on apple varieties, the effects of temperature on pollen tube length and pollen germination percentage

were investigated in a laboratory setting. Using a pollen germination medium, these parameters were assessed at intervals of 5 °C between 5 and 45 °C. Variations in temperature had a major impact on tube development and pollen germination in all cultivars. With an average of 85.2%, The highest percentage of pollen germination ranged from 99.9% ("Shinano Gold") to 61.5% ("Green Ball"). Averaging 855.1 µm, the maximum pollen tube length ranged from 716.5 µm ("Tsugaru") to 989.8 µm ("Arisoo") (Zebro et al., 2023). Plant reproductive organs are more sensitive to extreme temperatures than vegetative organs (Guo et al., 2019; Radović et al. 2019). According to earlier research on olive trees, excessive temperatures during flower development can drastically diminish fruit set (Benlloch-González et al. 2018). Reduced fruit set at severe temperatures is a result of reduced pollen viability and germinability in a number of crop species (Tolessa and Heuvelink 2018; Yang et al. 2019; Shenoda et al. 2021). Güclü et al. (2018) tested four temperature regimes in studies to determine pollen delivery in naturally growing blackberries. They found that 20 °C was the ideal temperature for both pollen germination and tube growth, and that as the temperature rose, the rate of pollen germination and tube length fell. Pollen germination investigations on the strawberry varietiesToyonoka' and Nyoho' revealed that both kinds' germination rates dramatically dropped at 30°C (Ladesma and .Sugiyama, 2005). The best temperatures for germination and tube development were found to be 20 and 25°C in a An investigation of the elongation of pollen tubes and pollen germination in sweet cherries at different temperatures (Koyuncu and Güçlü, 2009).

Since fruit farming is a perennial agricultural activity, it is greatly affected by climate change. Global warming could raise temperatures during winter, and these changes are expected to harm regions that grow various fruit species, grapevines, and nuts (Şahin et al., 2015). Besides the negative effects of high temperatures on bee activity—which is crucial for pollination and fertilization—they also harm fruit production by reducing pollen viability, affecting morphological consistency, and decreasing pollen germination and pollen tube length. Studies have shown that high temperatures negatively influence healthy pollen development, pollen germination, and fruit set, which are the initial stages of fruit formation. Additionally, it has been noted that high temperatures have a greater detrimental impact on tube development and pollen germination rates than low temperatures (Kakani et al., 2002). Temperatures in Turkey are increasing each year. Heat waves, caused by high temperatures and humidity during summer, have become more frequent, longer, and more intense. Rising nighttime temperatures will worsen urban heat islands, and the resulting thermal stress will pose existential threats. Rainfall has steadily decreased since the 1970s. Climate

change and conditions across Turkey will create regional disparities. These changes will affect water sources, natural vegetation, agricultural potential, and human health and well-being. The risk of forest fires will rise. Insect populations will grow, negatively impacting the food chain and other organisms. Diseases and general health issues will increase, and some species will face extinction. Climate change and drought are ongoing and will continue to be serious issues in Turkey. The recent extreme temperatures and water shortage must be addressed urgently, and our water resources must be managed efficiently. Significant changes should be made in how we consume power, water, and other natural resources. Infrastructure improvements are needed to reduce losses in water and electricity distribution. Renewable energy sources (wind, solar, geothermal, etc.) should replace fossil fuels. Efforts to increase afforestation should be expanded, and measures to prevent forest fires should be put in place. Agricultural policies need reevaluation. Proper crop patterns should be established based on current developments. Illegal water use should be stopped, and groundwater consumption should be monitored. Major water projects such as dams and reservoirs should be planned. An integrated and sustainable approach to water management should be adopted. Stress management in fruit farming should be intensified, and breeding of varieties and rootstocks suited for extreme conditions should be promoted.

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