NEW APPROACHES IN AGRICULTURAL, FORESTRY, AND AQUATIC SCIENCES

THEORY, METHOD, AND PRACTICE

Editor: Prof. Dr. Türkay TÜRKOĞLU

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Chapter 1

An Overview of the Ecological Importance of the Carbon Cycle, Aquatic Ecosystems and Aquatic Plants.

Hatice TEKOĞUL[1]

ABSTRACT

Carbon is one of the fundamental building blocks of life and exists in a continuous cycle within ecosystems. The carbon cycle encompasses complex biochemical processes occurring between the atmosphere, terrestrial, and aquatic ecosystems. The fact that approximately 70% of the Earth's surface is covered by water makes the role of aquatic ecosystems in global carbon and oxygen cycles even more significant. Aquatic plants and phytoplankton, thanks to their high photosynthetic capacity, absorb large amounts of carbon dioxide (CO_2) from the atmosphere, produce oxygen, and contribute to maintaining ecosystem balance. However, human-induced activities such as fossil fuel use, indiscriminate fertilization, deforestation, and water pollution disrupt the natural balance of matter cycles, leading to climate change and ecosystem degradation. This study examines the carbon cycle, the global importance of aquatic ecosystems, and the roles of aquatic plants in carbon sequestration, oxygen production, and phytoremediation processes

Keywords: Carbon theory, aquatic ecosystems, aquatic plants, photosynthesis, phytoremediation

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1. INTRODUCTION

Water, oxygen, nitrogen, phosphorus, and carbon are inorganic substances that are the basic components of life. These substances constantly circulate between living and non-living environments. This circulation is called the matter cycle. The cycle begins with energy from the sun and photosynthesis and continues throughout the food chain. Many substances found in nature are produced or consumed by living organisms, thus ensuring the continuity of life (Ribeiro, K., et al.,(2021). Carbon, represented by the symbol C in chemistry, is the fourth most abundant element in the universe. It forms the basis of life because it is found in the structure of biological molecules such as proteins, fats, carbohydrates, and DNA. The ability of the carbon atom to form four stable chemical bonds makes it possible to create complex organic structures. This property distinguishes carbon from other elements and allows it to play a central role in ecosystems. Bryndin, E. (2024) Battin, T. J., et al (2009)

In nature, carbon is constantly circulating between the atmosphere, hydrosphere, lithosphere, and biosphere. This circulation is called the carbon cycle. Disruption of the natural balance of the carbon cycle leads to global climate change and serious disruptions in the functioning of ecosystems. The balance of ecosystems is maintained by the ratios of these substances. An excess or deficiency of a substance in a region affects the ecosystem balance.

2. THE CARBON CYCLE AND ECOSYSTEM BALANCE

Aquatic plants (macrophytes), besides their beautiful appearance, are a very important part of nature. The carbon cycle begins with the uptake of CO_2 from the atmosphere by plants through photosynthesis. Aquatic plants (macrophytes) are important components of the carbon cycle in global ecosystems. Considering that their habitats, water bodies, cover three-quarters of the Earth, they absorb much more carbon dioxide from the atmosphere through photosynthesis than terrestrial plants. The carbon converted into organic matter as a result of photosynthesis is transferred to other living things through the food chain. Microscopic aquatic plants and similar microscopic photosynthetic organisms are the most fundamental and powerful component of carbon sequestration on a global scale. As a result of respiration, decomposition, and combustion processes, carbon returns to the atmosphere as carbon dioxide. Tekoğul, H., et al. (2023a)..

This cycle works in a balanced way under natural conditions. However, the excessive amount of CO_2 released into the atmosphere as a result of the intensive use of fossil fuels disrupts the speed and balance of the carbon cycle, causing global warming and climate change. This disruption in the carbon cycle also has negative effects on the oxygen balance.

Approximately 70% of the Earth's surface is covered by oceans, lakes, rivers, and other bodies of water. These vast areas play a fundamental role in regulating the global climate system. Water bodies absorb and dissipate heat, regulate the water cycle by controlling evaporation and precipitation processes. Furthermore, aquatic ecosystems contribute to the conservation of biodiversity by providing habitats for millions of micro and macroorganisms. Photosynthetic activities within these ecosystems constitute a significant portion of global oxygen production (Pokorný, J., & Květ, J. (2003)..

Aquatic plants (macrophytes) and phytoplankton, thanks to their high photosynthetic efficiency, take up large amounts of CO_2 from the atmosphere and convert it into organic carbon compounds. The constant presence of dissolved carbon dioxide in aquatic environments and the homogeneous distribution of light in the water column increase the photosynthetic capacity of aquatic plants.

Some aquatic plants and amphibian species can utilize not only CO_2 but also bicarbonate (HCO_3^-) ions as a carbon source in the carbon cycle. In this way, they achieve primary production by using dissolved carbon dioxide (CO_2) and bicarbonate (HCO_3^-) in the water. Algae and emergent macrophytes (e.g., reeds, rushes) play a significant role in this production. Some of this carbon is retained in plant biomass for a long time, while some passes into the sedimentary layer as dead plant material. They take carbon from the atmosphere or dissolved carbon dioxide in the water and store it as organic matter in plant tissues (Sand-Jensen, K., et. al., (1992)).

As we all know, aquatic plants (such as reeds, rushes, water lilies, duckweed, elodea, etc.) perform photosynthesis just like terrestrial plants, converting CO_2 (carbon dioxide) + H_2O (water) → Organic carbon (sugar, cellulose, biomass) + O_2 (oxygen). Seagrasses, along with coastal plants such as sea samphire and salt marsh vegetation, are part of "blue carbon ecosystems." The concept of blue carbon encompasses not only seagrasses but also freshwater marshes. Some ecosystems, particularly deltas, lake bottoms, marshes, and reedbeds, are systems with high long-term carbon storage capacity.

It is estimated that seagrasses cover only 0.2% of the global ocean floor, but store 10–18% of marine carbon.

In particular, root and rhizome systems contribute to carbon sequestration in sediment by transporting carbon to the bottom of the lake(Photo 1). The journey of this acquired carbon in the plant begins. As plants grow, they store carbon in the stem, leaves, and roots, and also bury some of it as organic carbon in underwater sediments. Because this buried carbon dissolves very slowly in the bottom layers where oxygen is scarce, carbon can be stored for centuries. They can store 3–5 times more carbon per unit area than tropical rainforests. For example: One hectare Reedbeds can store approximately 1–3 tons of carbon per year.

This amount is close to that found in some forest ecosystems. This characteristic allows aquatic plants to become dominant, especially in CO_2-rich aquatic environments.

Human activities negatively impact nitrogen and phosphorus cycles, particularly carbon. For example, the discharge of agricultural and domestic waste into water leads to increased levels of substances like nitrogen and phosphorus. This can lead to algal blooms, which are prevented by plants that consume excess substances in the water. Excessive fertilizer use in agriculture leads to the transport of nitrogen and phosphorus into aquatic environments, causing problems such as eutrophication, algal blooms, and dissolved oxygen depletion. Aquatic ecosystems play an active role in both carbon sequestration and carbon recycling processes (Gandhi, N., et al., (2013), Lal.(2015)). Just as forests on land act as "carbon sinks," aquatic ecosystems have a similar function—and in some cases, can be even more effective. Deforestation reduces carbon sequestration by decreasing photosynthetic capacity, leading to an increase in the amount of CO_2 in the atmosphere. Water pollution disrupts the functioning of matter cycles by exceeding the natural decomposition capacity of ecosystems.

Phytoremediation is an environmentally friendly treatment method based on the principle of aquatic plants absorbing and removing excess nutrients and pollutants from wastewater. Due to its low energy requirements, lack of chemical use, and additional benefits to the ecosystem, it stands out as a sustainable ecosystem management solution (Tekoğul, 2023b; Tekoğul, 2023c; Stefanakis vd., (2014). Tekoğul, 2023d).

Aquatic plants improve water quality and support ecosystem balance through oxygen production and habitat creation during phytoremediation processes.

When aquatic plants die or shed their leaves, this organic matter is broken down by microorganisms (bacteria, fungi). Decomposition and carbon recycling occur. As a result of decomposition, gases such as CO_2 and methane (CH_4) are released back into the water or atmosphere.

Photo 1: Aquatic ecosystems (original)

3.CONCLUSION

This process is important in terms of heterotrophic respiration and greenhouse gas emissions.

Aquatic plants are one of the natural solutions that mitigate this effect by sequestering carbon. They also purify water, provide habitats, and stabilize sediment (providing a multi-functional ecosystem service). The carbon cycle and other matter cycles are vital for the sustainability of ecosystems. Aquatic plants reduce the amount of CO_2 in the atmosphere. They slow down climate change, prevent coastal erosion, and create habitats for marine life. Aquatic ecosystems and aquatic plants play a central role in maintaining global carbon and oxygen balances. To rebalance matter cycles disrupted by human activities, sustainable resource use, the widespread adoption of environmentally friendly technologies, and the protection of natural ecosystems are necessary.

The carbon cycle and other matter cycles are vital for the sustainability of ecosystems. Aquatic ecosystems and aquatic plants play a central role in maintaining global carbon and oxygen balances. To restore balance to matter cycles disrupted by human activities, sustainable resource use, the widespread adoption of environmentally friendly technologies, and the protection of natural ecosystems are necessary.

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Chapter 2

Electroculture In Viticulture: Biophysical Basics, Applications, And Future Perspectives

Ruhan Ilknur GAZIOGLU SENSOY[1], Ozgur BOR[2]

1. Introduction

1.1. The Importance of Viticulture and Modern Production Approaches

Viticulture is an important part of the agricultural economy and occupies a large area of land, about 7.4 million hectares in the world (FAO, 2023). Grapes are a high-value commodity with multiple ends that use wine, raisin, grape molasses and table grapes. "Today, viticulture is not simply about quantity but also quality, sustainability and environmentally friendly production systems" (Bramley et al., 2011). Treatment concepts are based on IPM and include biological control, mating disruption, mass trapping, use of semiochemicals and natural products, biotechnological solutions, energy-saving solutions (Alina & Marinela, 2024; Moahmmed & Messaoud, 2024), as well as precision viticulture, remote sensing systems, sensor-assisted irrigation, biotechnological innovations and renewable energy. Such innovations are driven by a desire to better manage resources within the vineyard, and in response to the growing challenges of climate change.

1.2. Definition and History of the Concept of Electroculture

Electroculture is a new type of farming that uses electric currents or electromagnetic waves to stimulate growth, development, and the general abundance of plants (Briggs, 1926). The idea originates in the late 18th century from experiments in France and England (Matthews, 1922; Phoenix, 1929). In the beginning of 20th Century, the "Electroculture Committee of the Ministry of Agriculture" undertook the systematic investigations on the influence of electric currents on plants. Currently, studies in this field concern a number of application methods, such as electrostatic fields, electromagnetic fields, pulsed

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electric fields (PEF), and electro-priming (Cao et al., 2025; Morales et al., 2021).

1.3. New Technological Approaches to Increasing Agricultural Productivity

The increasing world population and the influence of climate change are demanding new technologies in agricultural production (FAO, 2023). In this sense, electroculture can be considered as a sustainable and energy-saving technique that reduces the dependence on chemical inputs. A lot of research has reported positive effects of electrical stimulation on seed germination, photosynthesis (Phirke et al., 1996), and stress tolerance (Fromm & Fei, 1998). These results show that electroculture increases the quantity of seeds and, presumably, improves ecological sustainability.

1.4. Why Electroculture Has Become a Topic of Discussion in Viticulture

In the production of wine, factors such as drought, soil exhaustion and phytopathological pressures (powdery mildew, downy mildew and Botrytis) have severely limited production effectiveness in recent years (Bramley et al., 2011). Toward that end, current speculation is considering the possible effects of electroculture techniques on efficiency of water usage, activity of soil microbes, and plant defense mechanisms, based on research with diverse plant species and theoretical considerations. The potential applicability of electroculture to viticulture is being identified as a line of enquiry worth pursuing (Ahmed et al., 2025).

2. The Scientific Foundations of Electroculture

2.1. Interaction of Electromagnetic Fields with Biological Systems

Electromagnetic field has a strong impact on plant physiology at the level of cells. Plant tissue is very sensitive to external electrical field, and this sensitivity mainly arises from ion transport and electrical potential difference across cell membranes (Cao et al., 2025). Such fields may influence ion channels, protein synthesis, photosynthetic enzymes, and mitochondrial function. Studies have indicated that low frequency magnetic fields can promote the growth and root formation in some plants species in certain experimental conditions (Phirke et al., 1996).

2.2. The Role of Electrical Potentials in Plant Physiology

Plants produce intracellular and extracellular electrical potential shifts when they are stimulated by the environment. These “resting and transient electrical potentials” are vital for the regulation of numerous plant physiological processes, such as water movement, nutrient transportation, and hormone transport (Takamura & Volkov, 2006). Spontaneous “action potentials” in plants can be induced by mechanical contact, light, temperature gradients and ionic changes in the environment. It is believed that electroculture treatment can induce such physiological responses in plants as natural electric signals (Sensoy et al., 2022).

For example, research on model plants has revealed that application of weak direct current (DC) has the potential to stimulate ion flow in plant roots and increase water and mineral intake. These changes in physiology have been correlated to enhanced photosynthetic performance in model plants (Lee & Oh, 2021).

2.3. Electrostatic, Electromagnetic, and Electrokinetic Mechanisms

The phenomena of electroculture could be considered as three fundamental processes: electrostatic, electromagnetic, and electro–kinetic action.

*Electrostatic Effec*t: This process enhances ion uptake by the seed or plant through increasing the presence of electric charges on the surface of seeds/plants. An effect was reported on an increased germination, mainly for some plants and in some experimental conditions (Dannehl, Sun et al., 2024).

Electromagnetic Effect: The time-varied magnetic fields induce an electric field or potential difference within the cell (membrane) that causes the opening ion channels. This may improve the enzyme activities and oxygen delivery in the plant (Kovacic & Somanathan, 2010; Lewicka et al., 2015; Morales et al., 2021).

Electrokinetic Effect: This process is related to electrical field effects on the mobility of ions in the system of soil-water-plant. In particular, previous reports demonstrated a stimulatory effect of low-voltage direct current (DC) on the growth of beneficial bacteria like Pseudomonas in the rhizosphere (Olszanowski & Piechowiak, 2006).

2.4. Effects of Electrical Stimuli on Ion Transport, Photosynthesis, and Water Balance

Electroculture is an inquiry into the effects of electric fields or currents applied to tissue on the growth, water and nutrient absorption, and general physiology of plants. Plants produce biological electrical signals naturally in response to environmental changes. This type of signals works as a modulator of intracellular ion flows and as a coordinator of distant tissue responses, including action potentials and variation potentials. Intercellular communication is also regulated by such electrical signals, which are essential to stress-induced intercellular and inter-tissue electrical waves. These molecules, for example, act in photosynthesis and in systemic acclimation (Szechyńska-Hebda et al., 2017).

Studies in well-controlled experimental systems with model plants such as *Arabidopsis thaliana* demonstrated that electroculture treatment methods promote water/mineral absorption, raise tissue electrical charge, and enhance plant development through the induction of auxin accumulation. This study suggests that application of electrical field could change the various metabolic activities in plants (Lee & Oh, 2025).

Furthermore, electrical signals in plants have also been associated with photosynthesis; because electrical stimulation may regulate gas exchange and stomatal functioning, it can also have impact on CO2 intake and photosynthetic responses. For instance, there is evidence that electrical signals propagate in poplar and affect photosynthetic responses (Silke et al., 2005).

Nevertheless, the scientific consensus on electroculture efficacy is not clear-cut; positive physiological effects have been divergently reported in some works, but the general recommendation of the scientific community is the completion of more strictly controlled studies to allow for these effects to result in general and repeatable outcomes (Anonymous, 2023). It has been reported that the application of electroculture could enhance plant tolerance to environmental stresses such as drought, salinity and high temperature (Cárdenas-Flores et al., 2025).

In conclusion, biophysical data on how electrical signals affect plant physiology are accumulating; and ion transport, water balance and photosynthesis are among the processes that can be associated with electrical signals. However, although beneficial effects of electroculture treatment at an agricultural level have also been reported, in this context stronger experimental support including mechanism-based elucidation of physiological and biochemical pathways is necessary to confirm the general applicability and reliability of electroculture treatments (Szechyńska-Hebda, 2017).

3. Agricultural Electroculture Applications: Overview

3.1. Experimental Studies Conducted on Field Crops

The influence of such fields and electromagnetic techniques on the processes of the production of a plant has also been considered in agriculture. The majority of the research related to pre-planting seed treatments using weak EF or magnetic field has been concentrated on seed treatment before planting and soil EF applications of low intensity.

Results of field experiments conducted in Greece for three years showed that durum wheat seeds treated with pulsed electromagnetic fields (PEMF) prior to sowing exhibited higher germination, superior leaf area, chlorophyll content, rate of photosynthesis, and stomatal c. as compared with control. This resulted in an increased dry weight of plant and the yield as well, with the rise of amylase activity as a possible factor contributing to the effects (Katsenios et al., 2016).

Corn plant-related laboratory experiments also revealed that the exposure of corn plants to a LIEF could enhance their production in terms of the leaves, the stems, and the roots. This growth was due to the electric-field-induced enhancement in root electro-motion of nutrients Solís, 2023.

Other results revealed that electromagnetic and electric field treatments had a positive effect on rice seed germination and early root growth after 48 hours of treatment, under certain intensities and frequencies of the electrodes. These results confirm the efficacy of the classic pre-seed treatments derived from "magneto-priming" or low-intensity field treatments (Alshalwi, 2017).

In general field experiments at the turn of the twentieth century, high-voltage electric charges applied to forage crops, including wheat, oats, and barley, increased yields by more than 20 percent on average over the uncharged control areas. Conversely, the results were dependent on the application protocol and environmental conditions (Pelesz & Fojcik, 2024). Several research reports indicate that electroculture treatments are linked with increases in yield for a range of field agriculture crops when rigorously performed according to protocols.

3.2. Electroculture Experiments in Vegetable and Fruit Species

Reports on the influence of electromagnetic and electric field treatments on vegetables and fruits are few, and were mainly concentrated on magnetic field treatments, electric field application, or magnetic/electric field-based water/irrigation pretreatments. These applications aim at investigating possible beneficial effects on growth, yield, quality, and shelf-life.

Electroculture is a sustainable method of stimulating the growth of plants using electromagnetic fields. A single research, pure copper electroculture antenna was applied on okra, and the result showed that okra treated with electroculture produced a total fruit count 2.3 times higher than the control. There was a slight reduction in unit fruit weight, but the continuity of production and fruit set was enhanced significantly. This result indicates that electroculture can be considered a potential technique for yield improvement without requiring energy or chemical resources (Atanda, 2025).

There is research on tomatoes (*Solanum lycopersicum*) which found that magnetic fields and magnetic water had similar positive impacts on growth parameters including leaf, stem, and root biomass, as well as leaf area and yield. In addition, some studies have shown that magnetic water treatments improve nutrient mobilization and uptake in the soil. These results also suggest that magnetic field and electro-water pretreatment could stimulate growth and yield of vegetables (Pelesz & Fojcik, 2024).

Magnetic field applications have been associated with increases in the number of fruits, fruit weight and total yield; but to what extent seems to be dependent on the field strength (Kumar et al., 2025). In addition, the impact of electric and magnetic fields on fruit quality and shelf life can be attributed to their ability to retard the rate of ripening, respiration and ethylene generation, ultimately resulting in longer storage life. High voltage electric field treatments have been shown to decelerate softening of fruit tissue and to yield better quality with respect to firmness,β-carotene, lycopene and sugar content. Research has shown that electric and magnetic fields can also positively affect post-harvest longevity, as evidenced by a diminished rate of aging and slower degradation of quality in fruits and vegetables during storage (Saletnik, 2022).

3.3. Electromagnetic Waves and Their Effects on Seed Germination

Seed germination is one of the plant processes that was most rapidly influenced by electrical stimulation. It has been shown that EM waves act on the seed coat, enabling water absorption and speeding up metabolism (Pohl & Todd, 1981).

Studies on tomato (*Solanum lycospericum* L.) seeds have revealed that exposure to magnetic fields induces an increase in both the rate and speed of germination. Pre-treatment of seeds of tomato with magnetic fields improved the germination percentage and reduced the germination period (El-Yazied, 2011).

It has recently been suggested that magnet ease in germination and seedling growth. For example, shining magneto-priming seeds with neodymium magnets found the highest germination and water stress tolerance (Abhary & Akhkha, 2023).

Moreover, further investigation showed that the static magnetic fields during the germination stages of various vegetables (tomato, lettuce and arugula) have an influence on germination traits, but some magnetic intensities showed adverse effects (Koukounaras, 2023).

Studies with water or seeds exposed to magnetic fields have highlighted an enhancement in germination due to salt stress and a decrease in average time of germination (Samarah et al., 2021). Similarly, studies conducted under various magnetic field intensities and exposure periods recorded increases in germination rate, root length, stem length, leaf length and width (Feizi, 2012).

The activity of enzymes like amylases and the process of water uptake have been suggested to be associated with the effects of magnetic and electromagnetic field exposures on germination. Electromagnetic stimulation of seeds that if treated may have a good effect on germination through change of ion content or metabolism. EMF treatment accelerated the germination and activated biochemical processes in wheat, a cereal crop (Urnukhsaikhan et al., 2025). In a study on a filler crop, jute (*Corchorus olitorius* L.), treated seeds showed higher germination, seedling growth, and biochemical attributes.

Species, field intensity, and exposure time are among the factors that determine the effect of EM waves on seed germination. However, despite the general consensus that magnetic and EMF treatments may improve germination success, further elucidation of the mechanism, along with more controlled studies, is needed in order that these results may be applied in practical agriculture.

3.4. The Relationship Between Soil Electrical Conductivity and Microbial Activity

Soil electrical conductivity (SEC) is a proxy of soil salinity and concentration of other ionic components in the soil solution, which is positively related to soil health and nutrient status. SEC affects many processes such as microbial activity, mineralization of organic matter, nutrient cycling and soil aggregation. The dependence of plant growth and the soil flora on a relationship between electrolyte conductivity and microbial activity is a rather strong one. This article highlights the ability of low-frequency currents to enhance nitrification in the soil microbiome (Morales et al., 2021).

The electrical conductivity of soil is increased by an increase in dissolved salt concentration in soil. But too much salinity can have a deleterious effect on microbes. Microbial metabolism is higher in TEI -moderate soils, as the organic matter breaks down faster in these soils (Kim et al., 2016).

Moisture content of soil and salt are two of the major factors that shape microbial community structure and function. Soil conductivity measurements, which can be used as indirect indicators of microbial activity and soil diversity (Zhao et al., 2024).

There were significant relationships between enzyme activities in soil and electrical conductivity. These enzymes are markers of microbial metabolism and may also contribute to increasing the availability of nutrients for plants (Tunc & Demir 2021).

In high organic matter soils, TAE values around the values for maximum microbial activity are found. In farming applications, TAE measurements can provide a rapid assessment of the soil quality for deciding on fertilization and irrigation treatments to maximize microbial activity. On the other hand, elevated values of TSE are associated with high salinity or over-fertilization, which reduce the bacterial diversity. It is only when the equilibrium is met that the biological activities and nutrient conversion in soil can maintain at the maximum level.

To sum up, soil EC is an important indicator of microbial activity and thereby soil health. Moderate TEC values are correlated with the highest microbial densities and enzymatic activities. Knowledge of this correlation is therefore important for the strategies for sustainable agriculture and soil management. These results open the route for considering electroculture as a novel means for biological fertilization and sustainable soil management."

3.5. Different Results Obtained According to Energy Type and Dose

In soils containing abundant organic matter, moderate apparent electrical Total Soil fertility is an inherent property of soil as the energy of soil processes, closely connected with the dynamic balance between mineral and organic components of soil. Applicability of different types of electro energy (e.g., direct current (DC), alternating current (AC), pulsed electric fields (PEF), and electrostatic energy) and of various intensities is subject to so called dosing principles. Low-voltage fields promote growth while high-voltage or long treatment time can induce stress in plants (He, 2024; Baydilli, 2025).

Electrical treatment, such as electric fields, electric currents, and discharge plasma, can promote or suppress plant growth, depending on treatment method. Direct and indirect electrostimulation may increase yield through genomic alterations, enzyme activation, photosynthesis enhancement, and nutrient absorption (Wang, 2022).

Hence, the energy density, duration and frequency parameters in electroculture should be finely tuned. At the right parameters, these treatments are usually successful in promoting growth and stress tolerance in plants.

4. Electroculture Applications in Viticulture

4.1. Initial Experimental Studies Conducted in Grape Vineyards

The idea of electroculture was first mentioned in farming writing in the 1790s and featured in Abbé Pierre Bertholon's 1783 treatise, "De l'électricité des végétaux," which addressed the influence of atmospheric electrical potentials on plants. Until the birth half of the twentieth century, however, no systematic experimental work in vineyards was undertaken. These initial studies were mainly concentrated on electrode-based soil stimulation and approaches of electric atmospherization simulation.

Initial studies inspired by this work suggested that electrical stimulation might increase plant growth in viticulture and might have a positive effect on several quality attributes of the plant.

4.2. Effects of Electrical Stimulation on Vine Physiology

Water Uptake and Photosynthesis

Grapevine is a species of plant with physiology that is susceptible to water and environmental variations. In vitro experiments on model plants have demonstrated that applications of low-voltage direct currents may affect the process of water uptake and cause positive effects on photosynthetic efficiency (Lee & Oh, 2025). It is also discovered that ions can be facilitated to be transported by electrical stimulation within xylem and phloem, regulating the leaf water potential (Lee & Oh, 2021).

In studying the energy absorption characteristics of grapevine cuttings during electrical contact, both the water environment and the cut environment were considered by scientists. This work investigated the dependence of energy absorbed available tissue in a cutting pattern (phloem, xylem, pith and bark) on various parameters including the distance l between electrodes, the applied voltage U, and the time of exposure (τ). Results showed that current density increased as a function of time in the humid environments while it decreased in the dry ones. This aligns with the grapevine's needs as by reducing leaf wilting, it facilitates the support of metabolism, especially in warm and drying conditions (Markaev et al., 2023).

Vine Root Development

The study of direct current (DC) on rooting in *Vitis champini* cv. Ramsey cuttings revealed that the DC application at 30–60V significantly improved the rooting rate, root number, root length and root weight. A 3-h treatment with 60V DC significantly increased the rooting rate by 122% and the number of roots by 100%, confirming its utility as a technique for propagation of grapevine rootstocks via cuttings (Köse, 2007).

Alternating current (AC) treatment effects on yield and root growth of “Kishmish Black” grape were investigated in the study of Berdishev et al. The results showed that the electrical treatment given to the cuttings before planting enhanced the root development by 15–20% and increased the rate of survival in seedlings by 20–22%. Positive responses were also recorded for the length of main shoot, as well as root number and root length. These data indicate that AC treatment can be used as an efficient way to improve the productivity of grapevine seedling and speed up the root growth so that the vine can be more effectively supplied with H_2O and nutrients.

Leaf Surface and Chlorophyll Synthesis

It has been reported that electroculture treatments can have a positive effect on photosynthetic pigments such as chlorophyll a, chlorophyll b and carotenoids. These pigments are very important to the metabolic activity of plants, their potential to use light energy and their stress tolerance. Nevertheless, to date, there are not enough studies dealing with this issue in viticulture.

4.3. Effects of Electroculture on Yield and Grape Quality (Sugar, Acid, Phenolic Compounds)

In grape growing, the composition of the fruit is just as important as how much you get, when talking about fruit quality. Electrical stimulation positively regulates sugar accumulation, phenolic compound synthesis and a suitable organic acid metabolism in grape berry (Saletnik et al., 2022).

In particular, electrical stimulation raised sugar concentration (Brix) and levels of anthocyanins and resveratrol in grape berries. It is attributed to enhanced vine growth, development and photosynthesis via up-regulation of sugar and phenolic compound biosynthesis related genes triggered by electrical stimulation (Mikami et al., 2017).

Furthermore, electrical stimulation increased berry size by promoting cell division in the vineyard-grown Shine Muscat and Pione grapes. Analyses of micrographs demonstrated that the increase in berry size is caused by an increase of cell numbers, while cell elongation did not contribute much to the berry growth. Expression analysis showed up-regulation of the KIN-5C and NUP88 genes and growth that is Stimulated by electricity (Mori et al., 2022).

4.4. The Potential of Electromagnetic Applications Against Phytopathological Effects

The applications of electroculture showed some good results in protecting vines from disease in a number of works. Electric fields have been found to decrease the gray mold, grapevine downy mildew, and botrytis diseases occurrence in vineyards. This protective effect is correlated with an increased expression of the β-1,3-glucanase and PR1 genes in plants and therefore the SA-dependent defense pathway is triggered. These results suggest that ES could be a green tool to elicit plant defenses against biotic stresses (Mori et al., 2021).

The spread of downy mildew (*Plasmopara viticola*) and powdery mildews (*Uncinula necator*) tends to be explosive, particularly under moist conditions. It has been reported that electromagnetic waves induce electrical polarization within the leaf surface, and inhibit the spore germination (Morales et al., 2021). In some field experiments high reduction of Botrytis infection up to 20 % was observed in Turkish in case of application of the electric current (Ali et al., 2022). These observations imply that electroculture could be a valid alternative to chemical inputs in phytopathological control.

4.5. Electroculture and Sustainable Vineyard Management: Potential Contributions to Reducing Pesticide and Fertilizer Use

Investigations on model plants have revealed that a weak electrical current stimulation promotes the absorption of some macronutrients, such as Ca^{2+}, Mg^{2+}, and K^{+} (Lee & Oh, 2021). These physiological responses should be considered as the mechanistic points of view facilitating the efficient development of plant nutrition strategies, but their influence on fertilizer reduction in vineyard should be confirmed by direct experiments.

A further sustainability notion associated with electroculture is the strengthening of plant resilience and immunity. Plants' natural defenses can be triggered by electrical stimulation and under stress they can become more tolerant of pathogens, potentially leading to a decrease in pesticide applications, according to some research. In addition, certain reports indicate that the exposure of controlled electric fields may trigger an increase in production of secondary metabolites in plant cells or tissues, which accumulate natural defense related components that are thought to contribute to the enhancement of resistance in plants to infectious diseases (Chier et al., 2025).

Electroculture is a method in which electrical currents are used. Typically, electroculture devices are based on electrical principles and use low voltage, making their energy consumption very low. Such low-energy electrical treatments can be combined with renewable energies, i.e. solar and wind energy (Moahmmed & Messaoud, 2024).

The application of renewable energy-based systems for farming is considered a potential approach to mitigate carbon emissions, and to reduce the external dependence and the operational expenditures for long term. Several reports suggest that low-energy electrical and electromagnetic field treatments can have effects on biological processes in plant systems, and these findings are interpreted in conceptual and comparative terms with respect to sustainable agriculture (Vian et al., 2016). Electroculture, in this regard, is a breakthrough farming technique.

Recent reviews have pointed out that electroculture applications have potential to affect soil micro-ecological processes and the activity of soil microbial communities, which in turn could indirectly influence nutrient cycling for plants. This is proposed to be able to sustain the ecosystem level practice in vineyard (Ahmed et al., 2025).

Thus, we speculate that electroculture might be used to reduce fertilizer and pesticide application in the future by influencing plant nutrient uptake and defense responses. But more field-based, controlled and mechanistic studies are required to enable translation of electroculture into real-world vineyard production.

5. Technological Approaches and Application Systems

5.1. Types of Electroculture Devices

The devices and systems for electroculture applications can be classified into various technologic architectures according to the source of energy, method of application, and its influence on plant organism. These well-established practices are conceptually divided into methodologies based on direct electrical stimulation (such as in electro-antennas, atmospheric antennas and soil-electrode systems), and others based on exposure to magnetic or electromagnetic fields (Vian et al., 2016).

Electro-antennas are usually passive and are made out of metal or carbon-based rods that rest on or above the plant canopy. These are known to produce low-level electrical effects by tapping into the naturally available potential electrical gradients in the air. These systems are classified as passive and they do not rely on an external energy source, which leads to a discussion on experimental and the traditional energetic applications of these systems although it is still uncertain how exactly they work (Goldsworthy, 2006).

The soil electrode method consists of introducing a direct current (DC) or an alternating current (AC) into the soil using electrodes placed in the plant root zone. These electrodes, which are usually made from copper, stainless steel, or carbon-based materials, are laid out parallel to the rows of plants. Previous experiments and recent reviews have shown that such exposures may affect root zone ion mobility, nutrient movement, and electrical potential gradients within the root zone (Murr, 1963; Vian et al., 2016).

The mechanisms by which static magnetic field or electromagnet effects act on plant tissue and irrigation water are physical and biological systems are affected by physical mechanisms of action via magnetic fields. Several reports have indicated that the physical attributes of water and the mobility of ions may be influenced by magnetic fields, resulting in indirect modification of water absorption and mineral nutrition in plants. In vitro studies specifically on magnetic field–plant interactions to date have been conducted to investigate the influence of magnetic field treatment on plant growth and physiology (Carbonell et al., 2011; Racuciu & Creanga, 2007).

All these forms of electroculture may be modified for specific plants, soils, climates, and potential end-use applications. The literature, however strongly highlight that the findings related to the performance of these systems are inconsistent and their use is regarded as largely experimental and at the research stage.

5.2. System Design: Frequency, Voltage, and Duration Parameters

Plant production responses to electroculture environments depend on technical parameters such as voltage, frequency, current density, and the exposure time of the electric stimulus. It is stressed in the literature that these factors may have either stimulating or stress-invoking effects on plant physiology (Murr, 1964; Vian et al., 2016).

Low-voltage DC treatments are generally thought to have a positive effect on plant growth and root development, but high-voltage treatments may cause electrical stress and disrupt cell membrane physiology (Murr, 1964). Therefore, in the electroculture studies in agriculture, low energies are overwhelmingly favored.

In alternating current (AC) treatments, frequency is thought to be associated with the movement of ions and electrical signal transduction processes in plants. In particular, low-frequency electric field is reported to offer primitive conditions for the applications with less thermal effect, thus is suitable for experimental application (Vian et al., 2016).

Pulsed electric field (PEF) processing involves short pulses of high energy to temporarily increase the permeability of cell membranes. The basic principle of this technique is to enhance the diffusion of ions and molecules through inducing reversible electroporation to the cell membrane. Although PEF systems have been examined to a limited extent for plant tissues, their modes of action are mainly explained by biophysical processes (Kotnik et al., 2015; Toepfl et al., 2007).

In applications of electroculture, the length of exposure and the number of repetitions is said to have a significant effect on the response of plants. The literature indicates that short-term and pulsed treatments are more efficient than long-term continuous treatment, although non-control applications can have negative consequences for plant physiology (Vian et al., 2016).

5.3. Energy Distribution and Safety Criteria in Vineyards

Vineyards are defined by their hilly slopes, large areas, and diverse soil composition inside, and energy management needs consideration when implementing electroculture in them. Uneven energy distribution may reduce the efficiency of the application and result in a safety hazard (Moahmmed & Messaoud, 2024).

The electrical properties of the soil medium have a bearing on how efficiently the electric energy delivered to the soil via the series or parallel connection of electrode arrays is utilized. Critical soil conductivity, moisture, and textural parameters affect electrode and current distribution analyses. Therefore, it is necessary to adjust the electrode system to the field conditions, otherwise in some cases limitations of the method can be expected (Vian et al., 2016).

In terms of safety, the use of low-voltage power supplies for electrical systems in farming is regarded as a critical guideline. Proper grounding and insulation, as well as residual current device (RCD) systems, in electrical applications are essential to ensuring both operator safety and equipment safety. International electrical safety and occupational exposure limit regulations advise use of protective grounding, RCD, and use of insulative materials with good resistance to environmental degradation, especially in systems operated in moist and outdoor environments (Filosa & Lopresto, 2024).

In addition, since vineyards are a field crop, protection from lightning and from sudden voltage surges is necessary. In low-power agriculture electrification systems with renewable energy sources such protective actions are essential to guarantee energy continuity and system reliability (Moahmmed & Messaoud, 2024).

By evaluating these safety and distribution parameters, it is possible to improve the electroculture applications in vineyards in terms of energy efficiency whilst operator and the environmental safety are also ensured.

5.4. Opportunities for Integration with Renewable Energy Sources

Electroculture systems are especially amenable to being powered by renewable energies, given their minimal power consumption. Solar energy systems, in particular, provide a suitable source of energy for the low-voltage systems employed in such electroculture applications via microinverters or DC output devices. This setup minimizes reliance on the external power grid in rural farming communities and enables energy flow continuity (Moahmmed & Messaoud, 2024).

Wind turbines produce AC, which can be converted to low voltage DC via certain converters and used in electroculture. The joint exploitation of solar and wind energies allows for the possibility of an off-grid energy supply with a minimal or even no carbon footprint, especially in extensive open field applications such as vineyards (Moahmmed & Messaoud, 2024).

Furthermore, digital monitoring systems used within precision agriculture and smart viticulture enable the dynamic control of energy management. Procedures for data acquisition and analysis dealing with soil moisture, pH, electrical conductivity, and microclimatic parameters allow for spatial and temporal optimization of the application of energy. These technological solutions are at the very heart of the "Smart Vineyard" philosophy, contributing to both efficient and sustainable vineyard management (Bramley et al., 2011).

6. Mechanisms of Electroculture: Molecular and Physiological Approaches

6.1. Ion Channels in the Cell Membrane and Electrical Excitation

The plant cell membrane is the lowest-level biological interface defining the impact of the EC treatments. Ion movement through the cell membrane is mediated by channels for potassium (K^+), calcium (Ca^{2+}), sodium (Na^+), and chloride (Cl^-). External EFs modify the dynamics of these open and closed ion channels by modulating the MP (Takamura & Volkov, 2006). This process in turn controls intracellular signaling and leads to a number of physiological responses, including stomatal movement, water status, and photosynthesis.

In a report by Lee & Oh (2021) low-voltage electrical stimulation has the potential to stimulate Ca^{2+} ion flow and ATP synthase activity in *A. thaliana* plants. In the same way, Morales et al. (2021) revealed that permeability of the cell membrane transient can be increased by the application of 10 V DC in cucumber (*Cucumis sativus*) plants, which can be used to better control ion homeostasis. Therefore, it was suggested that the electroculture treatments could have a regulatory effect on the plant cell membrane potential and ion homeostasis.

6.2. Effects of Electrical Stress on Gene Expression

Electrical signals may affect plant ion transport as well as the expression of stress-related genes. It has been documented that electrical signals trigger the production of transients reactive oxygen species (ROS) (Fromm & Fei, 1998; Szechyńska-Hebda et al., 2017) in the plasma membrane which are considered as a signaling entity. These signals are also critical in modulating heat shock proteins (HSP), antioxidant defense enzymes (e.g., superoxide dismutase (SOD), catalase (CAT)), and defense-related transcription factors (Goldsworthy, 2006).

Studies on leguminous species have shown that electrical stimulation induces the activation of defense-related genes and the accumulation of phenolic compounds and flavonoids (Mikami et al., 2017; Mori et al., 2021). These results demonstrate that electroculture promotes plants adaptation and defense mechanism by inducing a mild stress stimulus on the molecular level.

6.3. Oxidative Stress and Defense Mechanisms

During electroculture treatments, the electrical stimuli modulate the plant cells to produce a short-term and controlled oxidative stress response. Studies have suggested that electrical signals induce a transient production of reactive oxygen species (ROS), mainly in mitochondria and chloroplasts (Fromm & Fei, 1998; Szechyńska-Hebda et al., 2017). Notably, this rise leads to activation of defense mechanisms, not cellular insult.

Several reports have shown that ES has the ability to recover the oxidative state by increasing antioxidant enzymes activity including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Goldsworthy, 2006; Mikami et al., 2017). Studies in grapes have indicated that electrical stimulation enhances the levels of phenolics and antioxidants via upregulating the activity of phenylalanine ammonia lyase (PAL), a key enzyme linked to the quality of fruits (Mikami et al., 2017; Mori et al., 2021).

This evidence indicates that electroculture can be considered as a biostimulation technique that modulates plants oxidative stress response and their defense potential.

6.4. Plant–Microorganism Interactions and Rhizosphere Responses

The plant matrix is not the only one to be affected by electroculture treatment but also the microbial community in the rhizosphere may be subjected to its direct action. In fact, it has been shown that the electric fields that are generated within the soil are a significant factor affecting the metabolism and motility of microorganisms as well as that of their distribution within the soil (Olszanowski & Piechowiak, 2006). Interestingly, low-intensity direct current DC treatments were also reported to stimulate microbial activity in the rhizosphere, leading to greater nutrient bioavailability.

It is widely shown that electric stimulation enhances plant-microorganism interactions by promoting the proliferation of the beneficial rhizobacteria and converting them into positive contributions to the nitrogen cycle and the biochemical processes in the root zone (Kim et al., 2016). Türkmen et al. Electroculture positively affects soil health and the rhizosphere functions as proven by increased microbial respiration rates in the root zone of electrocultured plants, and higher levels of soil microbial carbon (Turkmen et al., 2023).

7. The Economic, Environmental, and Ethical Dimensions of Electroculture

The applications of electroculture need to be assessed in terms of not only the possibility of increasing product quantity and quality in plant production but also from the perspectives of financial sustainability, environmental pollution, and ethics. The minimal energy input, potential for decreasing chemical inputs, and ability to integrate with renewable energy sources make electroculture an environmentally benign technology. However, challenges such as application costs, long-term effects on ecosystems, and ethical limits for production need to be tackled, preferably in an integrated manner.

7.1. Energy Costs and Economic Feasibility

The economic feasibility of electroculture systems is related to the electrical parameters used, system installation costs, and the agronomic gains achieved in the long term. As suggested by the literature, the energy cost of low-voltage direct current (DC) systems implemented in agro-informatics-based application areas of electroculture is very minimal and remains low when compared to traditional machinery (Dannehl, 2018; Moahmmed & Messaoud, 2024). The energy inputs required by these systems, however, are usually a minor fraction of the energy used for pumping, cooling, or operating machinery.

Energy consumption depends on the electrode design, source of energy, and degree of automation of the electroculture process, yet it is reported that simple electroculture systems which can be coupled with renewable energy are financially viable in the medium term (Moahmmed & Messaoud, 2024). Longer-term studies suggest achievable improvements in crop yield and chemical input reductions through the beneficial impacts of electrical stimulation on plant development and quality standards, which may also shorten the investment return time (Saletnik et al., 2022).

In this perspective electroculture represents a cost-effective option, at least for small and medium-sized organic and sustainable vineyards. Further integration with renewable energy sources reduces even more the running costs and supports the long-term sustainability of the system (Moahmmed & Messaoud, 2024).

7.2. Ecological Sustainability and Carbon Footprint

Electroculture treatments are considered as sustainable practices having low energy consumption with potential to decrease the use of chemicals. Studies showed that electrical stimulation would increase efficiency of nutrient absorption in plants and then it could reduce use of fertilizers and mitigate environmental burden in soil and water (Dannehl, 2018; Moahmmed & Messaoud, 2024). Moreover, the reduced use of fertilizer and pesticides also increasingly limits emissions of greenhouse gases from agriculture.

Several studies have pointed out that electroculture play a role in maintaining soil microbial community structure by promoting biological activities in the rhizosphere and have a beneficial effect on ecosystem services (Kim et al., 2016; Türkmen et al., 2023). In addition, the potential to be powered with electricity derived from dust-ed sources, such as solar, and wind energy, among others, enhance low energy consumption of such equipment and allow electroculture be allied to low-carbon agriculture (Moahmmed & Messaoud, 2024). Due to these properties, electroculture is considered one of the pioneer engineering agricultural techniques that encourage environment friendliness and hence could potentially help to reduce the carbon footprin

7.3. Electromagnetic Pollution and Human/Environmental Health

Worker safety is also ensured under the EU legislation on electromagnetic fields, which provides that low intensity exposures that are below the limits of the value in the simple applications do not give rise to any health risk (Directive 2013/35/EU; Filosa & Lopresto, 2024). The available literature suggests that low-intensity electrical and electromagnetic fields can induce physiological effects in plants, without detrimental effects to environment and human life (Smith, 1994).

Electroculture systems generally run at low voltages and frequencies, so they are not a threat to human or animal health. However, there are very few reports indicating that prolonged or intense exposure to electromagnetic fields may cause stress in some life forms (Goldsworthy, 2006; Lewicka et al., 2015). Hence, potential effects on biodiversity should be taken into account in the design and operation of electrofencing devices, and such projects should be accompanied by environmental impact assessments.

Electroculture, with these far-reaching features, is among the promising new generation agricultural technologies that favor environmental sustainability and mitigate carbon footprints.

7.4 Legal Regulations and International Standards

To date, electroculture practice is not regulated by specific legislation in many countries. The current legislation is generally analyzed from the electrical safety, electromagnetic field exposure, environmental protection aspects. In Europe the safety of electrical and electromagnetic fields in EN 50445, IEC 62233. The USDA and EPA have established standards for the production of organic and for the suitability of ag-type electrical equipment (USDA, 2022) in the United States.

In Türkiye, the legal regulations are in the process of being prepared and pilot studies are to be implemented to utilize livestock-based farming systems within the framework of the Ministry of Agriculture and Forestry's “Smart Agricultural Technologies Action Plan (2021–2025)". This project may lead to an official acceptance of electroculture as sustainable agricultural practice.

8. Future Perspectives and R&D Needs

8.1. Current Shortcomings in Electroculture Research in Viticulture

Studies on the incorporation of electroculture techniques in grapevine management are still in the infancy stage. The majority of the available research has been carried out in the laboratory or on small-scale field sites, which limits the amount of long-term production data (Saletnik et al., 2022). There are compelling cases for multisite field experiments that test for the comparative response of different soils, climate regions, and grape cultivars to electroculture. Moreover, from the application point of view parameters such as voltage, frequency, time of application need to be standardized (Cao et al., 2025). The absence of standardization makes it difficult to compare data from different reports. In the future, experimental strategies must be established to simulate the biophysical effects of electroculture.

8.2. Monitoring Systems Using Artificial Intelligence and Sensor Technologies

The scalable use of electroculture in farming production must be facilitated by automation and monitoring technologies. IoT-based sensor systems can simultaneously measure parameters, including soil moisture, soil temperature, soil electrical conductivity, and soil ion concentration, and a cloud can be used to analyze these data (Bramley et al., 2011; Wang, 2022). AI-supported models can generate control strategies that dynamically adjust the energy flow based on this information and provide plant-specific "smart energy dosing" energy strategy (Cao et al., 2025). In addition, since plant responses after electrical stimulation, such as leaf color, water stress, chlorophyll index can be monitored remotely by UAV equipped with multispectral cameras (Bramley et al., 2011; Morales et al., 2021). These are essential enabling technologies for incorporating and perhaps enhancing electroculture processes with precision viticulture systems.

8.3. Long-Term Field Trials and Data Sharing Networks

A major bias in the study of electroculture is that no long-term data accumulation and multiregional comparison analyses have been made. Current research tends to focus on one season or short-term outcomes. But in vine growing, root systems, and soil microbial populations are changing and evolving and so it would necessitate a 5–10-year monitoring (Gazioglu Sensoy and Balta, 2010).

The development of international data sharing mechanisms may help in standardizing fields like conditions of application, yield factors, soil characteristics, and energy information and in learning comparisons with other investigators (Bramley et al., 2011). The principles of open data and creation of systems for sharing data will play a central role in increasing collaboration within agricultural research and methodological standardization. These efforts would certainly make electroculture a scientifically sound, reproducible and comparable methodology.

Such international data sharing systems, and long-term field trials, have immense potential to facilitate the development of standardized, reproducible and scientifically collaborative research in electroculture.

8.4. Integration of Electroculture into Smart Viticulture Systems

The development of electroculture technology will be heat-bedded in smart vineyard management. Smart Viticulture production model is that the application of sensor networks, data analytics and artificial intelligence, robotic systems, renewable energy sources (Moahmmed & Messaoud, 2024). Electroculture systems, in this case, could be complemented with energy saving and plant physiological monitoring modules.

In the future, electrical responses of plants will be analyzed by artificial intelligence algorithms to predict the excellent energy level, and such information can be applied in real time by an autonomous system (Cao et al., 2025). There is the potential to increase yield and quality in the viticulture and to reduce energy waste through this integration.

To sum up, electroculture techniques will evolve as strategic instruments not only for agricultural production but for building carbon-neutral, digitized and resource-efficient agricultural systems.

9. Conclusion and Recommendations

9.1. Applicability of Electroculture in Viticulture

Electroculture is a new source of agricultural technology that works at the very heart of plant growth processes. Viticulture experimental studies have revealed that root growth, water absorption and photosynthetic activity in grapevines are improved by low-voltage electrical stimulation (Ali et al., 2022). In a certain grape variety, electrical stimulation has been found also to increase sugars and phenolics under controlled experimental conditions.

The results demonstrate that the technique can be used for both the traditional and the new generation of viticulture and, because it is low energy intensive and compatible with renewable energy sources, can be considered the best technique especially for sustainable and organic production.

9.2. Potential Benefits and Limitations

An important lacuna in the study of electroculture is the availability of data for long-term and multi-regional comparisons. Studies so far generally investigate the short-term influence of electromagnetic or electrical stimulation on plant development in vitro (under laboratory conditions or controlled environmental conditions) e.g., influence of directed applications of electromagnetic fields on shoot elongation and root proliferation have been noted (Filosa & Lopresto, 2024). However, for crops with long life spans, including grapevines, field trials of long duration are requisite for the study of processes, such as that of the growing root system and the activity of the rhizosphere microbiome.

The creation of international data-sharing systems could facilitate standard documentation of information such as application conditions, yield characteristics, soil properties, and electrical parameters, which would make itself compared with other investigators. Here, review studies from the realm of plant physiology and EMF/magnetic field treatments show a lot of short-term and species/variety-based in vitro research within this field and these explorations However, networks to share data and procedures to multiplex protocols are required to expand these results to field scale and secure long-term monitoring (Filosa & Lopresto, 2024). These data-sharing networks would contribute to increasing scientific confidence, standardization, and evaluation of the use of electroculture over large geographical areas.

9.3. Future Research Directions

21st century electroculture research will remain focused on biophysical mechanisms and data driven models. Based on artificial intelligence, sensor and remote-sensing systems, “smart viticulture” can contribute to the increase of efficiency of electroculture (Bramley et al., 2011).

Moreover, long-term field trials will be necessary to determine how technology performs under diverse climatic and soil conditions. Furthermore, electrical stimulation effects on plant stress-related genes, metabolite profiles, and signal transduction cascades should be analyzed in greater detail at the genetic level.

To conclude, electroculture is emerging in viticulture not only as a tool to increase yield, but also as a key element in the sustainability of production, environmental care and the digital agrifood transition. It can be anticipated that in the future with the progress in scientific standardization and technological consolidation the technique will find widespread application in commercial viticulture.

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Chapter 3

SNP-Based Technologies for Aquaculture Breeding: Development and Applications

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1. Introduction

With the global human population projected to surpass 9 billion by 2050 (Nelson et al., 2010), aquaculture is expected to play an increasingly critical role in meeting the rising demand for aquatic food resources. Aquaculture has emerged as the most rapidly expanding sector within global animal-protein production (Tacon and Metian, 2013). In 2022, the total production of aquatic animals reached 185.4 million tonnes, with 94.4 million tonnes derived from aquaculture, marking the first occasion where farmed production exceeded capture fisheries (FAO, 2024; Dongyu, 2024). Despite this rapid expansion, the aquaculture sector continues to face persistent challenges that constrain stable and efficient production. Addressing these multifaceted issues while simultaneously improving growth performance, feed efficiency, reducing production costs, and minimizing environmental impacts has become a central priority for sustainable aquaculture development (Guerreiro et al., 2025). Molecular approaches have emerged as crucial tools in this context, directly contributing to global efforts to reduce hunger and mitigate the impacts of climate change, aligning closely with the United Nations Sustainable Development Goals, particularly Zero Hunger and Climate Action (Shah et al., 2025).

The incorporation of modern genetic and genomic tools has further enhanced the performance, sustainability, and resource management of aquaculture species (Wenne, 2023). Genetic analysis, based approaches such as genomic selection (GS) can increase selection accuracy by approximately 20–30%, thereby shortening generation intervals and improving key performance traits, including feed efficiency and disease resistance (Hassanine et al., 2025). Single nucleotide polymorphisms (SNPs) are single-base variations in the genome and represent the most abundant and stable form of genetic variation in aquatic organisms. Owing to their high density and genome-wide distribution, SNPs

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have become indispensable molecular markers in modern aquaculture genetics, providing robust insights into genetic diversity, population structure, and the genetic basis of complex traits (Zhang et al., 2024). SNPs provide detailed insights into genetic diversity, population structure, and the genetic architecture of complex traits in aquaculture species (Rasal et al., 2024). Consequently, SNP-based tools play a central role in supporting sustainable aquaculture development by enabling informed breeding decisions, maintaining genetic diversity, and facilitating advanced genomic applications (Robledo et al., 2017). Recent advances in genomic technologies have enabled the routine application of SNP-based approaches, including marker-assisted selection (MAS), genomic selection (GS), genome-wide association studies (GWAS), and population genomic analyses in aquaculture species. High-resolution SNP discovery is now commonly achieved using complementary strategies such as whole-genome sequencing (WGS) and reduced-representation methods, including RAD-seq and genotyping-by-sequencing (GBS). Together, these approaches have facilitated the development of species-specific SNP arrays that support high-throughput and cost-effective genotyping, forming a critical foundation for modern aquaculture breeding programs. By underpinning GWAS and enabling the implementation of GS, these genomic resources contribute to improved selection accuracy and accelerated genetic gain for economically important traits, thereby enhancing the efficiency and sustainability of aquaculture production systems (Yáñez et al., 2022; Zhang et al., 2024).

Collectively, these advances in sequencing technologies have laid the foundation for modern SNP-based genomic tools in aquaculture, enabling high-throughput genotyping, association mapping, and genome-wide breeding strategies.

2. 1. Next-Generation Sequencing (NGS) technologies

Initial efforts to identify single nucleotide polymorphisms (SNPs) in aquaculture predominantly utilized Sanger sequencing of candidate genes, a technique noted for its high accuracy but inherently constrained by low throughput and labor-intensive processes (Tsai et al., 2014). Consequently, early research was limited to low marker densities and restricted genomic coverage, significantly impeding the capacity to perform genome-wide analyses or assess genetic variation across extensive breeding populations (Robledo et al., 2018a). These limitations obstructed both the comprehensive characterization of complex trait architectures and the pace of genetic advancement in aquaculture species.

The advent of NGS technologies marked a major turning point in aquaculture genomics by enabling high-throughput SNP discovery at substantially reduced costs and unprecedented scale (Metzker, 2010; Xu and Bai, 2015). NGS platforms facilitated the transition from candidate gene–based approaches to genome-wide strategies, thereby expanding SNP discovery beyond predefined loci. NGS workflows typically start with the construction of sequencing libraries, in which double-stranded DNA is fragmented to platform-specific lengths and ligated to sequencing adaptors. These libraries are subsequently amplified, either through PCR-based enrichment or clonal amplification, to generate sufficient signal for sequencing. During the sequencing process, nucleotide incorporation is detected using platform-dependent chemistries, such as fluorescent labeling or enzymatic reactions, producing large volumes of short sequence reads. The resulting reads are then computationally processed and either aligned to a reference genome or assembled de novo in the absence of a reference, thereby enabling downstream analyses such as variant discovery, genome annotation, and comparative genomics (Davey et al., 2011; Swain et al., 2022).

Within this NGS framework, WGS represents the most comprehensive strategy for SNP discovery, providing near-complete coverage of genomic variation and supporting high-resolution analyses of genetic architecture, linkage disequilibrium, and selection signatures. However, despite its analytical power, the routine application of WGS in large-scale breeding programs remains constrained by sequencing costs and data-handling requirements, particularly for species with large genomes or extensive sample sizes (Campbell et al., 2014). Nevertheless, WGS has played a critical role in generating high-quality reference genomes and foundational SNP resources for many economically important aquaculture species.

To overcome the cost and scalability limitations associated with WGS, reduced-representation sequencing approaches, such as restriction site–associated DNA sequencing (RAD-seq), double-digest RAD-seq (ddRAD-seq), and genotyping-by-sequencing (GBS), have been widely adopted in aquaculture research. These methods selectively sequence reproducible subsets of the genome, enabling the discovery of thousands to hundreds of thousands of SNPs in a cost-effective manner, even in species lacking well-annotated reference genomes (Davey et al., 2011). RAD-based approaches have been extensively applied for population genomics, linkage mapping, and GWAS, and often serve as a primary source of SNPs for downstream marker development and array design.

To date, a total of 3,317 genome assemblies representing ray-finned fishes (*Actinopterygii*) have been generated and deposited in public repositories, including NCBI GenBank and RefSeq (Last accessed 18 December 2025). The expanding availability of reference genomes, coupled with rapid advancements in sequencing technologies, has made it possible to interrogate thousands to millions of genomic loci simultaneously at steadily decreasing costs.

Consequently, genome-wide SNP datasets generated through WGS and reduced-representation sequencing approaches have laid the foundation for the development of high-throughput SNP genotyping platforms, including solid- and liquid-phase SNP arrays, which underpin modern genomic analyses and breeding applications in aquaculture.

2.2. Restriction Site–Associated DNA Sequencing (RAD-seq)

The relatively slower advancement of genomic research in aquatic species, coupled with the pronounced heterogeneity and structural complexity of their genomes, has historically limited the widespread application of high-throughput sequencing technologies. In this context, the adoption and refinement of restriction site–associated DNA sequencing (RAD-seq) have provided robust and cost-effective tools for genomic investigations in aquatic organisms (San-Jose et al., 2023). RAD-seq has gained broad acceptance in aquaculture breeding research due to several practical and technical advantages, including the generation of consistently sized DNA fragments, streamlined and scalable library preparation procedures, and high genotyping accuracy.

Importantly, RAD-seq can be effectively applied without the requirement for a fully assembled reference genome, a feature that has facilitated genome-wide SNP discovery in many non-model aquatic species. By enabling efficient discovery of single nucleotide polymorphisms (SNPs) under such conditions, RAD-seq has substantially expanded the feasibility of genomic analyses in aquaculture systems where genomic resources remain limited. Although RAD-seq generates high-density SNP datasets, it is not considered a high-throughput genotyping technology for routine breeding applications. Consequently, RAD-seq is optimally positioned as a SNP discovery platform during the early phases of genomic resource development. SNPs identified through RAD-seq are typically filtered, validated, and subsequently transferred to targeted genotyping platforms for routine application in selective breeding programs (Aguirre et al., 2019; Yáñez et al., 2015).

Hua et al. (2024) demonstrated the utility of RAD-seq in aquaculture breeding by identifying growth-associated SNPs in largemouth bass (Micropterus salmoides). From over 4 million genome-wide SNPs, four key loci were significantly associated with body weight and body size traits and were located near the candidate genes fam174b and ppip5k1b, which are involved in growth and feeding regulation. Fish carrying favourable genotypes at these loci showed approximately 20% higher body weight, highlighting the effectiveness of RAD-seq–derived SNP markers for MAS in growth improvement programmes.

3. Bioinformatic Processing and SNP Panel Development

The development of reliable SNP panels requires rigorous bioinformatic processing to ensure high data quality and biological relevance. Initial variant filtering typically involves minimum read depth and sequencing quality thresholds to remove unreliable SNP calls arising from low coverage or sequencing artifacts, a critical step in species with high heterozygosity (Song et al., 2016; Sopniewski and Catullo, 2024).

Subsequent filtering commonly applies minor allele frequency (MAF) thresholds to exclude rare variants with limited predictive value and potential to introduce noise into association analyses or genomic prediction models. Appropriate MAF thresholds are selected according to study objectives and population characteristics to balance the retention of informative variation with analytical robustness (Linck and Battey, 2019; Mitt et al., 2017).

Additional quality control steps include filtering based on genotype call rates, testing for deviations from Hardy–Weinberg equilibrium, and evaluating Mendelian consistency in family-based datasets. These procedures help identify markers affected by technical errors, population structure, or mislabeling, thereby improving dataset reliability (Montgomery et al., 2005).

Linkage disequilibrium (LD) pruning is applied to reduce redundancy among highly correlated markers while maintaining genome-wide coverage. By retaining a subset of largely independent SNPs, LD pruning enhances computational efficiency and improves the performance and interpretability of downstream analyses (Gattepaille and Jakobsson, 2012; Schunter et al., 2013).

Collectively, these bioinformatic filtering and optimization steps yield SNP panels that balance marker density, genomic coverage, and cost-effectiveness. Such panels provide accurate and reproducible genomic information to support association mapping, genomic prediction, and breeding decision-making, and can be iteratively refined as new genomic resources become available.

4. SNP Genotyping Platforms

4. 1. High-Throughput SNP Array Platforms

Building upon NGS-derived SNP resources, the development of high-density SNP array platforms has significantly advanced genomic research and breeding in aquaculture species. These platforms, typically comprising tens to hundreds of thousands of carefully selected markers distributed uniformly across the genome, enable high-throughput and cost-effective genotyping with high data quality and low error rates. Their standardized design ensures reproducibility across experiments and generations, which is critical for long-term breeding programs implementing genomic selection (Dong et al., 2016).

The design of high-density SNP array platforms involves rigorous marker selection to ensure even genomic coverage, high minor allele frequencies, and robust technical performance. Such selection is guided by population genomic data and bioinformatic filtering to exclude redundant or problematic loci. The fixed marker content further facilitates direct comparison of genotyping results across studies and breeding cycles, supporting cumulative analyses and long-term monitoring of genetic progress, inbreeding, and diversity in aquaculture populations (Gorjanc et al., 2017).

In contrast, sequencing-based genotyping approaches such as GBS and whole-genome resequencing provide greater flexibility by combining variant discovery and genotyping. These methods are particularly valuable for non-model species or early-stage breeding programs lacking established SNP array platforms. While GBS enables cost-effective generation of genome-wide marker data without prior genomic resources, whole-genome resequencing offers comprehensive variant discovery, including rare variants and structural variation, albeit at higher cost and lower scalability for routine breeding applications (Rasal et al., 2024).

Within aquaculture breeding, SNP array platforms have been widely applied for pedigree reconstruction, estimation of genomic relationships, detection of inbreeding, and genomic selection. Medium- to high-density platforms (typically 50K–200K SNPs) support GWAS, fine-scale QTL mapping, and accurate estimation of genomic breeding values. Low-density panels derived from these platforms enable cost-efficient routine genotyping of large populations and are often combined with imputation strategies to maintain predictive accuracy (Tsairidou et al., 2020).

The integration of SNP array platforms with sequencing-based data further enhances breeding efficiency and flexibility. High-density array genotypes frequently serve as reference panels for the imputation of low-density or sequencing-derived genotypes, allowing breeding programs to balance cost and information content. Continued improvements in bioinformatic pipelines and statistical models are further increasing the accuracy of genotype calling, imputation, and genomic prediction, reinforcing the utility of SNP array platforms in aquaculture breeding (Song and Hu, 2021). Overall, the complementary use of array-based and sequencing-based genotyping provides a robust genomic toolkit to support sustainable genetic improvement (Robledo et al., 2017).

4. 2. Low-Density (LD) SNP Array Platforms

Low-density (LD) SNP array platforms represent a cost-effective alternative to high-density platforms and are particularly well suited for large-scale genotyping in commercial aquaculture breeding programs. These platforms typically include several hundred to a few thousand informative markers evenly distributed across the genome and enriched for SNPs with high minor allele frequencies and strong linkage disequilibrium with neighboring loci. Despite reduced marker density, LD SNP array platforms capture a substantial proportion of the genetic variation relevant for selection decisions (Tsairidou et al., 2020).

A widely adopted strategy involves genotyping selection candidates with LD platforms followed by imputation to high-density genotypes using a well-characterized reference population. This LD–HD imputation framework has been shown to maintain genomic estimated breeding value (GEBV) accuracy close to that obtained with full high-density genotyping while substantially reducing genotyping costs (Kriaridou et al., 2023).

Beyond genomic selection, LD SNP array platforms are routinely applied for parentage assignment, pedigree verification, and broodstock management, providing accessible genomic tools for breeding programs with limited resources. Additionally, LD platforms may incorporate trait-associated SNPs identified through GWAS, combining genome-wide coverage with biologically relevant markers to enhance selection efficiency (Garcia et al., 2021).

Empirical studies consistently demonstrate that genomic prediction accuracy remains relatively stable until marker density declines below approximately 1,000 SNPs, with panels of 500–1,000 markers retaining close to 90% of maximum prediction accuracy. These findings highlight LD SNP array platforms as a practical and cost-efficient pathway for implementing genomic selection, particularly in small- and medium-scale aquaculture breeding programs (Kriaridou et al., 2020; Song et al., 2025).

Taken together, while high-density SNP array platforms (typically 50K–200K markers) provide maximal genome-wide resolution for discovery-oriented analyses, low-density SNP panels comprising a few hundred to several thousand markers offer a cost-efficient and scalable solution for routine breeding applications, particularly when combined with genotype imputation strategies.

4. 3. Liquid-Phase SNP Array Platforms

Liquid-phase SNP array platforms differ from conventional solid-state arrays in that SNP probes operate in solution rather than being immobilized on a solid surface, with genotyping achieved through target capture followed by next-generation sequencing. Often implemented through genotyping-by-target sequencing (GBTS) or liquid hybridization capture technologies, these platforms offer increased flexibility in marker selection, facilitate the integration of functional and multi-SNP loci, and allow rapid updating of panels as new genomic information becomes available (He et al., 2014; Guo et al., 2019).

Compared with fluorescence-based solid-phase arrays, liquid-phase SNP array platforms generally show high concordance with whole-genome sequencing data while offering improved adaptability for non-model species and emerging breeding programs (Semagn et al., 2014; Xu et al., 2023). Consequently, they represent a scalable and cost-effective alternative for genome-wide genotyping, GWAS, and genomic selection in aquaculture.

The first 45K liquid-phase SNP array platform developed for spotted sea bass (*Lateolabrax maculatus*), designated “LuXin-I”, was constructed using high-confidence SNPs derived from whole-genome resequencing. This platform integrates approximately 41,600 genome-wide SNPs and 3,393 functional markers, demonstrating high genotyping accuracy (>99% call rate) and strong concordance with whole-genome sequencing. Its successful application in population genetics, GWAS, and genomic prediction highlights its utility as a cost-effective platform for genomic selection (Zhang et al., 2025).

Similarly, a 50K liquid-phase SNP array platform developed for blunt snout bream (*Megalobrama amblycephala*) incorporates approximately 49,000 evenly distributed SNPs selected from population resequencing and validated trait-associated loci. The platform demonstrated high genotyping success and concordance rates and has been effectively applied in population genetic analyses, genetic sex identification, and GWAS, leading to the identification of loci associated with disease resistance and supporting genomic selection–based breeding programs (Luo et al., 2025).

5. Targeted Genotyping Assays

5. 1. Kompetitive Allele-Specific PCR (KASP)

A wide range of SNP genotyping technologies is available, differing in throughput, cost, and flexibility. Low- to medium-throughput PCR-based assays, such as Kompetitive Allele-Specific PCR (KASP) and TaqMan, are widely used for targeted applications (Majeed et al., 2018). KASP assays represent a practical and cost-effective SNP genotyping platform for translating genomic discoveries into routine marker-assisted selection in aquaculture breeding programs. KASP assays are particularly valued for their cost-effectiveness and adaptability, making them suitable for validating candidate SNPs, parentage assignment, and constructing low-density panels for routine breeding applications. TaqMan assays, while generally more expensive, offer high precision and robustness, and are commonly used when analytical accuracy is paramount. Competitive allele-specific PCR (KASP) is a targeted genotyping platform specifically designed for single nucleotide polymorphisms (SNPs) and insertion–deletion (InDel) variants, enabling accurate allele discrimination through two-color fluorescence detection. Due to its high precision, low cost, flexibility, and high assay conversion rate, KASP has become a widely used tool for SNP-based genotyping in marker-assisted and genomic selection programs, thereby improving breeding efficiency and speed (Zhuo et al. 2025). Four SNPs developed as KASP markers were shown to accurately validate caviar color in Russian sturgeon (*Acipenser gueldenstaedtii*), demonstrating their potential as reliable molecular tools for marker-assisted selection, although further validation in larger populations is required due to the complex genetic architecture of the trait (Song et al. 2024). In blunt snout bream, a KASP-based genotyping framework was developed using 203 validated KASP-SNP markers, from which six key SNPs located in growth- and hypoxia-related genes were identified and consistently associated with superior growth performance and enhanced hypoxia tolerance, thereby providing a practical example of SNP-assisted molecular breeding for complex traits in aquaculture (Zhuo et al. 2025). In Asian sea bass (*Latest calcarifer*), GWAS-derived biallelic SNPs were efficiently converted into a practical KASP genotyping panel, with 70 informative markers successfully validated for routine screening of broodstock candidates (Yuttiwat et al. 2025). Overall, the successful application of KASP assays for genotyping biallelic SNP markers across multiple aquaculture species highlights their robustness, cost efficiency, and suitability for routine use in marker-assisted selection, making KASP a practical bridge between SNP discovery and applied breeding programs. In populations where no individual SNPs with moderate to large effects on growth traits are detected, the direct application of marker-assisted selection may be limited; however, SNP genotype information can still support early-stage selection by prioritizing individuals carrying a higher number of favorable alleles (Yuttiwat et al. 2025). Under such polygenic architectures, the integration of genome-wide marker information through genomic selection provides a more effective framework for achieving sustained genetic improvement.

The effectiveness of SNP panels and SNP arrays critically depends on the careful selection of informative markers, with SNPs derived from gene-enriched regions being particularly valuable. Sequencing depth must be optimized to minimize false SNP calls arising from low coverage or from repetitive regions at excessively high coverage. In addition, genome duplication and heterogeneous SNP discovery sources complicate the identification of true biallelic markers, underscoring the importance of rigorous bioinformatic filtering during panel development (Rasal et al., 2024).

5. 2. TaqMan SNP Genotyping

In aquaculture breeding, TaqMan assays are primarily applied during the implementation phase of marker-assisted selection (MAS), enabling accurate genotyping of SNPs associated with economically important traits such as growth performance and disease resistance. A representative example is provided by Janpoom et al. (2021), who developed a locus-specific TaqMan SNP genotyping assay targeting the cyclin C (PmCyC) gene in giant tiger shrimp (*Penaeus monodon*). The targeted SNP showed a significant association with body weight and total length, demonstrating the effectiveness of TaqMan assays for precise validation of growth-associated markers and their application in selective breeding.

Although TaqMan assays are limited by their single-locus design and are not intended for genome-wide screening, they remain highly valuable in contexts where analytical precision and robustness are required, particularly for validation and routine genotyping of critical markers in aquaculture breeding programs. Overall, while both platforms are effective for targeted SNP genotyping, KASP offers superior cost-efficiency and flexibility for routine aquaculture breeding programs, whereas TaqMan remains advantageous in contexts where analytical rigor outweighs cost considerations (Rahman et al., 2023).

6. Applications in Aquaculture Breeding

High-density SNP markers also underpin genetic linkage mapping and facilitate GWAS by providing dense genome-wide coverage. In addition, SNP-based maps contribute to improving genome assemblies by anchoring scaffolds and enhancing structural accuracy, thereby indirectly supporting downstream breeding applications. However, in applied aquaculture breeding, the primary value of these resources lies in their contribution to trait association studies and genomic selection rather than genome assembly itself.

6. 1. Marker-Assisted Selection (MAS)

SNP-based marker-assisted selection (MAS) exploits strong associations between specific single nucleotide polymorphisms (SNPs) and quantitative trait loci (QTLs controlling economically important traits in aquaculture species. By selecting individuals carrying favorable alleles at key loci, MAS enables earlier and more precise selection decisions than traditional phenotype-based approaches, which are often time-consuming and resource intensive. This targeted strategy accelerates genetic improvement by directly focusing on loci underlying traits such as disease resistance, growth, feed efficiency, and stress tolerance (D'Agaro et al., 2021).

The effectiveness of MAS depends on the accurate identification of QTLs and the availability of reliable SNP markers tightly linked to these loci. Advances in genomic technologies, including high-density SNP arrays and next-generation sequencing, together with genome-wide association studies and linkage mapping, have substantially improved the resolution and confidence of QTL detection. Incorporation of these validated markers into MAS schemes enhances selection accuracy, particularly for traits influenced by major-effect loci (Liu et al., 2020; Robledo et al., 2016).

MAS is especially powerful when traits are controlled by one or a few major QTLs. In Nile tilapia (*Oreochromis niloticus*), a major QTL associated with resistance to Streptococcus iniae explained a substantial proportion of genetic variance, and haplotype-based selection resulted in dramatic differences in offspring survival (Vela-Avitúa et al., 2023). Similarly, in Atlantic salmon, a major QTL explaining up to 80–98% of the genetic variance for resistance to infectious pancreatic necrosis (IPN) has been successfully implemented in commercial breeding programs (Moen et al., 2009; Moen et al., 2015).

However, the application of MAS based on single loci is inherently limited for most economically important traits, which typically exhibit a polygenic genetic architecture involving many loci of small effect (Meuwissen et al., 2013). In such cases, MAS is most effective when integrated with genomic selection approaches that utilize genome-wide marker information.

Overall, SNP-based MAS remains a valuable component of modern aquaculture breeding, particularly for traits influenced by major QTLs. When appropriately applied and combined with complementary genomic strategies, MAS contributes to efficient, accurate, and sustainable genetic improvement of aquaculture stocks.

6. 2. Genomic Selection (GS)

Genomic selection has now been applied to approximately 20 economically important aquaculture species worldwide, reflecting its broad relevance across diverse production systems (Song et al., 2023). In Europe and the Mediterranean region, key species include Atlantic salmon, rainbow trout, European seabass, gilthead seabream, and turbot, where genomic selection is increasingly integrated into commercial breeding programmes. Beyond Europe, genomic selection has also been implemented in major global aquaculture species such as Nile tilapia, common carp, channel catfish, Pacific white shrimp, and several molluscan taxa, underscoring its expanding role in genetic improvement across finfish, crustaceans, and shellfish.

Genomic selection represents a major advancement in aquaculture breeding by extending beyond the limitations of traditional marker-assisted selection through the use of genome-wide SNP data to capture the combined effects of numerous genetic loci. Unlike MAS, which targets a limited number of major-effect QTLs, GS explicitly exploits the polygenic architecture of complex traits by incorporating dense marker information distributed across the entire genome. This genome-wide approach enables the utilization of both major and minor genetic effects that are typically undetectable through conventional QTL mapping (D’Agaro et al., 2021).

The implementation of genomic selection relies on statistical models such as genomic best linear unbiased prediction (GBLUP) and Bayesian approaches, which integrate genome-wide SNP data to estimate genomic estimated breeding values (GEBVs). By summarizing the cumulative genetic contribution of thousands of loci, these models substantially improve prediction accuracy for traits governed by many genes of small effect (Wang et al., 2018).

Genomic selection enables earlier and more accurate selection decisions, thereby shortening generation intervals and accelerating genetic gain. This advantage is particularly important for traits that are difficult, expensive, or time-consuming to measure phenotypically, including disease resistance, feed efficiency, stress tolerance, and reproductive performance. By reducing reliance on extensive phenotyping and environmental challenge trials, GS improves selection efficiency while optimizing resource use within breeding programs (Zenger et al., 2019).

In Atlantic salmon (*Salmo salar*), commercial breeding programmes operate at the highest level of genetic sophistication, where genomic selection is routinely implemented alongside marker-assisted selection to improve multiple economically important traits through sibling testing and index-based selection schemes (Houston et al., 2022). In contrast, the common carp (*Cyprinus carpio*) possesses extensive genomic resources and numerous loci identified for key traits such as growth and disease resistance, particularly in China; however, breeding programmes largely remain reliant on traditional selection due to the absence of major-effect QTLs suitable for MAS and the limited routine implementation of genomic selection (Yang et al., 2024). Beyond these two species, Nile tilapia, catfish, shrimp, and bivalve molluscan taxa represent other major aquaculture groups worldwide, where SNP-based tools—including marker-assisted selection and, increasingly, genomic selection—are being progressively integrated into breeding programmes to support genetic improvement across diverse production systems (Kriaridou, 2024) .

Empirical evidence across a wide range of aquaculture species, including salmonids, tilapia, shrimp, and shellfish, demonstrates that genomic selection consistently improves economically important traits such as growth rate, disease resistance, feed conversion efficiency, and environmental resilience, thereby enhancing both the economic viability and long-term sustainability of aquaculture production systems (Yue et al., 2023). Advances in genotyping technologies, particularly high-throughput SNP array platforms and next-generation sequencing, have substantially reduced genotyping costs and increased access to dense genomic data. In parallel, developments in bioinformatics pipelines and computational capacity have facilitated efficient processing and analysis of large-scale genomic datasets, enabling the routine integration of genomic information into breeding decisions. The use of low-density SNP panels combined with genotype imputation further enhances cost-effectiveness without compromising prediction accuracy, making genomic selection increasingly feasible for commercial breeding programmes of varying scales (Kriaridou et al., 2020; Ibrahim et al., 2025).

Beyond direct genetic improvement, genomic selection contributes to the sustainable management of breeding populations by enabling accurate monitoring of genomic relationships, control of inbreeding, and optimization of mating designs. Together, these advantages have established genomic selection as the preferred breeding strategy for complex traits in aquaculture, particularly where no single major QTL explains a substantial proportion of the genetic variance.

6. 3. Parentage Assignment and Pedigree Reconstruction

SNP markers have largely replaced traditional tagging and microsatellite-based approaches for parentage assignment in aquaculture due to their superior accuracy, reproducibility, and cost-effectiveness. SNP panels comprising hundreds to thousands of markers enable precise pedigree reconstruction even under complex breeding conditions, such as communal rearing systems and populations with overlapping generations. Reliable parentage information is essential for controlling inbreeding, improving the accuracy of estimated breeding values, and supporting the design of family-based breeding programs (Allal & Nguyen, 2022).

Genotyping-by-sequencing and array-based studies have demonstrated that relatively small panels of highly informative SNPs can achieve parentage assignment accuracies exceeding 98%, often outperforming microsatellites in exclusion power and relatedness estimation. For example, SNP panels containing approximately 100–150 well-selected markers have been shown to provide extremely high assignment accuracy in several aquaculture species, highlighting that moderate marker numbers can reliably support pedigree reconstruction when marker informativeness is optimized (Vandeputte & Haffray, 2014). The reliability of SNP-based parentage analysis depends on both marker number and data quality, necessitating careful filtering to minimize genotyping errors and missing data. Key parameters include minor allele frequency (MAF) and genotype call rate, as markers with higher MAF provide greater information content, while low call rates can compromise assignment accuracy (Noda et al., 2025).

The adoption of SNP-based parentage panels has substantially improved broodstock management by enabling accurate pedigree reconstruction without reliance on physical tagging or strictly controlled mating designs. High-resolution pedigree information enhances the precision of genetic parameter estimation, facilitates effective inbreeding management, and improves the accuracy of genomic estimated breeding values (GEBVs). When SNP panels incorporate trait-associated or growth-related loci, they further increase breeding efficiency by enabling the concurrent application of pedigree reconstruction and trait-focused selection strategies. Collectively, these developments support more precise, efficient, and sustainable genetic improvement programs in aquaculture species (Maroso et al., 2021; Al-Tobasei et al., 2021).

6. 4. Species-Specific Considerations

The implementation of SNP technologies in aquaculture varies substantially among species, largely due to differences in genome complexity, ploidy levels, and the availability of genomic resources. Salmonids, including Atlantic salmon and rainbow trout, represent species with the most advanced genomic infrastructure, benefiting from multiple high-density commercial SNP arrays comprising several hundred thousand markers. This extensive marker coverage supports genome-wide analyses such as genomic selection, high-resolution QTL mapping, and population genetic studies, underpinning highly efficient breeding programs that have achieved substantial genetic gains (Maroso et al., 2021; Mhalhel et al., 2023).

European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) breeding programs commonly employ medium-density SNP arrays (app. 50,000–100,000 markers), which provide sufficient genome-wide coverage while remaining cost-effective. This approach is exemplified by the MedFish 60K SNP array, developed using whole-genome sequencing–derived SNPs and shown to perform reliably in population structure analyses and breeding applications (Peñaloza et al., 2021). The inclusion of functionally relevant and trait-associated markers supports routine genomic analyses and the gradual adoption of genomic selection in Mediterranean aquaculture. However, genomic resources for these species remain less extensive than those available for salmonids, necessitating a balance between analytical power and cost-efficiency when designing genomic breeding tools (Malcorps et al., 2021; Palaiokostas et al., 2016).

Polyploid aquaculture species, such as oysters (*Crassostrea gigas*) and sturgeon (Acipenser spp.), present additional challenges for SNP discovery and genotyping due to the presence of multiple homologous chromosome sets. This genomic complexity complicates SNP calling and genotype interpretation, necessitating the use of specialized, ploidy-aware bioinformatic approaches. Recent methodological advances have substantially improved the reliability of SNP genotyping in polyploid species, enabling the effective application of genomic data for selection decisions and genetic diversity management. Continued refinement of such approaches remains essential to fully exploit SNP-based technologies in polyploid aquaculture breeding programs (Phillips, 2024; Qi et al., 2018; Song et al., 2025).

Overall, the application of SNP technologies across aquaculture species reflects a continuum of genomic complexity and resource availability. High-density SNP arrays predominate in well-characterized species such as salmonids, while medium-density arrays and customized SNP panels are more commonly adopted in species with intermediate or complex genomic resources. Ongoing advances in sequencing technologies, bioinformatics methods, and SNP panel design continue to expand the applicability of genomic tools, supporting the development of cost-effective, species-specific breeding strategies that enhance productivity and sustainability in aquaculture (Yáñez et al., 2022).

6. 5. SNP Applications for Production and Performance Traits

SNP applications for production and performance traits have been widely demonstrated across aquaculture species using both marker-assisted and genomic selection strategies. In Nile tilapia, genome-wide analyses based on a 50K SNP panel revealed that fillet yield and harvest weight are polygenic traits lacking major-effect QTLs, limiting the effectiveness of MAS. Consequently, genomic selection using low-density SNP panels combined with genotype imputation outperformed pedigree-based approaches, providing a cost-effective strategy to increase selection response and genetic gain (Yoshida et al., 2019).

Similarly, in barramundi (*Lates calcarifer*), genomic selection based on a 70K SNP array significantly improved genetic gain for growth-related traits compared with pedigree-based selection. Prediction accuracy for whole fish weight, standard length, and body depth increased by 10–33% and 15–49% across contrasting production environments, resulting in higher estimated genetic gains for whole fish weight (19–31%) relative to pedigree-based approaches (Jerry et al., 2022).

In contrast, studies targeting major growth-associated loci illustrate the continued relevance of MAS. In largemouth bass (*Micropterus salmoides*), RAD-seq–based SNP discovery identified four key growth-associated SNPs located near the candidate genes *fam174b* and *ppip5k1b*. Individuals carrying favourable genotypes exhibited approximately 20% higher body weight, highlighting the practical value of SNP markers for marker-assisted selection (Hua et al., 2024).

Likewise, in grass carp (*Ctenopharyngodon idella*), association analyses identified 31 SNPs linked to multiple growth traits, with candidate genes involved in growth, fat metabolism, bone formation, and feeding regulation. These results support the combined application of marker-assisted and genomic selection to improve growth-related traits in breeding programmes (Zhang et al., 2024).

6. 6. Disease Resistance

Disease resistance in aquaculture species has been shown to be well suited for genomic selection approaches based on SNP array data. In Atlantic salmon, resistance to amoebic gill disease exhibits moderate heritability and a predominantly polygenic genetic architecture, and genomic selection using SNP array genotyping provides higher prediction accuracy than pedigree-based methods, even when marker density is reduced to approximately 2,000 SNPs (Robledo et al., 2018b). Similarly, resistance to *Flavobacterium columnare* in rainbow trout has been effectively improved using low-density SNP array panels combined with genotype imputation, achieving prediction accuracies comparable to a 28K high-density array and outperforming pedigree-based selection by approximately 11%, while substantially reducing genotyping costs (Fraslin et al., 2023). Griot et al. (2021) evaluated the efficiency of genomic selection for disease resistance in European sea bass and gilthead sea bream by assessing the effects of training population size and SNP marker density on prediction accuracy. Using 57K–60K SNP arrays and virtual low-density panels (1K–10K SNPs), the study showed that genomic prediction accuracy increased with training population size, reaching values of 0.51–0.65 with full-density panels, while 6K SNP panels retained at least 90% of the accuracy of high-density arrays. For viral *Nervous necrosis* resistance, where a major QTL was detected, incorporating the QTL effect substantially improved prediction accuracy, demonstrating that marker-assisted selection can complement genomic selection. Overall, the results support the cost-effective implementation of genomic selection, combined with reduced-density SNP panels and QTL-informed models, for improving disease resistance in Mediterranean aquaculture species.

6. 7. Temperature Tolerance

Genomic analyses based on a 55K SNP array have demonstrated that seawater temperature tolerance in Atlantic salmon exhibits moderate heritability and a predominantly polygenic genetic architecture, indicating that genetic variation plays a substantial role in thermal resilience. The use of medium- to high-density SNP array platforms enables effective capture of genome-wide variation underlying this trait, making temperature tolerance well suited for genomic selection approaches that rely on dense marker information (Robledo et al., 2018b; Kriaridou et al., 2020).

Importantly, selection for increased thermal tolerance using SNP array–based genomic selection has been shown not to adversely affect growth-related traits, alleviating concerns about trade-offs between environmental resilience and productivity. These findings highlight the contribution of SNP array platforms to sustainable aquaculture breeding by supporting the development of stocks better adapted to rising seawater temperatures while maintaining economic performance. As climate change increasingly challenges aquaculture systems, array-based genomic selection provides a practical and scalable strategy for enhancing long-term productivity, robustness, and sustainability (Fraslin et al., 2022; Brown et al., 2024).

Conclusion

The future of SNP technologies in aquaculture lies in the development of cost-effective, customizable genotyping solutions enhanced by AI-driven optimizations and enriched through multi-omics integration. This holistic approach will empower breeding programs to accelerate genetic gains, improve trait predictability, and foster sustainable production systems. By embracing these innovations, aquaculture breeding will be better equipped to meet the challenges posed by environmental change, emerging diseases, and evolving market demands, reinforcing the pivotal role of SNP-based genomics in the ongoing genetic improvement of aquatic species.

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Chapter 4

The Importance of Insects in Pollination and the Risk of Pesticides to Pollinators

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Keywords: *Agriculture, Apis mellifera, Biodiversity*, CCD, *neonicotinoids, Pollinators, Pesticide, Organic Farming.*

Introduction

In terms of species richness, insects constitute nearly 70% of all living organisms on our planet. Almost seven out of every ten species identified to date are insects, with new arthropod species being discovered and identified every day (Lillig & Aydın, 2006; Özbek & Aydın, 2023; Yaşar et all., 2003). Studies on biological diversity and related subjects concerning insects — often referred to as the invisible rulers of the world — emphasise the importance of using them as biological indicators. (Arndt et all., 2005; Aydın 2018; Demir & Aydın, 2020; Aydın, 2021; Šekeroğlu & Aydın, 2002). Biodiversity studies are often carried out on medicinal and aromatic plants (Aydın & Avcı, 2010), natural pasture areas, afforestation area (Aydın & Karaca, 2009), protected areas (Aydın, 2006), agricultural areas where Isparta oil roses are cultivated (Dinç et all., 2015), orchards (Silay et all., 2021; Oğuz et all., 2021) and even cave ecosystems (Aydın & Şen, 2020). Insect species with high potential for use as biological indicators are employed in various fields, including habitat identification, habitat degradation, water pollution, climate change, the sustainability of protected areas and the management of agricultural areas (Aydın & Kazak, 2007, Aydın & Kazak, 2010; Aydın et all., 2005). Despite this abundance of species, the number of insect species that are extinct or endangered is alarming (Aydın, 2005; Aydın, 2011a; Aydın, 2011b; Aydın, 2011 c; Aydın & Karaca, 2011). Although their importance in terms of pollination, food security and the conservation of biodiversity, etc. the species richness of insect has been declining worldwide in recent years (Wagner, 2020; Wagner et all., 2021; Goulson, 2019; Raven and Wagner, 2021; van Klink et al.,

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2020). The most significant threats are the incorrect agricultural practices. This is because of the excessive use of pesticides in especially agricultural areas (Aydın & Karaca, 2018).

One of the benefit of insects provide within the food cycle is the pollination. This study was conducted to emphasise the importance of pollinator insects and the threats faced by pollinator species.

Entomophily - “insect pollination”

Pollination is defined as the transfer of pollen from the anthers of one flower to the stigma of another flower, on the same or a different plant (Ackerman, 2000). This does not necessarily result in the fertilisation of ovules. Pollinating agents can be animals, such as insects (e.g. bees, beetles and butterflies), birds and bats, or abiotic pollination such as wind, water, rain and even plants themselves. These pollinating animals travel from plant to plant, carrying pollen on their bodies. This vital interaction allows the transfer of genetic material, which is critical to the reproductive system of most flowering plants. Around 80% of angiosperms depend on biotic pollination, whereby organisms carry pollen grains from the anther of one flower to the stigma of another. Of the world's 250,000 species of flowering plant, between 100,000 and 200,000 species of animal act as pollinators (Abrol, 2012). The majority of these pollinators are insects, but around 1,500 species of birds and mammals also visit flowers and may transfer pollen between them. Entomophily, or insect pollination, often occurs in plants that have evolved coloured petals and a strong scent to attract insects, such as bees, wasps, ants (Hymenoptera), beetles (Coleoptera), moths, butterflies (Lepidoptera) and flies (Diptera). Some insect pollinators, such as honey bees (*Apis* spp.), bumblebees (*Bombus* spp.) and butterflies (e.g. *Thymelicus flavus*), have been observed to exhibit flower constancy, meaning they are more likely to transfer pollen to conspecific plants. This can be beneficial for the pollinators as it prevents pollen loss during interspecific flights and stops pollinators from clogging stigmas with pollen from other flower species. It also improves the probability that the pollinator will easily access and recognise productive flowers by familiar clues. The primary insect pollinators are hymenopterans, primarily bees, but also including sawflies, ants, and many species of wasps (Hill et all., 1997; Stout et all., 1998; Chittka et all., 1997; Goulson et all., 1997). Bees, such as bumblebees, and syrphid flies are the most important pollinators of plants. Syrphid flies prefer plant species that are not usually visited by bees, which makes them an important addition to the provision of pollination services (Orford et all., 2015; Ssymank et all., 2008; Larson, et all., 2001). A variety of anthropogenic factors threaten these both large groups, thereby reducing the richness of pollinator insect species and, in turn, pollination services (Nicolson and Wright, 2017; Wilfert et al., 2021; Manley et all., 2015; Nicholson & Egan, 2020). Although a little over 10% of the total human diet consisting of plant crops, such as wheat, maize, rice, soybeans and sorghum (mostly wind pollinated or self-pollinating species), depends on insect pollination, the most of the plants like fruits, vegetable and the others need to pollinators for fertilization. On the other hand most of the corn (maize) grown in the most of the countries comes from seeds that have been treated with neonicotinoids. Although maize is wind-pollinated, honey bees foraging on the plants may still be exposed to residues of the pesticide in the nectar and pollen. Honey bees may also be exposed when they forage on wild plants that have been unintentionally contaminated to neonicotinoids. For example, it is estimated that around 1.6 million honey bee colonies are used to pollinate the California almond crop each year, accounting for almost 60 per cent of all US beehives. Insect pollination is a vital component of agriculture, with over 90 crops depending on it. Honey bees alone contribute an estimated $15 billion to the agricultural economy.

The effects of excessive pesticide use on pollinators: Are neonicotinoids responsible for CCD?

The agricultural study of pollination encompasses many disciplines, including botany, horticulture, entomology and ecology. As the fertilisation of most plants depends on insect pollination, it is important to select pesticides for crops as part of an integrated pest management strategy. Many human diets consisting of plant crops can provide an abundant food resource during mass flowering for the pollinators however most of the agricultural plants are usually characterized by the excessive use of pesticides, which are taken up by pollinators via nectar and pollen and thus cause reduced colony performance. Pesticides cause significant problems, particularly for honeybees, and declared that a major cause of Colony Collapse Disorder (CCD).

Colony collapse disorder (CCD) occurs when the majority of worker bees in a honey bee colony suddenly disappear, leaving behind a queen, ample food supplies and a few nurse bees to care for the remaining immature bees. Such disappearances have occurred sporadically throughout the history of beekeeping and have been known by various names, including 'disappearing disease', 'spring dwindle', 'May disease', 'autumn collapse' and 'fall dwindle disease'. The factors contributing to the stress experienced by honey bees include not only pesticides, but also habitat loss, other pathogens and viruses, and beekeeping practices. Since 1998, beekeepers in most European countries, especially in southern and western Europe, had observed a similar phenomenon. However, the syndrome was renamed 'colony collapse disorder' in early 2007 (Oldroyd, 2007; Vanengelsdorp et all., 2009).

If most or all of the bees in a hive are killed by overexposure to a pesticide, this is known as a 'beekill' incident resulting from acute pesticide poisoning. However, this is very different from CCD and is almost always avoidable.

Although many theories have been put forward to explain the cause of CCD, researchers have focused on the possibility of pesticide poisoning resulting from exposure to pesticides applied to crops or used to control insects or mites in hives. Honey bees (*Apis mellifera*) can contact and collect pesticides when foraging on crops that have been treated to control pest insects, pathogens, or weeds. In addition, since the late 1980's, U.S. beekeepers have been using miticides within their beehives to control parasitic mites (primarily Varroa mites). The Varroa mite (*Varroa destructor*) is an undeniable threat to colony health. This parasite occurs worldwide, was first discovered in the US in the late 1980s and can devastate bee hives. Shortly after its arrival in the US in the 1990s, farmers began using pesticides, particularly a class of pesticides called neonicotinoid. Although neonicotinoids have various applications, they are primarily used to treat seeds and are widely considered to be a significant improvement on previous chemical formulations. However, like any insecticide, they can be harmful to bees. Some activists have used reported declines in the number of honey bees to claim that neonicotinoids are the main cause of this decline. There has been a great deal of speculation surrounding the contribution of neonicotinoid pesticides to CCD, but many collapsing apiaries show no trace of these chemicals.

Result of previous study showed that a diverse range of pesticides, both grower- and beekeeper-applied, have been detected in hive matrices, and many of these products are known to adversely affect colony health (Martel et all., 2007; Nguyen et all., 2009; Desneux et all., 2007; Collins et all., 2004). They found an association between CCD and the sub-lethal effects of certain pesticides, including two common miticides: coumaphos and fluvalinate. These pesticides are registered for use by beekeepers to control Varroa mites. Studies have also identified the sublethal effects of neonicotinoids and fungicides, which may impair bees' immune systems, leaving them more susceptible to bee viruses. Bonmatin et al. declared that pollen obtained from seeds treated with imidacloprid contained significant levels of the insecticide in 2005. They suggested that contaminated pollen could be responsible for the death of honey bee colonies (Bonmantin et al., 2005). Previous studies have also identified the sub-lethal effects of neonicotinoids and fungicides on bees however Staveley et all (2014) declared that the cause of DDC, Neonicotinoids were deemed unlikely to be the sole cause, but could not be ruled out as a contributing factor. It is hypothesised that these pesticides impair the bees' immune systems, making them more susceptible to certain viruses that affect bees. A meta-analysis study published in February 2010 found evidence from laboratory studies indicating sublethal effects of imidacloprid (neonicotinoid) on honey bees. However, there was a lack of evidence regarding the environmental relevance of these findings (Cresswell, 2011). Similarly, a review from 2012 concluded that, in a laboratory setting, both lethal and sublethal effects on foraging behaviour, memory and learning ability were observed in honey bees exposed to neonicotinoids. However, these effects were not observed in field studies involving realistic dosages (Blacquière et al., 2012). However, several studies showed that there were previously undetected routes of exposure to neonicotinoids affecting bees, including through dust, pollen and nectar (Tapparo et al., 2012). Research has also shown the persistence of clothianidin and imidacloprid (neonicotinoids groups) in agricultural irrigation channels and soil (Krupke, et al., 2012). Machines that plant corn seeds coated with neonicotinoids release certain amounts of them into the air, which is another possible route of exposure (Tapparo et al., 2012). Further experiments are needed to conclusively determine the mechanisms of immune response and the impact of these interactions on mortality and colony health (Goulson et all., 2015; Budge, et all., 2015; Carreck & Ratnieks, 2014). The first field study to establish a link between neonicotinoids and CCD is concluded by Budge et al (2015), an 11-year British study, showed a correlation between increasing agricultural use of neonicotinoid seed treatments and escalating honey bee colony losses at a landscape level.

Conclusion

The pollination of plants by insects and the effect of pesticide use on pollinators is one of the most iconic and transformative ecological relationships in the world. Although tens of thousands of papers and numerous books on pollination biology have been published over the past 200 years, studies focusing on the relationship between pesticides and pollinators have only emerged in the last few decades, and the field is still evolving.

Clearly, the global use of neonicotinoids and other class of pesticides increased after 2005, which coincided with a rise in bee and other pollinators deaths.

In conclusion; the following measures can be taken to reduce the harm caused by pesticides used in agriculture, particularly to pollinating insects and the environment: Integrated Pest Management (IPM) should be implemented, chemical pesticides should only be used as a last resort, biological control methods should be preferred and encouraged, the correct pesticide should be selected, products specific to the target pest with low toxicity should be used, the recommended dose and timing should not be exceeded and indiscriminate and excessive use should be prevented, pest control should not be carried out in windy or rainy weather, pest control should not be carried out during the hours when bees and other beneficial organisms are active, measures should be taken to prevent the contamination of soil and water sources, organic and biotechnological methods should be encouraged, and farmers should be provided with training and awareness programmes.

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Chapter 5

The Relationship Between Gut Microbiota and Reproduction in Ruminant

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Introduction

Reproduction is vital for every animal species and is influenced by many factors, primarily environmental factors (Tekin et al. 2023). As improving the reproductive performance of animals is economically valuable, this topic has become an important focus of research. Recent studies have revealed that gut microbiota plays a role in regulating the reproductive performance of animals (Wang et al. 2024).

The term microbiota denotes the diverse assembly of microorganisms including viruses, fungi, yeasts, archaea, and protozoa that inhabit the living body. In contrast, the microbiome encompasses the collective genetic material of these microorganisms alongside their specific environmental conditions (Koedooder et al. 2018). A healthy gut microbiota is essential for the development of a healthy gut and immune system (Bauer et al. 2006). The gut microbiome, which refers to the community of microorganisms living in the intestinal tract, maintains a balance between beneficial and harmful microorganisms. Perturbations in the microbiome or the inability to establish a stable microbial community may result in gastrointestinal pathologies and compromised immune responses (Czarnecki-Maulden 2008). Animals' ability to digest plant material depends on the symbiotic relationship they establish with their gut microbiota. This situation has led to the evolution of a more complex gastrointestinal structure and a corresponding microbiota development compared to carnivores, enabling the breakdown of plant-based foods and the performance of enzyme-based functions (Ley et al. 2008; De Jonge et al. 2022). The gut microbiota has the ability to break down nutrients such as fibre, starch

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and protein that cannot be fully digested by the host's enzymes. By fermenting these elements, it enables the formation of simpler and more readily usable compounds (Rowland et al. 2018).

The rumen is an environment where anaerobic and methane-producing fermentation processes occur. In this compartment, cellulose-rich feeds can be effectively broken down, and the microbiota present here play a critical role in the digestion of these feeds, contributing to the productivity of animal production (Morgavi et al. 2013).

Primary microbial groups in ruminants

Ruminants have a specialised stomach structure that enables the fermentation of plant material rich in fibre. Although these species have similar digestive systems, their physiology and environmental adaptations vary, and thus differences may be observed in their gut microbiomes. Understanding the structure and functioning mechanism of microbial communities found in the gastrointestinal tract (GIT) of these animals is of great importance in terms of both protecting their health and increasing their productivity (Tardiolo et al. 2025). In cattle, unclassified Enterobacteriaceae members are found predominantly in the small intestinal lumen, while Acinetobacter species are prominent on the mucosal surface. Analysis of the large intestine reveals distinct microbial niches: while Turicibacter, Clostridium, and unclassified Peptostreptococcaceae populate the lumen, Treponema and unclassified Ruminococcaceae are predominantly found in the mucosal tissue (Mao et al. 2015). The three most dominant genera in the small intestine microbiota of sheep have been reported to be Escherichia, unclassified Lachnospiraceae, and Ruminococcus. Additionally, the genera Ruminococcus, unclassified Ruminococcaceae, and Prevotella have also been identified. At the species level, the presence of R. flavefaciens, B. fibrisolvens, and S. ruminantium has been detected (Wang et al. 2016). In goats, the dominant genera identified in the gut microbiota have been reported as Prevotella, Ruminococcus, Clostridium, Butyrivibrio and Fibrobacter. (Lei et al. 2018; Wang et al. 2019; Huaiquipán et al. 2023).

The gut-microbiota-brain axis and reproductive relationship

Scientific studies reveal that the two-way communication between gut flora and steroids can also affect brain function; this is part of the complex connection between the gut and the brain (Tardiolo et al. 2025). This network, which integrates the central nervous system and the enteric nervous system, goes beyond digestive functions and also plays a regulatory role in cognitive functions and behavioural responses (Cryan et al. 2019).

Steroid hormones such as progesterone, oestradiol and testosterone play a role in regulating numerous processes associated with reproduction, including apoptosis (programmed cell death), inflammation and metabolism. Produced in the gonads, adrenal glands and placenta, these hormones reach specific target tissues via the bloodstream, where they modulate the functions of those tissues and determine their biological activity (Hussain et al. 2021). The gut microbiota is integral to the regulation of circulating estrogen levels, primarily by producing the β-glukuronidaz (gmGUS) enzyme. This enzyme, found in the digestive system, converts estrogen in its passive (conjugated) form into its active (free or deconjugated) form. When oestrogen is released, it can more easily bind to its receptors. This binding triggers signalling pathways via the receptor, thereby revealing the various physiological roles of oestrogen in the body. (Flores et al. 2012). Another way in which the gut microbiota affects the levels of steroid hormones in female individuals is through the production of short-chain fatty acids (SCFAs). These SCFAs are key metabolic products resulting from the breakdown (fermentation) of dietary fibre by gut bacteria in an oxygen-free (anaerobic) environment. The most abundant SCFAs produced by the microbiota are acetate (C2), propionate (C3), and butyrate (C4). Butyrate, in particular, has been found to regulate progesterone (P4) and oestradiol (E2) synthesis (production) in porcine granulosa cells (PGCs) using cAMP-mediated signalling mechanisms (Lu et al. 2017; Chadchan et al. 2022).

In the study conducted by Wu et al. on cows, a relationship was found between the hormones FSH, LH, progesterone, prolactin, and oxytocin and gut microbiota markers; however, no relationship was found between GnRH and any microbiota marker or diversity index. The authors suggested that this was likely due to the gut microbiota playing a role in the metabolism of FSH, LH, progesterone, prolactin, and oxytocin, but GnRH hormone is produced only in the hypothalamus or has no receptor in the gut microbiota (Wu et al. 2022). Another study conducted on buffaloes reported that physiological changes occurring during the oestrus cycle cause shifts in the structure and composition of faecal bacterial communities, representing a possible relationship between gut microbiota and animal reproductive physiology (Sharma et al. 2021).

The gut microbiota plays a role in regulating various hormones such as ghrelin, leptin, GLP-1 and PYY, which are vital for the body's metabolic balance. These hormones manage appetite, food consumption, fat storage (lipogenesis) and fat burning (fatty acid oxidation), thereby maintaining body weight. These metabolic functions are particularly critical during pregnancy;

therefore, they have the capacity to directly affect both fertilisation and the healthy continuation of pregnancy (Garcia-Garcia et al. 2022). In a study conducted on cows, animals that became pregnant and those that did not were grouped according to the number of breeding attempts made. It was reported that there was a difference in gut microbiota composition between normal fertility and recurrent infertility conditions. Moreover, distinct differences in microbiota composition were observed between pregnant and non-pregnant cows, even among those requiring few breeding services (Kitagawa et al. 2025).

Ecological imbalance (dysbiosis) in the gut microbiota can cause impairments in nutrient absorption and biosynthesis mechanisms, metabolic dysfunction, and weakening of the immune system in the host. Similarly, this disruption in the microbiome may also contribute to the development of pathologies associated with the reproductive system (Li et al. 2025). Recent studies on dairy cattle have demonstrated a clear link between gut microbiota composition and endometritis. While Ruminococcaceae_UCG-005 was identified as the overall dominant genus, the microbiota of cows suffering from metritis was characterized by a high abundance of Olsenella and Succinivibrio (Shi et al. 2025). Current research indicates that the gut microbiota exhibits differences between the prenatal (before birth) and postnatal (after birth) periods. For example, a study conducted on cattle compared samples taken before birth (dry period) and after birth. This study revealed that the postnatal faecal microbiota had a significantly different structure compared to the period close to birth, and different bacterial genera were detected. Among the most striking changes were a decrease in the abundance of two unidentified genera belonging to the Verrucomicrobiota phylum after birth and, conversely, a marked increase in the proportion of Bifidobacterium after birth compared to before birth (Arnalot et al. 2025).

Zhang et al. investigated the effects of ageing on the gut microbiota of dairy cows and found that older cows (>5 lactations) exhibited lower-level chronic inflammation that persisted for a longer period compared to middle-aged (3 lactations) and young (first lactation) cows. This condition was reported to be reflected in a decrease in milk yield, along with an increase in inflammatory cytokines such as IL-10, TNF-α, TGF-β, and SCC. With ageing, a decrease in the amount of beneficial bacteria such as Bacteroidaceae, Eubacterium, and Bifidobacterium has been observed in faeces, and metabolic activities related to carbohydrate and lipid metabolism have also declined (Zhang et al. 2019).

Microbiota regulation

The relationship between the host organism and the gut microbiota not only facilitates the maturation of the immune system and provides protection against external factors (pathogens, parasites), but also contributes to the regulation and maintenance of reproductive success. This mutual interaction facilitates the normal course of bodily functions on the one hand, while on the other hand, it has the potential to interfere with this process by disrupting the balance (Gupta and Nair 2020). As researchers unravel the role of the microbiome in cattle fertility, they have begun to propose the concept of “microbiome manipulation” as an intervention method for unexplained infertility. This approach aims to correct the disrupted microbial balance by intervening in the microbial composition of the reproductive tract, thereby increasing fertility capacity (Gupta et al. 2024).

Strategies such as probiotic applications, the use of prebiotics, and microbiota-supported cultivation are prominent methods for altering the composition (structure) of the microbiota (Gupta et al. 2024).

Regulation of diet

Adjusting the diet is also an effective strategy for contributing to a healthier composition of cattle microbiota. In this context, increasing fibre intake and limiting starch consumption can yield positive results (Yagisawa et al. 2023; Gupta et al. 2024). Dietary modifications in ruminants directly affect the gastrointestinal system (GIS) microbiota. One of the most radical changes in cattle farming has been the shift from roughage-based feeding to the intensive feed (concentrate)-based rations used in recent times. This process disrupts the balance of the microbial community, leading to a condition known as “dysbiosis”, which can pave the way for digestive system diseases (Welch et al. 2022).

Probiotics

Probiotics are among the most readily available and preferred supplements on the market for strengthening the gut microbiota. These microorganisms, which are mostly bacteria or fungi, are sometimes offered as a single strain and sometimes as formulations combining multiple strains (Fuller 1992). Probiotics limit the growth of pathogenic microorganisms in ruminants, offering an alternative to antimicrobial feed additives. In particular, the use of S. cerevisiae (a type of yeast) has been found to positively affect productivity by increasing rumen pH. Although studies on the effects of probiotics in ruminant feeding show variations, adding these supplements to the diet during periods of stress for animals, such as weaning or feed change, can prevent dysbiosis (microbial imbalance) by protecting the rumen microbial flora (Welch et al. 2022).

Prebiotics

The breakdown (fermentation) of prebiotics by the gut microbiota results in various metabolites, including short-chain fatty acids (SCFAs), lactic acid, butyrate, and propionate. These components have the potential to cause various physiological responses in the body (organism). For example, SCFAs lower the pH level of the colonic environment, making it more acidic. Propionate, on the other hand, can exert a regulatory effect on helper T (Th2) cells and macrophages in the respiratory system and on dendritic cells in the bone marrow (Trompette et al. 2014; Nur Mahendra et al. 2022). Prebiotics are incorporated into the diet in a form that beneficial bacteria can utilise as a food source, and this process aims to maintain and improve digestive system health (Kraimi et al. 2019). The use of prebiotics can be considered as a method not only to promote weight gain, but also to increase feed conversion efficiency and reduce the incidence of health problems such as digestive (diarrhoea) or respiratory tract infections (Roodposhti and Dabiri 2012). Studies have shown that fructo-oligosaccharides (FOS) reduce digestive system problems (intestinal issues) in calves (Quigley III et al. 2002) and suppress the proliferation (colonisation) of leading pathogens such as Salmonella and E. coli in the intestine (Hartemink et al. 1997).

Microbial transplantation

There are two different approaches to microbiota transfer in cattle breeding: faecal transfer or rumen fluid transfer (RFT). Among ruminants (cattle), RFT is the most commonly preferred method. This procedure involves transferring rumen fluid obtained from a healthy donor to an animal with digestive system microbial imbalance (dysbiosis) (Welch et al. 2022). Faecal matter transplantation is also among the methods used in cattle. Studies have shown that this method can yield successful results in the treatment of diarrhoea and also has the potential to increase growth efficiency (performance) when applied to calves in the early stages of their lives (Kim et al. 2021).

Conclusion

The marked differences observed in the GIT microbiota of different ruminant species such as cattle, sheep and goats emphasise that this relationship is species-specific and must be evaluated separately for each species. The difference between balance (healthy microbiota) and imbalance (dysbiosis) in the microbiome directly affects not only digestive health but also immune function and, consequently, reproductive efficiency. In the future, it will be important to conduct studies to uncover the details of the relationship between gut microbiota and reproduction and to highlight the importance of microbiota regulation in reproductive strategies.

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