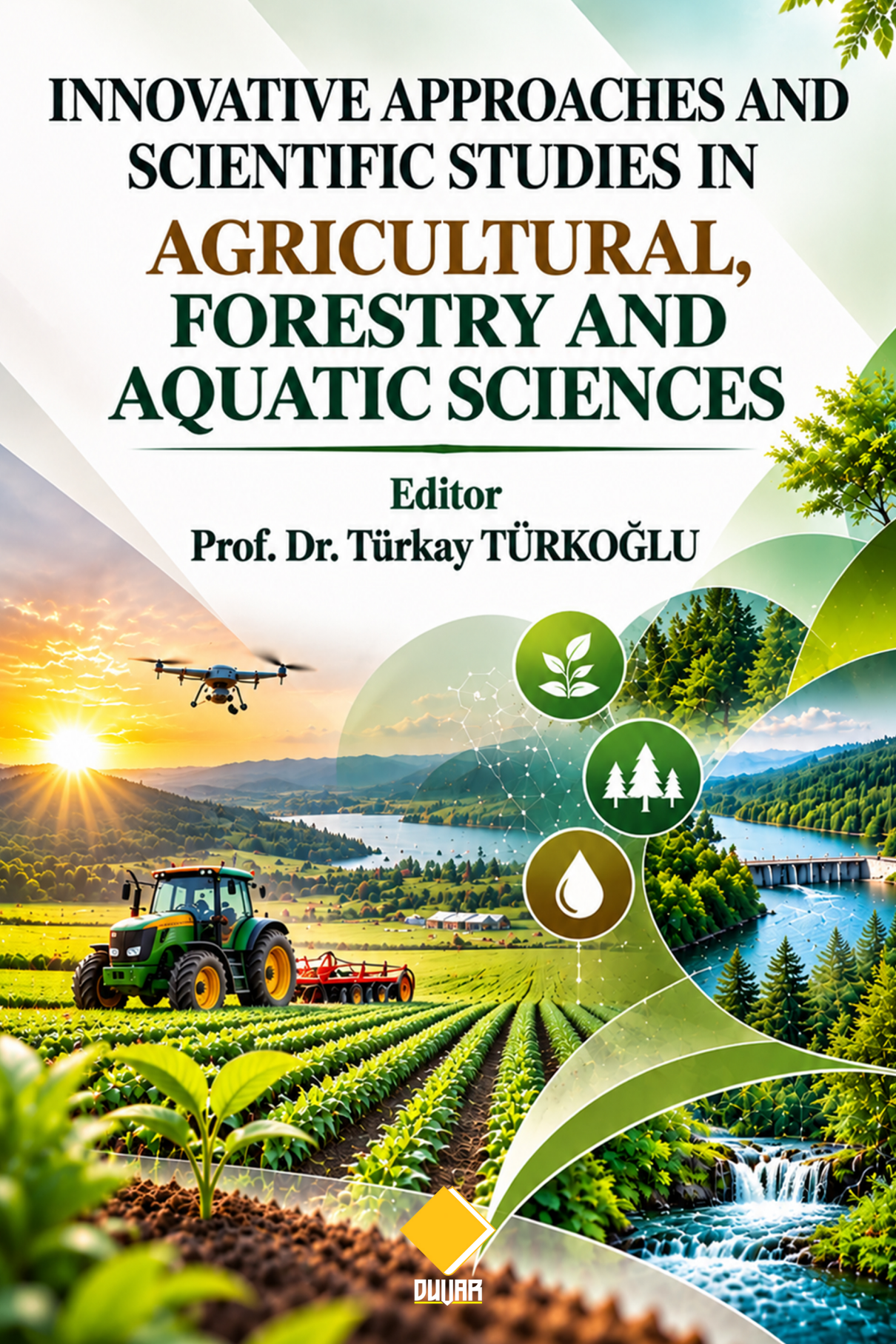
INNOVATIVE APPROACHES AND SCIENTIFIC STUDIES IN AGRICULTURAL, FORESTRY AND AQUATIC SCIENCES

Editor
Prof. Dr. Türkay TÜRKOĞLU



DUVAR

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Editor

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Chapter 1

Polyploidy Breeding in *Vitis* species

Arif ATAK[1,a]

INTRODUCTION

A large proportion of cultivated plants have emerged through natural hybridization, selection, mutation, or polyploidy (Şehirali and Özgen 2010). A significant number of cultivated plants possess an alloploid structure. Allopolyploid occurs through hybridization between two species with distinct genomes, followed by doubling of the chromosome number (Fig.1).

Polyploidy arises naturally in two ways:

a. Somatic doubling occurs due to abnormalities during mitosis in meristematic cells.

b. During meiosis, chromosome sets fail to separate, resulting in gametes that carry twice the expected number of chromosomes.

To induce polyploidy in plants artificially, a chromosome-doubling method is employed. The chemical most frequently used by plant breeders for this purpose is colchicine. Derived from the roots of the autumn crocus (*Colchicum autumnale* L.), colchicine is a potent alkaloid-based toxin; it is a colourless substance that dissolves in alcohol, chloroform, and cold water, but is insoluble in hot water or ether. When applied to plant tissues, colchicine inhibits the formation of spindle fibers during the metaphase stage of mitosis. This prevents the replicated chromosomes from being pulled toward the cell poles, thereby preventing the chromosome number from doubling (Köksal 1999).

As in many other areas, tissue culture offers significant advantages to plant breeders in producing polyploid plants. By incorporating specific chromosome-doubling chemicals into the nutrient medium, polyploid plants can be generated under *in vitro* conditions. One of the most practical methods for obtaining polyploid plants under in vitro conditions involves adding colchicine to the culture medium and allowing the explants to develop in this environment for a certain period (Griesbach 1990; Rose et al., 2000; Vainöle and Repo 2000).

The cell structures of tetraploid plants are larger than those of diploid plants. All cultivated grapevines belong to the genus *Vitis*. The genus *Vitis* consists of two subgenera: *Euvitis*, which has a chromosome number of 2n = 38, and

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Muscadinia, which has 2n = 40. Traditional *Vitis vinifera* cultivars are diploid (2n = 2x = 38), whereas polyploid grapevines specifically triploids (3n = 57) and tetraploids (4n = 76) (Kuliyev, 2020).

The objectives of grapevine breeding programs include improving yield and quality, developing seedless varieties, enhancing disease resistance, adjusting harvest timing (earliness or lateness), and increasing tolerance to stress conditions such as drought and cold. As a perennial, cross-pollinating plant, the grapevine presents several challenges, including a long wait for the first harvest (2–5 years), inbreeding depression, and the occurrence of somatic mutations or chimeras (Atak, 2022). In Türkiye, breeding programs—particularly those focused on table grape varieties—are conducted using techniques such as clonal selection and hybridization. Although hybridization, which aims to develop new varieties, is a lengthy process, it sometimes fails to fully meet evolving consumer and market demands. Given that grapevine species and varieties have been propagated vegetatively for many years, it is clear that a high accumulation of mutations exists within these populations. Furthermore, the variations observed in grapevine cultivars—which can be significant at the clone level—may stem from environmental conditions or, if they are of a permanent nature, from somatic mutations. Today, many economically important grapevine cultivars have been developed through the identification and selection of such somatic mutations. Various researchers have noted that natural mutations in grapevines can lead to the formation of tetraploid structures (Kunter et al., 2011). Examples of such cultivars include "Leopold III" (derived from Alphonse Lavallée), "Muscat Canon Hall" (from Muscat of Alexandria; Olmo, 1937), "Chasselas Gros Coulard" (from Chasselas; Branas and Truel 1965), large-berried tetraploid forms of the Italian cultivar Barbera (Botta et al., 1988), and "Centennial" (derived from Razakı; Kim et al., 2002).

Polyploidy refers to the condition in which a diploid organism has an excess of chromosomes as a result of the addition of one or more sets of chromosomes. Efforts have been made to obtain new triploid or tetraploid polyploid genotypes in grapevines and other plant species through various treatments or mutations that increase chromosome numbers. However, a high success rate could not be achieved with these techniques, and sequence-specific mutations were mostly dependent on chance (Orhan Özalp and Ergönül, 2013).

Polyploidy breeding in *Vitis* species (grapevines) is a highly valuable crop improvement strategy for overcoming the challenges posed by climate change, diseases, and pests. By manipulating chromosome numbers, breeders can

introduce desirable traits such as increased berry and organ size, enhanced fruit quality, seedlessness, and superior tolerance to biotic and abiotic stresses.

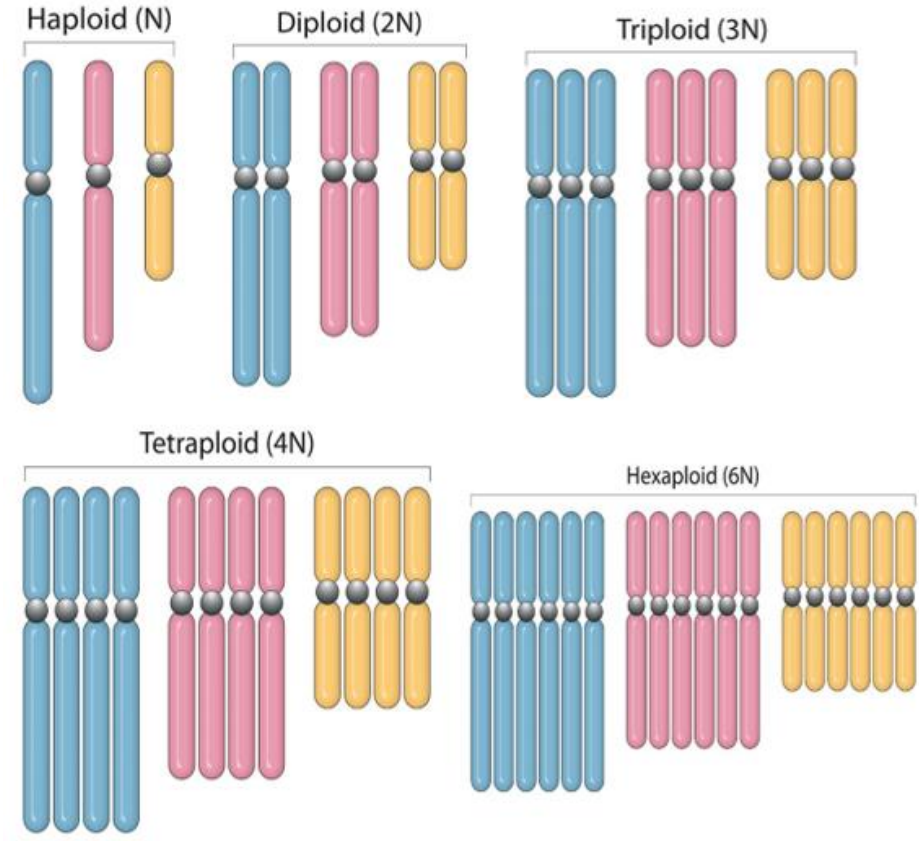


Figure 1. Schematic representation of polyploidy based on chromosome number

Methods of Polyploidy Induction in Grapes

Chemical treatments for obtaining polyploid genotypes are applied under both in vivo and in vitro conditions (Fig. 2). Furthermore, polyploid genotypes can be produced by crossing parents—either from the same species or different species—that possess different chromosome numbers. Artificial chromosome doubling is primarily achieved using antimitotic agents (chemical mutagens) that disrupt spindle fiber formation during cell division, arresting mitosis or meiosis (Notsuka et al., 2000; Eng and Ho, 2018)).

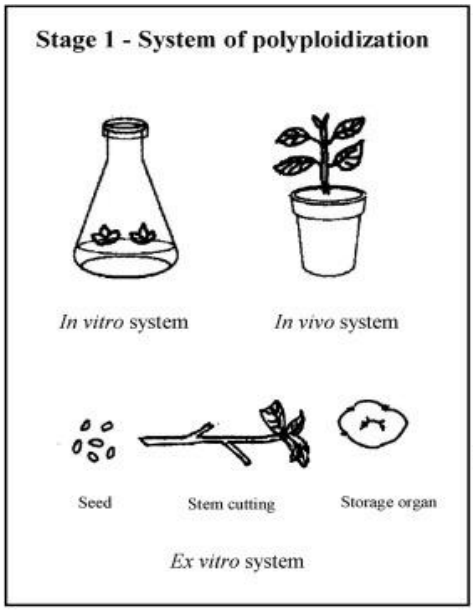
Chemical Agents: Colchicine is the most widely used mutagen. Other agents, like oryzalin, are also applied to minimize toxic side effects (Petersen et al., 2003). One of the most widely used methods to induce polyploidy in plants is the application of colchicine to the apical meristem, but it is reported to be not very

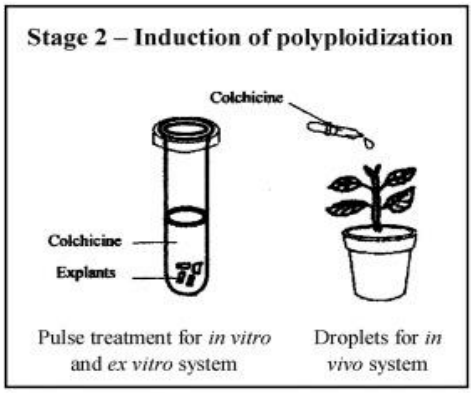
effective in grapevines. Furthermore, increasing doses of colchicine have a negative impact on the survival and embryogenic potential of grapevine embryogenic cell clusters (Rassoulli and Mahmoodzadeh, 2005).

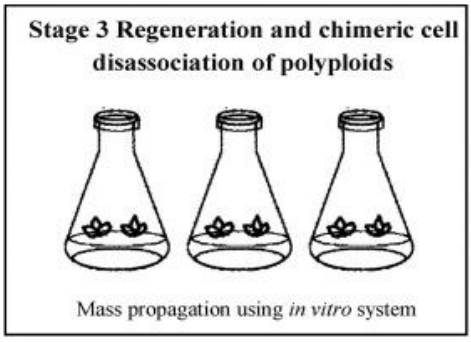
Many studies have been conducted to obtain polyploid genotypes using different *Vitis* species and cultivars via interspecific cross-breeding (Fig.3). Efforts have been made to obtain tetraploid genotypes, particularly in American grape rootstocks (*Vitis rupestris* and *Vitis riparia*) and *Muscadinia* species.

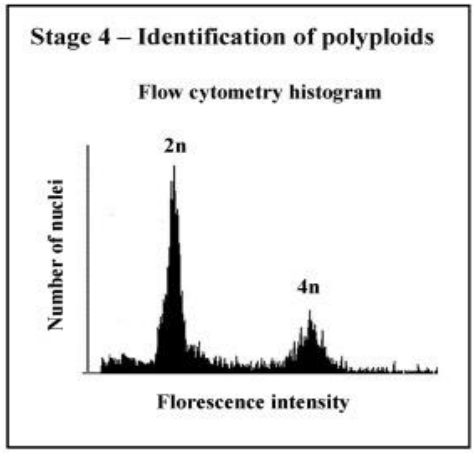
In Vitro Breeding: This procedure is applied to various *Vitis* explants or somatic embryos within a sterile laboratory environment. In some cases, success rates as high as 38% can be achieved (Fig.4).

Somatic embryogenesis is a method preferred by many researchers for micropropagation of various plant species (Fig.5). It is also used to remove many phytopathogens that have infected various plant organs under in vitro conditions (Yang et al., 2006). However, abnormalities may be encountered during somatic embryogenesis due to some somaclonal variations. These mutations are desirable in some cases as they sometimes allow the formation of polyploid forms. In particular, induced polyploidy and natural polyploidy are frequently used to obtain new genotypes for polyploidy breeding studies. It has been reported that new polyploid genotypes exhibit more resistant structures under many biotic and abiotic stress conditions.









Stage 5 – Assessment of polyploids

Apparatus	Parameter
Ruler	• Plant height • Leaf size • Internode length • Root length • Fruit diameter
Weighing machine	• Fruit weight
Colour chart	• Floral colour • Leaf colour
Light microscope	• Stomatal size • Chloroplast number in guard cell • Oil glandular
Transmission Electron Microscope (TEM)	• Cell cellular organelles • Cell subcellular organelles
Scanning Electron Microscope (SEM)	• Stomatal size • Leaf surface wax
Photosynthesis equipment, Chlorophyll meter	• Photosynthesis capacity
Liquid chromatography (LC), Gas chromatography (GC), Mass spectrophotometer (MS)	• Primary metabolites • Secondary metabolites • Bioactive compounds

Figure 2. Schematic illustration chromosome doubling in Horticulture crops. (Eng and Ho, 2018)

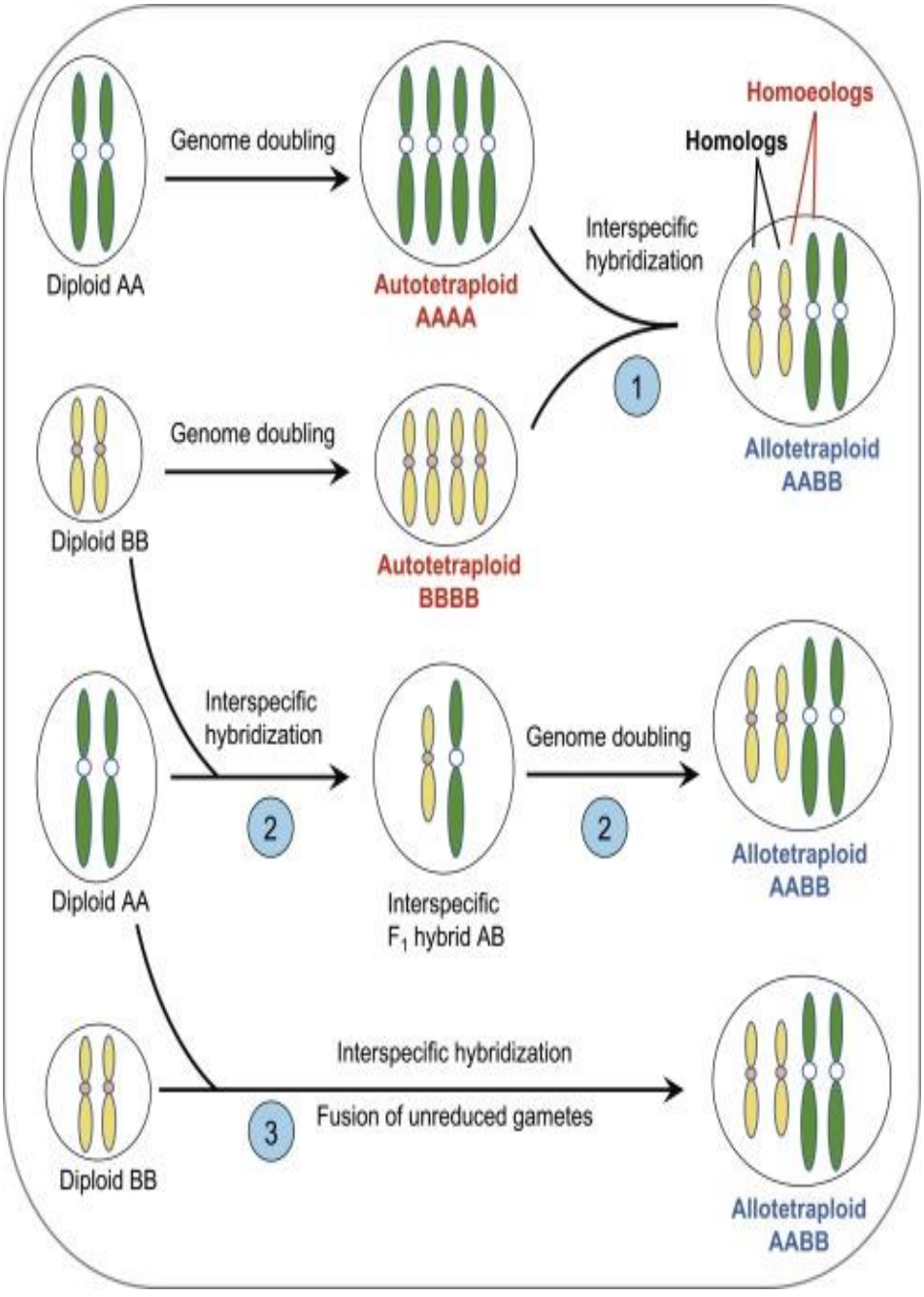


Figure 3. Schematic representation of polyploid hybrid breeding via interspecific cross-breeding (Li and Chen, 2022).

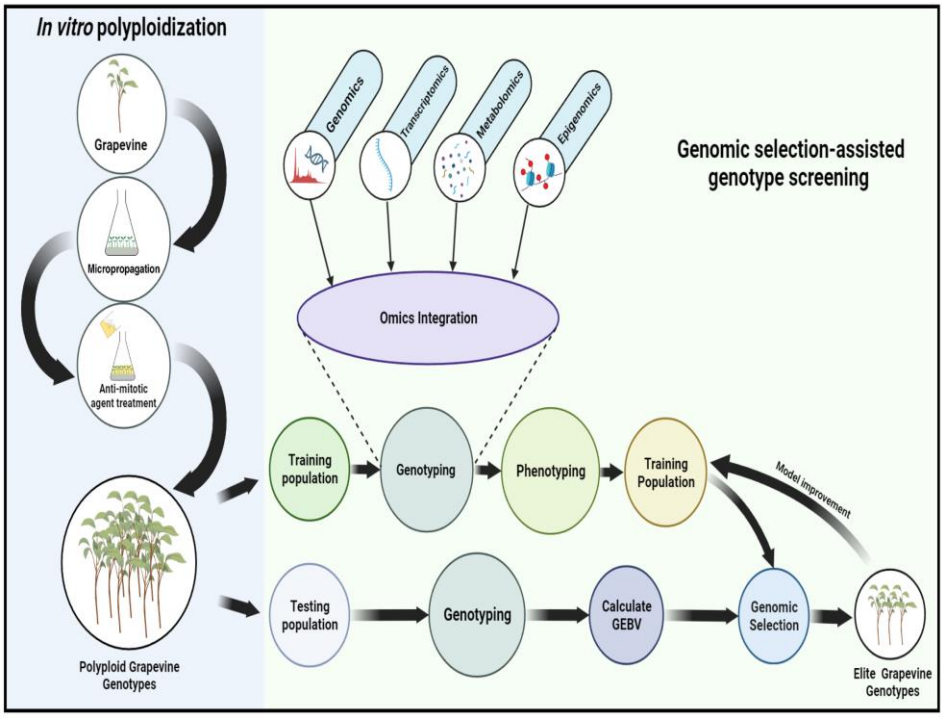


Figure 4. In vitro polyploidization in plant species (Bharati et al., 2023).

(In Vivo + In Vitro Breeding): The stages of this method and its schematic representation are provided in Figure 5.

Identifying and Verifying Ploidy Levels

Once vines are treated, breeders use a multi-step framework to screen and confirm successful polyploidization:

Morphological & Cyto-anatomical Screening: Polyploid genotypes typically present distinct physical markers. Compared to diploids, they exhibit decreased stomatal density, increased stomatal size, larger pollen grains, and an increased number of chloroplasts within guard cells.

Flow Cytometry: The gold-standard method for rapid, high-throughput verification of nuclear DNA content to differentiate diploids, triploids, tetraploids, or unstable mixoploids (Fig.6).

Chromosome Counting: Microscopic examination (such as the tip-end slicing of root or shoot tips) confirms the exact physical chromosome count (2n = 4x = 76) across cell layers (Fig. 7)

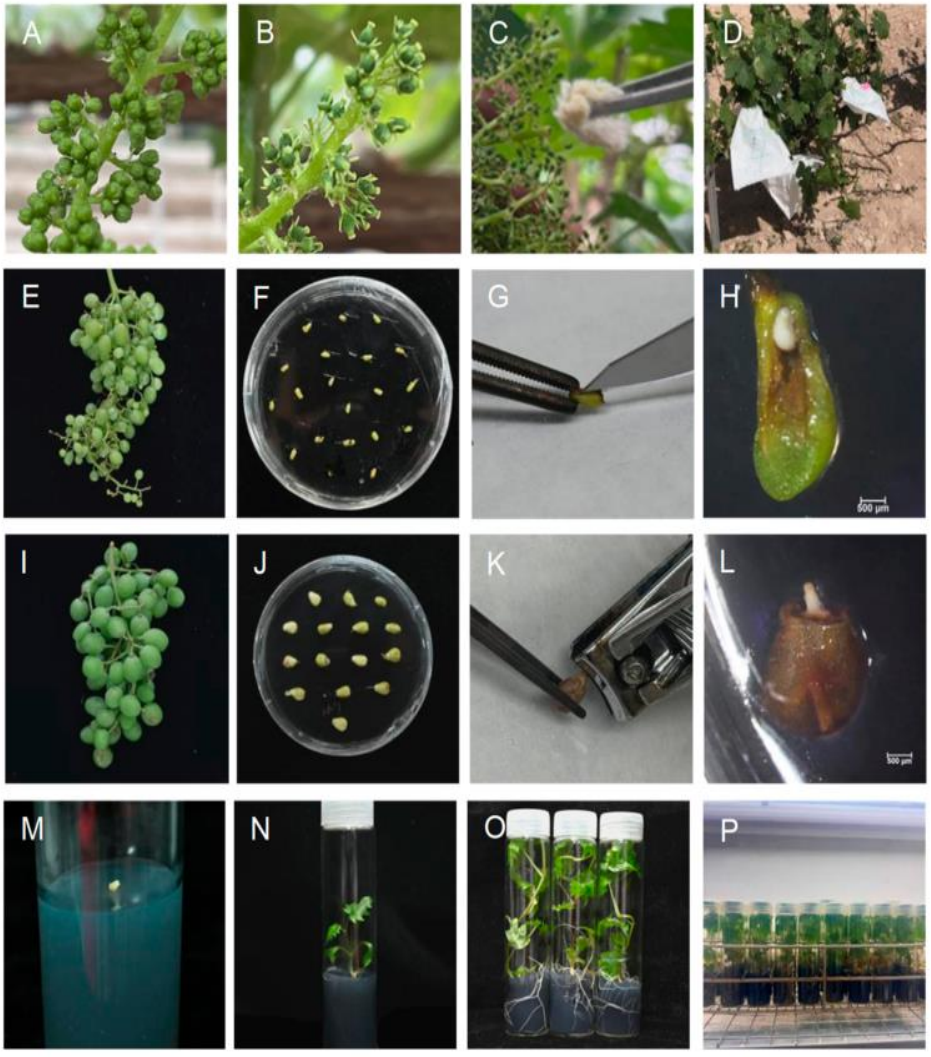


Figure 5. Stages of in vivo hybridization of polyploid parents and in vitro embryo culture: (A) Inflorescence with intact stamens; (B) Inflorescence with stamens removed (emasculated); (C) Pollination; (D) Bagging; (E) Polyploid hybrid fruits derived from a seedless grapevine as the female parent; (F) Ovules cultured on a nutrient medium; (G, H) Dissection of ovules; (I) Hybrid fruits derived from a seeded grapevine as the female parent; (J) Ovules cultured on a nutrient medium; (K, L) Excision of the tip of an ovule; (M–P) Embryos developing into complete plantlets via embryo germination culture (Chen et al., 2026).

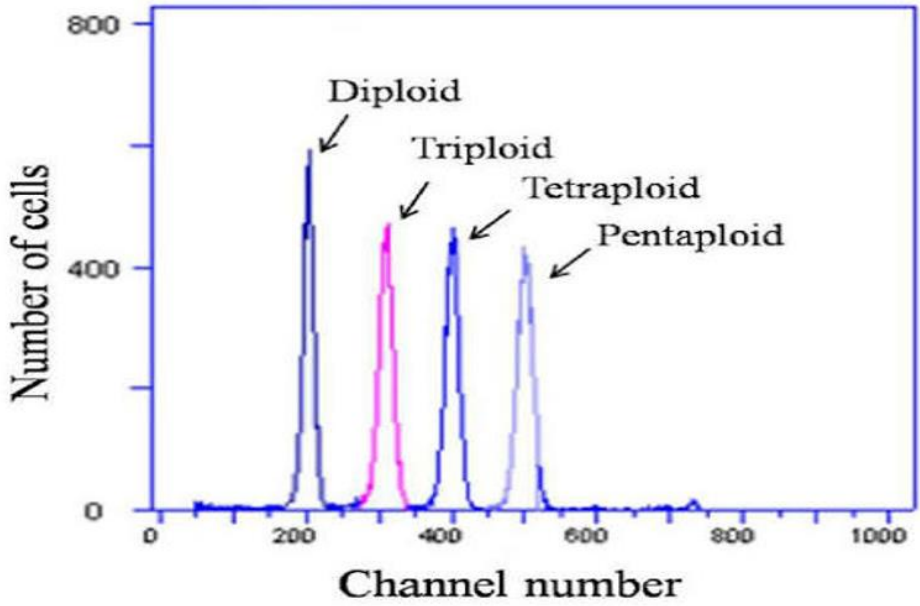


Figure 6. Flow cytometry profiles of cellular DNA content of the diploid, triploid, tetraploid, and pentaploid genotypes (Yu et al., 2014).

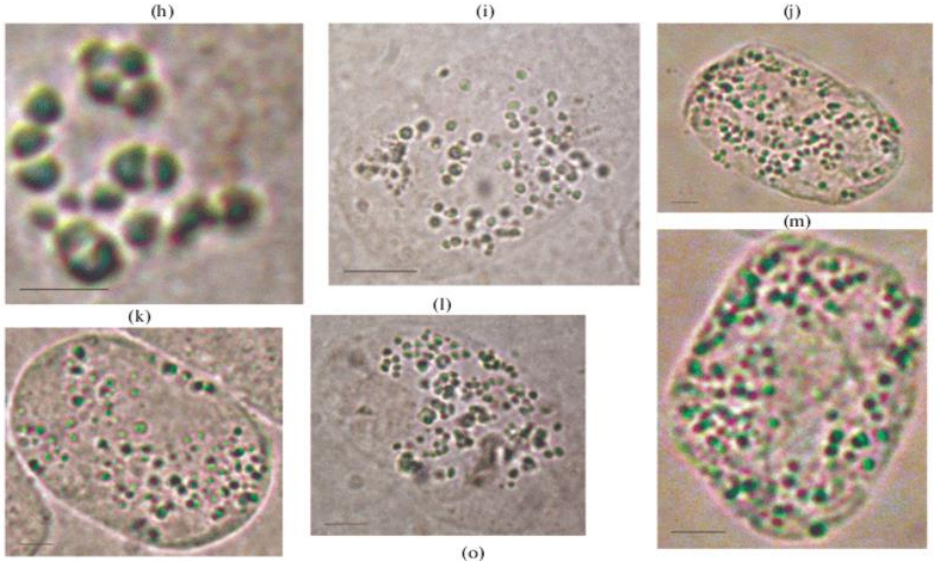


Figure 7. Meristematic tissue of grape plants. (h) haploidy (n = 19); (i) triploidy (3n =57); (j,k,l,mp) tetraploidy (4n = 76). Bar = 5 μm (Klimenko, 2019)

POLIPLOID BREEDING STUDIES in *VITIS* spp.

Seedless table grapes are the most preferred grapes by the consumers. Stenospermocarpy and parthenocarpy are the natural causes of seedlessness in breeding studies (Zhao et al., 2009; Zhang et al., 2011). The most significant characteristic of seedless grapes are their small sized and seedless fruits (Li et al., 2015). Polyploidy is an alternative way to obtain seedless and large sized new table grape cultivars. Kyoho, Pione, Olimpia, Heukgoosul are some of the tetraploid grape varieties. Triploid grapes of varieties Honey Seedless, King Dela, and Mirai were obtained by hybridization of these tetraploid cvs. with diploid genotypes (Orhan Özalp and Ergönül, 2013).

Seedlessness is generally desired by breeders and has become an increasingly important issue, especially for researchers in table grape breeding in recent years. Because the demand for seedless grape cultivars is much higher than seeded cultivars. However, there exists some mating obstacles in crosses between diploid and tetraploid grape cultivars. Embryo rescue or embryo recovery technique has been used with increasing success rate in recent years to overcome these obstacles (Xi et al., 2023).

There are few studies on embryo rescue from an interspecific cross between diploid and tetraploid grape species. There was a very limited number of studies on cross-breeding and embryo recovery between diploid and tetraploid grape cultivars, including interspecies, but these studies have begun to increase with the techniques and technologies developed in recent years (Yang et al., 2007; Doyğacı et al., 2024).

Breeding efforts were undertaken to produce triploid grape varieties in various programs aimed at developing seedless grape varieties. Polyploidy in grape varieties was first reported in California in 1914 (Olmo, 1952). Although polyploidy has been utilized in grapevine breeding programs since 1914, the development of triploid and autopolyploid genotypes has attracted significant attention in recent years. Plants with increased ploidy levels may exhibit superior quality compared to certain original diploid forms in terms of berry size, leaf size, and phylloxera resistance (Kara and Yazar, 2022).

Various studies have been conducted to develop new triploid and tetraploid genotypes with larger, seedless berries from different *Vitis* species (Luo et al., 1995). It has been reported that tetraploid grapes obtained through colchicine treatment exhibit poor vegetative growth, low cold resistance, and fail to meet desired yield levels. So cross-breeding with different polyploid level is the better alternative to have polyploid hybrids. Triploid genotypes generally possess seedless berries and a vigorous plant structure. Researchers have reported obtaining superior hybrid genotypes through triploid breeding programs in grapevines. Notably, larger berry formation is frequently observed in triploid

individuals. Consequently, there has been a recent increase in research regarding both natural and artificial polyploidy (Atak, 2022).

Despite these efforts, the commercial utilization of polyploid genotypes remains far below desired levels. While polyploidization can alter certain plant phenotypes, it does not affect the expression of most fundamental varietal characteristics. These advantages enable polyploidy breeding programs to improve key traits such as quality, yield, and tolerance to stress conditions (Sattler et al., 2016).

Cross-breeding between cultivars/genotypes with different ploidy levels is an effective method for creating new germplasm. Most of the triploid fruit plants are sterile and their fruit is seedless. Triploid breeding researchers have presented a new method for seedless grape breeding that enables high sterilization rates and the production of parthenocarpic fruits, ultimately facilitating the achievement of desired results. Polyploid cultivars have reshaped the fresh fruit market, particularly in East Asia, where large-berried table grapes are highly prized (Ateş et al., 2026; Chen et al., 2026).

The embryo rescue technique may prevent the early-stage abortion of triploid young embryo, so triploid plants can be produced (Fig.8). There are few studies on embryo rescue from an interspecific cross between diploid and tetraploid grape species. There was a very limited number of studies on cross-breeding and embryo recovery between diploid and tetraploid grape cultivars, including interspecies, but these studies have begun to increase with the techniques and technologies developed in recent years (Li et al., 2020). The first research aimed at developing triploid grapes using embryo culture was conducted by Okamoto et al. (1993) and Yamashita et al. (1993).

Polyploid plants are known to have advantages over diploid plants. Polyploid plants are among the most important advantages, with their ability to tolerate harmful mutations with larger structures also high heterozygous structures (Tossi et al., 2022). Polyploid seedless varieties transform into a polyploid structure as a result of certain errors during meiosis, and this is common in grapevines. It also allows the reproduction of hybrid genotypes in a sterile structure by doubling the genome (Sinski et al., 2014).

In some studies, on somatic embryoids and polyploidization of shoot tips in seedless grape cultivars, non-chimeric autotetraploid plants have been reported. Various studies on the effects of colchicine on proembryogenic cells have reported that it can induce the regeneration of true utopolyploids. In addition, researchers have identified and published the most effective protocols for inducing polyploidy (Liu et al., 2024).

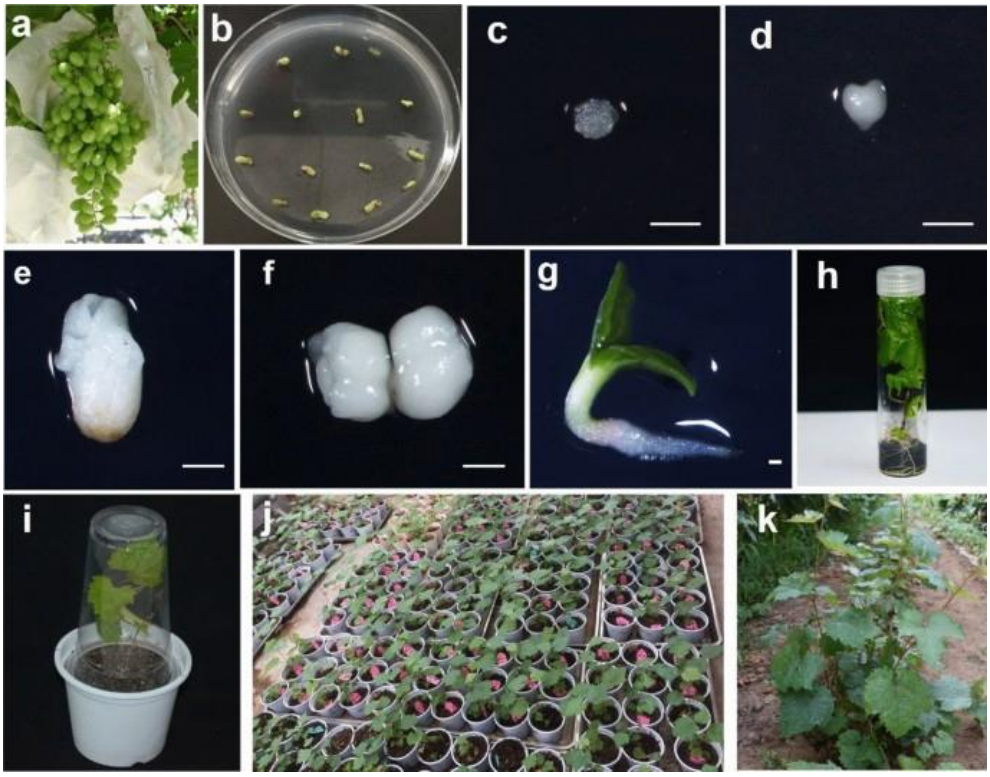


Figure 8. The embryo rescue method for obtaining new, seedless grape progeny (polyploids) (Li et al., 2020).

Researchers have not yet fully elucidated the genetic and physiological mechanisms underlying polyploidy in plants. Furthermore, this method can enhance the adaptation of artificial tetraploid (4x) grapevine rootstocks to biotic and abiotic stress conditions in *Vitis* species (Singh et al., 2022).

Research on synthetic polyploidy in plant species remains quite limited. Due to the high adaptability of polyploid varieties to various stress conditions, this subject has become a significant area of focus in breeding programs (Ruiz et al., 2020). Additionally, the importance of polyploid varieties and rootstocks and their use in production is steadily growing in the context of sustainable agriculture (Sobhanian et al., 2026).

Today, thanks to advancements in technology and new biotechnological approaches, it has become possible to generate functional mutations in a wide range of polyploid species—including grapes—through the use of sequence-specific nucleases (Zaman et al., 2019). Given their many advantages, polyploid grapes are expected to become even more widespread in the coming years.

CURRENT BOTTLENECKS AND MODERN SOLUTİONS

- Despite its advantages, traditional polyploidy breeding faces deep constraints.
- Artificial tetraploids can initially exhibit weak vegetative growth, low winter hardiness, and yields below desired levels.
- Furthermore, managing the massive volume of unique genotypes generated requires years of labor-intensive and costly field screening.
- To solve these problems, modern programs are integrating Genomic Selection (GS) with Multi-Omics data (genomics, transcriptomics, and metabolomics). By using statistical and machine learning models (such as GBLUP and BLASSO), researchers can now computationally predict elite polyploid performance and breeding values at the seedling stage, thereby skipping decades of manual phenotypic screening.

CONCLUSION

In the breeding of various *Vitis* species, the focus is primarily on traits such as seedlessness, berry size, high quality, early or late harvest, and tolerance or resistance to various stress conditions. In recent years, there has been increased global emphasis on developing new triploid and tetraploid grapevine varieties. In particular, the emergence of new biotechnological applications has made it more feasible to maintain various polyploid varieties and combine desired traits within them. Given their superior performance—as noted above—polyploid varieties (mainly interspecific hybrids) are expected to become more prevalent on market shelves in the near future.

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Chapter 2

From Smart Agriculture to Smart Villages: Opportunities, Limitations, and the Future of AI-Driven Rural Development

A Critical Review of the Literature and a Proposed Conceptual Model

Aytürk KELEŞ[1], Ali KELEŞ[2]

Abstract

Although the potential contributions of artificial intelligence (AI) to rural development are often discussed in terms of agricultural productivity, its implications extend well beyond agricultural production. This chapter examines the transition from smart agriculture to a smart village approach by considering agricultural applications alongside the local economy, education, healthcare, energy, natural resource management, and public services. Drawing on the relevant literature, it proposes an integrated conceptual model that examines technological opportunities and implementation challenges within a common framework. The review discusses how digital inequalities, investment and maintenance costs, data security, digital skills, and institutional capacity condition the benefits that can be derived from technology. Its central argument is that AI can bring about meaningful changes in rural life only when supported by accessible infrastructure, ongoing training and technical assistance, appropriate financing, transparent data governance, and local participation. In developing implementation recommendations for Turkey[3], the chapter considers regional differences in production structures and existing resources. The proposed model is grounded in the literature and should not be treated as an empirically validated model of impact.

Keywords: artificial intelligence, smart agriculture, smart villages, rural development, digital divide, sustainability

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1. Introduction

For many years, rural development policies have centered on increasing production, improving physical infrastructure, and raising incomes. Yet the conditions shaping the future of rural life extend far beyond agricultural production indicators. Population ageing, the migration of young people to cities, difficulties in accessing education and healthcare, climate-related pressures, rising input costs, and inequalities in digital connectivity have emerged as interrelated challenges. Rural development must therefore be assessed through its economic, social, environmental, and institutional dimensions together (European Commission, 2021; OECD, 2020; World Bank, 2016).

1.1. How Should Rural Digital Transformation Be Assessed?

The success of digital transformation cannot be explained merely by the number of devices installed in a rural settlement or the number of people registered on an application. The essential questions are which needs a technology addresses and what it changes in everyday life. For example, a sensor that monitors soil moisture can support water management if its readings are accurate, the information reaches farmers in time, and the recommended irrigation schedule can be implemented with available resources. Similarly, online sales channels may provide access to new buyers; however, their anticipated economic benefits remain limited if transportation and secure payment problems persist. Digital transformation therefore calls not only for the installation of technical equipment but also for a rethinking of service delivery and institutional practices.

1.2. An Approach Starting from Local Needs

Before choosing technologies for rural areas, it is necessary to understand what local residents need. The priorities of producers, cooperatives, municipalities, relevant public bodies, young people, and women may not always coincide. When these differing perspectives are excluded at the design stage, even costly systems may fail to gain sufficient acceptance. Accordingly, needs assessments should cover not only existing technical infrastructure but also production practices, connection quality, the costs of acquiring and maintaining devices, language and literacy requirements, and the availability of technical support. Such an approach makes it possible to select technology that fits local conditions rather than forcing a settlement to adapt to a ready-made solution.

Artificial intelligence (AI) can be used to identify recurring patterns in data from different sources, predict particular developments, and inform decision-making. In agriculture, its applications include examining images for signs of disease, forecasting crop yields, determining irrigation timing, and monitoring animal health. Rural development, however, cannot be reduced to activities carried out in fields or livestock enterprises. Marketing and logistics, education, healthcare, energy, transportation, disaster preparedness, and local government services are also essential components of rural life (FAO, 2022; Liakos et al., 2018; OECD, 2026).

Although the concepts of smart agriculture and smart villages are closely related, they do not have the same scope. Smart agriculture focuses on supporting production processes through data and digital tools. The smart village approach, by contrast, seeks to connect these capabilities with healthcare, education, local economic activity, environmental protection, and public participation. Thus, the presence of an automated irrigation system alone does not make a settlement a smart village. What matters is how technology interacts with people's skills, institutional arrangements, and local decision-making mechanisms (Naldi et al., 2015; European Commission, 2021).

This chapter discusses the economic, social, environmental, and governance-related opportunities that AI may offer in the transition from smart agriculture to smart villages, as well as the circumstances in which these opportunities may be constrained. It then presents a conceptual model that can be evaluated through future empirical research. The review draws on international academic studies and policy documents issued by relevant institutions. Because no field research was undertaken for this chapter, the proposed relationships are treated not as demonstrated effects but as hypotheses and possible mechanisms requiring further investigation (Klerkx et al., 2019; Assimakopoulos et al., 2024).

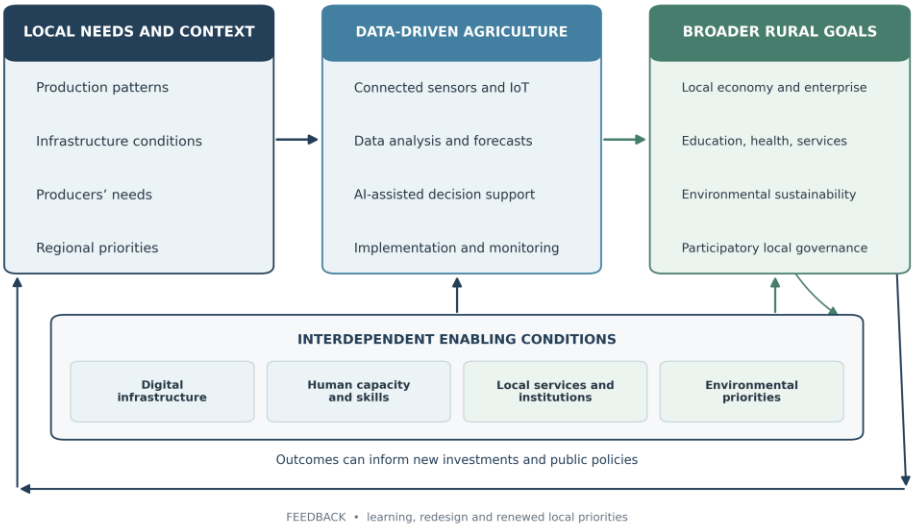


Figure 1. An AI-Based General Framework for the Transition from Smart Agriculture to Smart Villages

Figure 1 summarizes how data-driven solutions developed in agriculture may be linked to the broader objectives of rural development. Digital infrastructure, human resources, local services, and environmental priorities are interdependent components of this transition. The process is not unidirectional: local needs shape technology choices, while implementation outcomes may, in turn, influence new investments and public policies.

2. Conceptual Framework: Smart Agriculture, Smart Villages, and Rural Development

2.1. Smart Agriculture and Artificial Intelligence

Smart agriculture can be defined as an approach that supports production-related decisions using sensors, satellite imagery, geographic information systems, connected agricultural machinery, and data analytics. The Internet of Things (IoT) enables data to be collected and shared through connected sensors. Keleş and Keleş (2018) draw attention to the security and privacy issues associated with this technology, alongside its areas of application. AI can jointly assess information from these sources to classify features in images, identify relationships, and generate predictions. Examples include identifying suspected plant diseases from leaf images, estimating irrigation requirements using soil moisture and meteorological records, and comparing production data from different periods (Klerkx et al., 2019; Wolfert et al., 2017; Kisliuk et al., 2023).

The Data Chain and Decision-Making Process

The operation of smart agricultural applications can be understood as an interconnected data and decision chain: collecting measurements, checking and cleaning data, analyzing them, translating findings into decision recommendations, and monitoring implementation outcomes. A problem at any link in this chain can diminish the ultimate benefit. Data from a poorly calibrated sensor may mislead an analysis, while a statistically accurate recommendation may be incompatible with a farmer's budget or equipment. Evaluation should therefore address not only model performance but also maintenance requirements, ease of use, and tangible outcomes under field conditions.

Farmers' Knowledge and the Role of Technology

The knowledge farmers acquire over many years complements, rather than conflicts with, AI-based decision support. Through experience, producers learn about the history of their land, soil behavior, local climatic variations, and the seasonal occurrence of pests. Interpreting numerical data alongside this knowledge may make recommendations more actionable. It is also important for a system to explain its reasoning in understandable terms, communicate uncertainty, and allow farmers to adjust recommendations when necessary. In decisions with substantial financial consequences, human responsibility and final decision-making authority must not be left ambiguous.

The outputs of AI models are closely linked to the quality of the data on which they rely and the conditions under which they were developed. A solution designed for a particular crop, climate, or farm size may not perform equally well in another region. Applications should therefore undergo local testing before being scaled up, with user experiences collected regularly and model errors monitored so that necessary adjustments can be made (Khanna et al., 2024; Liakos et al., 2018).

2.2. The Concept of the Smart Village

In this chapter, a smart village is understood as a rural settlement that seeks to use digital technologies beyond agricultural production to expand local economic opportunities, improve access to essential services, protect natural resources, and strengthen rural residents' participation in decision-making. The characteristics of such a settlement are determined not only by its devices or connectivity capacity but also by the people and local institutions capable of making effective use of these resources (Naldi et al., 2015; Hernandez et al., 2024).

Connectivity infrastructure, smart production, local entrepreneurship, education and healthcare, energy and natural resource management, local governance, and inclusion are among the main domains of a smart village. Their relative importance, however, may differ between settlements. In an area dependent on livestock farming, herd health and pasture conditions may be central concerns, whereas in a village with tourism potential, digital visibility, visitor management, and preservation of cultural heritage may be more pressing (Naldi et al., 2015; OECD, 2020; European Commission, 2021).

2.3. The Multidimensional Nature of Rural Development

Assessing rural development solely through improvements in agricultural yields may obscure changes in other domains. From an economic perspective, producers' incomes, input costs, market connections, and employment should be considered; socially, attention should be paid to access to education, healthcare, and other services, as well as opportunities for participation. The environmental dimension encompasses water and energy consumption, soil health, and biodiversity, while the governance dimension emphasizes interinstitutional coordination, transparency, and accountability. AI may help improve these indicators, but no automatic relationship between technological progress and development should be assumed (OECD, 2020; United Nations, 2015).

3. Applications of Artificial Intelligence in Smart Agriculture

3.1. Potential Advantages of AI-Based Agriculture over Conventional Agriculture

In conventional farming, farmers' observations and experience remain primary sources of information for decision-making. Some practices, however, rely on observations from only a limited number of locations or on predetermined schedules. AI-supported agriculture can combine satellite imagery, sensor measurements, and weather data to detect changing conditions earlier and reconsider when interventions should be made. Its main potential benefits are summarized below:

- Needs-based input use: Irrigation and fertilization can be adjusted to the requirements of different areas of land, potentially limiting unnecessary consumption of water, fertilizer, and energy.
- Opportunities for early intervention: Timely identification of signs of disease, pests, water stress, and abnormal growth may enable producers to take preventive action sooner.

• Support for production planning: Joint interpretation of weather conditions, soil characteristics, and plant growth can provide more up-to-date evidence for decisions on sowing, crop management, harvesting, and labor.

• Reduced workload and costs: Automating repetitive measurement and record-keeping tasks may allow labor and machinery to be directed toward activities where they are most needed.

• Site-specific decisions: Rather than applying the same treatment across an entire field, different interventions can be planned for areas with varying soil, moisture, and climatic conditions.

These benefits do not arise automatically when a technology is installed. Outcomes depend on measurement reliability, uninterrupted access to electricity and connectivity, maintenance costs as well as initial investment, digital skills, and the suitability of recommendations for local production conditions. AI should therefore be regarded as decision support that complements, rather than replaces, producers' expertise.

3.2. Precision Agriculture and Resource Efficiency

In precision agriculture, AI supports irrigation, fertilization, and production management by jointly interpreting satellite data, meteorological information, soil moisture measurements, and indicators of plant development. This avoids assuming that all parts of a field have identical needs and allows more detailed recommendations regarding where, when, and how much water and other inputs should be applied (FAO, 2022; Liakos et al., 2018; Wolfert et al., 2017; Mandal et al., 2024).

Nevertheless, adopting precision agriculture tools does not necessarily reduce water or fertilizer use under all conditions. Sensor accuracy, interruptions in connectivity, maintenance costs, and producers' ability to follow recommendations directly affect outcomes. The value of an application must therefore be examined not only in terms of predictive performance but also in relation to total operating costs, farmers' experience, and environmental effects (Khanna et al., 2024; OECD, 2026).

3.3. Livestock Farming and Herd Management

Sensors and analytical systems used in livestock farming can regularly provide information on animal activity, feed intake, milk production, and various health indicators. AI applications that identify unusual changes in these data may prompt farmers or veterinarians to investigate at an earlier stage. Such an alert, however, does not constitute a definitive diagnosis. These systems

should support timely intervention rather than replace veterinary assessment (FAO, 2022; OECD, 2026).

The adoption of these technologies cannot be explained solely by herd size or the economic scale of an enterprise. Reliable electricity, internet access, repair services, and users' capabilities also influence uptake. For small farms, jointly purchasing devices, accessing services through cooperatives, and using regional technical advisory services are possible ways of sharing costs. The economic and operational effects of these arrangements nevertheless require separate evaluation.

3.4. Adaptation to Climate Change and Agricultural Resilience

Examining historical production records alongside weather forecasts, drought indicators, and satellite observations can help assess climate-related agricultural risks. AI-supported forecasts may inform crop selection, planting periods, irrigation schedules, and disaster preparedness. All forecasts, however, involve uncertainty. This uncertainty should be communicated clearly to users, and the findings should be compared with local observations, expert assessments, and producers' experience (FAO, 2022; World Bank, 2019).

More accurate forecasts do not, by themselves, imply greater capacity to adapt to climate change. Irrigation and water-management infrastructure, insurance mechanisms, producer organizations, access to finance, and public support are important prerequisites for translating information into action. Without these resources, even a producer who receives an early warning may find it difficult to take the necessary precautions (Liakos et al., 2018; Wolfert et al., 2017).

4. The Role of Artificial Intelligence in Other Dimensions of Rural Development

4.1. Local Economy and Entrepreneurship

For rural businesses, AI and other digital tools do more than organize production; they may also change how products reach markets. Demand forecasting, online promotion, product classification, inventory control, and transportation planning are examples of applications in this area. Cooperatives may reach wider customer groups through shared sales channels, exchange demand information with their members, and make information about production processes more visible. Such traceability may support consumer trust (World Bank, 2019, 2021; OECD, 2020; Schroeder et al., 2021).

Digital marketplaces, however, may also impose new burdens on producers. Platform commissions, increased competition, transportation requirements, and

the concentration of sales data among a small number of intermediaries may reduce anticipated gains. An increase in sales volume alone may therefore be misleading. Assessment should also consider producers' net income, their bargaining power vis-à-vis buyers, and their degree of dependence on a single platform.

4.2. Education, Healthcare, and Social Services

In rural areas, online learning environments, educational content tailored to individual needs, and distance education may offer new ways to access knowledge. In healthcare, remote consultation and digital tools supporting clinical decisions may likewise complement existing service networks. These options should not, however, be regarded as complete substitutes for teachers or healthcare professionals. Their purpose is to provide additional, usable channels for accessing services, particularly where access is difficult (Hernandez et al., 2024; Miao & Holmes, 2021).

Accessibility and Inclusion in Service Delivery

The existence of a digital service is not the same as being able to access it in practice. Applications for education and healthcare may become more inclusive if they function with slow connections, are easy to use on mobile phones, provide offline content when needed, and offer appropriate language options. Software cannot, however, eliminate structural problems such as staff shortages or inadequate transport. Conventional service channels should therefore remain available to people who require face-to-face support.

Sustaining Digital Skills

Developing digital skills is not a process that ends with a single introductory training session. Support units through which users can report difficulties, regular hands-on training, and updated user guides contribute to the continued operation of systems. Training should be adapted to age and previous experience and should address personal data protection, the recognition of online fraud, and the verification of digital information. Schools, public education centers, universities, and cooperatives can work together to provide such support.

Particularly in healthcare and education, personal data protection, information security, the possibility of algorithmic error, and unequal access must be addressed seriously. People with limited connectivity or little experience using digital tools may be excluded from new services. Accordingly, the expansion of digital channels should not eliminate alternative ways of

obtaining services or submitting requests (Jakku et al., 2019; Eastwood et al., 2019).

4.3. Energy, the Environment, and Natural Resource Management

AI can also be used in rural energy and environmental management. Potential applications include forecasting electricity generation from renewable sources, balancing energy consumption throughout the day, identifying water losses in distribution networks, and planning waste-collection routes. Considering irrigation requirements and energy use together may support operational planning, particularly on farms with high energy costs (OECD, 2020; United Nations, 2015).

Assessing the net environmental contribution of these applications requires looking beyond the savings achieved during operation. The resources used to manufacture equipment, the energy required to run a system, infrastructure needs, and electronic waste generated at the end of its useful life must also be considered. This provides a more balanced assessment of environmental effects across the technology's life cycle, from installation to end-of-life disposal.

4.4. Local Governments and Digital Governance

Local authorities can use data analysis to identify underserved areas, prioritize infrastructure investments, map disaster-related risks, and assess requests submitted to public offices. However, the reasons underlying technology-generated recommendations and the decisions based on them must be understandable. Citizens' opportunities to correct missing or inaccurate data, contest decisions, and participate in planning must be protected. Leaving choices that directly affect rural residents solely to outside technology providers or experts may weaken local ownership (European Commission, 2021; OECD, 2020).

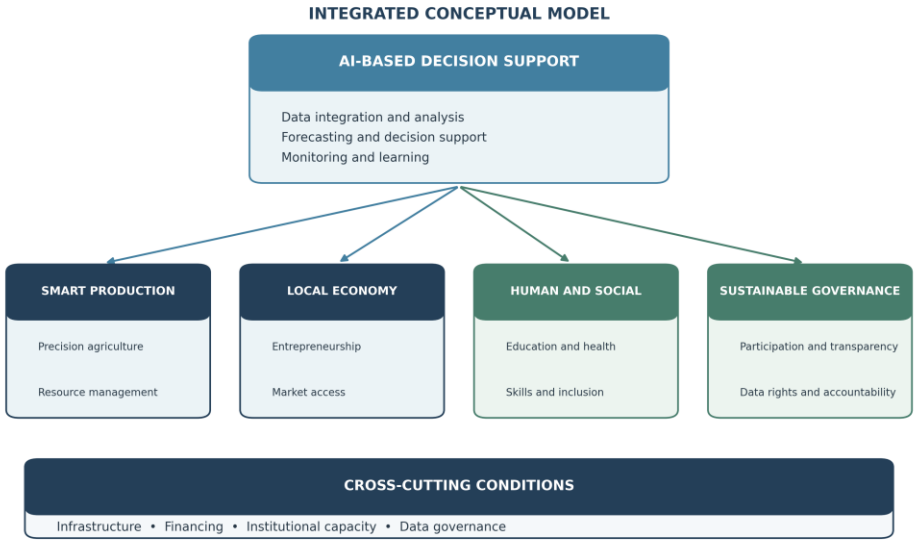


Figure 2. An Integrated Conceptual Approach to AI-Based Rural Development

Figure 2 positions AI not as an independent source of development outcomes but as decision support connecting different domains of rural life. Shared digital infrastructure, adequate human resources, sustainable financing, institutional involvement, and rules governing data are fundamental conditions affecting every application area. The outcomes cannot therefore be explained solely by the technical capabilities of the algorithms employed (Jakku et al., 2019; Eastwood et al., 2019).

5. The Turkish Context: Opportunities and Regional Differences

When developing a smart village approach for Turkey, it would be a mistake to treat rural areas as homogeneous. Production patterns, farm sizes, land and transport conditions, connectivity, educational services, and the capacity of local institutions vary across regions. The same digital solution cannot therefore be expected to produce comparable results in every settlement. A more appropriate starting point is to identify regional needs, involve producers in planning, and assess solutions through limited-scale pilot implementations (European Commission, 2021; Trendov et al., 2019; World Bank, 2021).

5.1. Designing Applications Around Local Differences

Rural settlements in Turkey differ considerably in climate, topography, principal sources of livelihood, and access to services. This diversity calls for locally determined implementation priorities. Where crop production

predominates, irrigation management, disease monitoring, and harvest timing may take precedence. In livestock-oriented areas, feed costs, herd health, pasture use, and access to veterinary services may be more important. In settlements known for rural tourism or handicrafts, digital promotion and access to sales channels for small businesses may be priority needs.

5.2. From Pilot Projects to Scalable Solutions

Pilot implementations provide an opportunity to observe how a technology performs in a particular context. Positive results in one settlement, however, do not prove that the solution will be equally successful elsewhere. Reproducibility under different conditions, maintenance and operating expenses, institutions' ability to assume long-term responsibility, and user participation are therefore important. Success indicators should be defined before a pilot begins; where feasible, comparison with a similar settlement should be undertaken, and both favorable outcomes and failures should be documented. Such an approach can help assess the effects of public spending more rigorously.

For example, livestock-rearing regions could develop projects involving early health alerts, feed planning, milk quality, and cold-chain monitoring. Satellite imagery and geographic data could be used to assess pasture conditions. In regions where crop production is prominent, priorities could include early disease detection, irrigation scheduling, yield forecasting, and input management. Rural settlements earning income from tourism could also consider digital promotion and visitor-flow management.

Ağrı and its surrounding areas, where livestock farming and pasture use are important, may be considered among potential locations for needs-based pilot projects. Nevertheless, it would be inappropriate to claim that a specific solution would be effective there without examining up-to-date production data, existing infrastructure, and producers' access to technology. Any region-specific proposal should be supported by official statistics, field interviews, and assessments from relevant stakeholders.

6. The Transition from Smart Agriculture to Smart Villages

Smart agriculture may provide a strong starting point for the digital transformation of rural settlements, but wider use of technology on farms does not imply that broader development will occur automatically. Linking production data with cooperative activities, local marketing, educational services, and public planning may extend its influence. Such integration requires interoperable systems, clear rules for data sharing, and meaningful user

participation in decision-making (Klerkx et al., 2019; Assimakopoulos et al., 2024).

6.1. A Phased and Flexible Transition

The transition toward smart villages need not follow the same mandatory sequence of steps in every settlement. In one area, reliable internet access and digital literacy may be the most basic needs; elsewhere, improving a cooperative's online sales capabilities or managing energy expenses may be more urgent. Plans developed in response to local conditions should therefore be reviewed at regular intervals. Rather than maintaining an application solely because an investment has already been made, it may be more appropriate to modify it or adopt an alternative when expected benefits fail to materialize.

6.2. Shared Infrastructure and Cooperation

Small producers may not always be able to bear the full costs of advanced devices, software subscriptions, and maintenance services individually. Cooperatives, producer associations, municipalities, and relevant public agencies can share this burden through jointly used equipment, data services, training, and technical assistance. Establishing a partnership is not sufficient on its own, however: decision-making authority, responsibility for equipment maintenance, and rights of access to data must be defined from the outset. The real value of cooperation is evident not only in the initial investment but also in keeping systems operational over time.

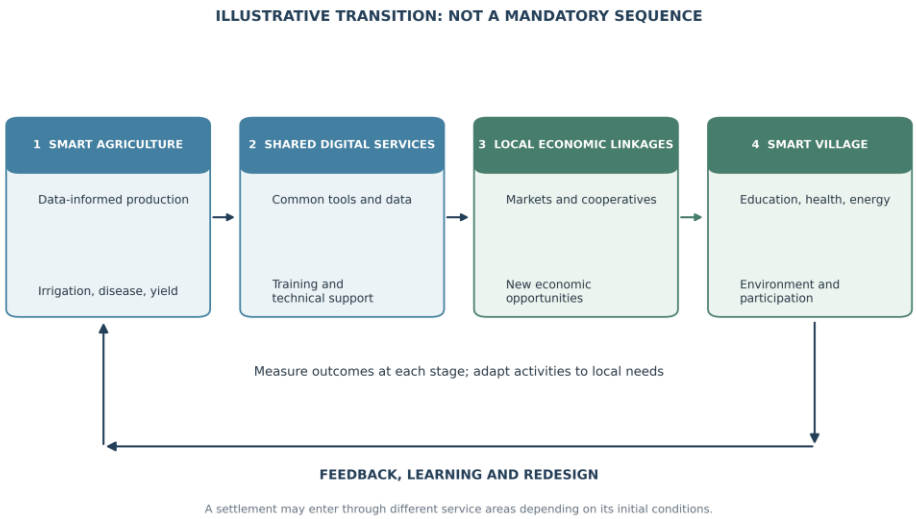


Figure 3. Possible Stages in the Transition from Smart Agriculture to Smart Villages

The four stages shown in Figure 3 should not be read as a development sequence that all villages must complete in the same order. Healthcare or energy services may improve in a settlement where agricultural digitalization remains limited; another settlement may face different obstacles at the outset because of basic connectivity problems. The purpose of the diagram is to make possible links between domains visible and to emphasize that outcomes should be monitored at every stage so that applications can be revised in response to feedback.

7. Enabling Factors, Limitations, and Ethical Considerations

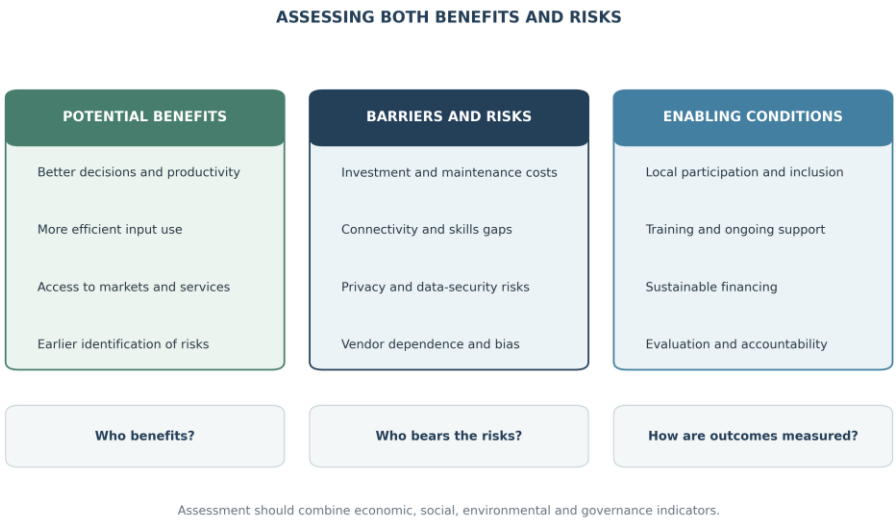


Figure 4. Opportunities, Barriers, and Implementation Conditions for AI-Supported Rural Development

Figure 4 presents the potential benefits of AI use in rural development alongside associated risks and summarizes the conditions needed to sustain implementation.

Understanding AI's role in rural development requires examining its possible gains together with its limitations. Reliable electricity and internet access, user training, financial resources, maintenance and technical support, coordination between institutions, and local participation may facilitate technology adoption. Conversely, high investment costs, insufficient or inaccurate data, privacy concerns, inadequate digital skills, and dependence on a single provider may undermine a system's long-term operation (Naldi et al., 2015; OECD, 2020; European Commission, 2021).

7.1. Indicators for Measuring Success

It is insufficient to measure the success of smart village projects solely by the number of systems installed or the total number of application users. Economic assessment may cover operating costs, net income, crop losses, time required to reach markets, and employment opportunities created. Social assessment should consider access to services, participation in training, the involvement of women and young people in decision-making, and user satisfaction. Environmental effects can be monitored through water and energy

consumption, input use, soil conditions, and waste volumes. The indicators to be used and methods for measuring them should be decided before implementation begins.

7.2. Monitoring Risks and Benefits Together

The benefits of digitalization and its potential risks should be considered within the same evaluation process. Automation may accelerate certain tasks while changing the nature of work. Digital sales channels may open access to new customer groups but also increase producers' dependence on a single platform. Likewise, data-driven recommendations may enable more efficient resource use, yet incomplete or erroneous measurements may cause losses for some enterprises. User experience, economic costs, and environmental outcomes should therefore be monitored together, and applications should be modified when problems arise.

Who uses data, and for what purposes, is as important as the technology itself. It should be clear who collects information on land characteristics, production volumes, animal health, and commercial transactions, with whom it is shared, and how long it is retained. When producers lack a say over the use of their own data, the economic value generated by digitalization may not be distributed equitably among the parties involved. Trust, transparency, and benefit-sharing are therefore central to data governance (Bronson & Knezevic, 2016; Jakku et al., 2019; Eastwood et al., 2019). Keleş and Keleş (2018) highlight security and privacy risks associated with IoT devices. Data protection should consequently extend to sensors and communication networks as well.

Ethical assessment cannot be restricted to the technical accuracy of algorithms. It must also ask who bears the cost of an incorrect recommendation, whether different groups of producers benefit equitably, whether decisions are explainable, and whether mechanisms for appeal exist. In particular, when people are automatically classified or prioritized in public services, human oversight, transparency, and accountability must be maintained (Bronson & Knezevic, 2016; Eastwood et al., 2019; Khanna et al., 2024).

Table 1. Potential Benefits and Limitations of AI Applications for Rural Development

Application area	Potential contribution	Main limitation / risk	Suggested evaluation indicator
Precision irrigation	Better planning of water and energy use	Sensor costs, data errors, maintenance needs	Water use, yield, total operating cost
Animal health monitoring	Support for early warnings and herd management	False alarms, connectivity problems, lack of expert support	Disease records, response time, animal welfare
Digital marketing	Greater market access and product visibility	Platform commissions, logistics, competition	Net producer income, sales continuity, commission share
Distance education	Improved access to learning resources	Unequal access to devices and connectivity, digital skills	Participation, completion, learning outcomes
Smart energy management	Planning energy use and generation	Investment costs, technical maintenance, hardware life span	Energy consumption, costs, outage duration
Data analysis in public services	Better identification of needs and allocation of resources	Privacy concerns, bias, limited transparency	Service access, decision time, user satisfaction

8. A Proposed AI-Based Smart Village Model

The smart village model proposed in this chapter consists of five interrelated components: digital infrastructure, smart production, the local economy, human and social capacity, and sustainable governance. Its underlying premise is that these components do not operate independently. For example, agricultural data can contribute to cooperative planning only if more than the data themselves are available: arrangements for interinstitutional coordination and data sharing must also be defined (Naldi et al., 2015; OECD, 2020).

The first component is a digital foundation comprising internet and electricity access, appropriate devices, and technical support. The second encompasses agricultural production and natural resource management. The third covers marketing local products, rural enterprises, and diversification of income sources. The fourth concerns digital skills, education, public participation, and knowledge exchange between generations. The final component brings together principles of data protection, transparency in decision-making, local participation, and environmental responsibility (Jakku et al., 2019; Eastwood et al., 2019).

Table 2. Dimensions of the Proposed Smart Village Model and Potential Monitoring Indicators

Dimension	Example indicator	Data source / method	Consideration
Digital infrastructure	Reliable connectivity and service continuity	Infrastructure records, user surveys	Distinguish coverage from actual use
Economic	Net income, input costs, market access	Business records, producer interviews	Account for inflation and market conditions
Social	Access to educational and healthcare services	Service records, user surveys	Disaggregate by age, gender, and income
Environmental	Water and energy use, resource conditions	Meters, field measurements, remote sensing	Consider seasonality and weather conditions
Institutional	Cooperative participation and interinstitutional coordination	Meeting records, interviews	Examine the quality of participation as well
Inclusion	Benefits received by women, young people, and small producers	Disaggregated surveys and interview data	Averages may conceal inequalities

Because the model's domains influence one another, concentrating investment on a single component may be insufficient. Even where internet connectivity is available, systems may fall into disuse if technical assistance is unavailable when faults occur. Training alone may not sustain an application if users cannot afford devices or subscriptions. Similarly, uncertainty about rights to use production data and the benefits available to farmers may undermine trust. Infrastructure, financing, training, data management, and local participation should therefore form parts of a common plan. Budgets should cover not only installation but also maintenance, updates, and user support.

To test the proposed model, conditions in pilot settlements should be documented before implementation, and subsequent changes should be monitored systematically. Evaluation should not end once the technology has been installed and becomes operational; it should also encompass costs, incomes, access to services, and effects on social life. Where possible, comparisons with settlements sharing similar characteristics may help determine how much of the observed change is associated with the intervention.

The monitoring design should specify in advance who will collect each type of data, how frequently, and at what cost. Without regular measurement, it becomes difficult to establish an application's actual effects. A baseline value, intended change, data source, and responsible institution may be defined for each indicator. For example, irrigation water consumption can be measured using meters, user satisfaction through brief surveys, and market access through sales and delivery records. Data collection should not impose an unnecessary administrative burden on producers and should preserve the confidentiality of commercial information. In a small pilot, a limited number of meaningful indicators monitored consistently may be more useful than a long list that cannot be tracked.

9. Policy Recommendations and Directions for Future Research

Policies supporting rural digitalization should focus not on purchasing technology itself but on strengthening the local capacity needed to use it meaningfully. This requires improvements in reliable internet and electricity access, accessible technical support, and affordable devices. Hands-on digital skills programs for farmers, cooperatives, rural businesses, and local governments are also important. Training should go beyond operating tools to develop the ability to interpret data, recognize incorrect recommendations, and understand data rights (Trendov et al., 2019; Hernandez et al., 2024; OECD, 2024).

9.1. A Roadmap for Implementation

Before implementation, local problems should be assessed alongside available financial and institutional resources. Pilot projects can then be designed around a limited number of achievable objectives with clearly defined measures of success. Training, maintenance, technical support, data security, and long-term financing should be planned at the same time as equipment investment. Independent evaluation of pilot outcomes can support more informed decisions on whether to continue, revise, or discontinue a solution. This can prevent systems from depending solely on temporary project funding and strengthen the responsibilities of local institutions.

9.2. Priorities for Future Research

Future research should first compare which digital solutions can be used in different settlements and under what conditions. Longitudinal monitoring may reveal changes in income, costs, resource consumption, and access to services. Small-scale producers, women, young people, and people with limited digital experience should not be excluded from research. Publishing only successful applications makes it more difficult to understand the limitations of technologies. Cases in which expected outcomes were not achieved should also be documented and discussed. Conducting research in collaboration with local actors may help translate findings into practice.

The technologies used in pilot projects should be selected according to problems identified with local stakeholders, rather than the features of off-the-shelf products. Options for providing shared maintenance, data management, and technical assistance through cooperatives or regional service providers can also be examined. From a public funding perspective, focusing solely on initial equipment and installation costs is insufficient; the means of covering operating expenses, updates, user training, and maintenance must also be clarified (Miao & Holmes, 2021).

Support programs should take account of inequalities in access among producer groups. If large enterprises already capable of using technology benefit disproportionately from assistance, existing disparities in rural areas may widen. Shared equipment, installment-based financing, advisory services, and cooperative-based services are possible alternatives for small producers. The particular difficulties faced by women, young people, older adults, and users with limited digital skills should also be considered. Simplifying application procedures and clearly communicating eligibility requirements may improve access. Public procurement and project selection should consider

ongoing maintenance, usability, and long-term social benefits alongside technological innovation.

Three areas stand out for further investigation. First, research should compare infrastructure conditions across rural regions and the factors influencing the adoption of AI tools. Second, the effects of particular applications on income, agricultural yields, water consumption, and access to services should be measured in the field. Third, data rights, trust in algorithms, questions of fairness, and interactions between local knowledge and digital systems can be examined through interviews and other qualitative methods. Findings from such studies would provide a basis for testing and, where necessary, refining the conceptual model developed here.

10. Conclusion

AI offers new opportunities for decision support across many domains affecting rural development. Precision agriculture, herd-health monitoring, climate-risk assessment, digital marketing, distance education, energy management, and planning of public services are among these applications. The fact that such tools have become technically available, however, does not itself demonstrate that inclusive and sustainable rural development has been achieved (FAO, 2022; Khanna et al., 2024; OECD, 2026).

The chapter's central conclusion is that AI's effects can be understood only by considering digital infrastructure, people's knowledge and skills, financing, the capacity of local institutions, and data governance together. Applications designed without regard for local conditions, or without the views of different groups within the rural population, may deepen existing inequalities. The smart village approach should therefore begin not with technology itself but with people's needs and with institutions capable of responding to them (Trendov et al., 2019; Hernandez et al., 2024).

Responding to every rural challenge with a new AI system may be neither necessary nor economically justified. In some situations, revising irrigation schedules, ensuring regular maintenance of existing equipment, offering advisory services to producers, or coordinating public services more effectively may yield better results than a new digital investment. Technology options should therefore be compared in terms of expected benefits, total cost, implementation time, environmental burden, and local acceptance. When AI is chosen, the decision it will support, the data it will use, and the response to erroneous outputs should be specified in advance. In this way, it is possible to remain open to technological innovation while keeping expectations realistic.

The proposed model does not claim that the transition from smart agriculture to smart villages follows a single development path in every settlement. Rather, it recognizes that different regions may follow different paths in line with their production structures, institutional resources, and social priorities. In the next stages, the model should be developed through a systematic literature review, supported by verifiable data from Turkey, and tested in pilot field studies. Only such an assessment can enable an evidence-based discussion of AI's contributions to rural development without exaggerating its benefits or dismissing its potential entirely (Klerkx et al., 2019; Assimakopoulos et al., 2024).

Note: The figures, model dimensions, and monitoring indicators included in this chapter were developed by the author for conceptual purposes. They do not represent findings validated through field research; rather, they provide a framework for future studies.

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Figure note. The visuals and conceptual diagrams in this chapter were prepared to illustrate the author's proposals. The model and indicators are not empirically validated results but proposals that can be assessed through future field research.

Chapter 3

Biotechnological Advancements in Goldfish *(Carassius Auratus)* From Targeted Genome Editing to Gut Structure, Microbiota and Health Management

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1. INTRODUCTION

Goldfish (*Carassius auratus*) occupy an unusual position in aquatic science. Domesticated in China more than 1,700 years ago, the species has long since outgrown its purely ornamental status to become a cornerstone of a multi-billion-dollar global industry (Li et al., 2024; Bohra et al., 2026). Centuries of selective breeding have produced a remarkable diversity of body morphotypes, fin structures and pigmentation patterns. Recent market analyses place the global ornamental fish trade at roughly USD 7–14 billion in 2025, growing at about 6–8.6% annually, with the Asia-Pacific region dominant and Thailand a global leader in goldfish breeding (Market Reports World, 2026a, 2026b).

Alongside this commercial role, goldfish have become a standard laboratory model. Their hardiness, low-cost husbandry and well-characterised physiology suit them to work in nutrition, endocrinology, toxicology, developmental biology and immunology (Alesci et al., 2022; Ahmadifar et al., 2026). Their digestive physiology and immune architecture share many conserved features with other vertebrates, so findings in goldfish inform gut biology well beyond the aquarium, and the same applies to their close relatives, crucian carp and gibel carp. A note on terminology is necessary, because several English names are used for closely related fishes in the literature. In this chapter, "goldfish" refers to *Carassius auratus*, the domesticated ornamental form bred for colour and body shape. "Crucian carp" has two meanings. In European literature, it denotes *Carassius carassius*, a separate species. In Chinese aquaculture literature, however, it is more commonly used for the wild or farmed form of *C. auratus* itself, from which goldfish were domesticated in China. In this chapter, "crucian carp" is used in this second sense, so that crucian carp and goldfish are the same species at different stages of domestication rather than two different species; they interbreed freely and produce fertile offspring (Wang et al., 2013). "Gibel carp" or "Prussian

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carp" refers to *C. gibelio*, a separate species that was long treated as a subspecies of *C. auratus*.

Production has changed just as much as research. Contemporary ornamental aquaculture increasingly integrates molecular genetics (Omori & Burgess, 2024; Li et al., 2024), functional nutrition and intensive engineering. Modern frameworks aim at physiological performance, improving intestinal health and nutrient bioavailability through gut microbiota modulation (Wang et al., 2022; Feng et al., 2026) and adopting closed-loop recirculating aquaculture systems (RAS) for biosecurity and sustainability. Bridging classical breeding and biotechnological innovation is therefore essential if the sector is to meet rising demand for premium, resilient phenotypes.

Both the scientific and the commercial trajectory converge on the intestine. The gut is simultaneously the principal site of nutrient absorption, the largest mucosal immune surface in the body, and the habitat of a dense microbial community whose composition tracks diet, environment, host genotype and disease state. Targeted microbiome modulation has emerged as a practical tool against diet-induced inflammatory conditions (Feng et al., 2026).

This chapter accordingly covers six themes: the structural organization of the goldfish digestive tract and its mucosal immune compartment; the composition of the gut microbiota and the factors that shape it; dysbiosis, including infection and the antibiotic-driven microbiome collapse documented in the retail supply chain; dietary and microbial interventions, from probiotics and synthetic consortia to prebiotics and phytogenics; genome editing and colour phenotypes, where genetic and nutritional routes to coloration increasingly intersect; and, finally, priorities for future work.

2. STRUCTURAL ORGANISATION OF THE GOLDFISH DIGESTIVE TRACT

2.1 Gross Anatomy and Regional Histology

Goldfish, like other omnivorous cyprinids, lack a true stomach; the oesophagus connects directly to the intestine, which is conventionally divided into anterior, middle and posterior segments followed by the rectum (Mirjalili & Alavi, 2024). McVay and Kaan's 1940 study provided one of the earliest detailed histological descriptions of the goldfish (*C. auratus*) digestive tract, reporting that the pharyngeal epithelium is stratified with abundant mucous cells, transitions abruptly to simple columnar epithelium at the intestinal sphincter, and reverts to stratified mucous epithelium in the rectum (McVay & Kaan, 1940).

What distinguishes the goldfish gut is the regional patterning of its mucosal folds. Fold length is greatest in the rectum and shortest in the middle intestine,

while basal fold width and inter-fold distance increase from anterior to posterior intestine before decreasing again in the rectum (McVay & Kaan, 1940; Mirjalili & Alavi, 2024). These gradients are best read as functional specialisation: long, thin folds and tall enterocytes in the anterior intestine maximise absorptive surface, while the thicker muscularis of anterior and posterior segments supports mixing and propulsion.

Goblet cell density increases along the same axis, from anterior intestine towards the rectum (Mirjalili & Alavi, 2024). Comparative work across eight ornamental species reared under identical conditions confirms that goldfish, crucian carp and zebrafish (*Danio rerio*), all members of the Cyprinidae, sit at the efficient end of this spectrum, with significantly thickened muscularis and prominent mucosal layers, whereas poeciliids such as red swordtail and Mickey Mouse platy show very high goblet cell density (Dede & Çek, 2025; Xie et al., 2026).

The liver consists of hepatocytes in diffuse or radial arrangement around central veins, with conspicuous cytoplasmic vacuolation reflecting lipid and glycogen storage, and lacks the clear lobular architecture of higher vertebrates (Dede & Çek, 2025; Mirjalili & Alavi, 2024). Vacuolisation is notably pronounced in parrot cichlids (Xie et al., 2026), a reminder that hepatic condition is a useful, if non-specific, readout of dietary adequacy.

2.2 The mucosal immune compartment

Teleosts lack organised lymph nodes but possess diffuse mucosa-associated lymphoid tissues, of which gut-associated lymphoid tissue (GALT) is the most extensive. In goldfish, GALT occurs in both scattered and aggregated forms within the lamina propria and submucosa, with numerous intraepithelial lymphocytes in the basal epithelium (Mokhtar et al., 2026). The esophagus of the koi (*Cyprinus rubrofuscus*) likewise functions as a genuine mucosal immune site (Mohamed et al., 2025). At the receptor level, Al-Rikabi (2024) showed in goldfish skin-associated lymphoid tissue that alternative splicing determines whether a receptor carries an activating or inhibitory signalling motif.

Several effector populations have now been characterised at the cellular level in goldfish. Intestinal mast cells label positively for TLR-2, S100, VIP, serotonin and piscidin markers highly conserved between fish and mammals, indicating that they are active participants in mucosal immune responses (Alesci et al., 2022). Macrophages are distributed across mucosal, submucosal and muscularis layers, express CSF, CSF-R1 and BMP2, and show clear heterogeneity (Zaccone et al., 2026). BMP2-expressing macrophages in the muscularis externa suggest a role extending beyond host defence into neuromuscular regulation.

That suggestion is reinforced by ultrastructural evidence of direct contact between macrophages and enteric neurons and nerve fibres (Mokhtar et al., 2026). Together, these observations describe an integrated neuroimmune network in which enteric neurons, serotonergic signalling, goblet cells and antigen-presenting cells operate as a single regulatory unit. Any account of goldfish gut microbiota that treats the epithelium as an inert wall therefore misses half the system.

3. COMPOSITION OF THE GOLDFISH GUT MICROBIOTA

3.1 Dominant phyla and genera

The gut microbiota of goldfish and related cyprinids is shaped by both environmental and genetic factors. Wang et al. (2022) compared the intestinal microbiota of goldfish (*C. auratus var. Japonicus simplex*) and crucian carp (*C. auratus*) reared under aquaponic versus traditional farming systems, finding Proteobacteria dominant overall and more abundant under aquaponic conditions. A meta-analysis of carp and goldfish data sets identified *Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria* and *Proteobacteria* as consistently dominant, with the last three together contributing roughly 90% of total gut microbiota in many surveys (Choirunisa et al., 2025). That analysis found no significant difference in the *identity* of dominant phyla between fish species, but highly significant differences in their relative *abundance*.

Functional attributions differ by taxon. Proteobacteria participate in nutrient metabolism and carbon, nitrogen and sulphur cycling; Firmicutes contribute to lipid metabolism and digestive enzyme production; Bacteroidetes handle carbohydrate metabolism; Actinobacteria produce hydrolytic exoenzymes, antibiotics and bacteriocins; and Fusobacteria include members that inhibit pathogens and synthesise vitamin B12 (Choirunisa et al., 2025).

At genus level, *Cetobacterium* emerges repeatedly as the signature commensal of the goldfish gut. It reached 19.17% relative abundance in inbred goldfish lines (Wang et al., 2022), dominated the intestinal community alongside *Flavobacterium* regardless of diet type (Huang et al., 2024), and was significantly enriched in goldfish relative to seven other ornamental species (Xie et al., 2026). It produces vitamin B12 (Wang et al., 2022), which strengthens interactions within the gut microbiota and improves host resistance to infection (Qi et al., 2026).

3.2 Host genetics

Comparison of koi (*Cyprinus carpio*), goldfish and their reciprocal hybrids under shared rearing conditions provides an unusually clean test of host genetic

effects. Alpha diversity was highest in goldfish, followed by koi, and lowest in both hybrid crosses; beta diversity separated the groups into clear clusters, with both hybrid types resembling koi more closely than goldfish (Wang et al., 2022). Since environment and feed were held constant, the parental asymmetry points to inheritance plausibly mediated by the carnivorous dietary preference of koi versus the herbivorous preference of goldfish.

This principle that host identity, not environment, drives compositional divergence extends beyond a single species pair. Among eight ornamental species reared under identical husbandry, Xie et al. (2026) found broadly similar alpha diversity but markedly divergent genus-level profiles and predicted KEGG functions: goldfish were associated principally with quorum sensing and ABC transporter pathways, whereas crucian carp showed higher activity in amino acid biosynthesis and glycolysis.

3.3 Diet

Aydın and Çek (2026) reviewed mineral–microbiota interactions in aquaculture, highlighting that dietary minerals and gut microbiota interact bidirectionally to shape health, immunity, and performance in farmed fish, including zebrafish, a close relative of goldfish. Organic and nanoparticle forms of iron, zinc, magnesium, selenium, manganese, and copper generally offered higher bioavailability and more favorable gut outcomes than inorganic sources, with partial organic substitution (~50%) often yielding optimal results and enrichment of beneficial microbiota. However, current evidence remains limited by short trial durations and narrow species coverage.

Beyond minerals, the broader diet itself acts quickly on the goldfish gut community, though against a notably stable background. In juvenile goldfish fed pellets, live Tubifex worms (*Limnodrilus hoffmeisteri*), or both, gut communities diverged sharply by day 7, with Proteobacteria dominating in the pellet and alternating groups and Firmicutes in the Tubifex group (Huang et al., 2024). By day 14, however, these differences had largely collapsed, and all groups converged on *Cetobacterium* and *Flavobacterium*. The authors interpret this as a resilient core community that re-establishes itself after dietary perturbation, so live feeds such as Tubifex are a workable alternative in goldfish husbandry.

Other work has examined how specific dietary additives act more directly on host physiology and immune function. Mehrgan et al. (2022) fed goldfish (*C. auratus*) fry sodium butyrate (SB) and sodium propionate (SP), singly and combined, for 56 days. The combined 0.2% SB + 0.2% SP treatment produced the best growth rate, feed conversion ratio, lysozyme activity and immunoglobulin levels, with upregulated immune genes and reduced stress

markers, indicating synergistic enhancement of growth, immune function, and stress resilience.

Just as specific additives can support gut and immune health, specific ingredients can undermine it. Soybean meal, a common fishmeal replacement, induces enteritis in goldfish, with measurable damage to intestinal morphology, elevated serum D-lactate and diamine oxidase, and reduced expression of tight junction proteins (Feng et al., 2026).

3.4 Rearing environment and water chemistry

Because gut communities partly recruit from the surrounding water, husbandry system design shapes them directly. Comparing floating-raft aquaponics with conventional pond culture, Wang et al. (2022) found Proteobacteria significantly more abundant in aquaponic goldfish ($P = 0.019$), while Lactobacillaceae were enriched under traditional farming. Growth did not differ between systems, but survival was significantly higher in both aquaponic treatments ($P = 0.027$), plausibly because the plants lowered ammonia. Because aquaponics appeared to disfavour lactic acid bacteria colonisation, the authors recommend supplementing such systems with LAB.

Beyond the external rearing environment, the fish's own physiological state contributes as well. Short-term starvation in the closely related gibel carp (*C. gibelio*) reduced Chao1 richness without changing Shannon evenness, suppressed Proteobacteria including the opportunists *Aeromonas* and *Acinetobacter*, and increased Fusobacteriota and *Cetobacterium* (Liu et al., 2025).

Where husbandry and physiological state represent manageable or even adaptive pressures on the gut community, environmental contaminants can disrupt this resilience outright. In goldfish (*C. auratus*) exposed to zinc oxide nanoparticles (nZnO) for 14 days, nZnO accumulated heavily in the gut, causing villus shortening and inflammatory infiltration, while gut microbiota shifted towards fewer beneficial Fusobacteria and more pathogenic taxa (Wu et al., 2023).

A comparable pattern emerges with other environmental stressors. Zhou et al. (2021) investigated acute exposure of goldfish (*C. auratus*) to deltamethrin (DM), a widely used pyrethroid insecticide, at 0, 0.2, or 2 μg/L for 96 hours. Apoptosis and immune-related genes were altered in the kidney, while the gut showed reduced *Aeromonas* and *Cetobacterium* and increased *Akkermansia*. Even sublethal, environmentally relevant DM concentrations can therefore disrupt kidney gene expression and gut microbial balance.

Nitrogenous water-quality stressors produce a related, if more tissue-specific, effect. Ortiz et al. (2023) examined how sublethal concentrations of nitrite (0.1

mM and 1.0 mM) affect the tissue microbiomes of goldfish (*C. auratus*) over 2 months. While the water microbiome remained unchanged, the nasal, skin and gill microbiomes were all significantly altered; the nasal microbiome was the most sensitive. These shifts occurred without visible signs of stress, suggesting that sublethal nitrite can disrupt host-associated microbiomes and potentially increase disease susceptibility.

Beyond dissolved contaminants, particulate pollutants pose a distinct and increasingly recognised threat. Kim et al. (2025) investigated how polystyrene microplastics (0.5 μm and 2 μm) affect gill and intestinal health and pathogen susceptibility in goldfish (*C. auratus*). After two weeks, microplastics had accumulated in gill and gut tissues, indicating inflammation. The 0.5 μm particles triggered stronger immune- and oxidative stress-related responses than the 2 μm particles, and when challenged with *Edwardsiella piscicida*, fish exposed to 0.5 μm microplastics showed significantly reduced survival, highlighting a size-dependent risk of microplastic pollution for fish health.

Set against this run of contaminant-driven damage, not all external inputs are harmful; some can actively support gut and systemic health. Nuntakad et al. (2025) fed goldfish (*C. auratus*) diets containing *Rosa rubiginosa* (sweetbriar rose) petal powder for eight weeks. Inclusion at 20–40 g/kg significantly improved growth performance, feed efficiency, survival, skin redness and yellowness and antioxidant capacity and intestinal gene expression, and shifted gut microbiota composition, showing that rose petals can act as a functional feed additive enhancing health, immunity, and ornamental coloration.

4. DISEASE AND DYSBIOSIS

4.1 Ectoparasites, Bacterial and Viral Infection,

Taken together, these dietary and probiotic studies raise a broader question: how does the gut microbiome shape resistance to disease under real infection pressure? Recent studies address this from complementary angles: nutritional management, ectoparasitic challenge, and bacterial pathogenesis.

Aydın & Çek-Yalnız (2023) present zebrafish (*Danio rerio*), a close relative of *C. auratus*, as a widely used model organism, with established value for studying disease mechanisms in humans and identifying new therapeutic strategies. The authors compile the latest findings on bacterial infections in fish where zebrafish has served as the model.

The first disease-specific angle concerns nutritional management under viral challenge. Liu et al. (2025) examined the impact of short-term starvation on the gut microbiota and intestinal tissue of gibel carp (*C. gibelio*) during infection with Cyprinid herpesvirus-2 (CyHV-2), a pathogen that causes mortality of 90–100%

in goldfish, crucian carp, and gibel carp. Infection alone reduced the beneficial genus *Cetobacterium*, promoted the opportunist *Vibrio*, and damaged intestinal tissue. Short-term starvation prior to infection increased *Cetobacterium* while suppressing opportunists such as *Aeromonas*, and these protective shifts persisted after infection, preserving villus length and intestinal wall thickness. As feed withdrawal is already routine during outbreaks, these findings offer a mechanistic rationale for the practice.

Ectoparasitic infection presents a different kind of challenge to the gut-microbiome axis, one that originates outside the gut entirely. Bu et al. (2025) investigated the effects of the ectoparasite *Ichthyophthirius multifiliis*, the causative agent of white spot disease, on goldfish (*C. auratus*). Gill damage was severe and gill microbiota diversity fell, while gut community diversity remained largely stable, yet opportunistic pathogens such as *Aeromonas* and *Achromobacter* still increased within the intestine. Primary parasitism of an external surface thus raised the internal pathogen burden without registering as a shift in gut diversity, which argues for taxon-level rather than diversity-level monitoring in aquaculture.

Where ectoparasitism illustrates how an external challenge can quietly elevate gut pathogen load, bacterial infection shows how pre-existing gut community structure can determine disease outcome outright. *Aeromonas hydrophila* remains the principal bacterial threat to cyprinids. Qi et al. (2026) found that the clinical outcome of *A. hydrophila* infection in crucian carp (*C. auratus*) was tightly coupled to baseline gut community structure. Mild-symptom fish were enriched in *Cetobacterium*, *Pseudomonas*, and *Paraclostridium*, taxa that correlated negatively with infection severity and intestinal *Aeromonas* load and positively with barrier gene expression, whereas severely affected fish showed gut networks dominated by *Aeromonas*, downregulated barrier genes (*zo-1*, *occludin*, *claudin12*), and epithelial detachment around the intestinal villi.

That this relationship is causal was demonstrated through faecal microbiota transplantation (FMT): recipients of intestinal contents from mild-symptom donors showed milder clinical signs, lower *Aeromonas* loads, restored barrier gene expression, and markedly higher survival (66.7% versus 36.7% in untreated controls). A simplified synthetic community of the three protective taxa *Cetobacterium*, *Paraclostridium*, and *Pseudomonas* was then constructed and shown to enhance host resistance. Together with the studies above, these findings highlight microbiome-based therapies as alternatives to antibiotics and underscore that the gut microbiome is not merely a passive marker of fish health but an active, manipulable determinant of infection outcome.

4.2 Dysbiosis without a pathogen: the ornamental supply chain

In contrast to these beneficial dietary interventions, antibiotic exposure has repeatedly been shown to destabilize the goldfish gut microbiome, illustrating that disruption need not come from a pathogen at all. Jia et al. (2020) exposed goldfish (*C. auratus* Linnaeus) to environmentally relevant concentrations of tetracycline (0.285 and 2.85 μg/L) for 7 and 21 days. The proportion of tetracycline-resistant bacteria rose from 1.02% in controls to over 60% in the high-concentration groups, *Aeromonas* spp. diversity decreased while their tolerance increased in a time- and dose-dependent manner, and resistance genes, particularly efflux pump genes, became more abundant. Even low levels of tetracycline pollution can thus reshape gut microbiota and accelerate antibiotic resistance.

What Jia et al. demonstrated under controlled experimental dosing, the ornamental supply chain reproduces and dramatically amplifies under real-world conditions. Bohra et al. (2026) reported that chronic antibiotic use in the ornamental fish trade severely disrupts the gut microbiota of *C. auratus*). Retail-sourced goldfish from Hong Kong exhibited an extreme collapse in microbial diversity (mean Shannon index of 0.107 ± 0.141), far lower than in healthy controls or experimentally exposed fish such as those in Jia et al.'s study. The residual gut community consisted almost exclusively of *Cetobacterium* and *Vibrio*, with a near-total loss of beneficial commensals. The authors propose this as an explanation for the widely reported post-purchase die-off of retail goldfish and call for reduced antimicrobial reliance across the global ornamental trade.

5. INTERVENTIONS: FEEDING THE GUT

5.1 Probiotics

Aydın (2023) notes that in aquaculture, environmentally friendly feed additives such as probiotics, prebiotics, and synbiotics have been increasingly used as alternatives to antibiotics. Dead microbial cells encountered during and after probiotic production, or the components released when they break down, can still confer beneficial effects on the host, which has led to the terms "postbiotic" and "paraprobiotic." The review covers their definitions, production methods, and applications in aquaculture as more stable, infection-risk-free alternatives to live probiotics.

Complementing this focus on non-viable alternatives, Aydın & Çek-Yalnız (2019) note that probiotics are already widely used in aquaculture for growth promotion, stress tolerance, pathogen inhibition, and improving water quality, but far fewer studies have examined their effects on reproductive performance and gamete quality in fish.

Figure 1 summarizes the current applications and potential mechanisms of probiotics and their non-viable alternatives in aquaculture. Recent studies in goldfish further demonstrate the beneficial effects of *Escherichia coli* Nissle 1917, host-derived *Cetobacterium somerae*, and multi-strain bacterial consortia on intestinal health, antioxidant capacity, immune responses, microbial balance, and disease resistance (Figure 1).

Live and host-derived bacteria have in fact been the most successful intervention class studied to date. *Escherichia coli* Nissle 1917 fed to goldfish at 10^6–10^8 CFU/g for 80 days improved growth, raised gut lysozyme, immunoglobulin and total protein, upregulated *lysozyme*, *il-1β*, *iNOS* and *tnf-α*, and improved intestinal absorptive surface area and goblet cell density, with the best results and significantly higher survival after *A. hydrophila* challenge occurring at 10^8 CFU/g (Nofouzi et al., 2024).

Host-derived strains may be better still. *Cetobacterium somerae* LNUB571, isolated from fish, markedly alleviated soybean meal-induced enteritis over an eight-week trial: intestinal morphology improved, inflammation and serum D-lactate and DAO fell, and ZO-1, Occludin and Claudin-1 expression rose ($P < 0.05$) (Feng et al., 2026). Strain-level analysis confirmed successful colonisation even though total *Cetobacterium* abundance was unchanged, a point genus-level 16S surveys would have missed.

The synthetic community approach takes this further still. Guided by the FMT results described above, Qi et al. (2026) assembled a three-strain consortium of *Cetobacterium somerae* S3, *Pseudomonas stutzeri* J1 and *Paraclostridium bifermentans* M1 and fed it for 28 days. Intestinal SOD and CAT rose by 66.01% and 24.82%, barrier gene expression was upregulated, and survival after challenge improved from 32.5% to 60%. The proposed mechanism is metabolic complementarity, with B12 from *C. somerae* supporting biosynthesis in partner taxa and *P. bifermentans* generating short-chain fatty acids.

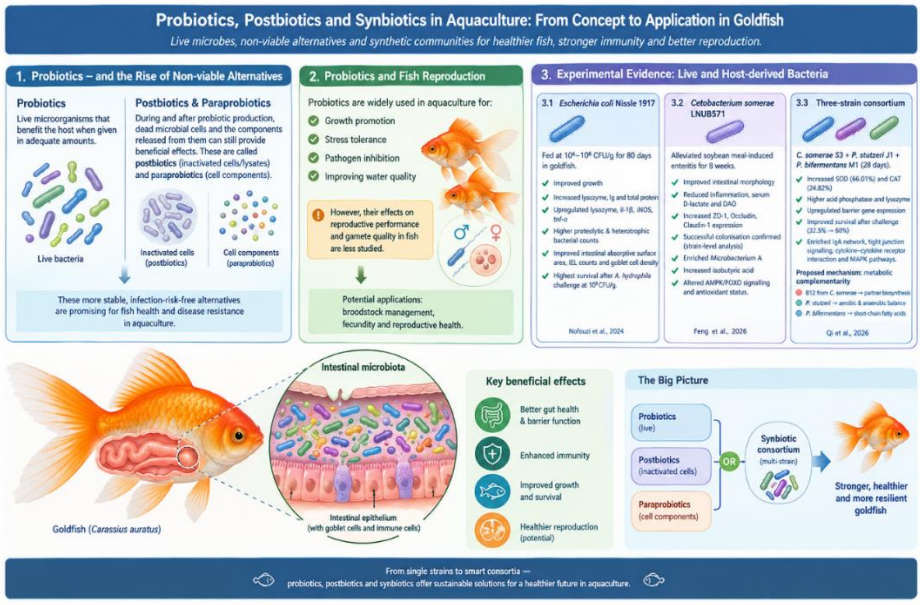


Figure 1. Overview of probiotics, postbiotics, paraprobiotics, and multi-strain microbial consortia in aquaculture, highlighting their effects on intestinal microbiota, gut barrier integrity, immune responses, growth, survival, and potential reproductive performance in goldfish.

5.2 Prebiotics and phytogenics

Concentrated mannan oligosaccharide (cMOS) fed for 60 days produced longer gill lamellae, more mucus cells and higher Muc-2 expression in goldfish,. After *I. multifiliis* challenge, cMOS-fed fish showed mortality of 10% against 43.33% in controls and higher IgT in gill and skin (Huang X. et al., 2022). The proposed mechanism, enhanced mucin secretion reducing ectoparasite adhesion, is consistent with the goblet cell gradients described in Section 2.

Where cMOS acts primarily through barrier and mucosal defence, plant-derived additives address the ornamental sector's dual demand for health and appearance. *Rosa rubiginosa* petal powder at 40 g/kg increased weight gain, raised skin redness and yellowness, improved serum antioxidant status, upregulated immune and growth genes, and shifted gut microbiota beta-diversity ($P = 0.017$) (Nuntakad et al., 2025).

Additive classes can also be combined, raising the question of whether their benefits are simply additive or genuinely synergistic. Chlorogenic acid (0.5 g/kg) combined with *Saccharomyces boulardii* (10^{10} CFU/g) over eight weeks produced the highest weight gain, the lowest feed conversion ratio, and the lowest mortality after *A. hydrophila* challenge (Ahmadifar et al., 2026). Synergy was specific rather than general: it appeared for *sod* and *gst* but not for *cat*, and *igf-1*, *mn-sod*, *il-6* and

tgf-β were unaffected. Combination products should therefore be evaluated endpoint by endpoint.

6. GENOME EDITING IN GOLDFISH

Alongside microbial and dietary approaches, genome editing has begun to reshape the ornamental phenotypes to which gut-health research is ultimately applied. Goldfish are allotetraploid, and their duplicated gene families complicate functional analysis. CRISPR/Cas9 disruption of both duplicated tyrosinase genes, *tyrA* and *tyrB*, eliminated melanocytes from the eyes and body surface and produced a golden goldfish, with no off-target mutations at predicted sites (Li et al., 2024). Broader reviews place this within a developing toolkit targeting *Tyr*, *Mc1r* and *Slc45a2* for stable, uniform colour modification, in contrast to the slow and genetically ambiguous results of selective breeding (Priyanka et al., 2025).

Editing is most useful when it can be applied quickly to causal genes, as shown by Yu et al. (2022). They combined CRISPR/Cas9 with artificial gynogenesis and temperature-induced sex reversal to establish homozygous mutants within two generations (approximately eight months), compared with 7–10 generations for conventional breeding. They confirmed *lrp2aB* as the causal gene for the dragon-eye phenotype and showed that albinism requires both *oca2* homeologs, which have subfunctionalized.

The same causal-gene logic has been applied to skeletal traits, particularly those governed by the *chordin* homolog *chdS*. Chen et al. (2022) asked whether *chdS*, already known to cause the twin-tail phenotype, also underlies the dorsal-finless phenotype of strains such as Ranchu. Backcrossing and injection of $chdS^{wt}$ mRNA into Ranchu embryos showed that the wild-type allele strongly suppressed dorsal-finlessness, indicating pleiotropic effects on the median fins. The authors proposed that $chdS^{wt}$ acted as a buffering (capacitor) gene that masked accumulating mutations until its function was lost in twin-tail lineages, which may explain the rapid fixation of both traits during domestication.

Whereas Chen et al. (2022) explained how *chdS* affects multiple fins, Lee et al. (2024) addressed why only a single allele, $chdS^{E127X}$, underlies the twin-tail phenotype. Two CRISPR/Cas9 alleles (CR0 and CR1) proved equivalent to E127X in penetrance, expressivity, and carrier viability, indicating that multiple truncating mutations could have produced viable twin-tail goldfish. The authors concluded that the absence of polymorphic alleles most likely reflects stochastic elimination or the lack of competing alleles in the common ancestor, rather than any advantage of E127X.

Beyond creating and explaining phenotypes, breeders also need practical tools to track them. Chen et al. (2026) validated variants in five genes, *Kcnk5b* (caudal fin

length), *Bace1-like* (scale transparency), *Lrp2aL* (eye morphology), *ChdA* (dorsal fin number), and *Lrp6S* (dorsal fin morphology), and converted them into Kompetitive Allele-Specific PCR (KASP) markers. The assays showed complete concordance with Sanger sequencing in 100 fish from six breeds and matched phenotypes in a further 102 fish from three farms. The authors cautioned that these loci are probably major-effect variants rather than sole determinants, and proposed the KASP toolkit for marker-assisted selection and germplasm purity control.

These lines of work are not independent. Pigmentation can be pursued genetically (Li et al., 2024; Priyanka et al., 2025) or nutritionally, via carotenoid-rich additives that simultaneously modulate immunity and gut microbiota (Nuntakad et al., 2025). Likewise, disease can be managed with antibiotics, at the cost of the microbiome collapse documented in retail supply chains (Bohra et al., 2026), or with microbiome-based strategies that raise survival without the same collateral damage (Qi et al., 2026; Nofouzi et al., 2024). Collectively, these advances demonstrate the growing potential of genome editing and molecular breeding to modify pigmentation, body morphology, and other ornamental traits in goldfish, while enabling rapid strain development and genotype-assisted selection (Fig. 2).

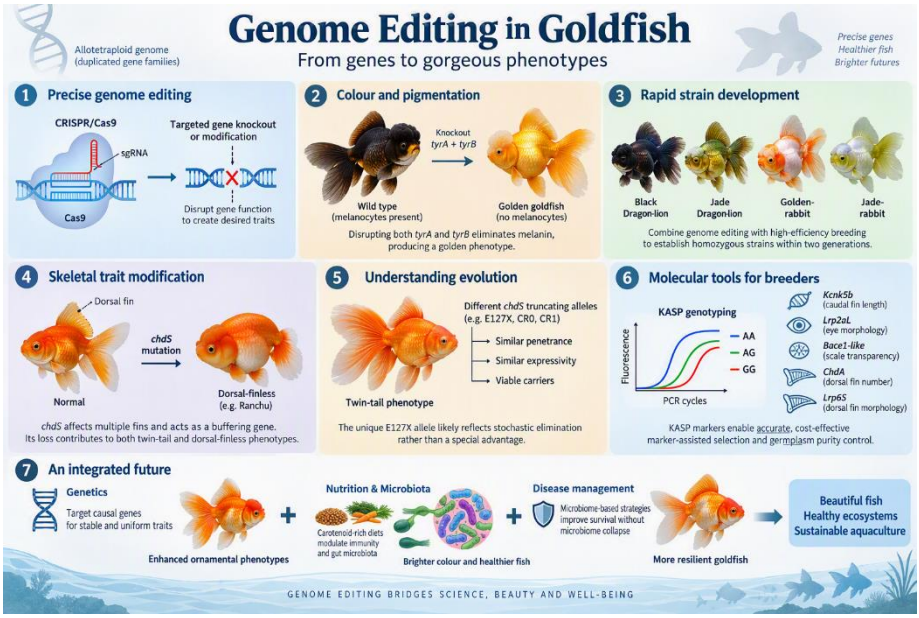


Figure 2. Genome editing in goldfish: from causal genes to ornamental phenotypes. Integration of CRISPR/Cas9 genome editing, accelerated breeding, functional genetic analysis, and KASP-based marker-assisted selection for the modification and tracking of pigmentation, eye, fin, and body-shape traits in goldfish.

7. CONCLUSIONS AND RESEARCH PRIORTIES

The goldfish has travelled a long way from ornamental curiosity to biotechnological model. This chapter followed that shift from three angles that seem unrelated at first: the structure and immunology of the gut, the microbial community it hosts, and the genetic tools now reshaping the fish's appearance. Read together, they point to one message. The goldfish is best understood, and best managed, as an integrated system in which anatomy, microbes, environment and genome continually influence one another.

The goldfish intestine is a regionalised organ. Its fold architecture, muscular gradients and goblet cell distribution reflect a division of labour between absorption, mixing and mucosal protection, and its epithelium is part of an active neuroimmune network, the setting in which the microbiota operates. That community has a conserved core, with *Cetobacterium* as its signature member, while its relative composition shifts with host genotype, diet, water quality and disease state. It is resilient to short-term dietary perturbation, but far less so to chronic pressures such as contaminants, pathogens and, above all, antibiotics, as the collapse of gut diversity in retail-sourced fish illustrates (Bohra et al., 2026).

Importantly, the microbiota is not merely a marker of health. Transplantation and defined-consortium experiments show that it is an active and manipulable determinant of infection outcome (Qi et al., 2026). This is the basis for the interventions reviewed here: host-derived probiotics, synthetic consortia, prebiotics, phytogenics and their combinations, which improve growth, mucosal defence and survival without the collateral damage of antibiotics. Because the same additives can also improve coloration, health and appearance can increasingly be pursued together. Genome editing and marker-assisted selection now allow pigmentation, body shape and fin morphology to be modified precisely and within a few generations rather than a few decades. Several gaps stand in the way of turning this knowledge into practice.

Longer and more realistic trials. Most feeding studies last only weeks and are run under laboratory conditions; long-term microbiome stability, probiotic colonisation and performance in commercial and recirculating systems remain largely untested.

Higher-resolution methods. Genus-level 16S surveys can miss strain-level colonisation entirely (Feng et al., 2026). Strain-resolved metagenomics, metabolomics and single-cell approaches should become standard, and functional predictions based on limited fish reference genomes should be interpreted with caution.

Mechanisms in vivo. Cross-feeding, signalling and niche partitioning within synthetic consortia have been inferred rather than measured, and how microbial metabolites interact with the enteric neuroimmune network is unexplored.

The supply chain as a research object. Antibiotic-driven dysbiosis has been documented in a limited number of samples. Systematic, multi-market surveillance is needed, together with studies on microbiome recovery and on alternatives to prophylactic antibiotics.

Linking genotype to gut. Almost nothing is known about whether edited or selectively bred lines differ in gut structure, microbiota or disease resistance. Answering this would connect the two halves of the field, alongside attention to animal welfare, biosecurity and regulatory frameworks for edited ornamental fish.

Standardisation. Common protocols for sampling, sequencing, reporting and product evaluation, and evidence that meets regulatory requirements, are needed before microbiome-based products can be adopted with confidence.

Meeting these priorities would allow the field to move from descriptive surveys to predictive, evidence-based management. The goal is a goldfish industry that is more sustainable, more welfare-conscious and better able to deliver the resilient, high-value phenotypes that its markets demand.

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