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Review Article

APPLICATIONS OF ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN PLANT TISSUE CULTURE

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ABSTRACT. Plant tissue culture is a cornerstone of modern plant biotechnology, enabling rapid clonal propagation, germplasm conservation, and the controlled study of morphogenetic and stress-related responses. However, the multifaceted and highly nonlinear nature of in vitro systems, involving complex interactions among genotype, culture media composition, plant growth regulators, and environmental conditions, often limits the efficiency and reproducibility of conventional experimental approaches. In recent years, artificial intelligence (AI) and machine learning (ML) techniques have emerged as powerful tools for modeling, predicting, and optimizing tissue culture responses, complementing and, in some contexts, outperforming traditional statistical methods. This review provides a comprehensive and critical synthesis of AI- and ML-based applications in plant tissue culture, with particular emphasis on artificial neural networks, support vector machines, random forest models, k-nearest neighbors, genetic algorithms, and hybrid optimization frameworks. We examine their use across key application domains, including micropropagation efficiency prediction, culture medium and hormone optimization, assessment of abiotic stress tolerance, secondary metabolite production, image-based phenotyping, and control of automated and bioreactor-based culture systems. The strengths, limitations, and data requirements of each modeling approach are discussed in the context of biological interpretability, model robustness, and experimental reproducibility. In addition, the review explores emerging directions such as quantum machine learning and explainable AI, highlighting their potential contributions while critically addressing current technical and practical constraints. Finally, we outline future perspectives for integrating AI-driven decision support systems into plant tissue culture research and production, aligning these advances with the broader framework of Plant Biotechnology 5.0. By consolidating methodological insights and identifying existing gaps, this review aims to guide researchers toward more efficient, transparent, and data-driven in vitro culture strategies.

Keywords: *Artificial intelligence; machine learning; plant tissue culture; in vitro optimization; predictive modeling*

INTRODUCTION

Plant tissue culture has been a cornerstone of plant biotechnology since the early twentieth century, enabling the regeneration of whole plants from isolated cells and tissues based on the principle of cellular totipotency [77]. This technological advancement has supported a wide range of applications, including the micropropagation of elite genotypes, conservation of genetic resources, production of pathogen-free planting material, and controlled synthesis of secondary metabolites [26,47]. Consequently, plant tissue culture has become an indispensable tool in fundamental research, commercial horticultural, and agricultural production systems.

Despite its widespread application, plant tissue culture remains a highly complex biological system in which morphogenic outcomes are governed by nonlinear interactions among genotype, culture medium composition, plant growth regulators, and environmental conditions. Traditional optimization strategies have largely relied on single-factor experimental designs and linear statistical analyses. Although historically valuable, these approaches are increasingly recognized as insufficient for capturing the multivariate and nonlinear nature of in vitro responses, often leading to prolonged trial-and-error experimentation and limited reproducibility [32].

Recent advances in digital agriculture and computational biology have accelerated the integration of artificial intelligence (AI) and machine learning (ML) into plant biotechnology research. AI-based approaches enable the analysis of large, multidimensional datasets, identification of hidden patterns, and modeling of complex biological interactions that are difficult to resolve using classical statistical frameworks [18,23]. In this context, machine learning algorithms such as artificial neural networks, support vector machines, and ensemble-based methods have been increasingly applied to predict morphogenic responses, optimize culture conditions, and enhance experimental consistency in plant tissue culture systems.

Accumulating evidence demonstrates that AI-assisted models can substantially improve the prediction of optimal in vitro conditions, reduce experimental workload, and enhance the efficiency and reproducibility of micropropagation protocols [64,74]. By simultaneously evaluating multiple input variables, these models provide more accurate estimations of complex responses related to shoot proliferation, rooting, and biomass accumulation, thereby reducing both time and material costs. As a result, AI-driven approaches are emerging as practical decision-support tools in modern in vitro production systems.

The growing adoption of AI and ML in plant tissue culture is driven by increasing system complexity, high biological variability, and the economic and temporal limitations of conventional optimization strategies. Beyond protocol optimization, machine learning has facilitated the identification of previously unrecognized relationships between environmental factors and plant developmental responses, expanding its relevance to stress physiology, secondary metabolite production, and molecular breeding [32,34]. The integration of AI into plant tissue culture aligns closely with the broader framework of digital agriculture, where data-driven decision-making and automation play central roles. This interdisciplinary convergence enhances scalability, reproducibility, and system-level understanding of in vitro production processes, while also contributing to the development of climate-resilient and stress-tolerant plant production strategies [23,28].

This review summarizes recent progress in using artificial intelligence and machine learning in plant tissue culture. It critically examines popular algorithms, their specific applications, advantages, and drawbacks. Additionally, it discusses methodological challenges and identifies research gaps, offering a structured foundation to guide future development of data-driven, intelligent tissue culture systems in plant biotechnology.

OVERVIEW OF ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING

Artificial Intelligence in Plant Biotechnology

AI refers to a broad class of computational approaches designed to emulate aspects of human cognition, including learning, pattern recognition, and decision-making, through data-driven algorithms. Rather than functioning solely as rule-based systems, modern AI frameworks extract knowledge from large and complex datasets to support adaptive and predictive decision-making across diverse scientific domains [80].

In agricultural biotechnology, AI has increasingly been adopted to address challenges associated with data complexity, system variability, and operational efficiency. Applications range from predictive modeling and automated control systems to advanced image-based analyses and decision-support platforms [20]. By enabling rapid processing of multidimensional datasets, AI-based systems substantially reduce the time required for data interpretation while improving analytical precision. Within the context of plant tissue culture, AI facilitates more accurate regulation of culture media, continuous monitoring of developmental processes, and objective assessment of production performance. In particular, image-based AI systems allow the detection and quantification of subtle morphological variations that are difficult to evaluate reliably through manual observation, thereby reducing operator-dependent bias and experimental error [49].

Machine Learning as a Core Component of AI

ML, a central subfield of AI, focuses on algorithms that learn patterns directly from data and iteratively improve their predictive performance without explicit rule-based programming. Unlike traditional computational approaches, ML models infer relationships among variables through exposure to experimental data, making them especially suitable for biological systems characterized by high variability and nonlinear interactions [31].

In plant tissue culture research, ML has been widely applied to optimize culture medium composition, predict micropropagation efficiency, and model quantitative growth parameters such as shoot proliferation and rooting responses. By enabling the simultaneous evaluation of multiple experimental variables, ML-based models accelerate the identification of optimal conditions and reduce reliance on labor-intensive trial-and-error methodologies. This capability has proven particularly valuable for improving experimental efficiency, conserving resources, and enhancing reproducibility in in vitro culture systems [41].

Deep Learning

Deep learning (DL) represents an advanced subset of machine learning that is based on multilayer artificial neural network architectures capable of automatically learning hierarchical feature representations from data. By processing information through multiple hidden layers, DL models can capture complex, nonlinear relationships and extract high-level abstractions from large and heterogeneous datasets, making them particularly effective for high-dimensional data analysis [60]. Within plant tissue culture research, deep learning has emerged as a powerful tool for image-driven applications that require high precision and objectivity. DL-based approaches have been applied to the automated classification of callus development stages, quantitative assessment of somatic embryogenesis, embryo counting, and image-based evaluation of morphological traits. These applications enable the rapid and standardized analysis of large image datasets generated under controlled in vitro conditions, substantially reducing subjectivity and operator-dependent variability associated with manual assessments [36]. The capacity of deep learning models to process extensive image repositories and generate consistent analytical outputs has significant implications for commercial micropropagation systems. By supporting real-time or high-throughput phenotyping, DL contributes to improved quality control, enhanced reproducibility, and greater scalability of tissue culture operations. As production systems increasingly rely on automated monitoring and data-driven decision-making, deep learning-based image analysis is expected to play a central role in the standardization and industrial-scale implementation of plant tissue culture technologies [71].

RATIONALE FOR THE USE OF ARTIFICIAL INTELLIGENCE IN PLANT TISSUE CULTURE

Plant tissue culture represents a highly complex and dynamic biological system in which the simultaneous interaction of multiple factors, including culture medium composition, plant growth regulators, genotype, environmental conditions, and stress stimuli, governs morphogenic outcomes. These interactions are inherently nonlinear and context-dependent, making the reliable prediction of in vitro responses and the efficient optimization of protocols particularly challenging. Classical experimental strategies, especially the widely used “one factor at a time” approach, are limited in their ability to capture such multidimensional interactions and frequently overlook subtle yet biologically significant interdependencies among variables [83].

Traditional statistical methods applied in tissue culture research typically assume that variables are linear and independent. While these approaches have contributed substantially to early protocol development, they are often inadequate for modeling the high-dimensional and nonlinear relationships characteristic of in vitro systems. As a result, protocol optimization often relies on prolonged trial-and-error experimentation, resulting in increased time, labor, and resource consumption, as well as limited reproducibility across genotypes and laboratories [36].

AI and ML approaches offer a robust alternative framework that can overcome these limitations. By simultaneously analyzing multiple input variables, AI-based models can capture complex interaction networks and identify patterns that remain inaccessible to conventional analytical methods. Algorithms such as artificial neural networks, support vector machines, decision tree-based ensembles, fuzzy logic systems, and evolutionary optimization techniques have demonstrated strong performance in modeling nonlinear biological processes and predicting in vitro developmental responses [49]. These models have been successfully applied to predict key outcomes such as callus induction, somatic embryogenesis, shoot proliferation, and rooting efficiency with high accuracy, even under variable experimental conditions.

Beyond predictive capability, the integration of AI into plant tissue culture offers substantial practical advantages. AI-assisted modeling significantly reduces experimental workload by narrowing the experimental search space and minimizing the number of wet-lab trials required to identify optimal conditions. This leads to measurable reductions in time, labor, and material costs, while simultaneously accelerating protocol development and facilitating rapid scale-up. Such efficiency gains are particularly valuable in the micropropagation of commercial crops and the in vitro production of medicinal and aromatic plants, where consistency, throughput, and cost-effectiveness are critical [20].

Significantly, AI-based models also enhance robustness and standardization in tissue culture workflows. By accounting for biological variability and unexpected fluctuations in culture conditions, predictive models improve outcome precision and support quality control in both research-oriented and industrial-scale production systems. This capability contributes directly to improved reproducibility, a persistent challenge in plant tissue culture applications.

Collectively, the integration of artificial intelligence into plant tissue culture represents a paradigm shift from linear, empirically driven experimentation toward a data-driven, predictive, and system-oriented framework. As experimental systems become increasingly complex and production demands continue to rise, AI-supported approaches are emerging as indispensable tools for optimizing culture conditions, reducing resource waste, and supporting sustainable large-scale plant production within modern agricultural biotechnology [41].

FUNDAMENTAL ARTIFICIAL INTELLIGENCE METHODS USED IN PLANT TISSUE CULTURE

Artificial Neural Networks (ANN)

Artificial neural networks (ANNs) are data-driven modeling tools that learn complex, nonlinear input-output relationships through interconnected layers of artificial neurons. Their capacity to capture multivariate and nonlinear interactions makes them particularly suitable for plant tissue culture systems, where morphogenic responses are jointly regulated by culture medium composition, plant growth regulators, and environmental conditions [85].

In contrast to classical regression approaches, which often fail to represent the multifactorial nature of in vitro processes, ANN models learn directly from experimental data and provide accurate predictions for key developmental traits, including shoot proliferation, rooting efficiency, callus induction, and somatic embryogenesis [24]. This predictive capability enables substantial reductions in experimental trial numbers, supporting the development of decision-support systems for protocol optimization.

ANN-based models have proven particularly effective in complex processes such as in vitro rooting, where small variations in hormonal balance or environmental conditions can produce disproportionate biological responses [31]. Despite their strong predictive performance, ANN models are often limited by reduced interpretability and sensitivity to data quality, underscoring the need for careful model validation in biological applications.

Support Vector Machines (SVM)

Support vector machines (SVM) are supervised learning algorithms widely used for classification and regression tasks involving high-dimensional and nonlinear datasets. By maximizing the separation margin between data classes and employing kernel functions to map nonlinear relationships into higher-dimensional spaces, SVM models provide robust predictive performance in biologically complex systems [25].

In plant tissue culture, SVM has been successfully applied to classify in vitro responses such as callus formation, shoot regeneration, and rooting success, as well as to predict quantitative traits including shoot length and biomass accumulation [64]. A key advantage of SVM lies in its ability to maintain high generalization performance with relatively small datasets, which is particularly relevant for tissue culture experiments where data availability is often limited.

Compared with ANN models, SVM is generally less prone to overfitting and requires fewer hyperparameters, although its performance remains sensitive to kernel selection and parameter tuning. These characteristics make SVM a reliable component of decision-support systems aimed at optimizing micropropagation protocols while enhancing reproducibility and operational efficiency. For example, Isak et al. [28] investigated the effects of cadmium (Cd) stress on the micropropagation of *Lycium barbarum* L. across three genotypes (ERU, NQ1, and NQ7) using multiple machine learning algorithms, including Multilayer Perceptron (MLP), SVM, Random Forest (RF), Gaussian Process (GP), and XGBoost. While SVM effectively captured nonlinear genotype-specific responses to Cd stress, MLP models achieved higher prediction accuracy, indicating a stronger capacity to model complex physiological responses under heavy metal stress conditions.

Random Forest (RF)

Random forest (RF) is an ensemble learning approach that integrates multiple decision trees to improve prediction accuracy and model stability. By combining bootstrap sampling with random feature selection, RF effectively captures nonlinear interactions among variables while minimizing overfitting, even in noisy or small biological datasets [2].

In plant tissue culture research, RF has been widely used to predict morphogenic responses such as shoot proliferation, rooting percentage, and biomass accumulation. A major strength of RF lies in its ability to rank variable importance, thereby identifying key factors, such as specific phytohormones, explant type, or nutrient components, that drive in vitro responses [29]. For instance, Aasim et al. [1] applied RF alongside other artificial intelligence-based models to optimize an in vitro regeneration protocol of Sorghum bicolor using direct organogenesis from mature zygotic embryos under varying cytokinin-auxin combinations. While RF demonstrated strong predictive capability for shoot count and shoot length (R^2 up to 0.786), Multilayer Perceptron (MLP) models achieved higher prediction accuracy, indicating a superior ability to capture complex nonlinear interactions among plant growth regulators.

Beyond prediction, RF contributes to experimental design optimization and quality control by revealing dominant variables and their relative influence on biological outcomes. Compared with ANN models, RF offers improved interpretability, making it particularly valuable for translational applications and commercial micropropagation systems where both accuracy and biological insight are required [64].

K-Nearest Neighbors (KNN)

K-Nearest Neighbors (KNN) is a non-parametric, instance-based learning algorithm that predicts class labels or continuous values by evaluating the similarity between an unknown sample and its closest neighbors in the feature space. Due to its model-free structure, KNN is particularly suitable for biological systems characterized by nonlinear relationships and heterogeneous data distributions [6].

In plant tissue culture research, KNN has been applied to both classification and regression tasks, including the prediction of callus induction, shoot regeneration, rooting success, and quantitative growth traits such as shoot number, shoot length, and root length [5,66]. Its ability to simultaneously consider multiple morphological parameters allows KNN to capture local response patterns associated with differences in phytohormone concentration and culture medium composition, making it useful for exploratory modeling and protocol screening.

However, comparative evidence suggests that KNN may exhibit lower predictive performance when modeling complex, multivariate in vitro datasets. For instance, Demirel et al. [16] investigated the micropropagation of *Aronia melanocarpa* under different culture media (MS and WPM) and varying indole-3-butyric acid (IBA) concentrations, using several machine learning models to predict key morphogenic parameters, including root and shoot traits. Their results demonstrated that XGBoost and SVM outperformed KNN, achieving higher R^2 values across most growth variables, while KNN showed comparatively weaker predictive accuracy. These findings indicate that although KNN can capture local similarities among observations, it may be less effective than tree-based ensemble or margin-based algorithms in modeling complex nonlinear interactions between culture medium, hormone dosage, and plant developmental responses.

Despite these limitations, KNN offers advantages in terms of transparency and ease of implementation, as predictions are directly derived from observed samples rather than learned model parameters [67]. Nonetheless, its sensitivity to feature dimensionality and distance metric selection, along with the increasing computational cost associated with larger datasets, restricts its scalability in high-throughput micropropagation studies [46,79]. Consequently, KNN is best employed as a complementary or benchmarking method within integrated modeling frameworks rather than as a standalone predictive tool.

KNN remains a useful and interpretable machine learning approach for preliminary analysis and comparative evaluation in plant tissue culture research, particularly when combined with more advanced algorithms such as XGBoost or SVM to enhance predictive robustness and decision-making accuracy.

Quantum Machine Learning (QML)

Quantum machine learning (QML) represents an emerging frontier that integrates principles of quantum computing with machine learning algorithms to address computationally complex optimization and classification problems. By exploiting quantum properties such as superposition and entanglement, QML has the potential to explore solution spaces more efficiently than classical machine learning approaches, particularly in high-dimensional, nonlinear, and combinatorial problems common in biological systems [3].

While early QML applications in plant science were largely conceptual or limited to quantum-inspired models, recent studies have begun to demonstrate its practical feasibility. Notably, Katırcı et al. [40] applied a hybrid quantum-classical learning framework to optimize in vitro shoot proliferation in common bean (*Phaseolus vulgaris* L.), a crop historically constrained by inefficient regeneration protocols. In their study, the combined effects of potassium nitrate (KNO_3), auxins (IBA and NAA), and explant type were evaluated, followed by optimization using both classical and quantum machine learning models. A custom quantum circuit incorporating RX, RZ, and Hadamard gates was designed to exploit quantum superposition and entanglement, resulting in superior predictive performance for shoot count classification (accuracy: 83%; F1 score: 84%) and comparable performance for shoot length prediction relative to classical models. These results highlight the capacity of QML to capture complex nutrient-hormone-explant interactions that are difficult to model using conventional approaches. Beyond predictive accuracy, the hybrid quantum-classical framework reduced experimental uncertainty and enhanced nutrient-hormone optimization, demonstrating tangible benefits for experimental design in plant tissue culture. Such findings extend earlier quantum-inspired applications in plant disease classification and genotype-phenotype prediction [4] and provide one of the first applied demonstrations of QML in in vitro regeneration research.

Despite these promising developments, QML remains at an early stage of adoption in plant biotechnology. Current limitations include restricted access to quantum hardware, scalability challenges, and the lack of standardized biological datasets optimized for quantum computation [42]. Consequently, QML should currently be viewed as a complementary approach rather than a replacement for classical machine learning methods.

Emerging evidence, including the work of Katırcı et al. [40], suggests that hybrid quantum-classical learning frameworks hold significant long-term potential for tissue culture optimization. Continued advances in quantum hardware, algorithm development, and the design of biologically relevant datasets are likely to position QML as a valuable component of next-generation data-driven plant biotechnology and micropropagation systems.

Genetic Algorithms (GA)

Genetic Algorithms (GA) are an evolutionary computation-based approach that imitates natural selection processes in order to solve complex optimization problems encountered in plant tissue culture. In this context, GA begins by operating on an initial population consisting of candidate solutions, represented as “chromosomes” which simultaneously explore different regions of the solution space [84]. Each chromosome represents a potential set of parameters, such as the concentrations of culture medium components, phytohormone combinations, and environmental conditions. The fitness function evaluates each candidate solution according to predefined criteria most commonly growth rate, shoot regeneration, rooting percentage, or biomass production and provides a performance metric aligned with the objectives of the tissue culture process [29]. Individuals with the highest fitness values are selected and subjected to crossover and mutation operations; these processes reorganize genetic information and increase diversity, thereby enabling the exploration of new regions of the solution space [54]. This intuitive and iterative process, free from excessive mathematical complexity, allows GA to effectively generate solutions within the broad, multidimensional, and nonlinear search spaces characteristic of biological systems [33]. The primary reason GA is highly suitable for optimization studies in plant tissue culture lies in its ability to successfully identify global optimal solutions in complex systems containing numerous interdependent variables. Plant tissue culture is characterized by nonlinear responses to nutrient composition, hormonal balances, and environmental parameters, and in such systems traditional trial and error approaches and classical optimization methods are often insufficient [29]. By contrast, the population-based structure of GA enables the simultaneous evaluation of numerous potential medium formulations or hormone combinations, thereby allowing efficient convergence toward highly balanced and productive solutions [34]. Indeed, studies have demonstrated that GA achieves high success in optimizing culture medium components and plant growth regulators to enhance micropropagation efficiency, shoot regeneration, and rooting percentage [9,29]. The combination of the algorithm's stochastic nature with a systematic search strategy enables the overcoming of local minima in which conventional methods frequently become trapped.

In plant tissue culture applications, GA has been widely used particularly to improve micropropagation processes. For example, GA-based optimization models have been successfully applied to determine the most suitable combinations of culture medium components and environmental conditions in order to maximize plant biomass production [29,33]. The development of GA-based decision support systems further increases the importance of this approach in large-scale commercial micropropagation processes, where dynamically adjustable medium components and precise control of external conditions are critical [9]. In addition, the integration of GA with machine learning techniques such as Artificial Neural Networks (ANN), Support Vector Machines (SVM), and Random Forest (RF) has paved the way for the development of hybrid optimization models. These integrated approaches combine the high predictive power of machine learning with the global search capability of GA, enabling the effective solution of multi-parameter optimization problems in plant tissue culture [23,34]. Such hybrid models enhance the robustness of predictions related to tissue culture responses and provide deeper insights into the interactions among growth-influencing variables [19]. Despite these advantages, GA also presents certain limitations and computational challenges. The selection of initial parameters such as population size, crossover rate, and mutation rate can directly influence convergence speed and solution quality. Moreover, due to the stochastic nature of GA, repeated runs may yield different results, necessitating multiple iterations to ensure the reliability and reproducibility of optimal solutions [84]. In particular, the increased computational cost of GA when integrated into hybrid models with other machine learning algorithms may impose constraints in real-time or large-scale industrial applications [66]. Nevertheless, the strategic use of GA in combination with modern high-performance computing and parallel processing techniques allows many of these limitations to be largely overcome and expands its applicability in high-throughput plant tissue culture optimization studies [33]. Owing to its ability to efficiently explore and optimize the complex parameter space inherent in plant tissue culture, GA has become one of the fundamental building blocks of decision support systems. GA-based systems capable of jointly harmonizing numerous inputs, such as phytohormone composition, mineral nutrient concentrations, and physical environmental conditions, facilitate the design of optimized protocols that enable consistent and high-quality plantlet production. This approach is of particular importance in commercial micropropagation applications, where the balance between efficiency, cost, and reproducibility is critical [8,9].

The integration of GA into plant tissue culture represents a transition from classical trial-and-error methods to a systematic, data-driven, and high-efficiency paradigm, making a strong contribution to maximizing the potential of tissue culture technologies.

In Table 1, the fundamental artificial intelligence and optimization methods commonly used in plant tissue culture (ANN, SVM, RF, KNN, and GA) are comparatively summarized in terms of their basic approaches, typical application areas, strengths, and limitations. This table provides a practical roadmap indicating which method may be more suitable for specific experimental scenarios, thereby assisting researchers in making systematic model selection decisions.

Table 1. *Comparison of the basic artificial intelligence methods used in plant tissue culture*

Method	Core Approach	Typical Applications in Plant Tissue Culture	Main Strengths	Main Limitations
Artificial Neural Networks (ANN)	Multi-layer, data-driven neural models	Micropropagation efficiency; shoot/root traits; callus induction; somatic embryogenesis	High accuracy for nonlinear systems; flexible input handling; strong optimization capability	Large data demand; low interpretability ("black box"); sensitive to tuning
Support Vector Machines (SVM)	Margin-maximizing hyperplanes with kernels	Regeneration classification; callus and rooting prediction; growth regression	Good generalization with small datasets; effective for high-dimensional data	Kernel-dependent; computationally demanding at scale; limited interpretability
Random Forest (RF)	Ensemble of randomized decision trees	Growth trait prediction; biomass estimation; key factor identification	Robust to noise; stable with small datasets; interpretable feature ranking	Increased computation with many trees; less transparent than single trees
k-Nearest Neighbors (KNN)	Distance-based instance learning	Callus, regeneration, rooting classification; shoot/root regression	Simple; model-free; highly interpretable	Curse of dimensionality; slow prediction; sensitive to k and metric
Genetic Algorithms (GA)	Evolutionary global optimization	Media and hormone optimization; hybrid ML–GA systems	Effective global search; avoids local optima; multi-solution evaluation	Parameter-sensitive; stochastic variability; high computational cost
Quantum Machine Learning (QML)	Quantum-enhanced learning frameworks	Exploratory optimization; disease classification; genotype–phenotype analysis	Potential efficiency for complex, high-dimensional problems	Hardware-limited; low scalability; mostly experimental

APPLICATION AREAS OF ARTIFICIAL INTELLIGENCE IN PLANT TISSUE CULTURE

Prediction of Micropropagation Efficiency

Micropropagation is one of the fundamental in vitro techniques that enables the rapid and controlled multiplication of plants, and its efficiency is of critical importance for the success of commercial and research applications [43]. Micropropagation efficiency is evaluated using parameters such as shoot proliferation rate, multiplication coefficient, shoot elongation, and successful regeneration, which directly affect the standardization of production [37]. In classical experimental and statistical approaches, predicting micropropagation outcomes is quite tricky due to the nonlinear interactions among numerous variables, including explant type, hormonal balance, culture medium composition, and environmental conditions. This complex structure limits the predictive power of traditional models, such as linear regression, and also makes trial-and-error-based processes time-consuming and costly [42]. Artificial intelligence and machine learning techniques offer a robust alternative for predicting micropropagation efficiency, as they can effectively model this complex, multivariate, and nonlinear structure. ANN, SVM, RF,

and KNN algorithms are effectively used to predict shoot proliferation, multiplication coefficient, shoot length, and regeneration success with high accuracy [42]. In particular, RF reveals determining factors through variable importance analyses, whereas ANN successfully models nonlinear interactions of hormones. Machine learning models also provide effective results in determining the most suitable combinations of plant growth regulators (such as auxin-cytokinin). As a result, the classical trial-and-error approach is largely reduced, the protocol development process is accelerated, and experimental costs are decreased. Comparative studies have demonstrated that artificial intelligence-based prediction models offer higher accuracy and reliability than classical regression methods. Moreover, these systems can be integrated into decision support systems in commercial micropropagation laboratories, enabling production processes to be carried out in a more consistent, faster, and more economical manner [42].
Artificial intelligence-assisted micropropagation efficiency prediction systems constitute one of the most tangible application areas of digital transformation in tissue culture and offer significant advantages in terms of scalability, quality, and efficiency in commercial production. Table 2 summarizes representative studies applying machine learning and artificial intelligence models to diverse in vitro culture systems, highlighting species-specific protocols, modeling approaches, and key biological outcomes.

Table 2. *Machine learning models and key biological outcomes reported in recent plant tissue culture studies*

Species	In vitro method	ML model	Main findings	Ref
Aquilaria malaccensis	Micropropagation (shoot multiplication) on MS medium with BAP, KIN, NAA.	XGBoost (XGB), RF, SVR.	XGBoost performed best for predicting shoot number and length. 0.5 mg/L BAP was most effective for shoot propagation.	[70]
Aronia melanocarpa	Shrub Plant Medium (SPM) with BAP for shoot induction and auxin combinations for rooting	RF, XGBoost, GP, MLP	Optimized callus-free micropropagation; RF and XGBoost achieved $R^2 > 0.95$ for shoot number and root length prediction	[82]
Camellia sinensis (Tea)	MS medium with different PGR combinations (BAP, GA_3); solid culture	RF, XGBoost, MLP	ML models effectively predicted micropropagation and rooting efficiency; RF and XGBoost outperformed other models for growth traits	[14]
Cannabis sativa L.	Micropropagation on DKW basal salts to model morpho-physiological disorders (basal callus, hyperhydricity, necrosis).	PNN (Probabilistic Neural Network), KNN (K-Nearest Neighbor), SVC (Support Vector Classification).	ML algorithms successfully classified nutrient-associated disorders. Specific nutrient imbalances (e.g., Nitrogen/Potassium ratios) were identified as key factors for disorder severity, enabling precision troubleshooting.	[65]
Crocus sativus L. (Saffron)	Temporary Immersion System (TIS) vs semisolid medium	RF, GP, SVM, Artificial Neural Network (ANN)	TIS enhanced microcorm production; RF achieved $R^2 \approx 0.81$ for microcorm number prediction	[76]
Crocus sativus L. (Saffron)	Callogenesis optimization on MS medium supplemented with sucrose, ABA, NAA, and 2,4-D.	XGB (Xtreme Gradient Boosting), GBR (Gradient Boosted Regression), RF, DT, AdaBoost, KNN.	XGB and GBR provided the best prediction performance for callus induction and proliferation. The most effective combination was 3% sucrose, 100 µM ABA, 2 mg/L NAA, and 4 mg/L 2,4-D.	[69]

Evolvulus alsinoides (L.)	Direct shoot regeneration from nodal explants on MS medium with PGRs (BAP, KIN, TDZ, IAA).	MLP (Multilayer Perceptron - ANN).	MLP achieved high predictive accuracy (R2≈0.99). The highest regeneration rate (90.83%) and shoot number (26.25) were obtained with 2 µM TDZ + 0.1 µM IAA.	[11]
Ferula assa-foetida L.	Somatic embryogenesis and shoot organogenesis using MS medium + TDZ, BAP, NAA.	DL: CNN (Convolutional Neural Network), MobileNet. ML: RF, SVM, kNN, XGBoost.	CNN (Deep Learning) achieved the highest accuracy (87%) in predicting optimal PGRs. Thidiazuron (TDZ) was identified as the key modulator for regeneration.	[45]
Fragaria × *ananassa* (Strawberry)	Solid MS medium with PEG-induced drought stress	RF, MLP, GP, SVM	RF showed highest accuracy (≈91%) in predicting drought stress effects; genotype-specific responses observed	[72]
Lavender Genotypes	MS medium with BAP/IBA ± activated charcoal	MLP, RBF, GP, XGBoost	ML models effectively predicted micropropagation and rooting efficiency; optimal BAP and IBA at 1 mg/L	[74]
Lilium akkusianum	Micropropagation from scale explants using MS medium + NAA, BA, and Meta-Topolin (mT).	MLP (Artificial Neural Networks), XGBoost.	Highest callus rate (83.31%) with 2.0 mg/L NAA + 0.5 mg/L mT. Best shoot number (4.0) with 2.0 mg/L mT + 1.0 mg/L NAA. Regenerated plants were genetically stable.	[78]
Lycium barbarum L. (Goji berry)	MS medium with Cd stress (0–500 µM Cd)	MLP, RF, Support Vector Machines (SVM), Gaussian Process (GP), Extreme Gradient Boosting (XGBoost)	MLP and RF showed highest prediction accuracy (R^2 up to 0.98) for growth and rooting traits under Cd stress	[28]
Myrtus communis L.	MS medium with PEG-induced drought stress (1–6%)	GP, SVM, RF, XGBoost	Genotype-dependent drought tolerance; ML models successfully predicted micropropagation and rooting efficiency	[12]
Myrtus communis L.	MS medium with Cd stress (0–500 µM), BAP for shoot induction, IBA for rooting	MLP, RF, XGBoost	White-fruited genotype showed higher Cd tolerance; MLP predicted shoot height (R^2 = 0.87) and root length (R^2 = 0.99) with high accuracy	[12]
Olea europaea L.	In vitro surface sterilization and shoot proliferation	Support Vector Regression (SVR), RF, XGBoost, GP, Elastic net	XGBoost showed highest accuracy in predicting optimal sterilization conditions and shoot growth	[63]
Passiflora caerulea L.	Callogenesis on MS medium with 2,4-D, BAP, NAA, IBA on leaf, node, and internode explants.	MLP (Multilayer Perceptron) + GA (Genetic Algorithm).	MLP showed high accuracy (R2>0.81). Optimization yielded 100% callogenesis on leaf explants with 0.52 mg/L IBA + 0.43 mg/L NAA + 1.4 mg/L 2,4-D + 0.2 mg/L BAP.	[30]
Phalaenopsis amabilis (Orchid)	Micropropagation (Modified MS) optimizing Nitrogen, Phosphorus, and Potassium levels.	ANN (Artificial Neural Networks) + GA (Genetic Algorithm).	Optimized nutrients (Double Nitrate, +2.2-2.5% K, +4.7-5.0% P) increased morphological traits by >50% and biochemical traits by >200%.	[48]

Tanacetum balsamita L	Semisolid Media and Temporary Immersion System	Multilayer Perceptron (MLP) and Random Forest (RF)	ML-based predictive modeling can enhance parameter optimization and phenotyping precision	[13]
Vicia spp.	MS medium with PEG-induced drought and temperature stress	RF, k-Nearest Neighbors (k-NNs), MLP, SVM	ML models effectively predicted germination, vigor index, and seedling traits under combined stress	[59]
Vigna unguiculata (Cowpea)	In vitro regeneration (MS medium + BAP) using pulse treatments on embryo explants.	Quantum ML: QSVC (Quantum Support Vector Classifier), VQC (Variational Quantum Classifier). Classical: SVC, RF, MLP.	Quantum ML models (QSVC, VQC) displayed superior accuracy and recall compared to classical models. Pulse treatment with 5.0 mg/L BAP was most effective.	[39]

Optimization of Culture Medium Components

In plant tissue culture, the balanced regulation of mineral elements, carbon sources, and vitamins in the culture medium plays a decisive role in cell proliferation, morphogenesis, and overall plant health. Due to the complex nature of plant metabolism and the multidimensional effects of medium components, classical trial-and-error-based approaches are time-consuming and costly [10,56,58].

Artificial intelligence and machine learning methods offer powerful and effective solutions for optimizing culture media. Methods such as ANN, RF, and GA enable the prediction of optimal nutrient compositions by successfully modeling the non-linear interactions among medium components [7,34,85]. In particular, GA ensures rapid and reliable optimization by iteratively identifying the most suitable combinations of media. These artificial intelligence-based approaches significantly reduce experimental duration and resource utilization while offering higher reproducibility and reliability. Consequently, the process of culture medium development has been transformed from classical empirical approaches to a modern framework based on data-driven and predictive models [10,57].

Determination of Hormone Combinations

The accurate determination of hormone combinations in plant tissue culture is of critical importance for maintaining the balance between shoot formation and root development. In particular, the auxin-cytokinin interaction constitutes the fundamental hormonal mechanism that determines the direction of regeneration, and this balance has been quantitatively demonstrated since early studies [38]. However, classical approaches, which rely on adjusting hormone concentrations through trial-and-error methods, are insufficient in systems characterized by multidimensional hormonal interactions [52].

Artificial intelligence-based methods provide powerful solutions for modeling these complex hormonal interactions. Artificial Neural Networks (ANN), Random Forest (RF), and Genetic Algorithms (GA) enable the prediction of optimal combinations by analyzing nonlinear relationships among hormones. While ANN models can estimate optimal hormone ratios by learning from experimental data, RF identifies critical hormones through variable importance analyses; GA, in turn, can evolutionarily explore the most suitable combinations within a broad parameter space [53]. These artificial intelligence-based optimization approaches significantly enhance the reproducibility and efficiency of micropropagation and regeneration studies, while reducing experimental time and labor compared with classical approaches. In addition, by taking into account the genotype-dependent variability of hormonal responses, they contribute to the development of more universal and reliable protocols [44].

Stress Physiology Analyses

Stress physiology analyses in plant tissue culture are of critical importance for understanding plant responses to adverse conditions such as drought, salinity, temperature extremes, and oxidative stress. These stress factors can directly affect in vitro regeneration and morphogenesis processes [81]. Although classical physiological and biochemical stress analysis methods provide important information, they are often labor-intensive, low-throughput, and insufficient in capturing nonlinear relationships among multiple traits [12]. These limitations have accelerated the integration of artificial intelligence and machine learning techniques into tissue culture studies [72].

Machine learning algorithms such as Artificial Neural Networks (ANN), Random Forest (RF), and Support Vector Machines (SVM) have emerged as powerful tools for modeling and predicting stress tolerance using high-dimensional data obtained from in vitro experiments [14,28]. These algorithms enable rapid analysis of stress response patterns based on physiological and morphological traits [12,74]. In particular, ANN can model complex relationships between growth regulators and tissue responses during callus induction with high accuracy, while RF and SVM allow reliable prediction of stress tolerance [14,85]. Artificial intelligence-based stress analysis approaches offer significant advantages such as higher accuracy, strong predictive capacity, and the ability to process large datasets within a short time. Consequently, early detection of stress becomes possible, optimization of in vitro culture protocols is accelerated, and the development of plant genotypes with high stress tolerance is supported [28,66]. Overall, the integration of artificial intelligence into stress physiology analyses in plant tissue culture provides a powerful and transformative approach for a deeper understanding and improvement of plant stress responses under controlled conditions.

Prediction of Secondary Metabolite Production

In plant tissue culture, secondary metabolite production is of great importance for medical and industrial applications, as it enables the sustainable and standardized production of high value-added phytochemicals under controlled in vitro conditions [21,74]. Since the biosynthesis of these compounds is regulated by complex regulatory mechanisms, reliable prediction of production levels is often not possible using classical approaches. Empirical and regression-based traditional prediction models fail to adequately represent the nonlinear and multidimensional interactions that influence secondary metabolite accumulation [25,35]. Therefore, classical methods remain insufficient for reliably predicting metabolite production under variable in vitro conditions. Artificial intelligence and machine learning techniques provide powerful solutions for modeling and predicting secondary metabolite production. These approaches enable highly accurate predictions by learning the complex relationships between culture medium components, environmental factors, stress conditions, and metabolite synthesis [28,35]. In this field, ANN, RF, and SVM are particularly widely used. While ANN models successfully simulate nonlinear metabolic dynamics, RF can identify critical variables in high-dimensional datasets, and SVM enables precise prediction of complex biochemical interactions through margin optimization [7,25,28]. Artificial intelligence-based prediction models offer significant advantages, particularly in the in vitro production of medicinal and industrial plants, by reducing experimental workload, enabling rapid screening, and increasing production consistency [24,29]. In this respect, machine learning makes strong contributions to achieving more efficient, economical, and sustainable secondary metabolite production in plant tissue culture [74].

Image-Based Callus and Embryo Analysis

In plant tissue culture, image-based analysis of callus and somatic embryos is of great importance as it enables the rapid, precise, and objective monitoring of morphological changes that support somatic embryogenesis and subsequent plant regeneration [61]. Conventional visual evaluations limit reproducibility and the quantitative assessment of developmental details due to their susceptibility to operator-dependent subjective interpretations [15]. Artificial intelligence-based approaches can automatically detect, classify, and quantify callus and embryo structures through computer vision, deep learning, and machine learning techniques. In particular, Convolutional Neural Networks (CNNs) demonstrate high performance in the segmentation of complex images related to callus formation and embryo development. Artificial Neural Networks (ANNs) and Support Vector Machines (SVMs) are effectively used for the classification of developmental stages and the prediction of tissue culture outcomes. These artificial intelligence-based image analysis systems significantly reduce manual evaluation time, increase the objectivity of measurements, and enhance the reliability of data used for the optimization of regeneration protocols [15].

Artificial Intelligence–Assisted Bioreactor Systems

In plant tissue culture, intelligent bioreactor systems are closed cultivation environments that automatically regulate optimal growth conditions for plants by integrating advanced sensor technologies with artificial intelligence algorithms. In these bioreactors, critical parameters such as pH, temperature, dissolved oxygen, and nutrient concentration are continuously monitored, and environmental conditions are automatically adjusted using real-time data [73]. In this way, the most suitable environment for in vitro plant development and metabolic activities is provided [50,51]. Conventional bioreactor systems, on the other hand, generally require manual control and therefore lag behind these intelligent systems in terms of efficiency. The analysis of sensor-derived data using artificial intelligence enables real-time decision-making processes in bioreactors. Machine learning algorithms such as ANN and RF can successfully model the complex and non-linear relationships between growth parameters and plant responses. By jointly evaluating historical and real-time data, the most appropriate conditions for plant development can be predicted, and production processes can be optimized with greater precision [51,62,76]. Control algorithms also play a central role in the automatic regulation of the bioreactor environment. Variables such as aeration rates, nutrient solution flow, and mixing can be dynamically adjusted based on feedback from sensors. This significantly enhances stability, reproducibility, and production reliability within the bioreactor environment [50,75].

The main advantages offered by artificial intelligence-controlled bioreactors compared with conventional systems include more precise monitoring, reduced labor costs, and higher productivity. Moreover, owing to their flexible structures that can be adapted to different plant species and production objectives, these systems significantly accelerate micropropagation processes and secondary metabolite production [50,62]. The use of intelligent bioreactors in large-scale micropropagation and secondary metabolite production has led to important transformations. Maintaining environmental conditions at optimal levels has increased plant regeneration rates and has enabled high and consistent yields in the production of bioactive compounds such as alkaloids, particularly in medicinal plants [17,75]. In conclusion, artificial intelligence-assisted intelligent bioreactor systems emerge as a strategic technology in the field of plant tissue culture, combining automation with advanced data analysis techniques to enhance production efficiency, improve quality, and enable large-scale applications.

In an artificial intelligence-assisted intelligent bioreactor system, sensor data such as pH, temperature, dissolved oxygen, and nutrient density are analyzed in real time, and the bioreactor environment is automatically adjusted according to the resulting outputs, as shown in Fig. 1. Through this closed-loop control mechanism, a more stable, efficient, and reproducible production process is achieved in in vitro plant production.

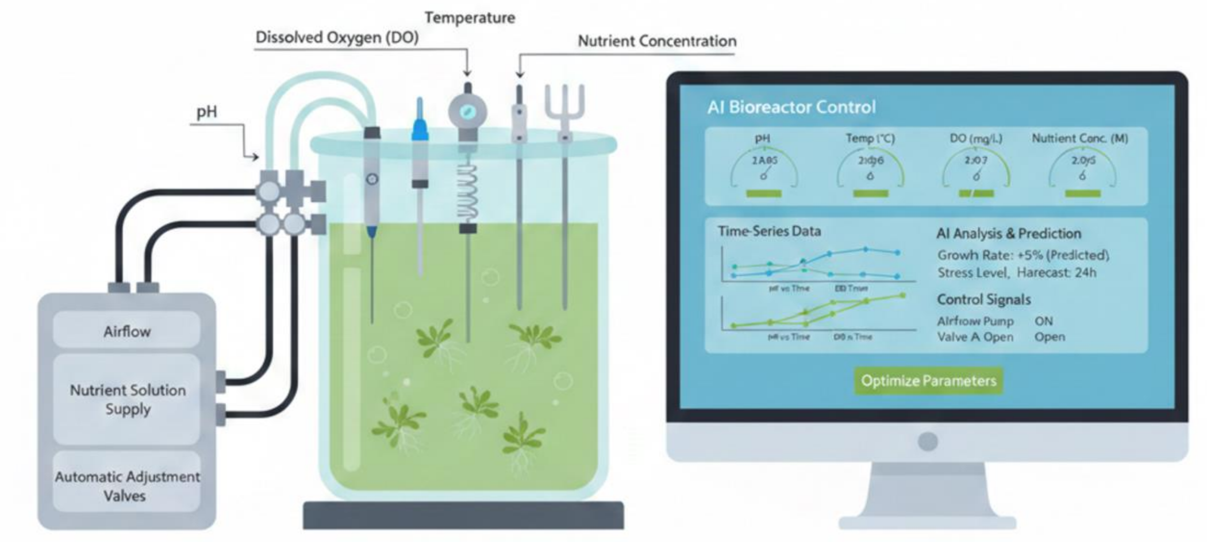

Fig. 1. *Artificial intelligence–assisted intelligent bioreactor system*

ADVANTAGES AND LIMITATIONS

The integration of artificial intelligence-based methods into plant tissue culture studies offers several significant advantages over classical approaches. One of the most significant benefits of these methods is their ability to achieve high predictive accuracy in complex and multivariable biological systems. By successfully modeling nonlinear relationships among experimental factors, the identification of optimal culture conditions becomes more reliable. Artificial intelligence models also enable a reduction in the number of experiments, thereby providing substantial savings in time, labor, and material use. Compared with trial-and-error-based classical approaches, their ability to generate results more rapidly significantly shortens the development process of tissue culture protocols. In addition, the simultaneous analysis of numerous variables allows the system to be evaluated holistically and contributes to a deeper understanding of complex biological relationships [22].

Nevertheless, artificial intelligence applications also have certain limitations. First, the development of reliable and generalizable models requires large quantities of high-quality data. Insufficient or unbalanced datasets can negatively affect model performance. Moreover, the effective use of these technologies requires a certain level of software and hardware infrastructure. High-performance computing systems, specialized software, and data processing tools often become necessary. In addition, the proper design, interpretation, and implementation of artificial intelligence-based analyses require interdisciplinary expertise. In the absence of sufficient knowledge in both plant biotechnology and data science, there is a risk of incorrect model construction or misinterpretation of results [27,55].

Artificial intelligence offers great potential in plant tissue culture studies, providing significant gains in terms of time, cost, and experimental efficiency. However, the effective and reliable use of these technologies depends on establishing an appropriate data infrastructure, having access to technical resources, and enhancing expertise.

DIGITAL TRANSFORMATION AND FUTURE PERSPECTIVES IN PLANT BIOTECHNOLOGY

The rapid advancement of digital technologies has brought about a fundamental transformation in the field of plant biotechnology. The integrated use of artificial intelligence, the Internet of Things (IoT), big data analytics, and automation systems has led to the emergence of the concepts of Agriculture 5.0 and, accordingly, Plant Biotechnology 5.0. This new paradigm aims to transform production processes into systems that are not only mechanical but also data-driven, predictive, and capable of self-optimization. In the future, the widespread adoption of fully automated micropropagation facilities in plant tissue culture and biotechnology applications is expected. In these facilities, all processes from the preparation of culture media to subculturing operations, from climate control to harvesting, will be managed by sensors, robotic systems, and artificial intelligence algorithms. Similarly, autonomous bioreactor systems will monitor environmental conditions in real time and self-regulate to ensure higher efficiency, standardization, and continuity of quality. In addition, artificial intelligence-assisted genetic selection and breeding systems will be able to analyze genotype-phenotype relationships much more rapidly and reliably using large datasets, thereby playing a far more effective role than classical methods in the selection of traits such as stress tolerance, yield, quality, and disease resistance. These developments will particularly accelerate the development of resilient plant genotypes under conditions of climate change. In conclusion, the process of digital transformation is reshaping plant biotechnology into a more efficient, predictable, sustainable, and scalable framework. Artificial intelligence, automation, and data-driven production approaches are expected to become standard in both academic research and commercial applications in the coming years [55,68].

CONCLUSION

The integration of artificial intelligence and machine learning into plant tissue culture research represents a significant methodological shift from traditional trial-and-error experimentation toward data-driven and predictive frameworks. As highlighted throughout this review, AI-based models have demonstrated substantial potential in capturing the complex, non-linear interactions among genotype, culture medium composition, plant growth regulators, and environmental conditions that govern in vitro morphogenic responses. Compared with conventional statistical approaches, these methods offer improved predictive accuracy, enhanced optimization capacity, and greater flexibility in handling multidimensional datasets. Despite these advantages, the effective application of AI and ML in plant tissue culture remains constrained by several critical challenges. Limited dataset sizes, insufficient standardization of experimental designs, and a lack of external validation often restrict model generalizability and biological interpretability. Furthermore, the "black-box" nature of many machine learning algorithms continues to raise concerns regarding transparency and reproducibility, underscoring the need for explainable AI frameworks and rigorous reporting standards. Emerging approaches such as hybrid optimization models, explainable machine learning, and quantum machine learning introduce promising conceptual advances, yet their practical implementation in routine tissue culture research is still in its early stages. These methods should therefore be viewed as complementary rather than replacement tools, requiring careful benchmarking against established techniques and biologically meaningful validation. Looking forward, the future of AI-assisted plant tissue culture will depend on the development of standardized, high-quality datasets, interdisciplinary collaboration between plant scientists and data scientists, and the integration of intelligent decision-support systems into automated and bioreactor-based platforms. Within the broader context of Plant Biotechnology 5.0, such advancements have the potential to enhance reproducibility, efficiency, and scalability of in vitro systems, ultimately contributing to sustainable crop improvement, conservation, and industrial plant production.

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