

A Survey of Machine Learning and Optimization Algorithm in Plant Tissue Culture [†]

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Abstract: With the increasing global population and agriculture facing numerous challenges due to climate change, finding sustainable solutions to food insecurity is crucial, as hunger and undernutrition continues to be a global challenge. Plant tissue culture has emerged as a promising technology for improving and multiplying crops rapidly. However, this technique produces extensive data due to the intricate interactions between genetic and environmental components, challenging traditional statistical methods. To address this, researchers are now employing Machine Learning techniques which excels in handling large, intricate datasets. Thus, current machine learning applications in plant tissue culture research are presented in this mini review.

Keywords: machine-learning algorithm; embryogenesis; breeding; optimization; breeding

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1. Introduction

Scientific and technological progress has revolutionized every aspect of human existence in the modern era. From personal well-being to agriculture, innovation has been critical in meeting the ever-increasing demands of the world's growing population [1]. As the global population continues to rise, the urgent need for a consistent supply of basic essentials particularly food, becomes more apparent. This demand has pushed agricultural advancement to the forefront, where higher yields, superior traits and resistance to biotic and abiotic stress and other desired agronomic traits have become critical. In this context, plant tissue culture has emerged as a technique for achieving these goals [2].

Plant tissue culture often referred to as micropropagation or in vitro culture is a method of growing plants in a nutrient-rich medium under regulated and sterile environment [3]. Explants, such as leaves, roots, and stems, are used to start plant cultures. Exploiting the totipotentiality of plant cells, these explants can generate into complete plants, yielding numerous plantlets. All the necessary prerequisites for the growth of the explants are supplied by the nutrient media [4]. Plant cultures find diverse applications, including the mass production of superior plants, genetic modification, preservation of germplasm, and production of disease-free plants. [5].

The development of plants in cultures is influenced by factors such as the composition of the nutrient media, plant genotype, age and explant type, plant growth regulators

(PGRs), level of phytohormones among others [6]. The complexity and unpredictability of data resulting from these variables pose challenges for analysis using conventional statistical methods like linear regression and ANOVA [7]. Machine learning (ML) techniques have come to be an effective technique for addressing this challenge [8], allowing computers to learn from data and experience, facilitating predictions and classifications [9]. These techniques include supervised learning in which models are intentionally trained on labelled datasets for accurate classification and prediction [9], unsupervised learning which discovers patterns in unlabeled data for analysis and clustering [10] and reinforcement learning which learns through action [11]. Recent advances in plant tissue culture research have employed ML models to interpret complex and nonlinear plant culture data [12]. Hybrid approaches that combine ML and optimization algorithms have been used to study the relationship between variables such as culture medium composition and plant growth traits enabling researchers to identify optimal inputs for maximizing plant biomass [12], [13]. This paper offers a succinct summary of the current and potential applications of ML algorithms in plant tissue culture research, laying the framework for applying this technology in plant improvement through plant culturing.

2. Review of Related Machine Learning Based Approaches in Plant Tissue Culture Processes

Artificial Neural Networks (ANNs) such as neuro fuzzy logic, generalized regression neural network (GRNN), probabilistic neural network (PNN), radial basis function (RBF), and Adaptive Neuro-fuzzy Inference System (ANFIS) are commonly applied in plant tissue culture research. Apart from ANN's, other ML algorithms such as support vector machine (SVM), random forest, and multilayer perceptron (MLP) are also used. These ML models can be improved using varying optimization algorithms like Symbiotic Organisms Search (SOS), Genetic Algorithm (GA), fast Nondominated Sorting Genetic Algorithm II (NSGA-II), and Nondominated Sorting Genetic Algorithm (NSGA), Multi-Objective Genetic Algorithm (MOGA).

2.1. Application of Machine Learning in Modelling and Optimizing Plant Culture Mediums

The Murashige and Skoog medium (MS) has long been used as a foundational medium for starting plant cultures [4]. Its use has however been reported to result into certain physiological challenges, potentially stemming from the concentrations of carbohydrate [14], PGRs [15] or other constituent of the basal media. Consequently, some cultures and plants are incompatible with MS, necessitating changes to its components [16]. Nevertheless, modifying the components of the culture medium can be challenging due to its complexity. Employing ML models streamlines this process, saving time and reducing costs. Several studies have employed ML algorithms to model and optimize plant culture media, offering the potential to enhance plant growth and productivity. These algorithms can forecast the ideal composition of culture media and can undergo further optimization using various techniques to boost their effectiveness. To optimize and forecast the most optimal hormonal combination for enhancing the growth of Garnem ($G \times 15$) rootstock in vitro, [13] employed an ANN paired with a GA to evaluate the impact of varying concentrations of different PGRs on certain growth parameters. Notably, the ANN-GA model attained 98% accuracy in predicting the optimal hormonal combination. Furthermore, the model suggested a combination of 1.02 mg/L of 6-Benzyleaminopurine (BAP) and 0.098 mg/L of Indole-Butyric Acid (IBA) for maximum proliferation of Garnem rootstock. Similarly, [17] conducted a comparative assessment of multiple ML models to model and forecast the in vitro development of cannabis as influenced by carbohydrate concentration and light quality. These included GRNN, MLP, and ANFIS. Each of these models was paired with four distinct optimization algorithms and although the disparities among the models were minimal, the GRNN-SOS pair outperformed all others across all assessed parameters. Likewise, in [18] MLP was used to predict the optimal medium composition

for the germination of some plum and apricot varieties. The MLP demonstrated a strong correlation between the expected and experimental result of all assessed growth parameters, underscoring the model's high level of performance.

Adequate mineral nutrition plays a crucial role in promoting the growth of cultured plants. Inadequate mineral supply can lead to morphological abnormalities and reduced survival rates [19]. Thus, finding the right mix of minerals is of paramount importance. Numerous studies have been conducted to refine this strategy. Employing ANN-GA, [12] developed an innovative nutritional medium: Yadollahi, Arab and Shojaeiyan medium (YAS) for enhancing the *in vitro* growth of Garnem. This optimized medium when compared with the traditional MS and woody plant (WP) mediums, yielded significantly greater fresh weight, shoot length, and dry weight. In another study, ANN-GA was utilized to build a new optimized medium (R medium) that promoted Kiwi berry *in vitro* growth and reduced physiological abnormalities better than previously used media. The GA predicted the optimal mineral mix to maximize all measured output [19]. The utilization of ML and optimization algorithms has brought about a transformation in analysis of large datasets, enabling researchers to reveal detailed patterns and hidden links between plant growth parameters and culture medium composition. This has enabled the tailoring of culture mediums compositions to the unique needs of various plants.

2.2. Machine Learning Application in *In Vitro* Sterilization

It is critical in the field of plant *in vitro* culture to ensure the development of healthy and viable plant material. However, the continuous problem of microbial contamination is a serious impediment to the healthy growth of plant tissue cultures. Therefore, *in vitro* sterilization is critical, involving the use of physical and chemical procedures to remove impurities [20]. Before now, *in vitro* sterilization was done using traditional techniques that were prone to errors, time-consuming and labor-intensive. Researchers have begun to explore the use of ML to enhance the efficiency and precision of the sterilization process, which involves the development of predictive models that can assist in selecting and combining sterilization agents and conditions optimally. This is achieved through the analysis of extensive datasets containing experimental outcomes. Numerous studies have showcased the effectiveness of ML and optimization techniques in elevating the quality of plant tissue culture sterilization, underscoring their potential to expedite and enhance the production of top-tier plant materials. In [21], a GRNN-GA approach was employed to forecast and enhance the concentration of disinfectants and immersion duration required for the sterilization of cannabis, aiming to improve its *in vitro* growth. The model exhibited strong performance, achieving an accuracy score exceeding 90%. During the validation process, the forecasted optimal combination of 0.008% hydrogen peroxide, 4.6% sodium hypochlorite, with an immersion time of 16.81 **min**, effectively eliminated contamination, yielding a contamination rate of 0%. In a similar context, [22] applied an MLP-NSGAI approach to optimize the *in vitro* sterilization process of chrysanthemum. Seven variables, encompassing AgNO₃, Nano-silver, HgCl₂, Ca (ClO)₂, NaOCl, H₂O₂, with immersion durations, were the input parameters used for predicting the contamination frequency and explant viability. The model exhibited an accuracy rate of over 94%. Furthermore, it indicated that using NaOCl concentration of 1.62% and immersing for 13.96 min, can result in 0% contamination frequency and 99.98% explant viability.

2.3. Machine Learning Applications in Somatic Embryogenesis

Somatic embryogenesis is the process of growing embryos from plant cells that are not typically involved in reproduction (leaf, stem, root, or epidermal cells). This process eliminates the requirement for sexual reproduction. The presence of somatic embryos signifies the capacity of plant cells to exhibit totipotency [23]. These somatic embryos find utility in a range of applications, including the cloning of superior cultivars, the creation of artificial seeds, genetic enhancement, and the production of secondary metabolites,

among other uses. In [15], a comparison was made between MLP and support vector regression (SVR) to assess their predictive accuracy for studying the impact of PGRs on somatic embryogenesis of chrysanthemum. SVR consistently outperformed MLP in all aspects. The greatest number of somatic embryos per explant (56.24) and the highest embryogenesis rate (99.09%) were achieved using a medium containing, 4.70 M kinetin (KIN), 9.10 M 2,4-dichlorophenoacetic acid (2,4-D) and 18.73 M sodium nitroprusside. [24] employed an image-processing approach along with an MLP model to forecast the optimal input combinations (sucrose concentration, 2,4-D concentration and explant age) for obtaining the best physical characteristics of embryogenic callus (perimeter, area, true density, roundness and Feret diameter) and the highest count of ajowan somatic embryos. The most effective parameters were found to be 1.5 mg/L of 2,4-D, 2.5% (*w/v*) sucrose, 0.5 mg/L of KIN, and 15-day-old explants, both in meas

ured and predicted somatic embryo production, highlighting the model's accuracy. Somatic embryos undergo different developmental stages, including globular, elongated, heart-shaped, expanded, torpedo-shaped, and cotyledonal phases. Due to the challenging, costly, and time-consuming process of selecting somatic embryos in embryo cultures, machine learning models are utilized to automate the classification of these embryos into various stages [24]. Techniques like image recognition are applied to streamline the identification of somatic embryos that are biologically suitable for transfer to another growth medium, as well as to determine which embryos should be excluded from further cultivation, as demonstrated in the research presented by [25]. Similarly, [26] used a penalized logistic regression model to classify the somatic embryos of some based on their transmittance, absorption, reflectance, or excitation spectra, predicting which ones had the potential to develop into healthy plants. The model demonstrated strong performance when applied to previously unseen somatic embryos with diverse genetic backgrounds.

2.4. Machine Learning Applications in Rooting and Acclimatization

Acclimatization and the process of *in vitro* rooting play pivotal roles in plant tissue culture [27]. Rooting is a crucial factor for the growth of plants [28]. Acclimatization happens when cultivated plants adapt to greenhouse or field conditions. Plantlets growing in controlled *in vitro* environments are exposed to specialized conditions designed to reduce stress and enhance plant growth. This results in plantlets with altered anatomy, physiology and morphology, necessitating gradual exposure to external condition [29]. Rooting and acclimatization primarily rely on the levels of auxin and sucrose [29]. In [30], an MLP-GA combination was utilized to predict and optimize the combination of inputs that would yield the best composition for promoting an optimal number of roots, nodes per plantlet, *ex vitro* leaves and height during grapevine acclimatization. The model performed well with a correlation between observed and predicted values close to one. In another study detailed in [31], neuro-fuzzy logic was applied to model the impact of light intensity and sucrose content/concentration on kiwifruit acclimatization. The model successfully identified optimum level and combinations of inputs to achieve maximum growth and development during *in vitro* rooting and acclimatization. In [32], a design of experiments (DOE) approach was used to create a five-dimensional IV-design space, coupled with a hybrid of artificial neural networks (ANN) and fuzzy logic to assess the effects of varying mineral concentrations in a Hoagland mineral solution on growth parameters (newly formed shoot length, total leaf number, leaf area, leaf chlorophyll content, and hardening efficiency) and three physiological disorders during the *ex-vitro* acclimatization of *Actinidia arguta*. The neuro-fuzzy logic effectively modeled all growth metrics and the occurrence of a physiological disorder known as leaf necrosis, showing a strong correlation between observed and predicted values. The 'IF-THEN' rule of the model also revealed that Mg²⁺ and Ca²⁺ played a positive role in enhancing certain growth parameters and preventing leaf necrosis but had opposing effects on leaf chlorophyll content. The addition of NO₃ to the media had a detrimental impact on some parameters, while NH₄⁺ in combination with Cu²⁺ or Mg²⁺ positively influenced several growth aspects.

3. Conclusions

This research looks into the various applications of machine learning and optimization algorithms in plant tissue culture. Multiple approaches have been used to optimize various parameters in plant tissue culture, which can be attributed to the processes' non-deterministic and complex nature. The use of ML in this field is attributed to their demonstrated success in analyzing massive amounts of datasets which allows the optimization of the process with fewer resources and less time. Future research could look into creating ML-based virtual simulations of tissue culture process to reduce experimental time and cost and also integrating ML with gene editing techniques to accelerate the development of new varieties.

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