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Bitki Biyoteknolojisi için Makine Öğrenimi Uygulamaları: Bitki Doku Kültürü Prosedürlerinin Yapay Sinir Ağları ile Modellenmesi

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ÖZ

Biyoteknoloji ve bilgi teknolojilerindeki gelişmeler son yıllarda katlanarak artmış ve bu ilerleme sayesinde fonksiyonel deneysel teknolojilerin geliştirilmesi mümkün olmuştur. Biyoteknolojik gelişmelerin hızlanması ile deneysel çalışmaları daha pratik, hızlı ve kolay tasarlamak, geliştirmek ve uygulamak kolaylaşmaktadır. Son yıllarda yapılan deneysel çalışmalar, gerekli analiz ve çıkarımların yapılmasına olanak sağlayacak büyük miktarda veri üretme potansiyeline sahiptir ve elde edilen veriler farklı şekillerde ve birçok veri tabanında saklanmaktadır. Araştırmacılar tarafından elde edilen bulguların doğru analiz edilmesi ve doğru çıkarımların yapılması, bu verilerin ve veri tabanlarında depolanan büyük miktardaki bilginin sisteme uygun şekilde entegre edilmesini gerektirmektedir. Bu makale, *in vitro* bitki doku kültürü çalışmalarından elde edilen verilerin saklanması, farklı kaynaklardan elde edilen verilerle bütünleştirilmesi, yeni bilgilerin elde edilmesi ve işlevsel olarak uygulanması konusunda çeşitli bakış açıları sunmaktadır.

Machine Learning Applications for Plant Biotechnology: Modeling of The Plant Tissue Culture Procedures with Artificial Neural Networks

Review Article

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ABSTRACT

Developments in biotechnology and information technologies have increased exponentially in recent years, and thanks to this progress, it is possible to develop functional experimental technologies. With the acceleration of biotechnological developments, it is becoming more and more practical, fast, and easy to design, develop and implement experimental studies. Experimental studies in recent years have the potential to produce a large amount of data that will allow the necessary analysis and inferences to be made, and the data obtained are stored in different ways and in many databases. Analyzing the findings obtained by the researchers correctly and making the proper inferences requires that these data and the large amount of information stored in the databases be integrated appropriately into the system. This article offers various perspectives on storing data obtained *in vitro* plant tissue culture studies, integrating it with data from different sources, obtaining new information, and applying it functionally.

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1. Introduction

As a result of the rapid increase in the world's population, the global food requirement also increases, but production remains insufficient to meet the required demand with factors such as the destruction of agricultural areas and climatic changes. Although approximately 2 billion people in the world do not have access to healthy, reliable, and sufficient levels of food (Gutu, 2020), it is seen that modern biotechnological developments should be used in agricultural practices, considering the current situation. For this purpose, it is necessary to develop high-quality and high-yield potential varieties resistant to biotic and abiotic stress factors to increase the yield obtained from existing agricultural foods. Although new varieties with different characteristics have been developed with breeding studies for many years, the low chance of crossing various plants due to high labor and long breeding times remains insufficient to solve the current food imbalance. With plant tissue culture techniques, new varieties with desired characteristics can be developed and reproduced much shorter than classical breeding methods.

To optimize a reproducible procedure with *in vitro* culture studies, different parameters such as the genotype of the plant used as the material, the explant source to be used in the culture, the content of the nutrient medium, the concentrations and different combinations of the plant growth regulators added to the nutrient medium were examined and evaluated, and the effects of these variables on the plant were determined. It is aimed to analyze the effect on the regeneration ability. The models included in the classical statistical techniques used in evaluating the obtained data are insufficient in evaluating the factors directly related to the plant and are not linear. Although classical statistical techniques evaluate the effect of independent variables on dependent variables with variance and linear regression-based models, more robust data systems should be used to determine complex relationships.

2. Problems in Modelling of Plant Tissue Culture Process

Plant tissue culture is the regeneration of different cells, tissues, and organs selected as an explant source by placing them in an artificial nutrient medium by taking advantage of the totipotency feature of plants (Bhojwani and Dantu, 2013).

In vitro culture studies are one of the fundamental techniques for plant propagation and breeding, enabling somatic embryogenesis, micropropagation, shoot regeneration, and production of plant-derived secondary metabolites (Hesami et al., 2018; Raj and Saudagar, 2019). The culture conditions, the physiological state of the explant, and the genotype are the main factors responsible for the regeneration success of the plant (Svetleva et al., 2003); optimizing the *in vitro* conditions suitable for the plant is the most critical step in the development of a routine regeneration protocol. Although many nutrient media are used in tissue culture studies, the most used nutrient medium is the nutrient medium with high salt concentration, called "MS," which was developed in 1962 by botanists Toshio Murashige and Folke K. Skoog (Murashige and Skoog, 1962). Various plant growth regulators are used to direct the regeneration as desired. The ratios of plant growth regulators to be added to the nutrient medium vary according to the purpose for which the plants are cultivated and their species (Sönmez, 2019). Murashige and Skoog (1962) spent about 5 to optimize 81 different macro, microelement, and different vitamin combinations to improve the "MS" nutrient medium; Hildebrandt et al. (1946) reported that more than 16000 procedures were required to develop a new culture medium.

The medium manipulated for a specific purpose contains many different components and combinations, so optimization takes time and requires high expertise (Phillips and Garda, 2019). The inclusion of computer technologies such as Artificial Intelligence (AI) in the process can make essential contributions to the realization of optimizations, which is the most basic requirement for success and can shed light on researchers on the way to discovering new information.

3. Application of Artificial Neural Networks *in vitro*-Based Plant Biotechnology

For many years, researchers have studied how to produce non-biological entities with human-like performance in understanding the task, analyzing it, and making logical inferences. As a result of the combination of information technologies and statistical science, Machine Learning, one of the systems obtained from studies, develops and applies computer algorithms with experience. While classical statistical methods focus on which results can be drawn from which data, Machine Learning also includes complex processes such as selecting the most valuable data, storing, combining, and determining appropriate calculation algorithms (Mitchell, 2006). Studies conducted in recent years show that modeling the complex and nonlinear relationships contained in the data with artificial intelligence technologies gives much better results than classical statistical methods in providing superior predictions (Landín et al., 2009; Gago et al., 2010).

Artificial Neural Networks (ANN) are one of the most important developments in recent years applied in various fields of science such as economics, health, and engineering (Hammerstrom, 1993; Lek and Guégan, 1999). Various studies and approaches have been reported on Artificial Neural Networks in plant science (Frossyniotis et al., 2008; Prasad and Gupta, 2008). In the reliable evaluation of biological processes, neural network technology, which uses complex mathematical functions to process and interpret unpredictable data sets, provides effective results (Karim et al., 1997; Prasad and Gupta, 2008). Studies reported that allow the estimation of the number and average length of shoots (Arab et al., 2016) and the number and weight of roots per plant (Mehrotra et al., 2008) in plant tissue culture.

3.1. Principal Structure of Artificial Neural Networks

Artificial Neural Networks (ANN), which consist of interconnected units called neurons, nodes, and sensors, are systems designed to simulate the information processing process of the human brain (Gago et al., 2010; Amiri et al., 2018). The artificial nerve cell in the system mimics biological neural networks and collects the signals it receives from a different cell, and when the accumulated signals exceed a certain threshold, it transmits the collected signals to a different artificial nerve cell (Ataseven, 2013). Inputs, outputs, weights, summation, and activation functions are the five essential elements of a nerve cell, called a process in engineering (Öztemel, 2003). In this system, all neurons except those connected with outer space to receive inputs and transmit outputs have connections only within the network and hidden layers (Anderson and McNeill, 1992). In a system consisting of layers, the input layer, which provides the reception of the information from the outside to the neural network, consists of the parameters affecting the problem, and the number of neurons in this layer is shaped according to the number of parameters. On the other hand, the output layer is responsible for exporting the information, and the hidden layers are located between the input and output layers (Çelik and Köleoğlu, 2022).

Following the study, the training algorithm to be selected includes two learning rules, with and without supervision (Ersoy and Karal, 2012). In the most preferred supervised learning model, a sample output is given to the network and compared with the output produced by the network, and the randomly given weights are changed in cycles until the difference between the outputs taken as errors is minimized (Anderson and McNeill, 1992). On the other hand, the unsupervised learning method is a learning method that has limited use and is constantly evolving in neurons without sample output (Anderson and McNeill, 1992). The complexity and interaction of variables in plant tissue culture applications, which is the subject of this article, make it challenging to optimize the process with traditional approaches. Therefore, artificial intelligence modeling and optimization applications have been used in different studies to model, predict and optimize this process.

3.2. Artificial Neural Networks in Plant Tissue Culture

Unlike statistical methods, artificial neural networks can be applied to any problem that requires a mathematical relationship between input and output variables (Zealand et al., 1999). In this way, complex and nonlinear systems such as biological processes can be modeled (Gevrey et al., 2003). The first applications of artificial neural networks in plant science included optimization processes. In various previously reported studies, neural network technology has been used to analyze the developmental stages of somatic embryos (Uozumi et al., 1993), defining the viability of plant cells. The system developed by Uozomi et al. (1993) was used to determine the appropriate time for transferring somatic embryos from the celery plant to the next culture stage. Variables such as length-width ratio, circularity, and area in the trained artificial neural network are obtained from cell culture images, and embryos at the globular, heart, and torpedo stages are determined. Even after training, the system successfully predicted the number of plantlets developing from the embryo (Uozumi et al., 1993). Honda et al. (1997) used neural network applications to determine the length of the shoots regenerated from the calli in the climatization study in which they will transfer the callus of the rice plant from the environment in which they develop to the sugar-free environment. Variables such as radius, width, and length in the digital images obtained from the somatic embryo were used as inputs, and the results of the neural network compared with the results of the multiple regression analysis estimated the shoot lengths with an error of 1.3 mm at a rate of 95% (Honda et al., 1997).

The first step is proper surface sterilization of the plant material used in plant tissue culture studies. The chemical used in sterilization, the duration of the application, and the type of explant affect the sterilization success. Therefore, the type and application time of the sterilant should be optimized for each plant species and different explants. Ivashchuck et al. (2018) used "Multilayer Sensor" and "Radial-Based Function" methods in their study with *Bellevalia sarmatica* (Pall. Ex Georgi) Woronow, *Nigella damascene* L., and *Echinacea purpurea* L., dipping times with different types of sterilants at different concentrations as inputs, explant viability, and percent contamination as output. The Multi-Layer Sensor models predicted functional sterilization (Ivashchuk et al., 2018). Murase and Okayama (2008) used artificial neural networks to model environmental conditions in plant tissue culture and modeled a system to determine the required temperatures with a 5% margin of error (Murase and Okayama, 2008). Callogenesis is a complex and nonlinear process affected by many factors (Hesami et al., 2018).

On the other hand, Mansouri et al. (2016), in their study with *Cuminum cyminum* L., variables such as area, diameter, lateral axis length, and density were used as input; they accepted fresh weight and callus volume as outputs and reported that the model they developed predicted the volume and fresh weight of callus precisely (Mansouri et al., 2016). Niazian et al. (2018) used artificial neural networks in their study with *Trachyspermum ammi* L. to determine the physical properties of embryogenic calli from explants of different ages, to which they applied different concentrations of kinetin, 2,4-Dichlorophenoxyacetic Acid (2,4-D), and sucrose. By determining the density, roundness, and area of the callus, they determined that 2,4-D changes the physical properties of the callus and has relatively the highest importance in this process (Niazian et al., 2018).

Kaur et al. (2020) determined different chitosan and salicylic acid concentrations as inputs, used multilayer sensors in their study with *Swertia paniculata* Wall., and reported that they accurately predicted the modeled in vitro secondary metabolite production (Kaur et al., 2020). Ruan et al. (1997) used a system they developed with an accuracy of 90% or higher with artificial intelligence technology to determine and model the morphological characteristics of somatic embryos in their study with carrots (Ruan et al., 1997). Zhang et al. (1999) succeeded by optimizing somatic embryogenesis and identifying normal and abnormal somatic embryos using a similar system (Zhang et al., 1999).

Hesami et al. (2019) took variables such as fructose, 2,4-D, sucrose, 6-Benzylaminopurine (BAP), glucose, and light as inputs to the model and optimized somatic embryogenesis in the system they modeled, and somatic embryo count was accepted as output data and was 92% (Hesami et al., 2019). On the other hand, Altuntaş and Kocamaz (2019) reported that the system had positive results in the determination of haploid seeds through the data set they obtained from haploid and diploid corn seed images in their convolutional neural network (CNN) studies (Altuntaş and Kocamaz, 2019).

4. Conclusions

In classical statistical methods, a limited number of factors known to be effective in evaluating various biological properties are used. Considering the complexity inherent in biological processes, generally, in systems where linear and simple variables are analyzed, the data obtained are insufficient to estimate the most accurate. Artificial intelligence applications can provide severe advantages in studies where many parameters such as plant tissue culture and optimum conditions vary from plant to plant, even in different genotypes within the same plant. With ML, appropriate data analysis methods can be determined in future studies. It is thought that the models developed in this way will save both labor and time and enable researchers to carry out systematic and efficient studies. With ANN, the correct and efficient analysis can be opened with the correct classification of appropriate data.

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

Contribution Rate of Researchers Statement Summary

Designed the article and wrote the paper: AK and DT.

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