

PREDICTION OF *CHLOROPHYTUM BORIVILIANUM* INVITRO REGENERATION USING DIFFERENT ARTIFICIAL NEURAL NETWORKS

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Abstract

Artificial neural networks (ANNs) were used in this study to assess the performance of Deep learning neural networks and Wide learning neural networks in predicting *Chlorophytum borivilianum* in vitro organogenesis. The effects of different compositions of macronutrients of MS media were analyzed for the micropropagation of *Chlorophytum borivilianum* using nodal explant to predict shoot organogenesis (outputs) by the application of different ANN architectures i.e., Wide neural network and Deep neural network. The number of hidden layers in a deep learning architecture is more than that of a WNN. The ANN architecture was trained using the Broyden-Fletcher-Goldfarb-Shanno (LBFGS) quasi-Newton algorithm. To determine which model was superior in predicting shoot organogenesis, we examined their respective accuracy percentages and R^2 values (the coefficient of correlation). When comparing the Regression neural network with the Wide neural network model, the former achieved a greater accuracy percentage (99.99991022) when predicting the total number of shoots. The Shoot length was more accurately predicted (97.58222034) by the WNN model. There is a slight discrepancy between the accuracy percentage and the R^2 value, but only at the decimal level. The results of nonparametric biological studies can be predicted with equal accuracy using either architecture.

Keywords: *Chlorophytum borivilianum*, Deep learning, Wide neural network, Macronutrients, *invitro* Regeneration

1. Introduction

Mineral nutrients, vitamins, and plant growth regulators are some of the many elements that interact throughout the process of adjusting and balancing the components of the medium [1].

The highest rate of reproduction appears to require unique cultural conditions for each species or cultivar. The nutritional needs of different plant species vary greatly. Macronutrients are required in greater quantities than micronutrients, vitamins, and growth hormones to ensure a plant's continued development and growth [2,3].

Adding a series of concentrations of a specific molecule to a universal base medium like MS, LS, or B5 can help determine the optimal composition of the final medium. A more efficient medium could be created by studying the connection between media nutrients and explant growth [4]. Therefore, we resort to mathematical and statistical methods like linear regression, logistic regression, and mixed models but they have limitations in calculating the non-linear and multi-variate biological data. Conventional modeling techniques may also become subjective to time and cost for assuming growth and final productivity patterns. Thus, to reduce these two major constraints (time and cost) during tissue culture practice, modeling systems that can simulate in vitro growth kinetics, thermodynamic limitations of culture conditions, and complex, but characteristic developmental patterns of biological systems are needed [5]. An artificial neural network, often known as an ANN, is a functional replacement that may be utilized for reliable evaluations of biological systems. The technology of neural networks estimates a variety of different difficult mathematical functions in order to process and infer a variety of sets of irregular input [6-8].

The tuber crop known as Safed Musli, scientifically known as *Chlorophytum borivillianum* Sant & Fern, has its origins in India and is a member of the Liliaceae family. The tubers of *C. borivillianum* have traditionally been employed in the treatment of impotence and as an aphrodisiac. The tuberous roots of Safed Musli have been proven to alleviate joint pain, arthritis, diabetes, and rheumatism [9]. Because of their usefulness in medicine, tubers are a lucrative crop. In addition, tubers have long been employed as planting materials, whereas the seeds are rarely used because of their low germination rate, low viability, and extended dormant period [10]. Clonal micropropagation of *Chlorophytum borivillianum* from dormant floral buds has been shown to be quite successful, however, shoot bases and stem disc preservation are other viable options [11].

Purohit et al. (1994a)¹² devised a methodology for laboratory-scale micropropagation of *C. borivillianum*, while Suri et al. (1999)¹³ reported in vitro plantlet regeneration. For improved in vitro shoot growth and high shoot biomass production of *C. borivillianum* [12,13], Elman back propagation neural network (Elman-BPNN) technology has been used by Rizvi et al., (2012)¹⁴ to predict the optimal culture environment at a small scale, in terms of sugar content and pH of the culture medium, inoculums density (number of shoot base explants/culture flask), and volume of growth medium/culture flask but no study has been done to predict the shoot biomass by changing the macronutrients concentration in nutrient media with the help of Artificial Neural network [14].

Therefore, this study developed different ANN models with a type of Deep neural network (Regression Neural Network) and Wide neural network (WNN) architectures to predict invitro shoot organogenesis of *Chlorophytum borivillianum* from nodal explants.

2. Material and Methods

2.1. Media preparation, culture establishment, and organogenesis of *Chlorophytum borivillianum* to gather data for model preparation

Different macronutrient compositions were used to design 42 variations of MS (Murashige and Skoog, 1962)¹⁵ medium containing 2.5 mg/l BAP (6-benzyl amino purine), 30 g/l sucrose, and 8 g/l Agar to promote the growth of micro-shoots. The concentrations of micronutrients and vitamins were consistent across all of the different culture media designs. The standard MS media concentration was termed “X” and the variations in other formulations were 0.58X, 1.42X, 1X, and 2X of the standard MS media composition. Media were poured into magenta boxes after adjusting the media with a pH of 5.7 before being autoclaved at 121 degrees Celsius for 15 minutes. The cultures were kept in a 25 ±2°C environment with a 16 h-photoperiod [16].

Chlorophytum borivilianum micro-shoots were obtained from Patanjali Nursery Haridwar. Tween-20 was used to sterilize the outside of the explants, and they were left to rinse for 15 minutes under running water. These explants were further sterilized in an aseptic environment with a 0.2% (w/v) aqueous mercuric chloride (HgCl₂) solution for 5 minutes, followed by three washes in distilled water to eliminate all residues of the HgCl₂[17].

The response surface method was utilized in order to determine the effect of the variable macronutrient quantity in media (inputs), and 42 samples were designed in accordance with this having an input range from 0-2. There were five magenta boxes used for each replication, and each jar had four explants. In order to train the ANN models, we first collected data from the actual experiments. The prediction model was built with inputs representing the various macronutrient compositions and outputs representing the shoot organogenesis data. A linear mix of input and output data was used to train the models.

2.3 Application of multilayer perceptron networks with distinct architectures to train, test, and validate the datasets.

MatlabR 2022a Software was used to build and interpret the different ANN models. MATLAB is an interactive platform for writing code and analyzing data using matrix operations [18]. Deep Learning Toolbox™ provides straightforward MATLAB® commands for constructing a deep neural network and establishing connections between its many layers. Different Multilayer perceptron (MLP) neural networks such as RNN and WNN with feedforward architecture were used to construct prediction models because of their ability to recognize nonlinear data [19]. Artificial neurons are the fundamental computational elements of neural networks; they take in signals with weights and output signals with the help of an activation function. The user can configure the multi-layer perceptron by setting its lengths and activation functions. The weight of neurons is initialized randomly using a dedicated method [20].

To construct the model, ML algorithms were trained on the data in order to make predictions about the target variables. Different levels of five different macronutrients (NH₄NO₃, KNO₃, CaCl₂.2H₂O, MgSO₄.7H₂O, KH₂PO₄) were used as input variables along with the control (MS media) and Shoot organogenesis (No. of shoots and Shoot length) were used as output variables to training the MLP. The available record for each output against the input data was used in the proportion of 80 and 20 to build prediction models and validate the built model. Although models were trained using train datasets, heuristics related to their performance with test datasets were also used to guide choices for building an optimal model. As a result of this, a good ANN model typically has similar performances with both train and test datasets. The goal of training models was to produce a formula that would capture key relationships between inputs and outputs [21].

Empirical methods were used to determine model network configurations, and the best-performing model was chosen for further study. Several statistical indices have been developed for assessing ANN model performance. Standard deviation, bias factor, mean absolute relative residual, mean relative percentage residual, accuracy factor, and coefficient of determination were computed [22]. The best models were chosen based on their accuracy percentage and R^2 values [23], which quantify the strength and direction of a linear relationship between anticipated and observed outputs (higher R values were deemed to be better). The upper limit of the coefficient of determination is 1, which is achieved when there is a perfect fit. On the other hand, R^2 does not have a lower limit, a value of 0 indicates an insignificant fit provided by the horizontal line $y = K$, where K is the average of the targeted value of all the training points, with small perturbations. Given that R^2 values below zero indicate a poorer fit than the average line. Consequently, it can be observed that the coefficient of determination remains unaffected by linear transformations of the distribution of independent variables. Furthermore, a prediction that yields an output value close to one is indicative of a good prediction, irrespective of the measurement scale of the aforementioned variables [24].

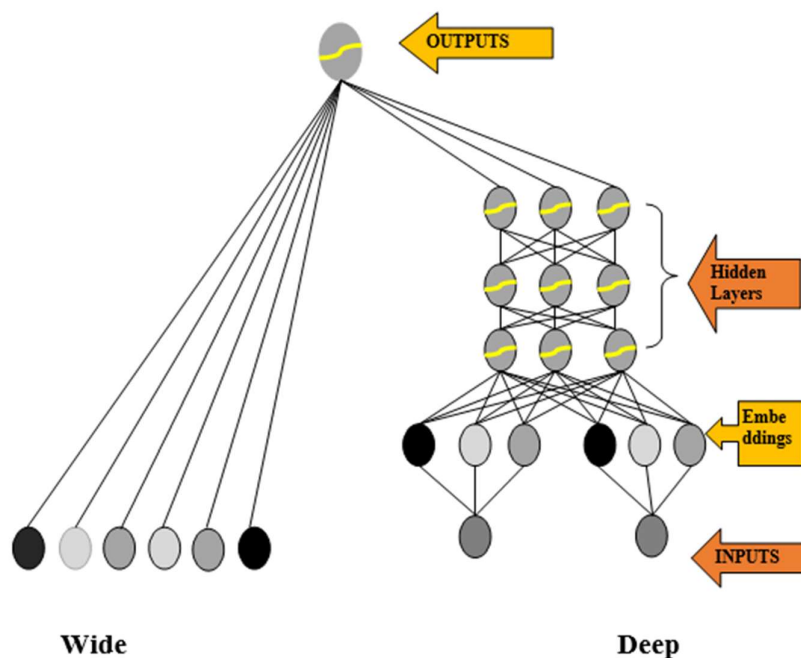


Figure 1: Basic architecture of Wide and Deep neural network

Deep learning Architecture i.e., Regression Neural Networks was found to be the best architecture to assess the best-predicted value against each input data. A Regression Neural Network object is a deep-learning neural network that has been trained for regression and features feedforward and complete connectivity. The initial layer of the neural network that is fully linked has a connection that goes to the network input (the predictor data X), and each layer that follows it has a connection that goes to the layer that came before it. After multiplying the input by a weight matrix called Layer Weights, each completely linked layer adds a bias

vector called Layer Biases. Each fully connected layer is followed by an activation function, with the exception of the very final layer (Activations and Output Layer Activation). The output of the network, in the form of predicted response values, is produced by the topmost layer of the network, which is fully connected. Limited-memory Broyden-Fletcher-Goldfarb-Shanno quasi-Newton algorithm (LBFGS) was used to train the ANN architecture. The LBFGS solver approximates the Hessian using a line-search algorithm. `fitnet(X,Y,'LayerSizes',[10 10])`. The activation function used is “relu” and "tanh" which specifies to create a neural network with two fully connected layers, each with 10 outputs. The first layer uses a rectified linear unit (ReLU) activation function, and the second uses a hyperbolic tangent activation function. Adjusting the depth and width of the architecture of a neural network is a frequent practice that can improve the performance of a neural network and better fit it to the computational resources that are available. Wide Neural Network is a subtype of Regression Neural Network with an extra layer in width. Standard activation functions are utilized by both broad and deep network architectures. To be more specific, the activation functions that are most frequently utilized include ReLU, sigmoid, swish, cosid, sine, tanh, and scaled hard tanh (Shtanh) [25,26].

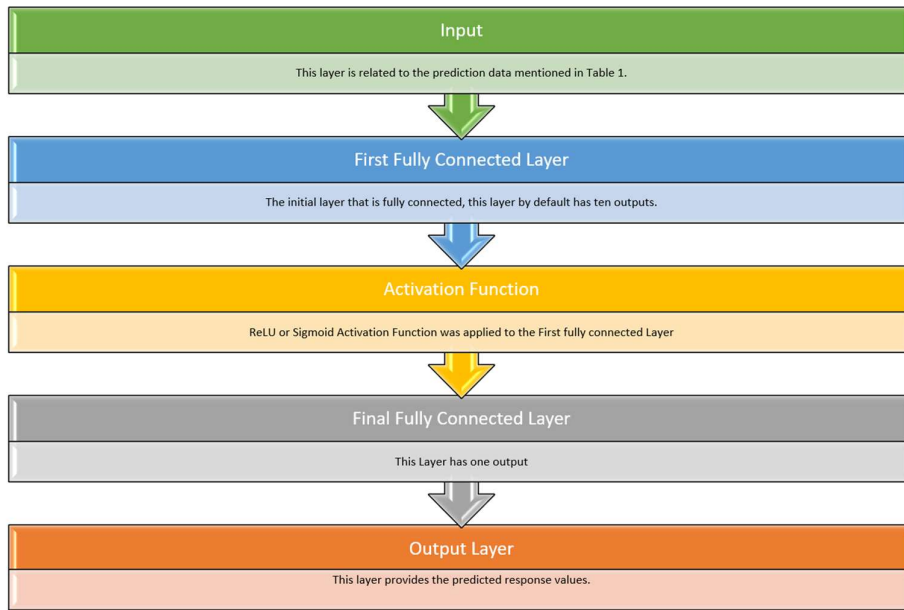


Figure 2: Default Regression Neural Network Model

3. Results

Empirical methods were used to figure out how networks should be set up, and statistics like Coefficient of determination, Accuracy factor, Root-mean-square residual (RMSR), Mean Square Error (MSE), and Mean Absolute Error (MAE) were used to measure how well models predicted the outcome of the experiment.

Regression Neural network models developed to predict the effects of varied nutrient composition are shown in Table 2.

Table 1: Comparison of Statistical indices between both models of Artificial neural networks.

Row	WNN_no_of_shoots	WNN_shoot_length	RNN_no_of_shoots	RNN_shoot_length
Accuracy percentage	99.82570316	97.58222034	99.99991022	97.52811881
RMSE	0.001742968	0.024177797	0.026772291	0.376182678
MSE	3.03794E-06	0.000584566	0.000716756	0.141513408
R-squared	0.999999927	0.999999887	0.999983762	0.992172534
MAE	0.000730307	0.000649414	0.003012868	0.056811613

In terms of predicting the total number of shoots, Regression neural networks performed better than Wide neural networks with accuracy percentage of 99.99, correct predictions for Shoot length were made using the WNN model with accuracy percentage of 97.58. The MSE in prediction of No. of shoots by wide neural network which is 3.03794E-06 is higher than error in RNN. The RNN model predicted a maximum of 20.34 shoots, with a corresponding maximum shoot length of 11.26 cm. In comparison, the WNN model predicted a maximum of 20.33 shoots, with a shoot length of 11.259. Figure [3] displays the graphical depiction of the predicted and actual response of the WNN and RNN models.

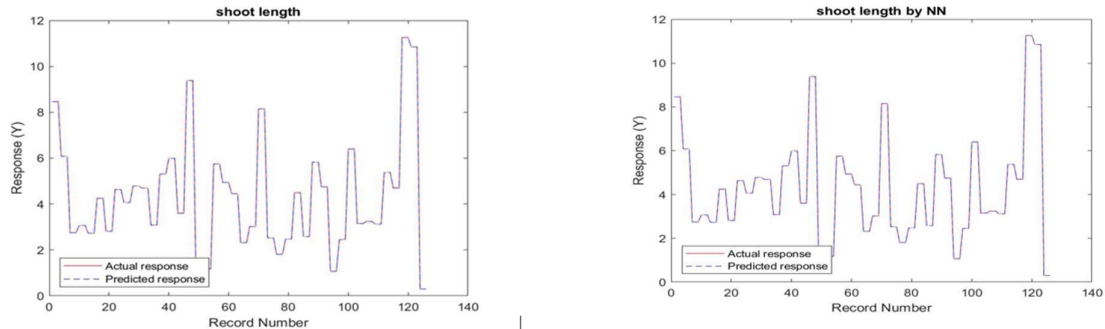


Figure 3: Graph showing the WNN and RNN models predicted and actual results.

Both models possess the capability to forecast shoot organogenesis. However, upon evaluating all parameters, it was determined that the deep learning architecture, specifically the recurrent neural network (RNN), exhibited superior performance in predicting this phenomenon.

Conclusion

The capacity of ANN models to interpolate unknown patterns makes them a useful tool for estimating in vitro growth rates of chlorophytum borivilianum. In addition, ANN methods may one day be able to take the role of time-consuming and resource-intensive experiments. It is apparent that both models exhibit a high degree of proficiency in predicting shoot organogenesis. A variety of prediction models can be developed through the utilization of Multilayer Perceptron models with varying numbers of hidden layers, trained using different algorithms. The two MLP models developed for this study have shown remarkable

performance in predicting shoot organogenesis. There is a clear indication of a significant level of expertise in forecasting shoot organogenesis but after assessing all the relevant factors, it was concluded that the deep learning framework, particularly the recurrent neural network (RNN), demonstrated exceptional efficacy in forecasting this phenomenon.

After identifying the respective strengths of each model, it is possible to construct a multilayer perceptron model that integrates a combination of wide and deep architectures. This approach can enhance the accuracy of predicting non-linear biological data. Through the process of simultaneously training a wide linear model, which is designed for memorization, and a deep neural network, which is intended for generalization, it is possible to merge the advantages of both models and make significant progress toward the desired outcome. The combined approach will be beneficial for addressing broad regression and classification tasks that involve sparse inputs, particularly those that feature categorical attributes with a high number of potential values.

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