In vitro micropropagation of *Chlorophytum borivilianum*: A Predictive Model Employing Artificial Neural Networks trained with a range of Algorithms

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Abstract: The formulation of plant tissue culture media continues to be a complex undertaking, primarily due to the intricate interplay of multiple components. Numerous factors (such as genotype, disinfectants, media pH, temperature, light, and immersion time) interact to affect the process of plant tissue culture. The artificial neural network is considered one of the most potent computational techniques that has emerged as a highly potent and valuable methodology for effectively representing intricate non-linear systems. This research paper focuses on the development of a predictive model for determining the number of shoots in response to different macronutrient compositions in the culture medium used for *in-vitro* micropropagation of *Chlorophytum borivilianum*. The study employs artificial neural networks (ANNs) trained with different algorithms to accurately predict the number of shoots and shoot length of the plant species. These algorithms include the Levenberg-Marquardt (LM), Scaled Conjugate Gradient (SCG), and Bayesian Regularisation (BR) backpropagation algorithms. A feed-forward backpropagation network was constructed with a single hidden layer consisting of ten nodes and two output units in the output layer. The input vector contained five elements. The transfer functions 'tansig' and 'purelin' were utilized for the hidden and output layers, respectively. In this study, the effectiveness of neural networks was tested by contrasting the outcomes with real-life data gathered from in-depth tissue culture experiments, which was named the target set. The comparative analysis of "Mean Square Error" and Pearson's correlation coefficient (R) were used to evaluate the effectiveness of networks for improved training initialization. The prediction ability of Levenberg-Marquardt was found superior to other training algorithms with an R-value of 9.92 also the output range of network ‘trainlm’ was closest to the empirical target range during the comparison of experimental target data ranges from wet lab practice.

Keywords: Artificial neural network, Bayesian Regularisation, Chlorophytum borivilianum, Levenberg-Marquardt, Scaled Conjugate Gradient (SCG)

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1. Introduction

In vitro micropropagation, a widely employed technique for plant propagation involves cultivating plant tissues in a controlled environment. The success of this technique largely depends on the composition of the culture medium, particularly the macronutrient content. Accurate prediction of the macronutrient composition is crucial for achieving optimal growth and development of the target plant species [1,22]. The majority of medicinal plants are going to be extinct if no steps are taken to preserve them. *Chlorophytum borivilianum* is a plant that is the epicentre of various medicinal properties and needs to be preserved with the boon of *invitro* micropropagation. A multilayer perceptron model with a feed-forward architecture has been applied to predict the shoot biomass of the mentioned plant [1,2].

Artificial neural networks (ANNs) have emerged as a prominent machine learning (ML) technique for the purpose of modeling and predicting intricate processes. ANNs are widely regarded as one of the most potent tools in the field, owing to their ability to effectively capture and represent complex patterns and relationships within data. They are computer simulations with biological inspiration that are used to carry out particular tasks. Neural networks are commonly shown as interconnected systems of "neurons" capable of performing computations by transmitting information through them. Artificial Neural Networks (ANNs) can be effectively utilized in machining processes to predict response parameters based on process parameters, provided that they have been appropriately trained. It is imperative to exercise caution and adhere to proper protocols while implementing Artificial Neural Networks (ANN) in these processes [3]. The design and implementation of an ANN must ensure that the set of input data leads to the intended output (either directly or through the use of a relaxation process). Various techniques can be employed to measure the magnitude of the connections. In other words, the weights can be predetermined based on prior information, or the neural network can be trained by inputting learning patterns and allowing the network to modify the weights based on a specified learning rule. An artificial neural network can be trained using supervised learning as well as unsupervised learning methods. Though there are other learning methods present, such as reinforcement learning methods, we will focus on supervised learning methods in this paper. The primary objective of a supervised learning algorithm is to ascertain a mapping function that effectively relates the input variable (x) to the output variable (y). There exist numerous types of artificial neural network (ANN) methods, which encompass perceptron, backpropagation, Learning vector quantization (LVQ), probabilistic neural network, Hopfield, and radial base network [4, 23]. The Back Propagation (BP) algorithm, however, is the most well-known and often employed learning technique for estimating the values of the weights. The Backpropagation Neural Network (BPNN) is a feedforward neural network utilizing chain rules. This approach utilizes a predetermined set of input and output values to determine the optimal weight and bias parameters for the neural network [5, 6]. The learning process consists of two distinct stages: the forward transmission of signals and the backward propagation of errors. The method terminates when the error function's value becomes negligibly small. The process chronology of the Backpropagation algorithm is shown in Figure 1. Traditional BP networks, however, have certain drawbacks, including slow convergence and a simple fall to the local minimum [7]. To mitigate the error associated with the backpropagation algorithm, various generalization methods have been utilized. These methods include Bayesian regularisation (BR) [8], Levenberg–Marquardt (LM) [9], and Scaled conjugate gradient [10]. These methods have been chosen due to their ability to achieve a lower mean squared error.

The Levenberg–Marquardt algorithm, also known as the LM algorithm, is a numerical optimization method that was independently developed by Kenneth Levenberg and Donald Marquardt. This algorithm is specifically designed to solve the problem of minimizing a nonlinear function. The observed phenomenon exhibits rapidity in its execution and demonstrates a consistent pattern of convergence [24]. This approach can be used to train small- and medium-sized issues in the field of artificial neural networks. In convergent situations, the Levenberg-Marquardt algorithm converges far more quickly than the steepest descent method, although it tends to be slightly slower than the Gauss-Newton algorithm. The fundamental concept

underlying the Levenberg-Marquardt algorithm is its utilization of a combined training process [11]. Bayesian regularisation is a novel approach that combines the principles of Bayesian methods and Artificial Neural Networks (ANN) to automatically determine the optimal regularisation parameters. This technique aims to enhance the performance and generalization capabilities of ANN models by incorporating Bayesian principles into the regularisation process[26]. By leveraging Bayesian regularisation, researchers and practitioners can effectively address the challenge of selecting appropriate regularisation parameters, which is a critical task in training ANN models. In contrast to the conventional methodology employed in network training, which entails selecting the optimal weight set by minimizing the error function, the Bayesian approach adopts a different perspective by considering the probability distribution of network weights. In light of the aforementioned observations, it can be deduced that the predictions generated by the network exhibit characteristics analogous to a probability distribution [12]. Another approach known as the scaled conjugate gradient (SCG), which was introduced by Moller in 1993, utilizes conjugate directions. However, unlike previous conjugate gradient algorithms that necessitate a line search at each iteration, the SCG algorithm does not. The SCG algorithm was developed to circumvent the laborious process of line search. It utilizes a step size that is determined by a quadratic approximation of the error function. This characteristic enhances the algorithm's robustness and reduces its dependence on user-defined parameters [13, 25].

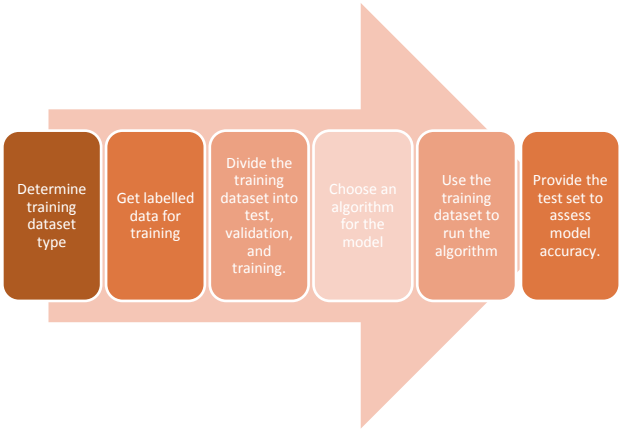

Fig 1: Several distinct stages comprise the procedure for supervised learning

The majority of medicinal plants are going to be extinct if no steps are taken to preserve them. *Chlorophytum borivilianum* is a plant that is the epicenter of various medicinal properties and needs to be preserved with the boon *of invitro* micropropagation [14]. A multilayer perceptron model with a feed-forward architecture has been applied to predict the shoot biomass of the mentioned plant. The primary aim of this research paper is to provide a thorough comparative analysis of the utilization of several artificial neural network training techniques in the prediction of no. of shoots and shoot length. This study investigates three training strategies for a multilayer perceptron (MLP) feedforward neural network: Levenberg-Marquardt (LM), Scaled Conjugate Gradient (SCG), and Bayesian Regularisation (BR) backpropagation procedures. The evaluation of the performance is conducted using various statistical metrics, including the mean square error (MSE), and Pearson's correlation coefficient (r). MATLAB is selected as the testing software for this purpose to carry out the necessary computations and visualizations.

2. Material and Methods

2.1 Compilation of Input data

The data used in this study was compiled after invitro propagation of *chlorophytum borivilianum* nodal explants grown in a Murashige and Skoog (MS) medium with different combinations of macronutrient concentrations ranging from 0.5 mg/l to 2.25 mg/l supplemented with 3% sucrose and 0.8% agar. All the explants were grown in a controlled environment of a 16h light/8h dark photoperiod at 25±2°C. The shoot length and number of shoots were noted after 20 days of culture establishment. The data retrieved from the experiments was used to train the Multilayer perceptron models.

2.2 Training the ANN models

Neural networks can be categorized based on their intended purpose, as well as their fundamental topology and the training method employed. The automation of commercial mass propagation of plants relies heavily on decision-making networks, which fall into the classification and clustering

models of neural networks [15]. The primary objective of this study is to evaluate the effectiveness of different training algorithms for neural networks (NN) in the field of plant tissue culture research. Among the different techniques available for neural networks, the specific approach employed in this study was the 'Backpropagation' (BPN) technique trained with Levenberg-Marquardt (LM), Scaled Conjugate Gradient (SCG), and Bayesian Regularisation (BR) algorithms.

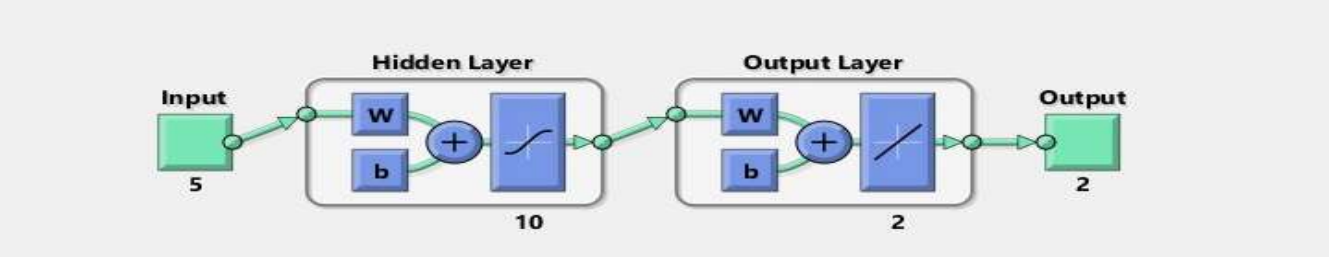

Fig 2: Artificial neural network model architecture

Feed-forward backpropagation-type network architectures were developed. Each network consisted of an input layer with five input nodes, a single hidden layer with ten nodes, and one output layer with two nodes trained with Levenberg-Marquardt (trainlm), Scaled Conjugate Gradient (trainscg), and Bayesian Regularisation (trainbr) functions, as shown in figure 2. The inputs to the ANN model were the varying concentrations of five media components, i.e., Ammonium nitrate, Potassium nitrate, Calcium chloride anhydrous, Magnesium sulfate, and Potassium phosphate monobasic. 42 combinations of the macronutrients were used to train the data. The datasets are categorized into three distinct subsets, namely the training set, validation set, and testing set. An altered transfer function between the hidden layer ('tansig') and the output layer ('purelin') has been implemented. Both the weight and the bias were initially set at random and were changed as the model was trained. It has been trained using a total of 250 epochs. The sigmoid function is used by the neuron set to calculate the weighted sum of its inputs. In the context of network analysis, the computed result is compared with the expected result to determine the accuracy of the network's performance. This comparison allows for the quantification of the discrepancy between the computed and expected results, which is commonly referred to as the error on the network. The error value is subsequently employed during the backward propagation step to modify the weights of neurons [16]. The output neuron generates the net output from the input neurons. Results from this study were compared with empirical data from thorough tissue culture experiments conducted to optimize different growth factors, allowing for a definitive conclusion to be drawn about the efficacy of neural networks.

3. Results and Discussion

The primary goal of this research was to evaluate the accuracy with which various backpropagation training algorithms predicted both the number of shoots and the length of shoots produced by *in vitro* propagation of *Chlorophytum borivilianum*. The evaluation of network efficiency for improved training initialization can be assessed through a comparative analysis of Mean Squared Error (MSE) and Pearson's correlation coefficient (r) for each trained network tabulated in Table 1.

Results	Levenberg-Marquardt	Scaled Conjugate Gradient	Bayesian Regularisation
MSE	1.82	1.91	1.91
R(Pearson's correlation coefficient)	9.92	9.189	9.81

Table1: Compilation of Mean Square Error and Pearson's correlation coefficient predicted by different algorithms

The Pearson correlation coefficient (r) is widely utilized as a primary method for quantifying the strength and direction of a linear relationship. The correlation coefficient is a statistical metric that quantifies the magnitude and direction of the association between two variables, with values ranging from -1 to 1[17]. The Mean Squared Error (MSE) quantifies the discrepancy between the estimated or predictive model and the observed values within a particular sample. The metric calculates the mean squared deviation between the anticipated values and the actual values, quantifying the disparity between the model's predictions and the observed data. The Mean Squared Error (MSE) is commonly employed as a metric for evaluating the performance of a model by comparing its predictions on the complete training dataset to the actual label or output value [18]. Accordingly, the efficiency of the network for training initialization including the training function was trainlm> trainbr >trainscg. The efficiency of trained networks for the least deviation from the target range was assessed by comparative observation between the experimental target data range and all trained network output ranges. Though the predictions made by all the MLPs trained with different algorithms were similar, the result predicted by the Levenberg-Marquardt algorithm was closer to the actual experimental results.

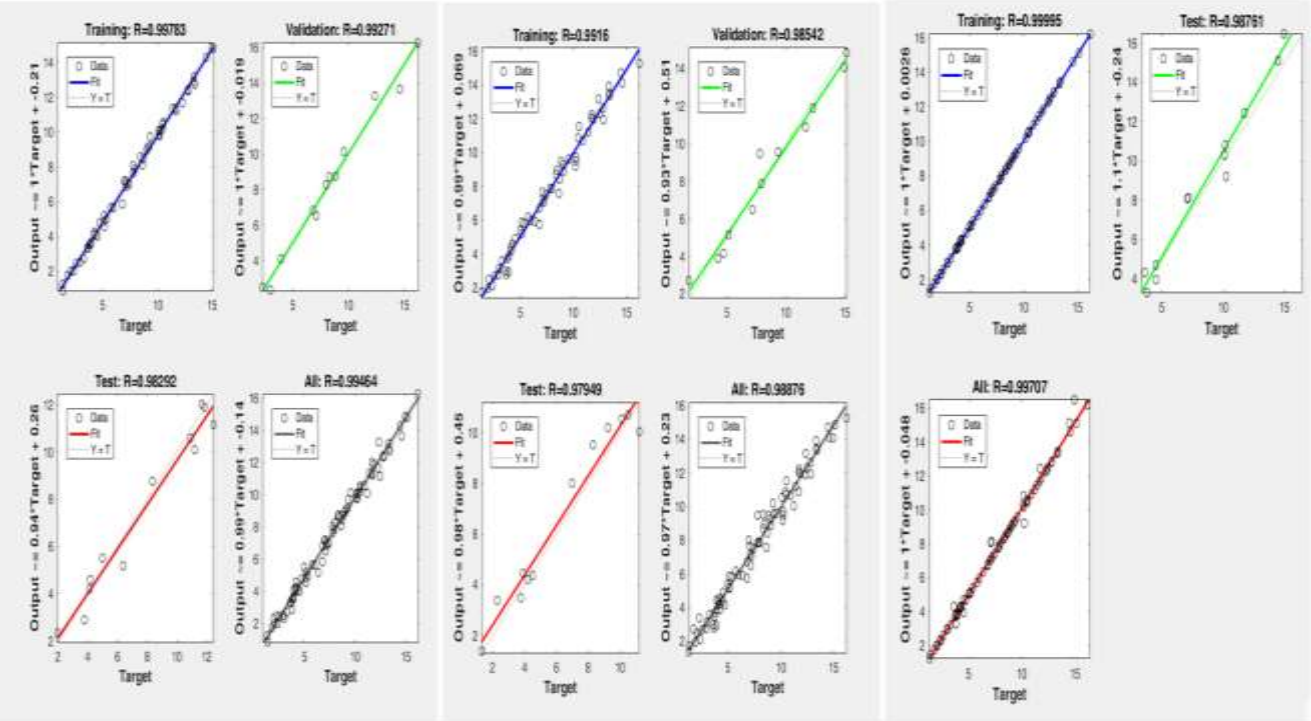

Fig 3: Regression plots as an output of Levenberg-Marquardt, Scaled Conjugate Gradient, and Bayesian Regularisation training algorithms

Hence, in the present investigation conducted on trained and tested datasets, it was observed that the trained network utilizing the "trainlm" transfer function, along with a three-layer feed-forward back propagation methodology, demonstrated proficiency in predicting optimal values for significant physical

parameters in the context of upscale culture. These parameters have a direct impact on biomass growth and, consequently, enhance overall productivity. The predicted values obtained in this study exhibit comparability to the values derived from experimental investigations as shown in Table 2. In conclusion, this approach offers a straightforward and unbiased means of making predictions in this domain and presents a neural network-based methodology for predicting the growth and productivity of in vitro cultured plants which can be used for successfully scaling up the cultivation process in larger bioreactors. Furthermore, neural networks have the potential to be utilized in the field of plant tissue culture in conjunction with other approaches to enhance the accuracy of results. These techniques may include image analysis, multiple regression modeling, and computer programming [19].

S.no.	**Inputs**					**Outputs**			
	Ammonium nitrate	**Potassium nitrate**	**Calcium chloride anhydrous**	**Magnesium sulphate**	**Potassium phosphate monobasic**	**Experimental Output**	**Bayesian Regularisation**	**Levenberg Marquardt algorithm**	**Scaled conjugate**
	1.42	1.42	1.42	1.42	1.42	11.86	11.897	11.859	11.197
	1.42	0.58	1.42	1.42	1.42	13.43	12.730	13.430	12.880
	0.58	0.58	0.58	1.42	0.58	12.47	11.168	12.469	12.448
	1	1	2	1	1	13.34	13.186	13.339	13.680
	1.42	1.42	1.42	0.58	1.42	10.24	10.060	10.240	9.636
	1.42	0.58	1.42	0.58	0.58	9.085	9.088	9.085	10.370
	0.58	0.58	0.58	0.58	1.42	10.45	10.186	10.450	11.723
	1	1	1	1	1	10.495	10.427	10.495	11.006
	0.58	0.58	1.42	0.58	1.42	12.8	12.435	12.799	14.116
	1.42	0.58	1.42	0.58	1.42	11.21	10.103	10.353	11.858
	2	1	1	1	1	8.73	8.474	8.729	8.036
	1.42	1.42	0.58	0.58	0.58	6.98	7.169	6.980	6.430
	1.42	1.42	1.42	1.42	0.58	10.91	10.592	10.909	10.578
	0.58	1.42	1.42	1.42	1.42	15.14	14.811	15.140	14.097
	1.42	1.42	0.58	1.42	1.42	9.58	10.145	9.435	8.883
	0.58	0.58	1.42	1.42	1.42	16.2048	16.285	16.204	16.043
	1	1	1	0	1	7.795	8.070	7.795	8.325
	1	1	0	1	1	7.81	7.660	7.809	7.702

0.58	1.42	1.42	0.58	1.42	11.42	11.363	11.419	10.670
1	1	1	1	0	10.17	9.866	11.669	9.655
0	1	1	1	1	14.48	14.305	14.480	13.811
1.42	1.42	0.58	0.58	1.42	8.31	8.723	8.3100	9.3197
0.58	0.58	1.42	0.58	0.58	11.68	12.0249	11.679	13.550
0.58	1.42	1.42	0.58	0.58	11.68	11.344	11.679	11.643
1	2	1	1	1	9.34	9.735	9.339	9.70
1.42	0.58	0.58	0.58	0.58	7.41	7.0235	7.409	6.448
1.42	1.42	0.58	1.42	0.58	8.64	8.097	8.639	8.0375
1	1	1	1	2	12.83	12.364	12.830	12.042
0.58	1.42	0.58	0.58	1.42	9.25	9.181	9.249	9.664
0.58	1.42	1.42	1.42	0.58	14.59	13.671	14.590	13.782
0.58	1.42	0.58	0.58	0.58	9.01	8.9481	8.995	8.317
1.42	0.58	0.58	1.42	0.58	8.44	8.586	8.440	9.224
0.58	1.42	0.58	1.42	0.58	10.52	10.558	10.519	10.768
1.42	0.58	1.42	1.42	0.58	12.31	11.678	12.309	12.505
0.58	0.58	0.58	0.58	0.58	10.21	9.771	10.209	9.514
1.42	0.58	0.58	1.42	1.42	10.11	10.083	10.110	9.251
1.42	0.58	0.58	0.58	1.42	8.84	8.755	8.839	8.761
1	1	1	2	1	12.36	13.288	11.823	12.874
1	0	1	1	1	11.73	11.227	12.229	13.134
0.58	0.58	1.42	1.42	0.58	14.96	14.886	14.960	14.731
0.58	0.58	0.58	1.42	1.42	13.34	12.905	13.340	12.971
1.42	1.42	1.42	0.58	0.58	8.36	8.768	8.3600	8.857

Table 2: Comparison of experimental output and outputs predicted by Artificial neural network models trained with different algorithms.

4. Conclusion

Neural computing presents a systematic and pragmatic methodology for representing the intricate patterns of growth and development in biological systems, both within and outside the context of in vitro experimentation. This approach offers a common endeavour that requires minimal time and utilizes the available information to its fullest extent [20]. The approach described in this study possesses several notable advantages. One such advantage is its independence from any prior knowledge about the structure or interrelationships between input and output signals [21]. The Levenberg-Marquardt algorithm has been identified as the most appropriate training algorithm for predicting the number of shoots. In the context of

artificial neural networks, the outcomes derived from these networks can serve as a fitness function for optimizing the process. The findings of this study have significant implications for the application of optimization algorithms in predicting the optimal macronutrient composition for achieving maximum shoot biomass.

The precise estimation of macronutrient levels has the potential to significantly reduce the financial burden associated with the formulation of nutritional media. The efficiency of prediction ability of Artificial Neural Networks (ANN) is heavily dependent on the selection of an appropriate training algorithm. The identification of the optimal training algorithm plays a crucial role in enhancing the training and prediction accuracy of plant in vitro physiological parameters. By incorporating suitable inputs into the algorithm, the prediction ability of ANN can be significantly improved. The utilisation of artificial neural networks (ANN) in predicting the optimal macronutrient levels for the growth of chlorophytum borivilianum has proven to be beneficial for both industrialists and researchers. This approach offers the potential to cultivate this plant species at a considerably reduced cost.

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Conflict of Interest

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