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# Artificial Neural Networks (MLP and RBF) as Tools for Weight Prediction of Orchid Synthetic Seeds Produced Using an Encapsulation Set-up

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**ABSTRACT**

A synthetic seed method involves processing encapsulated plant parts and any meristematic tissue which can develop into plantlets under *in vitro* or *in vivo* conditions. Various parameters and evaluation methods of 'one-variable-at-a-time' could be time-consuming, expensive, and inefficient. Thus, using process-modeling approaches including Multi-Layer Perceptron (MLP) and the Radial-Basis Function (RBF) can be required and beneficial for the prediction of synthetic seed weight. In the present study, two different types of artificial neural network (ANN) algorithms, the MLP and RBF models, have been developed to predict the weight of *Phalanopsis* orchid synthetic seeds using an encapsulation set-up especially developed for this purpose. Various topologies of ANN were configured based on different concentrations of sodium alginate (3, 4, and 5 (w/v)), calcium chloride (100,125, and 150 (mM)), and droplet falling height of sodium alginate (1, 1.5, and 2 cm) as input variables and the values of synthetic seed weights as output variables. Results showed that the RBF algorithm (R= 0.98 and SSE= 0. 13× 10-3) outperformed the MLP algorithm (R= 0.91 and SSE= 0.14× 10-3) owing to its better ability for predicting capsule weight. This study presented a machine learning-based approach for the classification of synthetic seeds. Algorithms for the extraction of capsule features have been developed, which are in turn used for training artificial neural network (ANN) classifiers. The outputs of ANNs were successfully applied herein to model the synthetic seeds production process, indicating that the appropriateness of the model equation in predicting orchid synthetic seed weight is mathematically integrated.

## Introduction

Synthetic seed technology is a popular method in plant biotechnology and agricultural science that can be defined as artificial encapsulation of explants for creating whole plants in vitro or in vivo (Magray et al., 2017). These synthetic seeds can retain their viability in terms of sprouting and

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conversion potential even after a considerable period of storage. The use of synthetic seeds helps the commercial propagation of rare and valuable plant species. The explants applied for encapsulation include somatic embryos, nodal segments, and protocorm-like bodies (PLBs). This kind of seed could be successfully planted in the field or greenhouse, having a potential for mechanical sowing at a commercial level, similar to regular seeds (Rihan et al., 2017). Another reason for using artificial technology is the fact that micro-propagules and somatic embryos are susceptible to drying unless coated with a hydrogel for planting in a greenhouse or mechanical sowing in the field (Chandra et al., 2018). Although various substances such as guar gum, sodium pectate, carrageenan, and agar have been studied as hydrogels for artificial seed production, sodium alginate is the most frequently used matrix because of its proper thickness, low toxicity to microorganisms, cost-effectiveness, bio-suitability, fast gelation, improvement in capsule structure and rigidity as well as better protection of explants against mechanical damage (Siraree, 2022). For the production of synthetic seeds, the explants are covered by sodium-alginate solution (0.5-5.0% w/v) consisting of liquid MS medium with sucrose. This is followed by adding a calcium chloride solution (30-100 mM) and then autoclaving. Since the desirable quality of capsules mainly depends on the gel matrix, they should consist of protective and nutritional agents which provide an appropriate micro-environment around the somatic embryos (Kocak et al., 2019).

Protocorm and PLBs are increasingly utilized by researchers as explants for the micropropagation of many rare and endangered orchid species (Antonietta et al., 2007; Chen et al., 2009; Fang et al., 2016; Gantait et al., 2015; Lee et al., 2013; Pradhan et al., 2014; Yeung, 2017). In most of these works, it was noted that not just the concentrations of the two gelling agents (sodium alginate and $CaCl_2.2H_2O$) but also the duration allowed for mixing can critically affect the structure, formation, roundness, rigidity, and flexibility of synthetic seeds. These factors, in turn, affect seed characteristics such as germination and storage ability (Oceania et al., 2015). In most cases, 2-3% of Na-alginate, when integrated with 75-100 mM calcium chloride solution, produced a desirable quality of synthetic seeds in many plant species (Reddy et al., 2012).

With 800 genera and 25000 species, orchids are one of the most fascinating of the ornamental plant families. Parallel to their commercial and economic importance, they exhibit an incredible range of diversity in size, shape, and color (Fay, 2018; Phillips et al., 2020; Wraith et al., 2020). In addition, orchids are of immense horticultural importance because of their roles in the ecological equilibrium of forests. Orchid flowers have emerged as prominent ornamental plants, in cut-flower production and potted plants, which fetch high prices in international markets (Sanghamitra et al., 2019). Orchids produce a large number of very minute and non-endospermic seeds, thereby limiting the chance of seed conversion into plantlets (Figura et al., 2021).

Artificial neural networks (ANNs) are defined as learning and computing systems based on experimental data that imitate some properties of neurological processing of the human brain based on computational techniques (Safari et al., 2021). ANN models can accurately use an unlimited number of input and output parameters to attain optimal system performance. They are data-driven, self-adaptive methods and can adaptively identify or model complex and nonlinear processes, thereby yielding information that can be used by another neural network. In addition, ANNs can be used as mathematic techniques to describe the relation between experiment inputs and outputs to statistically predict them in various processes. The main parts of each ANN structure consist of an input layer, a hidden layer(s), and an output layer of neurons (Tracey et al., 2011). The set of synaptic weights, the connections or architecture, and the transfer functions for each neuron determined the architecture of ANNs (Singh et al., 2020). Two of the more popular feedforward algorithms of ANN include multilayer perceptron (MLP) and radial basis function (RBF). In some cases, these constraints have led to modeling processes based on an artificial neural network (ANN).

Most applications of ANN consist of multilayer perceptrons used in deep learning. The basic MLP structure unit is a simple model of an artificial neuron in which each neuron output is connected to every neuron in subsequent layers connected in a cascade. All layers of the network are usually trained through a backpropagation algorithm to compute the weights of the network. By transforming input data into a desirable response, MLPs can generate approximations of nearly all segments of the input-output map and the performance of optimal statistical classifiers in difficult problems that cannot be analyzed linearly (Castejón et al., 2010).

RBF networks are approximate multivariable functions by linear combinations consisting of an input layer, a single hidden layer, and an output layer. In RBF architecture, there is a connection between each neuron of a layer and all of the neurons in the next layer, but not between the neurons on the same layer (Kopal et al., 2019). RBF applies radial basis functions (non-linear Gaussian) and non-linear sigmoid (or linear) functions in hidden layer neurons and an output layer, respectively. They generally use backpropagation functions for learning in the hidden layer and may usually be applied to approximate functions (Kopal et al., 2019).

Many studies reported the ability of different computational models based on MLP and RBF models to predict the changes in the quality parameters of crops during different processes (Baş and Boyacı, 2007; Mimouni et al., 2009; Youssefi et al., 2009). According to previous studies, the application of artificial neural networks has not been examined to predict synthetic seed weight as a critical factor for germination in orchids or other plants. The objective of this study was to evaluate the capability of ANN models (multilayer perceptron and RBF) to accurately predict the synthetic seed weight in *Phalaenopsis* orchid plants. Predictor variables of the models were sodium alginate concentration, calcium chloride concentration, and droplet falling height (DFH). The present research is part of a broader project aimed at investigating the potential of ANNs in predicting the physical properties of synthetic seeds. Artificial neural networks for weight prediction and quality detection of synthetic seeds are a suitable tool for the improvement of cultivation management and the avoidance of costly field surveys.

## Materials and Methods

### *Explants*

In this study, 2-3 mm protocorms of *Phalanopsis* (cv. 'Beijing') were used as an imported potted plant with very high ornamental value. These samples were utilized for making synthetic seeds. Protocorms were produced by seed culture (Mahdavi et al., 2018) in the Orchid Breeding and Propagation Laboratory, Horticulture Department, Aburaihan Campus, University of Tehran, Iran.

### *Encapsulation*

Protocorm encapsulation involved using three concentration levels of sodium alginate (3%, 4%, and 5% w/v) integrated into three concentrations of calcium chloride (100, 125, and 150 mm). A simple set-up was utilized to encapsulate protocorms of the *Phalanopsis* orchid (Fig. 1). This set-up included an alginate tank, connecting tube, flow-regulating pincher, and steel nozzle with an internal diameter of 0.38 cm. A perforated metal plate had holes 4 mm in diameter. The plate was used for creating droplets with suitable dimensions to completely cover the protocorm. It was placed on a beaker containing calcium chloride and a magnetic stirrer-heater was used for mixing the capsules into an integrated solution.

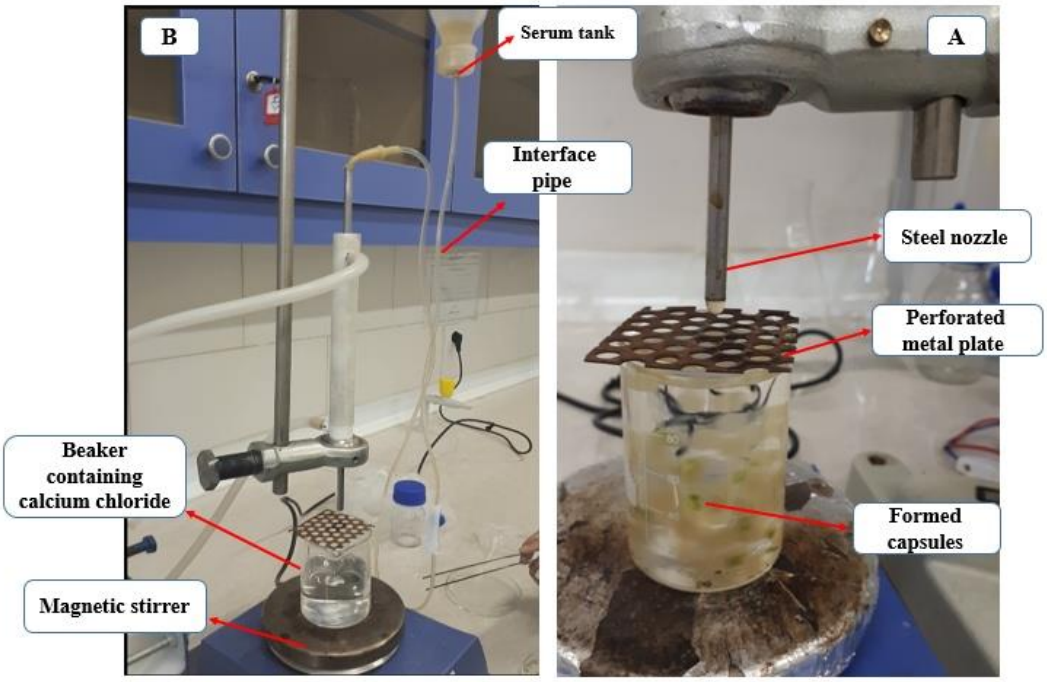


**Fig. 1.** a) encapsulation mechanism, b) formed capsules.

A single protocorm was placed on a layer of sodium alginate which was enclosed in a hole in the metal plate. The hole provided a suitable bed to fully cover the protocorm and had proper dimensions for orchid synthetic seeds. Sodium alginate flowed from the tank through a connecting tube to the nozzle and fell dropwise onto the perforated metal plate (**Error! Reference source not found.**). The capsules formed by dropping 2-3 drops into a hole of a metal plate containing the alginate layer. The droplet containing a protocorm fell into the calcium chloride solution, where the ion exchange reaction occurred and the capsules solidified. The height of the perforated plate from the surface of the complexion solution was adjusted by changing the volume of the calcium chloride to have different DFH values of 1, 1.5, and 2 cm. As a result, the performance of this set-up was such that the protocorm was positioned in the center of the capsules for a better provision of nutrition and protection (Fig. 1).

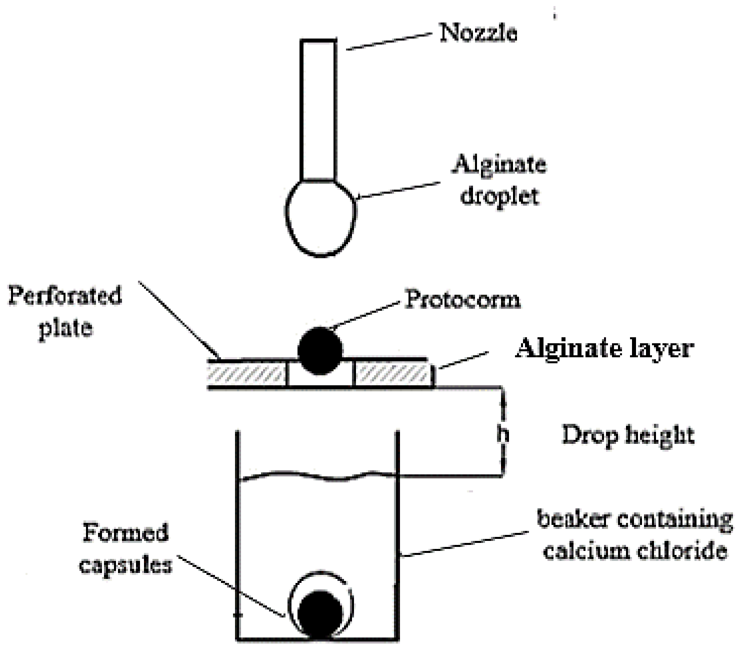


**Fig. 1.** Schematic of encapsulation for Phalanopsis orchid using the encapsulation set-up.

### *Statistical analysis*

In this study, synthetic seeds were placed into seven sterile Petri dishes and grouped into blocks, each block consisting of 5 samples from each treatment. The capsules were regularly weighed and the effect of sodium alginate concentration, calcium chloride solution, and gel DFH were examined on synthetic seed weight, using ANOVA by SPSS (version 16.0, SPSS Inc. USA). The mean values of treatments were compared using Duncan’s Multiple Range Test (DMRT) ($P \leq 0.05$).

### *Modeling*

Two effective feed-forward MLP and RBF neural networks were based on backpropagation. They were developed using experimental measurements to predict the changes in the weight values of synthetic seeds during the encapsulation process. The predicted values of performance for these models among all different values of training data were then compared. Fig. 2 represents a three-layered structure (MLP) that consists of 3 input layers, 1-20 hidden layers, and 1 output layer. The input layer accepts the data consisting of sodium alginate concentration, calcium chloride concentration, and DFH. The hidden layer processes these input data and finally the output layer presents the outputs of the model, i.e. the synthetic seed weight values. The training of RBF and MLP ANNs for the prediction of seed weights was implemented using 70% of the total data set. Subsequently, 15% of the data set was used for testing and the remaining 15% was used for validating the ANN performance. Data analysis of artificial neural networks was performed using STATISTICA 12 software to predict the response variable which was dependent on three independent variables. To evaluate the quality and reliability of the best network architecture, the lowest sum-square error (SSE) along with the highest coefficient of determination (R) were reported. In addition, the operational parameters of both MLP and RBF networks were, namely, the variable learning rate and tan-hyperbolic (tanh) for network training and network activation, respectively.

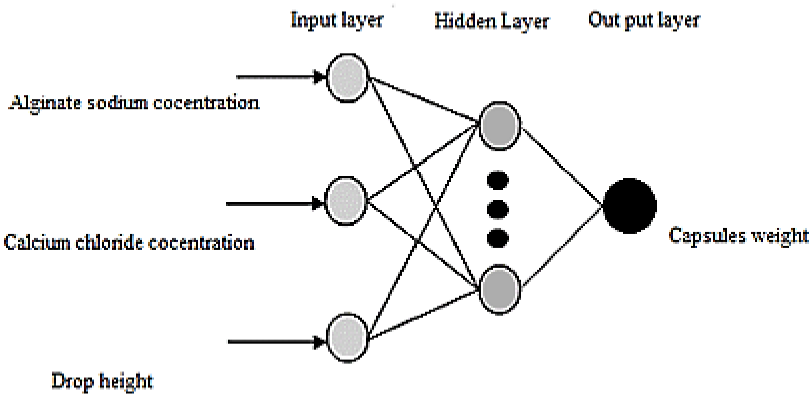


**Fig. 2.** Schematic depiction of ANN network in the present study.

## Results

### *Results of ANOVA for capsule weight*

Based on the results (
Table 1. **Results of ANOVA for capsule weight**), the analysis of variance showed that the effects of DFH and sodium alginate concentration, as well as DFH × sucrose, were highly significant on synthetic seed weight (p≤0.01). The calcium chloride concentration had no significant effect on capsule weight.

**Table 1.** Results of ANOVA for capsule weight

| S.V | df | MSE | F value |
|---|---|---|---|
| DFH | 2 | 0.012 | 4.839** |
| Sodium alginate concentration | 2 | 0.104 | 41.389** |
| Calcium chloride concentration | 2 | 0.004 | 1.566ns |
| DFH × Sodium alginate concentration | 4 | 0.006 | 2.399* |
| DFH× Calcium chloride concentration | 4 | 0.005 | 1.784ns |
| Sodium alginate concentration × Calcium chloride concentration | 4 | 0.006 | 2.223ns |
| Sodium alginate concentration × Calcium chloride concentration × DFH | 8 | 0.003 | 1.294ns |
| Error | 189 | 0.003 | |

| | |
|---|---|
| Total | 216 |

Differences between the weight values of artificial seeds of *Phalaenopsis* orchid, as affected by DFH × alginate concentration, are shown in plots of Fig. 3. As an example, in this plot, there was a significant difference among the means of capsule weights encapsulated in 4% alginate concentration at a DFH of 1.5 cm and those encapsulate in 3% alginate concentration with the same DFH. Meanwhile, the interaction effects of alginate concentration × DFH of 1 (cm) × 5% and 1.5 (cm) × 5% were non-significant. The error bar associated with each treatment on the plot indicated an estimate of the maximum and minimum weight of synthetic seeds. The 4% sodium alginate level with a DFH of 1.5 cm was found to be the most appropriate condition of encapsulation for the production of synthetic seeds, having maximum weight (0.164 g). These capsules were at least twice as heavy as the ones with a minimum weight of 0.054 g (encapsulated in 3% sodium alginate with a DFH of 2 cm) (Table 1). In Figure 4, a comparison of means showed a decrease in the DFH to the desired level (1.5 cm), resulting in a lower falling speed of droplets into the CaCl2 solution. Ultimately, this decreased the stresses on the capsules and helped increase the synthetic seed weight.

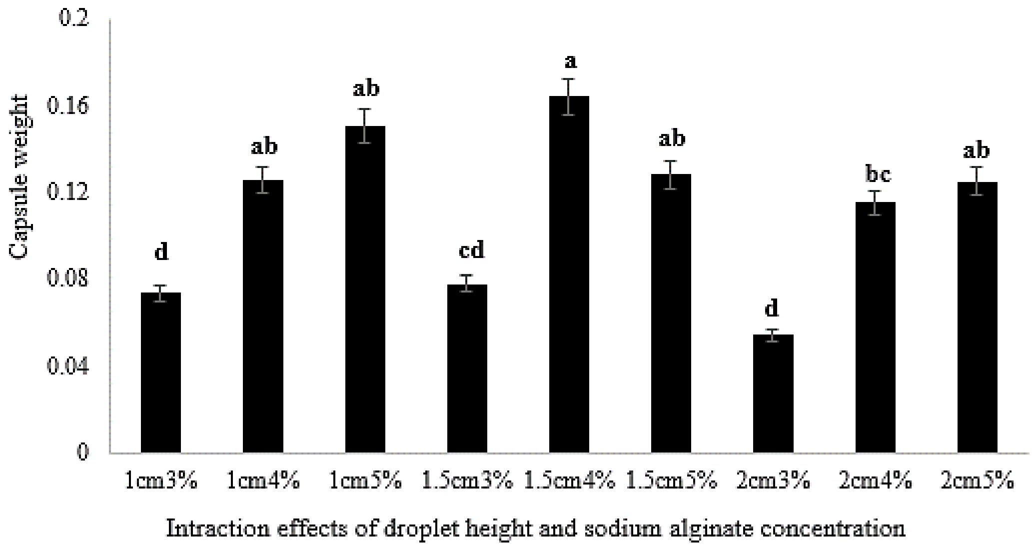


**Fig. 3.** Synthetic seed weight as affected by DFH × alginate concentration interaction.

## *Modeling of synthetic seed production process using MLP neural networks*

Multilayer perceptron (MLPs) and multilayer feedforward neural networks were utilized to model the synthetic seed production process. Alginate concentration, sodium chloride concentration, and DFH were considered as input data and the capsule weight was regarded as the output data. The model was evaluated based on performance indices (R and SSE in any topology) for artificial neural networks. In the MLP structure, the hyperbolic tangent function and identity function were adopted in the hidden and output layers, respectively. Table 2 shows the results of capsule weight prediction using artificial neural networks.

Results of qualitative analysis of the neural models showed that the best network was the topology with the 3-17-1 structure, where MLP had the lowest SSE (0.14 ×10E-3) and the highest correlation coefficient (R) was 0.91. The graph in Figure 5 indicated the cross-correlation of the predicted and measured values to check the prediction performance of the 3-17-1 MLP network which is acceptable. As shown in Figure 5, the best linear fit was indicated by a perfect fit (predicted equal to measured) as indicated by the red line. The lowest error and highest value of correlation coefficient were considered good indicators to check the prediction accuracy of MLP optimal structure (Fig. 5). To activate the hidden and output MLP layers, the hyperbolic tangent (Tanh) and identical functions were used, respectively.

**Table 2.** Results of capsule weight prediction using the MLP neural network

| Topology ( MLP) | R | | SSE × $10^{-3}$ | |
|---|---|---|---|---|
| | Train | Test | Train | Test |
| 3-17-1 | 0.91 | 0.99 | 0.14 | 0.19 |
| 3-10-1 | 0.90 | 0.99 | 0.16 | 0.28 |
| 3-20-1 | 0.85 | 0.98 | 0.24 | 0.31 |
| 3-3-1 | 0.82 | 0.96 | 0.27 | 0.42 |
| 3-16-1 | 0.81 | 0.93 | 0.29 | 0.54 |

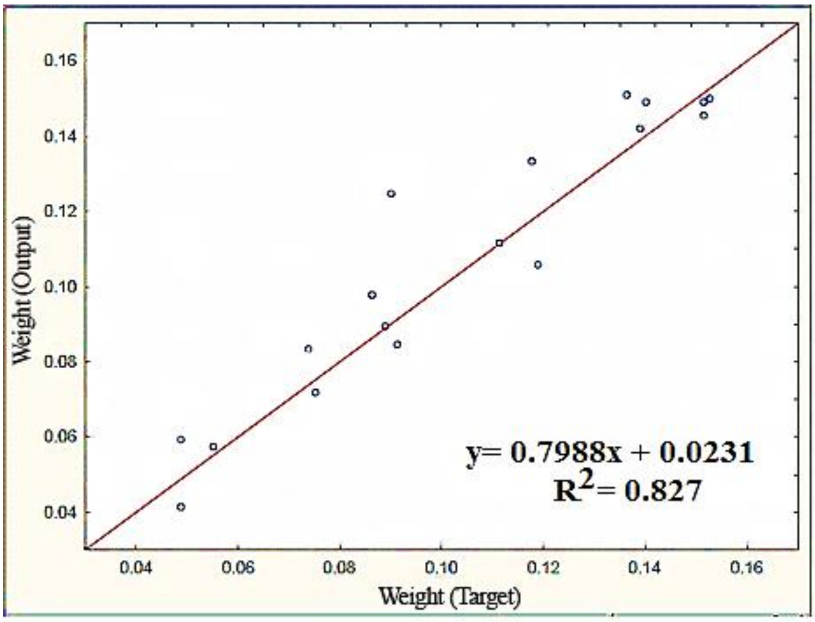


**Fig. 4.** Predicted vs. measured values of seed weight using 3-17-1 MLP neural network.

Three-dimensional modeling of the 3-17-1 MLP network was used for determining cross-correlations of the predicted and target values of capsule weight based on input parameters (alginate concentration interaction, calcium chloride concentration, and DFH) (Fig. 6-A, Fig. 6-B, and Fig. 6-C, respectively). According to the results (Fig. 6), the share of each input variable of the developed MLP model on the desired output (capsule weight) can be seen clearly.

## *Modeling of synthetic seed production processes using RBF neural networks*

In Table 3, several RBF models were developed and compared to select the best result based on maximal R and minimal SSE for the prediction of the capsule weight. The best performance was obtained by RBF topology of 3-15-1 with R=0.98 and SSE=0.13×10E-3, which represented a reasonable accuracy. It was found that the RBF neural network presented a more precise model than MLP algorithms, thereby providing higher accuracy and lower SSE. To activate the hidden and output RBF layer, the hyperbolic tangent and identical functions were used, respectively.

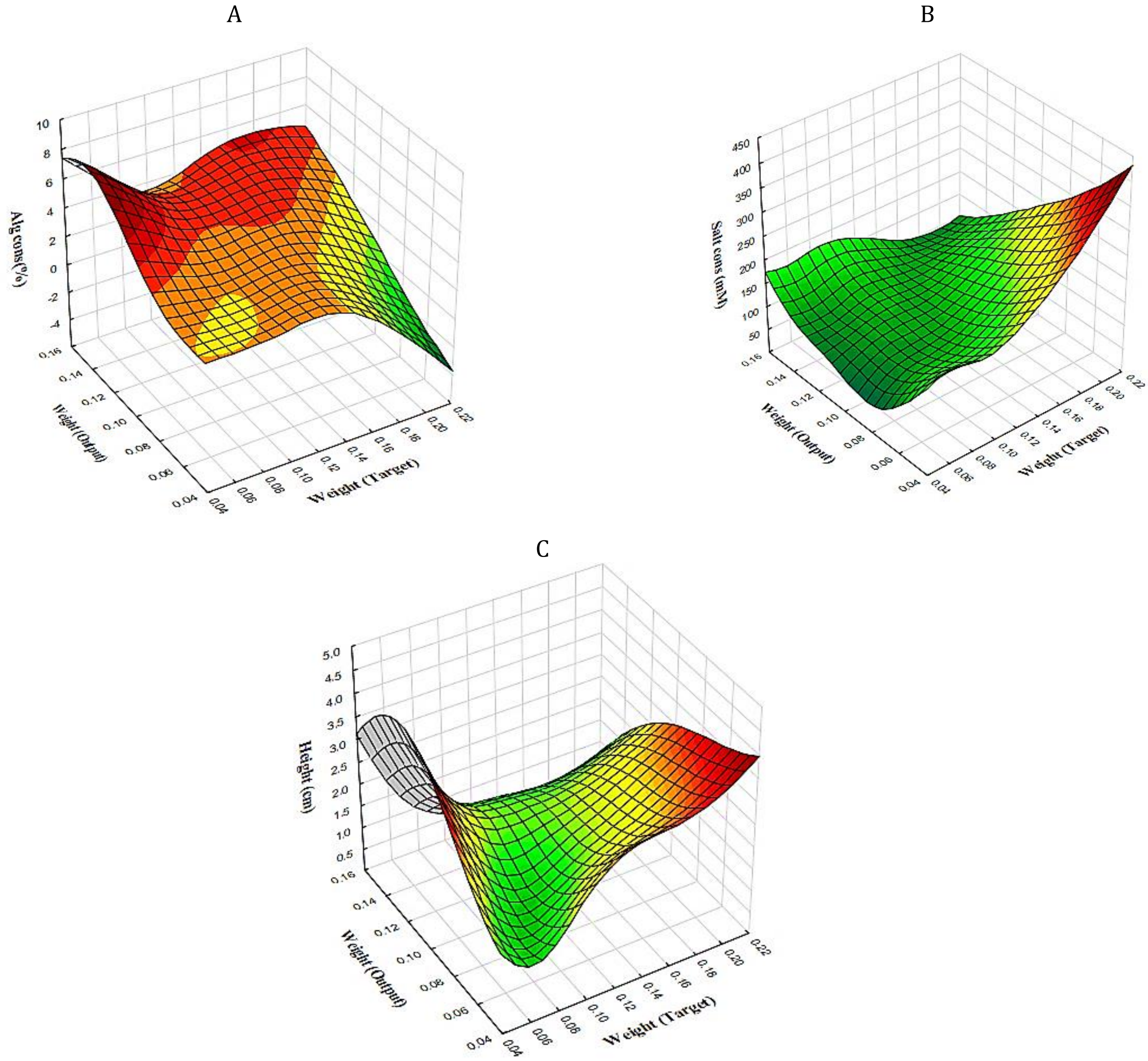


**Fig. 5.** Three-dimensional modeling of 3-17-1 MLP network for a) alginate concentration interaction, b) calcium chloride concentration, and c) DFH.

**Table 3.** Results of capsule weight prediction using the RBF neural network

| Topology ( RBF) | R | | SSE × $10^{-3}$ | |
|---|---|---|---|---|
| | Test | Train | Test | Train |
| 3-15-1 | 0.98 | 0.91 | 0.13 | 0.17 |
| 3-11-1 | 0.96 | 0.96 | 0.58 | 0.13 |
| 3-17-1 | 0.96 | 0.98 | 0.59 | 0.19 |

| | | | | |
|---|---|---|---|---|
| 3-18-1 | 0.92 | 0.97 | 0.12 | 0.30 |
| 3-15-1 | 0.91 | 0.99 | 0.14 | 0.15 |

Figure 7 presents the graphical output provided for the RBF models that plotted the predicted values (network outputs) versus the measured values (targets). As shown in Figure 7, the good cross-correlation of the predicted and target values indicated that the RBF model was most acceptable. RBF was applied to the dataset and was able to correctly predict capsule weight, with an efficiency rate of 96%. This is considered to be an excellent initial performance. However, it can be further improved by optimizing the network, changing the weights, and increasing the number of training datasets.

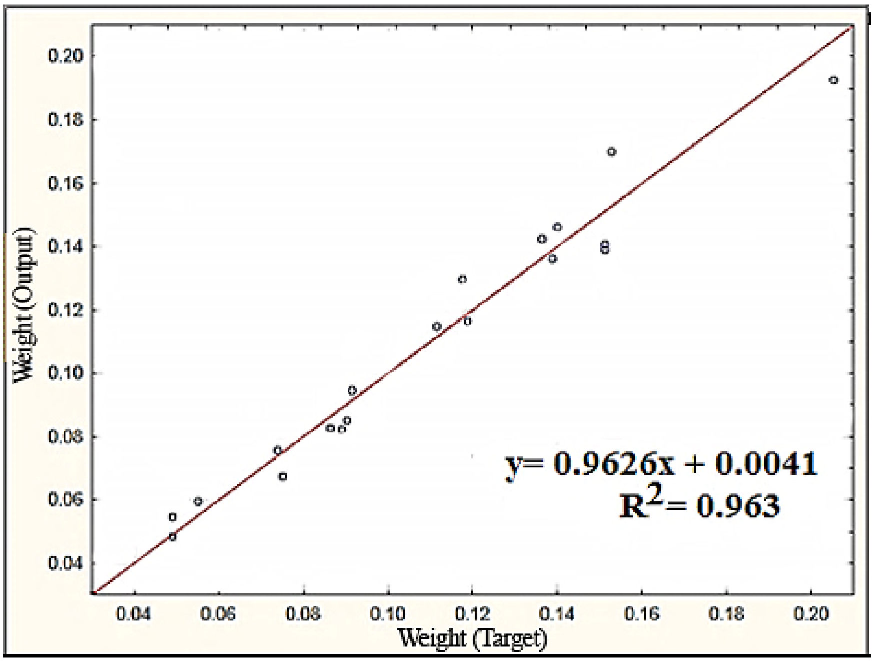


**Fig. 6.** Predicted vs. measured values of seed weight using the 3-15-1 RBF network.

To assess the predictive ability and validity of the RBF model and predict capsule weight based on the input parameters, the assessment involved alginate concentration interaction, calcium chloride concentration, and DFH (Fig. 8-A, Fig. 8-B and Fig. 8-C, respectively). Accordingly, 3D models were developed using the best network selected. A good level of accuracy was observed in the 3-15-1 structure, resulting in a much better RBF performance than MLP for predicting the capsule weight.

## Discussion

The physical properties of synthetic seeds affected their quality and performance. It is important to monitor these properties and predict them during production. This assists in optimizing the synthetic seed production process. In this study, two artificial neural networks, RBF and MLP were applied to model the encapsulation process and predict orchid synthetic seed weight. The input parameters were alginate concentration, sodium chloride concentration, and DFH. Meanwhile, synthetic seed (capsule weight) was selected as the output parameter. In Figure 4, it was revealed that increasing the concentration of sodium alginate to the desired level, not only prolonged the ion exchange time but also affected the quality of synthetic seeds, producing heavier capsules with greater firmness. The present finding was supported by Gantait et al. (2017) who similarly examined the effects of sodium alginate and CaCl2.2H2O concentration on synthetic seed quality (Gantait et al., 2017). The performances of the models were compared based on R and SSE indices. Using Artificial Neural Network (ANN) for this prediction, it was revealed that the optimal network was MLP topology as 3-17-1 with R=0.91 and SSE=0.14×10E-3, followed by RBF topology as 3-15-1 with R=0.98 and SSE=0.13×10E-3. To activate the hidden and output layers in both MLP and RBF models, the hyperbolic tangent and identical functions were utilized, respectively.

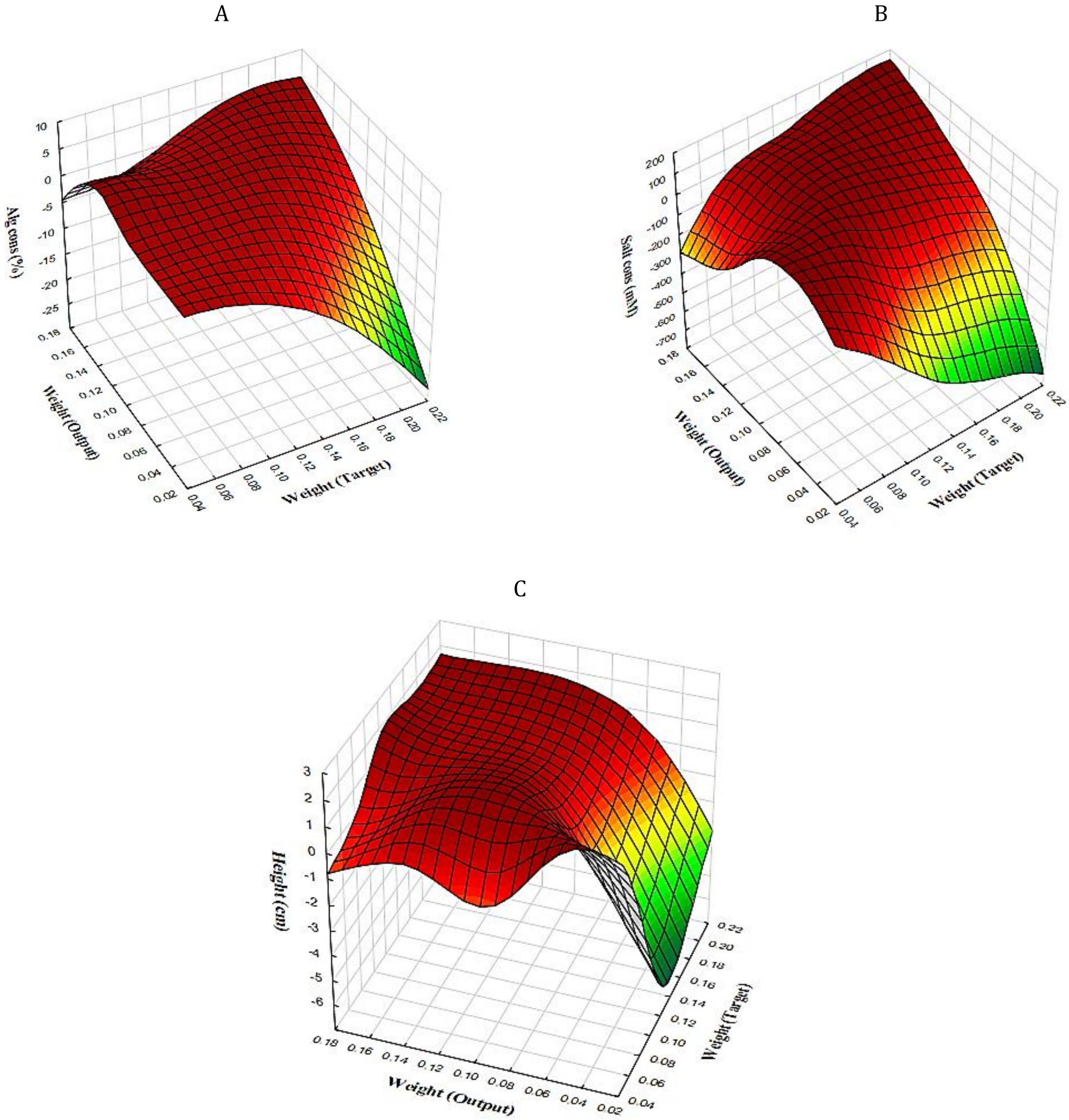


**Fig. 7.** Three-dimensional modeling of 3-15-1 RBF network for a) alginate concentration interaction, b) calcium chloride concentration, and c) DFH.

Similar results were reported by Tau et al. (2017) indicating that an artificial neural network can be a powerful tool in determining the correlation of measured and predicted variables in modeling blueberry anthocyanin extracts through the encapsulation process (Tao et al., 2017). According to the correlation of the predicted and measured values, associated with an optimal architecture, it can be said that MLP is a useful tool for the prediction of orchid synthetic seed capsule weight (Fig. 6). MLP and RBF algorithms were used in a similar study to determine the safety and integrity of leaf samples. The results clearly showed that the RBF Neural Network classifier had a better presentation, precision, and upgraded detection speed (Sumathi and

Karthikeyan, 2021). This study indicated better predictive capabilities of the RBF neural network to predict and model synthetic seed processing, compared to the MLP. In a similar case of research, the RBF and MLP techniques were successfully applied for the classification of healthy and infected tomatoes. The results showed 96% and 98% accuracy for the classification of healthy, powdery mildew (*Oidiumly copersicum*) and spider mite-infected plants, respectively (Ghaffari, 2010). The main characteristic of ANN models is their learning capacity. 'Property' means that when using a neural network, there is no need to program how the output is obtained, given a certain input. Rather, examples are shown of the relationship between input and output, and the neural network can thus learn the existing relationship between them via a learning algorithm. Once the neural network has learned to carry out the desired function, the input values can be entered and the neural network can calculate the output. This provides a quick and inexpensive method for classifying and predicting the characteristics of agricultural products. A similar study was conducted to develop an RBF neural network for estimating biomass and shoot length in plant cell cultures (Zielińska and Kępczyńska, 2013).

## Conclusion

The results confirmed the usefulness of RBF to predict the growth of plants under different *in vitro* conditions. It is concluded that ANN is a useful tool for predicting synthetic seed capsule weights and modeling the encapsulation process.

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### Conflict of Interest

The authors indicate no conflict of interest for this work.

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