Use of Artificial Neural Network in Predicting Survival and Growth Rates and Biochemical Characteristics in *Dendrobium* Sonia-28

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Abstract- *Dendrobium* sonia-28 is a popular orchid hybrid for its flowering recurrence and dense inflorescences which currently facing serious production problems due fungal diseases. In the present study, Protocorm like bodies (PLBs) of *Dendrobium* sonia-28 were subjected to different doses of gamma irradiation (10-200 Gy) followed by inoculation with various concentrations of *fusarium proliferatum* culture filtrate (CF) (2.5-20%). The results from these measurements were used in establishing an artificial neural network model meant to predict the result of more samples while being treated as carrying out laboratory measurements would be time consuming. CF and gamma irradiation were model inputs, while output was result value. The prediction performances of various neural network modelswere evaluated using statistical performance indices such as root of the mean squared error (RMSE), the mean squared error(MSE), and the multiple coefficient of determination (R2). The results show that the multilayer perceptron (MLP) neural network model with different nodes in the hidden layer was desirable for predicting results. Artificial Neural Networks analysis indicated that survival and growth rates of treated PLBs were dependent to treatment doses. Biochemical results revealed that the chlorophyll and total soluble protein decreased notably as the irradiation and inoculation concentration increases.

I. Introduction

In nature, plants may not be totally free from stresses and are expected to experience some degree of biotic and abiotic stresses(Ramoliya *et al.*, 2004). Furthermore, among the various environmental stresses, pathogens attack significantly limited the plant growth and development (Dehgahi*et al.*, 2015b; Dehgahi&Joniyas, 2016).

To combat stresses, plants exhibit several mechanisms which make them withstand the stress with the formation of new molecules and molecular mechanisms of stress tolerance. These variations are important in producing biotic stress tolerance in plants. However, the degree of tolerance differs from high to low length plants and also from plant to plant (Reddy *et al.*, 2006). In the 21st century, developing stable stress tolerance plants would be a challenge for plant scientists (Atkinson &Urwin, 2012).

In vitro selection method via fungal elicitors is preferable due to their low toxicity for plant cell cultures as well as their high efficiency (Yuan *et al.*, 2002; Dehgahi*et al.*, 2014). Establishment of an appropriate phytotoxin concentration that negatively affects cells, tissues, organs and whole plants increases the probability of obtaining disease tolerant lines (Daub, 1986; Dehgahi*et al.*, 2015a). *Fusarium* CF has been used widely in callus tests to select tolerant genotypes (Dehgahi*et al.*, 2014).

Fusarium CF was recognised as a suitable agent for the induction of mutants and selection of disease resistant plants (Modgil*et al.*, 2012).*In vitro* mutagenesis is a combination of *in vitro* culture and mutation induction, which provides the opportunity to increase variability of an economically important cultivar or used on plants in developing varieties that are agriculturally and have high productivity potential (Jain *et al.*, 1998; El-Beltagi*et al.*, 2011). Gamma application is preferred since it is not a threat for humankind and environment (Ulukapi&Nasircilar, 2015). Until now, some *Dendrobium* orchids have been successfully generated via mutagenesis and gamma irradiation in Malaysia (Ahmad *et al.*, 2006; Ariffin*et al.*, 2010).

Nowadays, the artificial neural network techniques have been widely used for predicting the nonlinear behaviour of many technical tasks (Ozer *et al.*, 2008). An artificial neural network does not require the accurate knowledge of relationships between variables as needed by traditional techniques.Thus,artificial neural network could be conveniently used for resolving issues whichmathematically, physically or structurally more are complicated. Vakili*et al.*(2015) stated that significant advantage of a neural network is that they could be updateable continuously when newdatais available. Furthermore, in contrast to an empirical equation which is created according to limited affecting parameters, logical concurrent relationships between large array of independent and dependent variable would be used in artificial neural network.

Consequently, the current study was undertaken to probe and investigate the effect of gamma radiation and CF concentrations as a mutagen for *Dendrobium* sonia-28 PLBs.Thus, in order to predict the survival and growth rates as well as chlorophyll and protein contents after stress, a suitable neural network program was developed which stress parameters and abovementioned results indicate inputs and outputs, respectively.

II. Materials and Methods

In vitro propagation of PLBs

The PLBs were cultured in semi-solid half-strength MS medium (Murashige&Skoog, 1962) supplemented with 2% (w/v) sucrose, 2.75g/L, Gelrite^{TM} (Duchefa, the Netherlands) and 1mg/L of benzylaminopurine (BAP; Duchefa, the Netherlands).

The cultures wereincubated at 25±2ºC under 16 hours photoperiod using cool white fluorescent lamps (Philips TLD, 36W, 150 µmol.$m^{-2}.s^{-1}$).

The pH value (CyberScan PC 510 pH/mV/Conductivity/TDS/°C/°F Bench Meter, Eutech Instruments, Singapore) was adjusted to 5.7-5.8 before autoclaving (STURDY SA-300VFA-F-A505, Sturdy Industrial Co. Ltd., Taiwan). The PLBs were then subcultured every four weeks.

Gamma radiation on PLBs

In order to determine PLB's gamma radiation sensitivity, four weeks old of 3-4 mm PLBs were irradiated with acute gamma irradiation at Agrotechnology and Bioscience Division, Malaysian Nuclear Agency, Bangi, Selangor. The source of gamma rays was Cobalt-$_{60}$. Hundred (100) PLBs were acutely irradiated with different doses of 0, 10, 20, 30, 40, 50, 60, 80, 100, 150 and 200 Gy (10 replicate containing 10 PLBs for each dose). PLBs were maintained at the above mentioned condition.

CF treatment of Dendrobium sonia-28 PLBs

The method reported by Tripathi*et al.* (2008) was used for the preparation of culture filtrate. In order to evaluate the effect of various severities and virulence of Fusarium wilt index. One hundred gamma irradiated PLBs were inoculated on MS medium containing 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20% CF and incubated for four weeks under a 16 hour photoperiod using cool white fluorescent lamps at 25±2°C. One hundred (100) additional PLBs (control) were cultured in Petri dishes, and incubated under the same conditions.

Determination of survival and growth rate of treated PLBs

Gamma irradiated PLBs surviving post CF treatment were selected after four weeks. At the end of selection process, PLB's survivability was scored based on colour of PLBs. The survival percentage of PLBs after selection process was calculated as follow:

$$\text{Survival (\%)} = \frac{\text{Number of survived PLBs}}{\text{Total PLBs cultured}} \times 100\%$$

Growth rate percentages were calculated using the following formula where FW indicates fresh weight (Sopalun*et al.*, 2010).

$$\text{Growth rate percentage (\%)} = \frac{\text{Final FW - Initial FW}}{\text{Initial FW} \times \text{Days of incubation}} \times 100\%$$

Determination of chlorophyll contents

The technique used by Harborne (1973) was used to evaluate the chlorophyll values of control and treated PLBs using following formula:

Chlorophyll a = 11.75 (A_{663}) - 2.35 (A_{645})
Chlorophyll b = 18.61(A_{645}) - 3.96 (A_{663})
Total chlorophyll = chlorophyll a+chlorophyll b

Determination of total soluble protein content of untreated and CF treated PLBs

The Bradford (1976) assay employs bovine serum albumin (BSA, Sigma, USA) as standard was used to determine the total soluble protein content of treated and control PLBs.

Artificial neural networks modelling

To create general relationships between variables in a given issue,an artificial neural networkhas been used. Artificial neural networkis considered as a model of artificial intelligence as well as it is consumed as somethingable to simulate the brain and nervous system of humans (Shahin *et al.*, 2008; Ozer *et al.*, 2008,). Artificial neural networkis made of a various processing elements,neurons or nodes. Feed-forward multilayer perceptron network which consisted back propagation algorithms, input and output layers, and some hidden layers is the most widely used artificial neural networks (Erzin*et al.*, 2009).

Habibagahi&Bamdad (2003) and Erzin*et al.*(2009) stated that although there is no connection among the neurons in the same layer, each neuron is fully connected with the next layer neurons. The output of each node could be defined by following Equation:

$\alpha = £\,(\sum^{n}_{i=1} P_i W_{j,i} + b_j)$

Where αindicates output of node *j*, P*i* indicates input from *i*th node, W*j,i*indicates the connection weight between *j*th node of the layer and ith node of the previous layer, b*j*is the bias at the *j*th node. Furthermore,£indicates the transfer function.

Moreover, Erzin*et al.*(2009) stated that although node transfer function is often expressed by sigmoid functions, depending on the nature of the variables, other functions including hyperbolic linear and tangent functions could be used.

Nodes number in the input and output layersindicate the number of input and output variables,respectively (Erzin*et al.*, 2009). Erzin*et al.*(2009) stated thatthe neural network systems are normally developed through dividing the available datasets into training, validation, and test subsets. A model is initially trained by using 70% of the data. However, 15% of the data has been used for validation process to minimizing over fitting. Trained model performance has been tested by the15% remaining data. Hence, for model constructing, the training data are used, however, the testing and validation data are used for accuracy controlling of the developed model (Erzin*et al.*, 2009). System error is defined as a difference between an experimental and network prediction value (Vakili*et al.*,2015). Furthermore, Sinha & Wang(2008) and Erzin*et al.*(2009) reported that a minimum network error is created by changing the nods number and weight in the hidden layer via cycles of trial and error during training. Various statistical performance indices are often used to analyse the developed network prediction performance. For the current research, mean squared error (MSE), root of the mean squared error (RMSE) and multiple coefficient of determination (R^2) have been used as the statistical criteria.

The above mentioned statistical models have been used to select optimum network as well as to quantify the predicted data accuracy from the neural network methods. In the following formulas, n indicates the data setnumbers, $k_{pi}$and $k_{mi}$are the predicted and measured results, respectively:

$RMSE=((1/10)\sum^{n}_{i=1}(k_{pi}-k_{mi})^2)^{0.5}$
$MSE=1/n\sum^{n}_{i=1}(k_{pi}-k_{mi})^2$
$R^2=1-(\sum^{n}_{i=1}(k_{pi}-k_{mi})^2/\sum^{n}_{i=1}(k_{pi})^2)$

According tothe methods, the optimum network would have the least MSE and RMSE, which the results of a perfect fit have zero MSE and RMSE. R^2can be used as the closeness of fit. While, R^2 =0 and R^2 =1 are the result of a poor and perfect fits, respectively.In this study, the abovementioned valueshave been calculated for different neural networks developed by differentnodes number in the hidden layer. Furthermore,separate optimum neural networkshave been selected accordingly.

In the current study, 2 variables effects on results characteristics have been considered.Hence, the trained neural network system included2 nodes in the input layer and one in the output layer. A total of 99 datasets have been used in this research. Networks with various nodes in the hidden layer were trained to find the optimum neural networks with the least error. MATLAB (2012) is the using software for modelling of the mentioned neural network which used the integration module in the computer application GENES.

III. Results

Effect of gamma irradiation and CF inoculation on PLBs survival rate

Gamma irradiation and CF inoculation at various doses showed a significant effect on survival rates of PLBs. Lower post-treatment survival was obtained when PLBs were treated with higher concentrations of gamma and CF (Figure 1).

The measured result has been compared with predicted results in different nodes of the hidden layers. Our results indicated that the proposed neural network with 10 nodes in the hidden layer (R=0.98172 and RMSE=3.5988×10^{-8}) was found desirable in predicting survival rate(Table 1, Figures 2, 3). The equality line(y=T) indicated network error. A system which showed zero error would have all plots positioned on the equality line. Hence, the plots deviation from the equality line shows system error. For the unknown properties, datasets from previous tests can be used and further tests can be carried out to determine whole results of samples through neural network models.

Effects of gamma irradiation and CF inoculation on the PLBs growth rate percentage

Interaction of various CF and gamma doses indicated higher doses of treatments was found to have significant effects on fresh weight of PLBs (Figure 1). Increasing CF concentration to 20% and gamma radiation to 200 Gydisplayed a greater reduce of growth rate in PLBs compared to PLBs inoculated with lower concentration of treatments. Furthermore, the neural network developed for 5 nodes in the hidden layer was found to be optimum as it has the least value of RMSE and MSE and the most value of R^2(Table 2, Figures 4, 5).

Effects of gamma irradiation and CF inoculation on the chlorophyll and protein contents

The effect of different CF and gamma doses on the physiological aspects of PLBs was studied. Results indicated that there was considerable reduction in chlorophyll and protein contents of the treated PLBs. However, higher reduction could be observed after higher doses of treatments.Moreover, the neural network developed for 12 and 7 nodes in the hidden layer were found to be optimum for chlorophyll and protein contents, respectively, as they have the least value of RMSE and MSE and the most value of R^2(Table 3, Figures 6, 7) and (Table 4, Figures 8, 9).

IV. Discussion

Results obtained by neural network model indicated higheraccuracy compared to the estimated and measuredresults.Moreover, Noh *et al.* (2006)and Gautam &Panigrahi (2007) obtained high accuracy through neural network analysis on corn plants (MSE = 0.03 and MSE = 0.066,respectively).

Similarly, Wang *et al.* (2009) analysed their results through the neural network model and stated that RGB components and growth and survival rates was not perfectly linear on assessing rape plant nitrogenconcentration.Furthermore, Moghaddam*et al.* (2011) verified that the MLPNN model canprovide more accurate estimations of sugar beet leafchlorophyll, in comparison tothe linear regressionmodel. Kumar *et al.* (2013) stated that gamma irradiation affects greatly on germination of nine popular rice varieties and their results indicated that increasing doses of gamma irradiation had significant effect on germination for the first seven days under laboratory conditions.

Vakili*et al.* (2015) reported that usingstress accurate dose in each project needsspecific measures, adequate management and precautions for avoidingany project failures. Limited budget and time and resolving stress problems resulted to improving different techniques. These methods should also be interpreted along with the fact that survival and weight rates and chlorophyll and protein contents decreases with higher stress doses. However, measuring the accurateresults is time and money consuming. By using artificial neural network techniques, survival, weight and physiological characteristics can be determined faster with reasonable accuracy.

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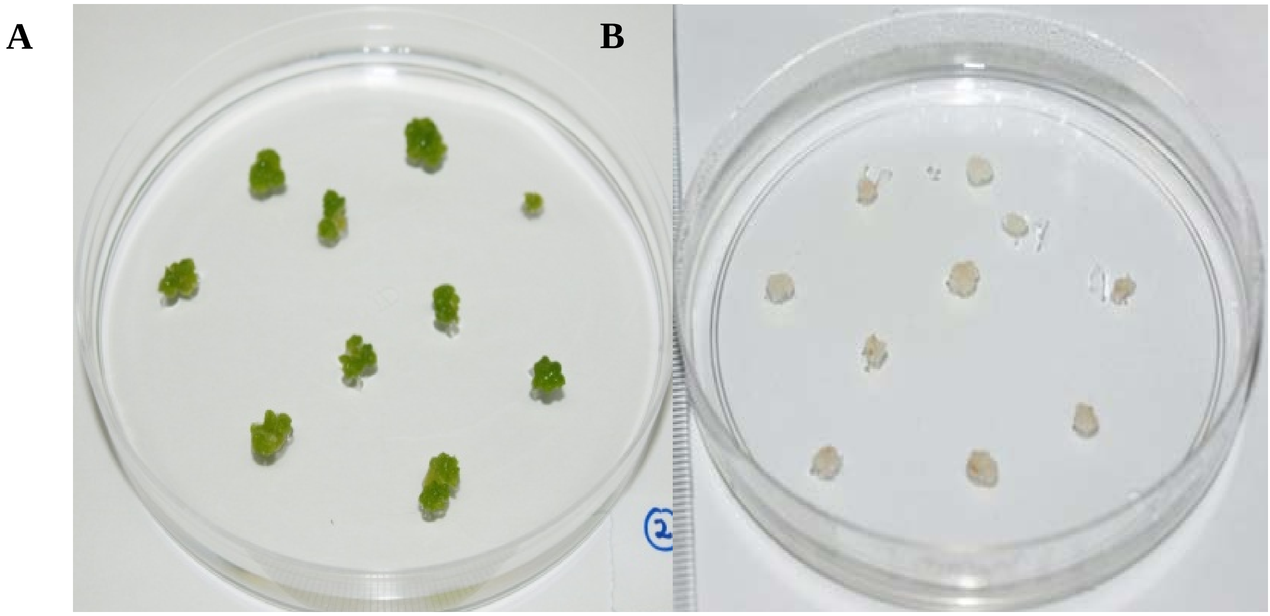

Figure1: Effect of gamma radiation and CF on *Dendrobium* sonia-28 PLBs after four weeks of inoculation. A (Untreated PLBs), B (200 Gy irradiated PLBs treated with 20% CF).

Table 1: Outcomes of various neural networks for *Dendrobium* sonia-28 survival rate results developed for the different number of nodes in the hidden layers

Number of nodes	RMSE	MSE	R	R^2
3	10.393×10^{-8}	9.798×10^{-15}	0.91028	0.828609678
5	6.2649×10^{-8}	5.6249×10^{-15}	0.95581	0.913572756
7	7.6001×10^{-8}	6.251×10^{-15}	0.94834	0.899348756
10	3.5988×10^{-8}	2.8297×10^{-15}	0.98172	0.963774158
12	4.3189×10^{-8}	3.7189×10^{-15}	0.97426	0.949182548
15	5.5604×10^{-8}	3.2676×10^{-15}	0.96472	0.930684678

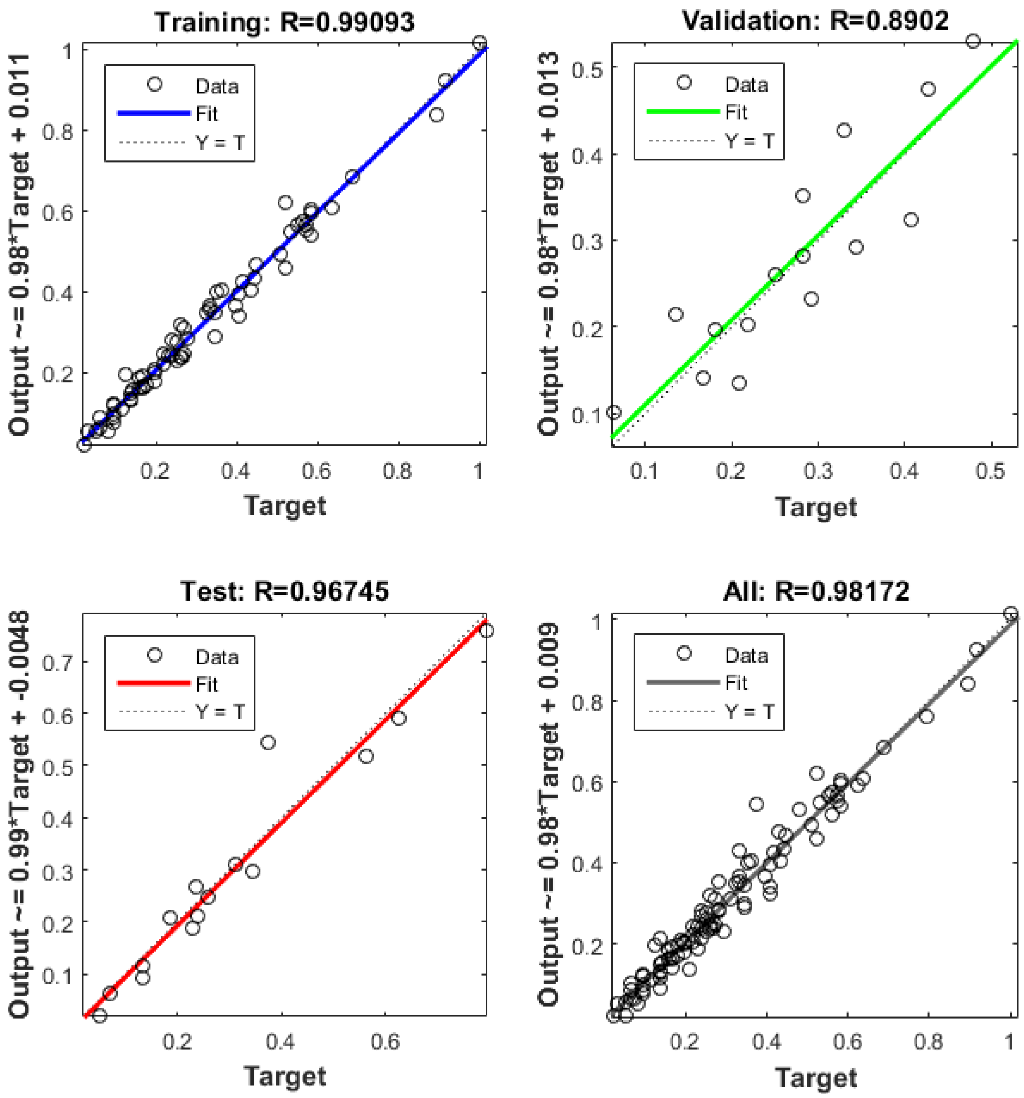

Figure 2: Plot of measured *Dendrobium* sonia-28 survival rate versus predicted survival rate from neural network modelling for 10 nodes in the hidden layers

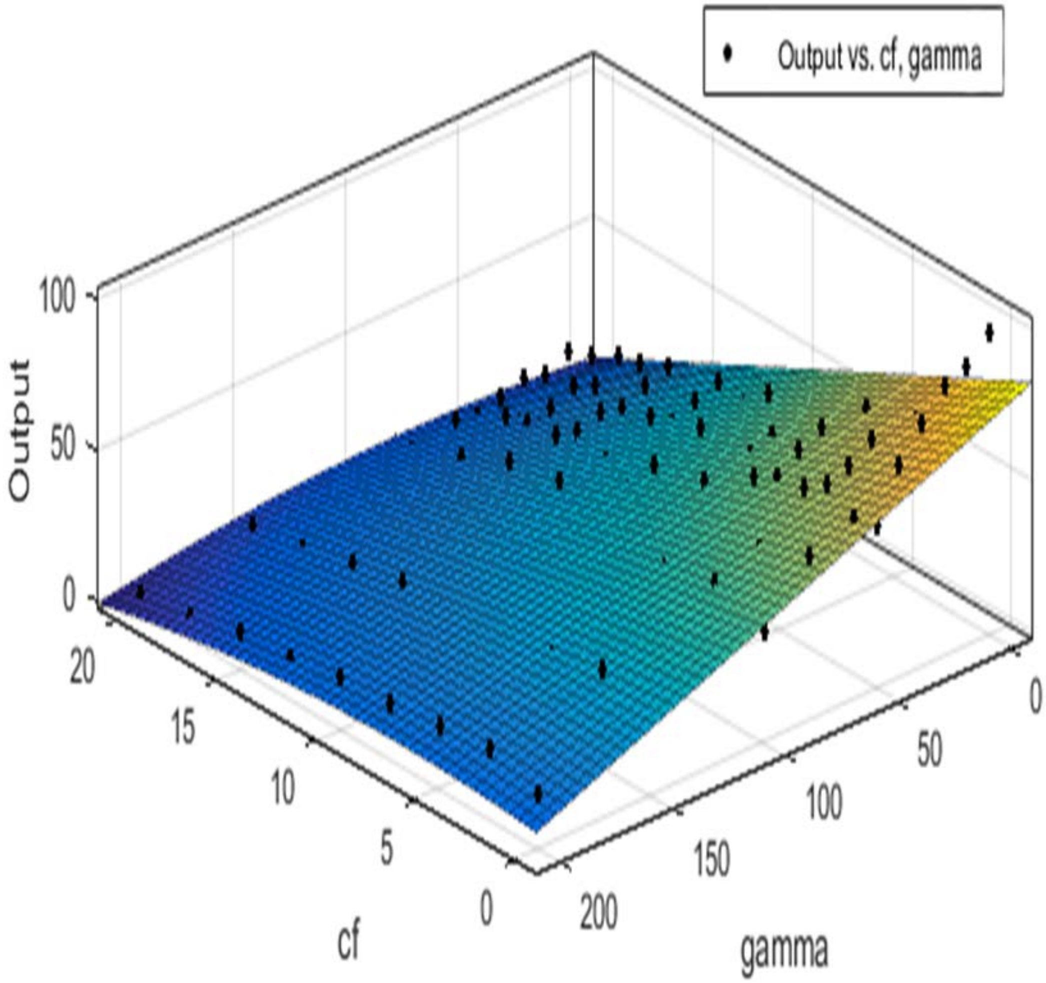

Figure 3: Estimated survival rates of *Dendrobium* sonia-28 based on different doses of gamma irradiation and CF inoculation.

Table 2: Outcomes of various neural networks for *Dendrobium* sonia-28 growth rate results developed for the different number of nodes in the hidden layers

Number of nodes	RMSE	MSE	R	R^2
3	4.6835×10^{-8}	3.968×10^{-15}	0.97321	0.947137704
5	3.1608×10^{-8}	1.6354×10^{-15}	0.98952	0.97914983
7	4.1032×10^{-8}	2.423×10^{-15}	0.97732	0.955154382
10	5.6365×10^{-8}	4.7195×10^{-15}	0.96456	0.930375994
12	7.1465×10^{-8}	6.8326×10^{-15}	0.94245	0.888212003
15	8.1376×10^{-8}	7.8534×10^{-15}	0.93679	0.877575504

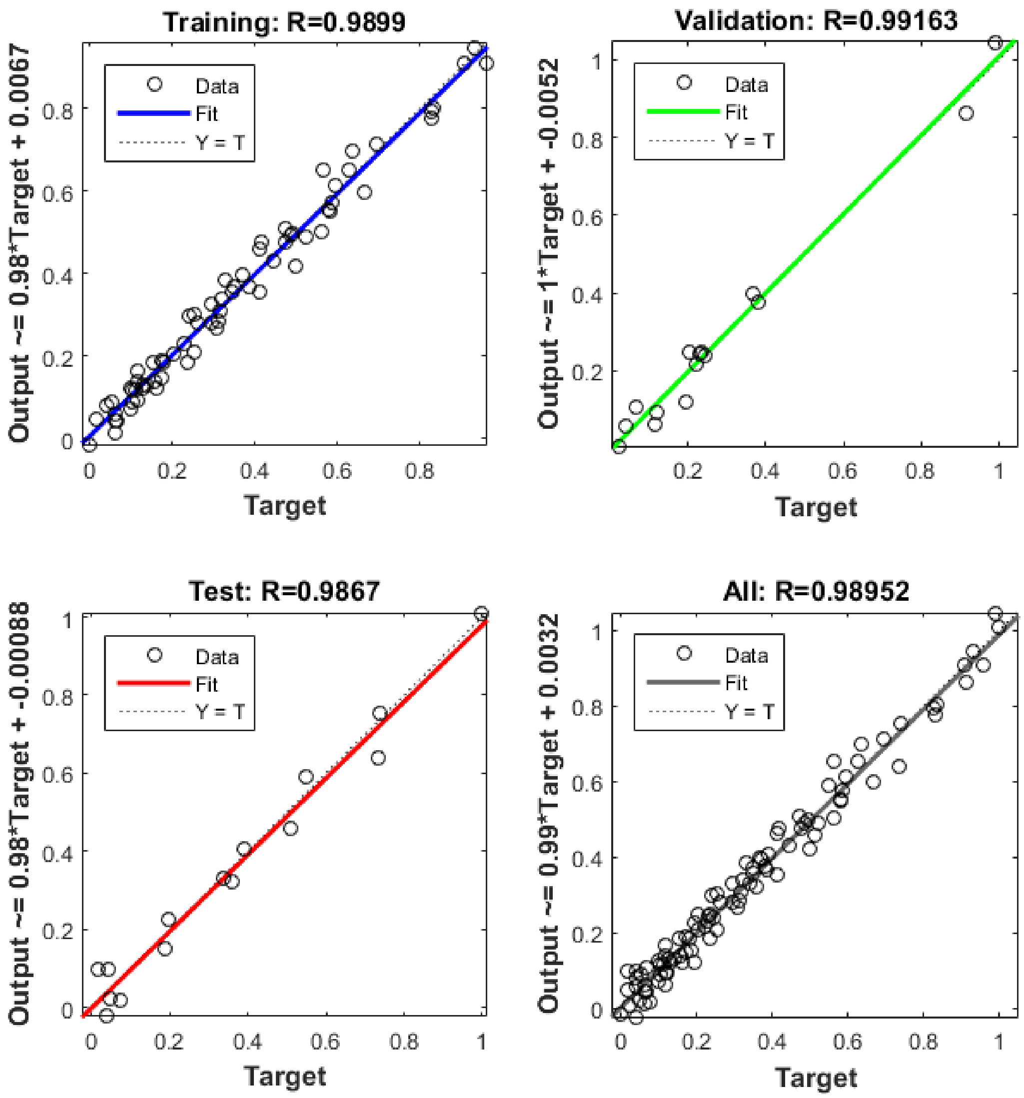

Figure4: Plot of measured *Dendrobium* sonia-28 growth rate versus predicted growth rate from neural network modelling for 5 nodes in the hidden layers

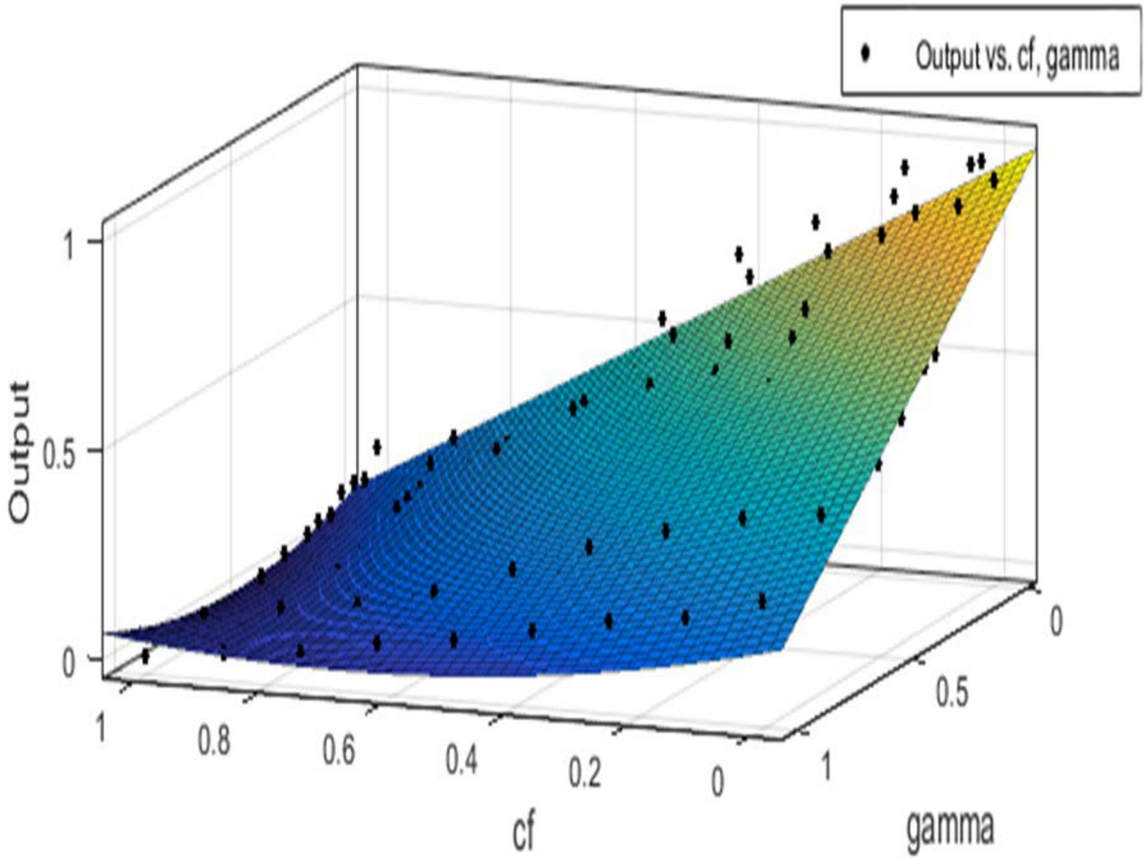

Figure 5:Estimated growth rates of *Dendrobium* sonia-28 based on different doses of gamma irradiation and CF inoculation.

Table 3: Outcomes of various neural networks for *Dendrobium* sonia-28 chlorophyll content results developed for the different number of nodes in the hidden layers

Number of nodes	RMSE	MSE	R	R^2
3	9.393×10^{-8}	8.609×10^{-15}	0.92025	0.846860063
5	8.2649×10^{-8}	7.9149×10^{-15}	0.93567	0.875478349
7	7.6012×10^{-8}	6.651×10^{-15}	0.94826	0.899197028
10	3.4988×10^{-8}	1.9195×10^{-15}	0.98183	0.963990149
12	3.0189×10^{-8}	1.0012×10^{-15}	0.99086	0.98180354
15	3.3604×10^{-8}	1.4652×10^{-15}	0.98432	0.968885862

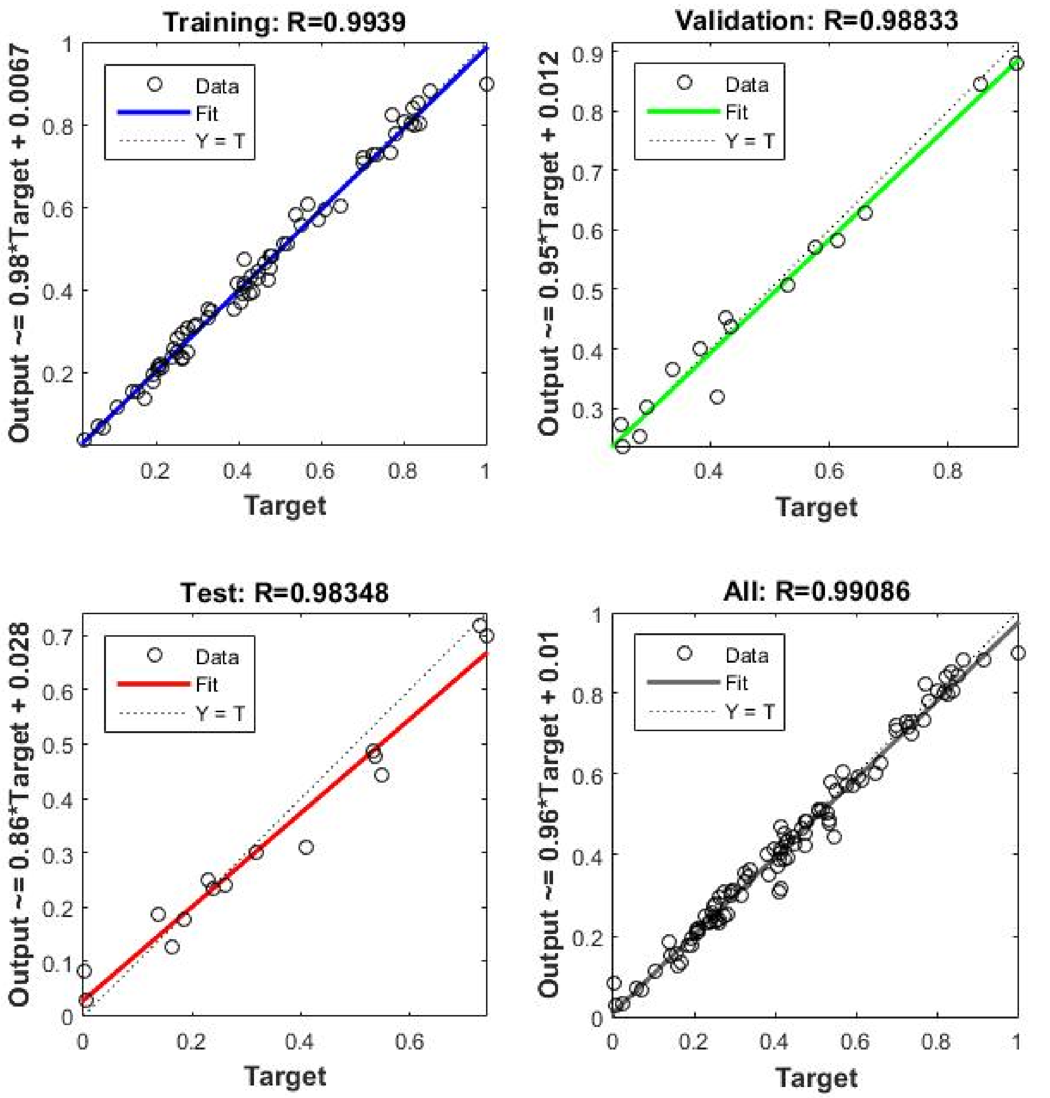

Figure 6: Plot of measured *Dendrobium* sonia-28 chlorophyll content versus predicted chlorophyll contents from neural network modelling for 12 nodes in the hidden layers

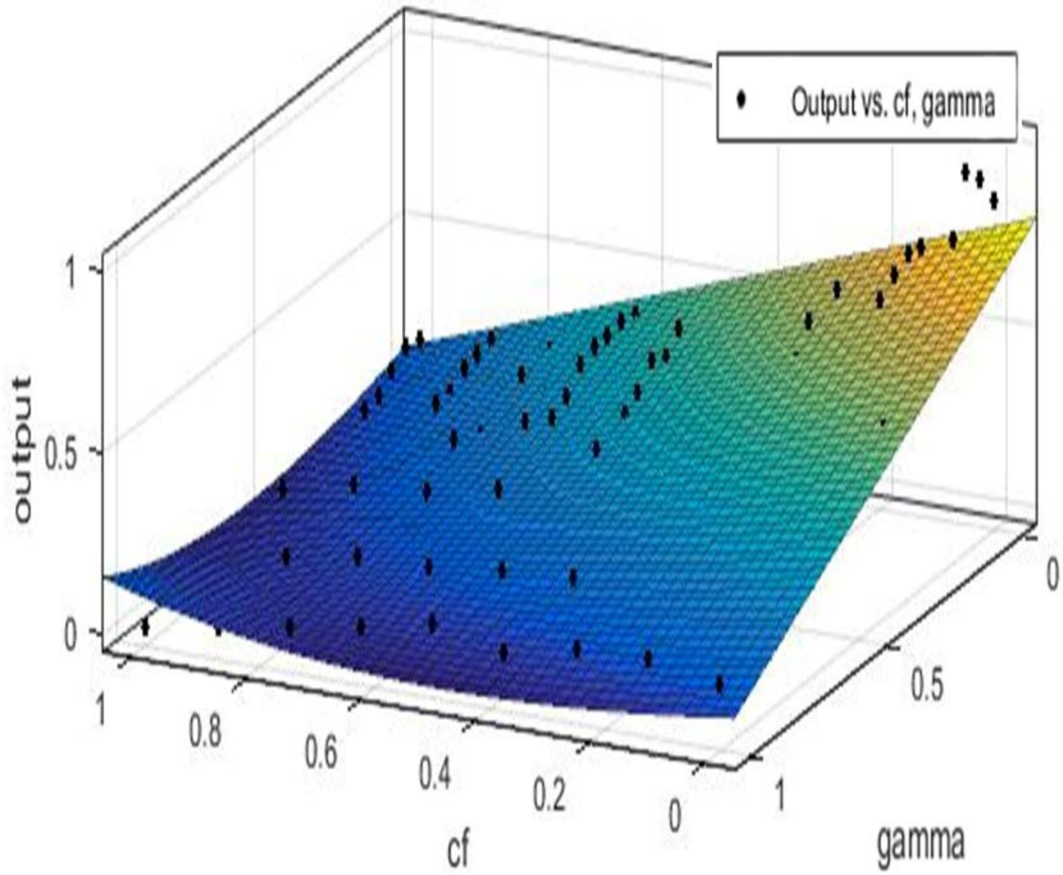

Figure 7:Estimated chlorophyll contents of *Dendrobium* sonia-28 based on different doses of gamma irradiation and CF inoculation.

Table 4: Outcomes of various neural networks for *Dendrobium* sonia-28 protein content results developed for the different number of nodes in the hidden layers

Number of nodes	RMSE	MSE	R	R^2
3	5.893×10^{-8}	4.909×10^{-15}	0.96022	0.922022448
5	4.2639×10^{-8}	3.5249×10^{-15}	0.97562	0.951834384
7	3.260×10^{-8}	1.751×10^{-15}	0.98778	0.975709328
10	3.5888×10^{-8}	2.4195×10^{-15}	0.98173	0.963793793
12	4.5189×10^{-8}	3.8289×10^{-15}	0.97345	0.947604903
15	5.7604×10^{-8}	4.8676×10^{-15}	0.96368	0.928679142

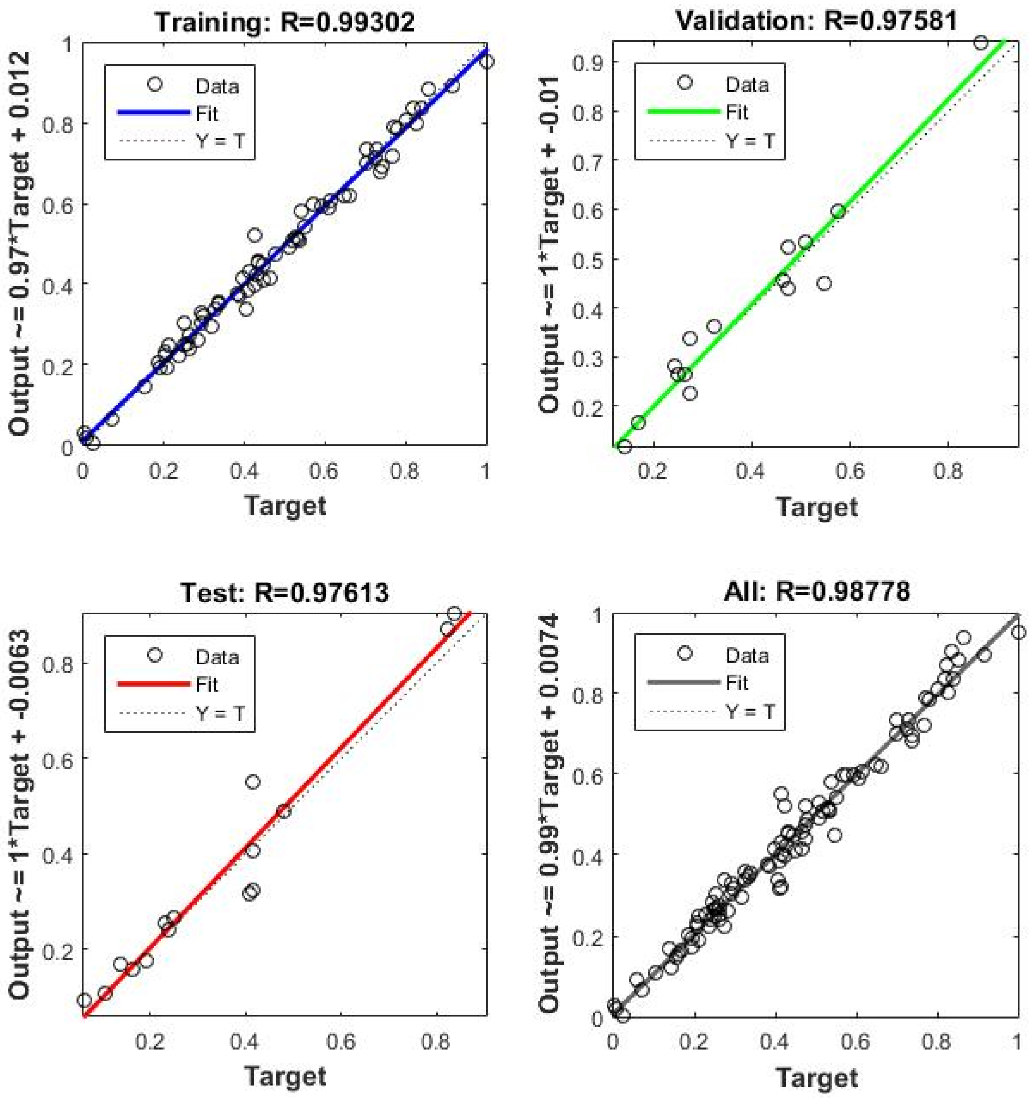

Figure 8: Plot of measured *Dendrobium* sonia-28 protein content versus predicted protein contents from neural network modelling for 18 nodes in the hidden layers

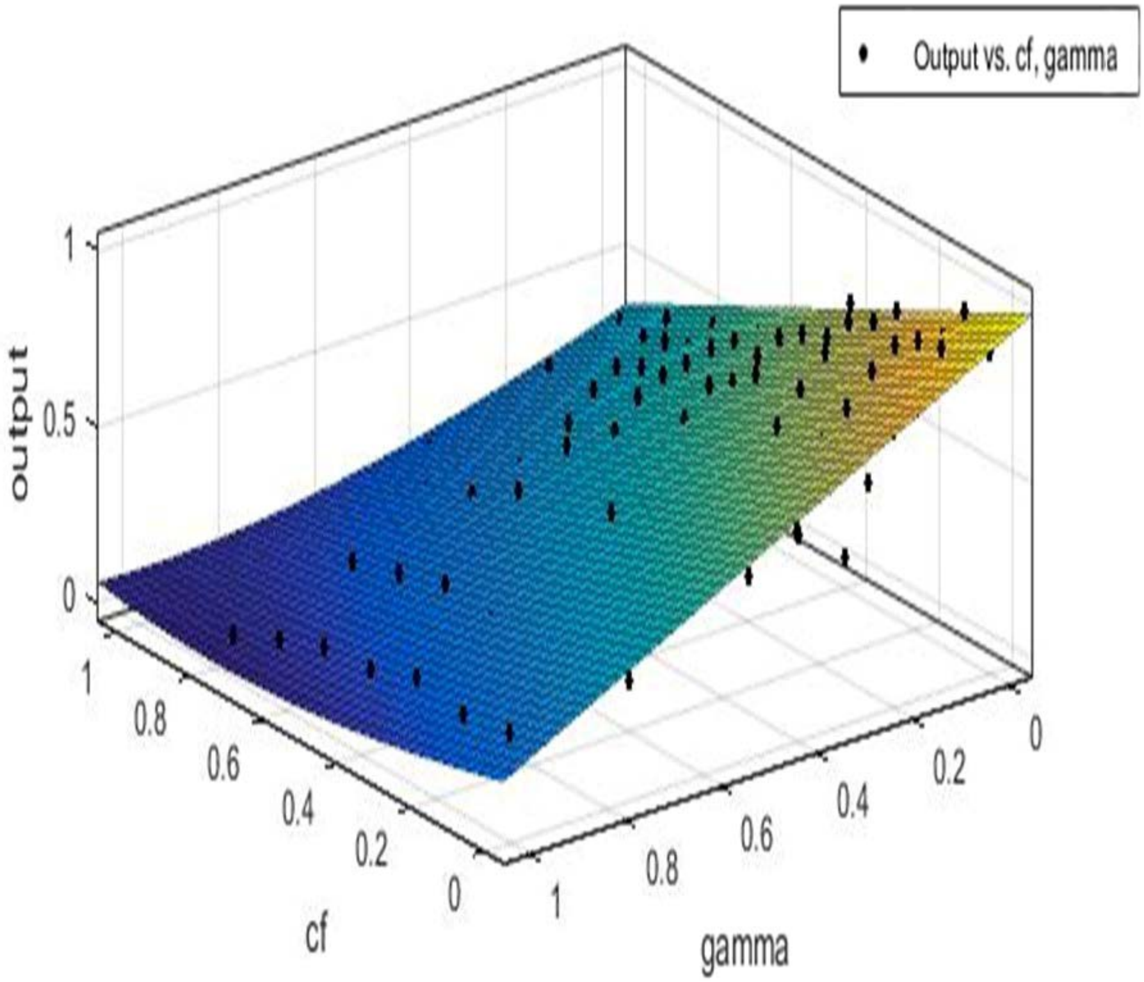

Figure 9: Estimated protein contents of *Dendrobium*sonia-28 based on different doses of gamma irradiation and CF inoculation.