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Optimisation of culture conditions for gesho (*Rhamnus prinoides*.L) callus differentiation using Artificial Neural Network-Genetic Algorithm (ANN-GA) Techniques

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Abstract

Gesho (*Rhamnus prinoides*) is a medicinal plant with antioxidant and anti-inflammatory activities commonly used in the ethnomedicinal systems of Africa. Using a three-layer neural network, four culture conditions viz., concentration of agar, duration of light exposure, temperature of culture, and relative humidity were used to calculate the callus differentiation rate of gesho. With the ability to quickly identify optimal solutions using high-speed computers, synthetic neural networks have emerged as a rapid, reliable, and accurate fitting technique. They also have the self-directed learning capability that is essential for accurate prediction. The network's final architecture for four selected variables and its performance has been confirmed with high correlation coefficient (R^2, 0.9984) between the predicted and actual outputs and the root-mean-square error of 0.0249, were developed after ten-fold cross validation as the training function. In vitro research had been conducted using the genetic algorithm's suggestions for the optimal culture conditions. The outcomes demonstrated that the actual gesho differentiation rate was 93.87%, which was just 1.86% lesser than the genetic algorithm's predicted value. The projected induced differentiation rate was 87.62%, the actual value was 84.79%, and the predicted value was 2.83% higher than Response Surface Methods optimisation. The environment for the growth of plant tissue can be accurately and efficiently optimised using a genetic algorithm and an artificial neural network. Further biological investigations will presumably utilise this technology.

Keywords In vitro culture conditions, Medicinal plants, Gesho, *Rhamnus species*, Mathematical modelling, Response Surface Methodology, Genetic algorithm, Artificial neural network

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Introduction

Gesho (*Rhamnus prinoides*) is a medicinal plant belonging to the family *Rhamanceae* [3, 11]. The plant occurs at an elevation ranging between 1400 to 3200 m along waterways, riparian forests, and peripheries of evergreen forests in central, southern, and eastern Africa. It is a small, dense, thick, East African evergreen shrub, which has huge socio-economic value among local communities in Ethiopia. The plant grows wild but is also widely cultivated in Ethiopia and its dried form is available in the local markets.

Rhamnus prinoides is a source of fruits, small timber, firewood, animal feed, ornaments, dyes, and oils. It is also used as a windbreak and live fence [8, 33], and to impart a unique bitter taste, aroma and flavour in the popular customary fermented Ethiopian beverages, tej and tella [22] (Tesfaye and Mulaw, 2017). In South Africa, the plant has magical significance. It is used to ward off evil eye from homes and crops and is believed to bring good furtune while hunting [28]. Ethiopian traditional medicine makes use of gesho to treat conditions like arthritis, back pain, pneumonia, rheumatism, flu, malaria, diarrhoea, indigestion, ringworm, and weariness [28, 36]. A complex variety of potentially beneficial biocidal substances, including geshoidin, quercetin, emodin, and different anthracene derivatives, are present in gesho. Studies indicate that the plant possesses antioxidant, anti-inflammatory, antibiofilm, antibacterial, antimalarial, antimycobacterial, and wound healing properties [33].

Owing to the pharmaceutical industry's interest in novel phytochemicals and bioactive compounds, researchers have investigated the development of in vitro culture protocols for medicinal plants. The importance of medicinal plant micro propagation in meeting pharmaceutical demand has grown exponentially [32]. Plant micropropagation refers to the process of using explants and allowing them to grow as undifferentiated or differentiated cells [6, 7]. One of its potential applications is the mass production of pharmaceuticals derived from plants in bioreactors, similar to the microbial fermentation process used to manufacture antibiotics [38]. Modeling approaches are useful for forecasting the growth of in vitro plant cultures. Accurate forecasts are difficult due to the variety of genetic and environmental influences, as well as the dynamic nature of biological processes. Furthermore, manipulating tissue growth necessitates comprehension and optimization. Modeling approaches are an important tool for modeling and evaluating complicated interactions, allowing for precise predictions of growth kinetics and dynamics. These models aid researchers in identifying optimal culture conditions, increasing biomass production, and improving metabolite synthesis. Researchers can achieve effective resource usage and desired outcomes in in vitro plant cultivation by combining modeling approaches with optimization algorithms.

The experimental process of optimisation is often carried out by focusing on one aspect at a time. While one factor is changed to determine the optimal response, others are maintained at the same level. In terms of their time behaviour, biological processes are incomprehensible. It is well understood that genetic and environmental factors play critical roles contributing to their functioning [26], these two variables exhibit high correlation. Diverse non-deterministic and non-linear biological processes are brought on by the variability within and among these limiting factors.

Plant tissues and cells cultivated aseptically in a regulated in vitro environment show similar developmental processes. Apart from its use in pharmaceutical, transgenic and other biological research, in vitro plant culture is typically designed for manipulating tissue growth and behaviour to quickly produce huge numbers of elite plantlets or for large scale production of beneficial metabolites.

As a result, it is critical to use appropriate modelling techniques rather than traditional analytical techniques to accurately predict and simulate kinetics of in vitro growth, thermodynamics limitations, and conversion of energy to mass [17]. A group of methods known as response surface methodology (RSM) can be used to analyse and improve issues in which multiple explanatory factors have an impact on a response. Although this method is widely employed in several mixing investigations, it has only a limited impact on the standardisation of micropropagation methods. Through applying the principle of slope rotatability, a prerequisite for evaluating the variance of a projected output at a point that remains constant with all points equally distant from the design center, RSM can be extended to situations where the error structures are correlated or heteroscedastic. RSM has been applied to the study of plants to improve the synthesis of secondary metabolites or enzymatic processes. RSM is also used as a substitute statistical method for in vitro analysis and for the optimisation of plant growth media [9, 10]. For accurate evaluations of biological processes, neural network technology provides a practical substitute. In order to process data sets, neural network technology uses approximate mathematical models. This technology employs algorithms to process information and make judgments in a manner that is similar to the organic network of human neurons. Neural networks use activation functions like sigmoid, tanh, and ReLU to introduce nonlinearity and approximate complex relationships. These functions, along with weights and biases, form the approximate mathematical

models within neural networks. The ReLU activation function is specifically employed in this work. It enables the network to process data, analyze relationships, and make predictions based on learned patterns. Given their incredible potential for learning, they are able to perceive and simulate complex nonlinear relationships between the input and output of a bioprocess [34]. The standard modelling tools, however, are ineffective for on-line monitoring due to the characteristics of regenerated plants and in vitro cell cultures, as well as the somatic embryogenesis process. ANN can be used to detect somatic embryo patterns, evaluate photosynthetic and photometric properties of regenerated plants, evaluate online biomass, and control secondary metabolite production. [1, 2]. The standard modeling tools, such as traditional analytical techniques, are ineffective for online monitoring in regenerated plants, in vitro cell cultures, and somatic embryogenesis due to their variations, high variability, complex and non-linear nature, and the absence of dynamic environmental factors. These tools assume linearity and steady-state conditions, which do not capture the dynamics and complexities of these biological systems. Alternative methods, like neural network-based modeling, are necessary to overcome these limitations and provide accurate modeling and monitoring capabilities. In dealing with the non-linear interactions that are frequently encountered in cell culture techniques, an ANN-based modelling approach has been found to be more flexible, effective, and versatile. The approach also has the notable benefit of not requiring any prior understanding of the relationships between input and output signals or how they are organized. Prior training is required for developing an artificial neural network (ANN) model. During the training process, the neural network is exposed to a dataset containing input–output pairs, and it learns to approximate the underlying relationship between the input and output through an iterative optimization process. The neural network adjusts its internal parameters, such as weights and biases, based on the provided training data to minimize the prediction errors. However, it's important to note that ANNs do not require prior explicit knowledge or understanding of the relationship between the input and output variables. Instead, the neural network learns and captures the complex patterns and relationships within the training data, allowing it to generalize and make predictions on new, unseen data. So, while prior training is necessary, ANNs can discover and model non-linear relationships and patterns even without prior explicit knowledge of the input–output relationship. ANN is fast gaining recognition as a preferred method for simulating and forecasting the intricate biological processes involved in in vitro plant regeneration. Neural computing offers a practical method for assessing in vitro plant cultures even with limited information [12, 15]. In this study, tissue-cultured gesho callus were differentiated under different culture conditions, including agar concentration, relative humidity, culture temperature, and light duration, all fixed at three levels and the non-linearity relationship between in vitro culture conditions and differentiation rate was predetermined using a three-layer neural network. The ideal culture conditions were then identified by employing a GA for global optimisation.

Materials and methods

Preparation of explants and callus induction

Young, growing tissues and the most suitable tissues were collected from gesho (*Rhamnus prinoides*) greenhouse-grown plants and chosen as sources for leaf explants. To avoid fungal contamination, the explants were prepared into an appropriate size of 1 cm^2 and washed with diluted Teepol for 1–3 min and rinsed with water more than three times before being treated with 0.01 percent bavistin for 1–2 min. Also, the explants underwent surface sterilisation with 0.8% w/v NaOCl for 15 min, a water rinse, and 0.1 percent mercuric chloride. After that, five rinses with double-distilled water were given to the explants. The pH of the callus induction medium was adjusted to between 5.6 and 5.8 and autoclaved at 120 °C for 20 min under 1 bar of pressure. The callus induction medium was composed of 30 g/l sucrose, 8 g/L agar supplemented with 2 mg/l BAP (6-Benzylaminopurine) and 2 mg/l IAA (Indole-3-acetic acid), and it contained 30 g/l sucrose. Four explants (1 cm^2) were placed in petri dishes containing 20 mL of MS (Murashige and Skoog medium), upper surface down, in an ad axial position on the solid callus induction medium, and cultured for 48 h in the dark before being exposed to a photoperiod of 16 h of light and 8 h of darkness until callus formation was evident.

Differentiation induction and computational networks

In a three-level, four-factor central composite design, the callus cultures were routinely transferred to differentiation medium (MS + 1.38 mL TDZ + 1.4 mL BAP) and cultivated under varied circumstances.

Design of experiment, Response Surface Methodology (RSM) and ANN based modelling

The effect of four independent process parameters (agar content, light exposure, culture temperature, and humidity) on differentiation was analysed using a Centre Composite design (CCD) [29]. Design-Expert® software was used for generating CCD combinations, RSM modelling, and statistical optimisation. In general, the

model can be generated by the software as presented in the Eq. (1) [25, 30].

$$G = \lambda_0 + \sum \lambda_i x_i + \sum \lambda_{ii} x_i^2 + \sum \lambda_{ij} x_i X_j \quad (1)$$

Equation (1) represents a mathematical model where G is the dependent variable and x_i represents the independent variable. The equation consists of multiple terms, each with a coefficient λ. The first term, λ_0, represents the constant or intercept term. The subsequent terms involve the multiplication of the independent variables x_i with their respective coefficients λ_i, the squared independent variables x_i^2 with coefficients λ_{ii}, and the interaction between different independent variables xi and xj with coefficients λ_{ij}. In summary, Eq. (1) is a polynomial equation that accounts for the linear, quadratic, and interaction effects of the independent variables on the dependent variable G. The λ coefficients determine the magnitude and direction of the influence of each term on the overall relationship between the independent variables and the dependent variable. The independent variables are given in Table 1

ANOVA (analysis of variance) was used to assess the model's suitability. The impacts of the independent factors on the response were then visualized using 3-D response surface plots [49].

The ANN model was developed by considering four different culture conditions as inputs and the rate of differentiation as the output. (Fig. 1). Figure 2 illustrates the schematic diagram of NSGAII optimisation process.

There were three experimental levels used: − 1, 0, and + 1. The range and levels of the process parameters examined in this study are displayed in Table 1. This experiment was set up in as randomized design with the factorial arrangement and three replications, each containing four explants for experimental validation in the plant tissue culture lab. Table 1 displays the factors and their levels for the CCD.

Table 1 Process parameter levels that were utilised in the experimental design

Independent variables	Levels		
	− 1	0	+ 1
Agar concentration (Y1, %)	0.3	0.5	0.9
Light duration (Y2, hours/day)	10	13	16
Culture temperature (Y3, °C)	16	28	40
Relative humidity (Y4, %)	55	70	100

Artificial neural network

By mathematically simulating the network structure of connected node cells, an artificial neural network is a type of computer programme that mimics how the brain learns. The respective layers that make up an artificial neural network's basic structure are the input, output, and hidden layers [24]. By varying the weights among the layers, the network can calculate complex correlations between the input and output variables. They function as "black box models" of significant variables whose linkages to other process elements are conjectured rather than declared or formally demonstrated [44]. Datasets for the input and output nodes are used to train an ANN

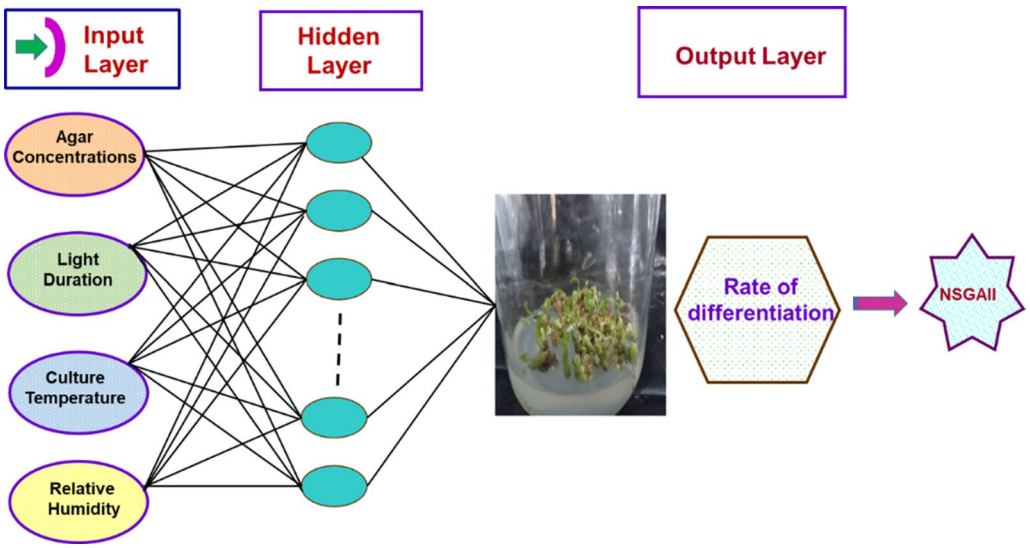

Fig. 1 The graphic illustration of the proposed for ANN method

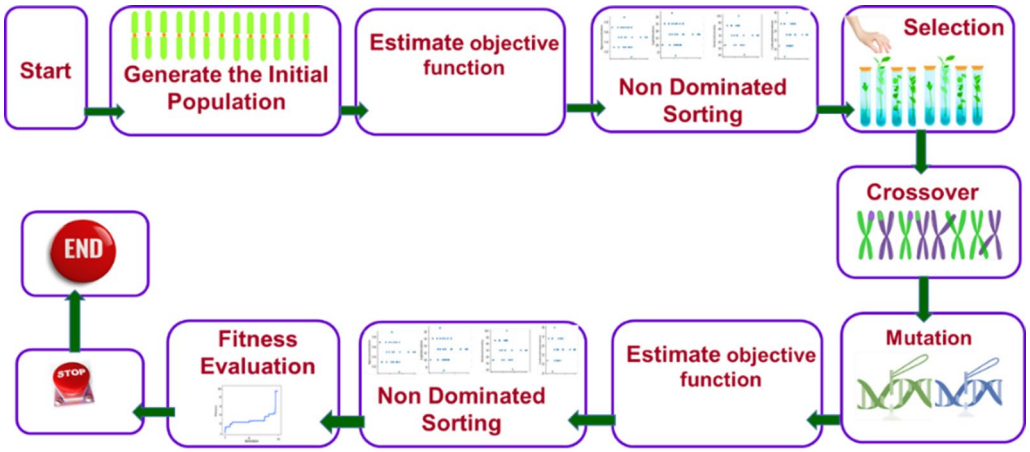

Fig. 2 The schematic diagram of NSGAII optimisation process

model. The neural network was built using the back-propagation approach, which is frequently used in literature. By propagating the error backwards through the network, the training method calculates the discrepancy between the output neurons' predictions and their actual outputs. Each new layer's weights are altered by the procedure [21].

The test data for the mentioned independent variables can be obtained by selecting specific combinations of the levels of each variable that were not used during the training phase. In this case, the levels for each independent variable are denoted as -1, 0, and + 1. To generate the test data, combinations of the levels (− 1, 0, + 1) for each variable (Y1, Y2, Y3, Y4) are chosen that were not included in the training dataset. Inputs that closely match the pattern an ANN has learnt can be used to anticipate the output. ANNs typically implicitly match the input vector (cultural condition) to the output vector, unlike regression-based response surface models that demand the definition of the models order (rate of differentiation). In this investigation, a nonlinear mapping between the concentration of the input variables (agar content, light exposure, culture temperature, and humidity) and the result variable (rate of differentiation) was made using an artificial neural network (ANN). The experimental data values utilised for the RSM simulation were used to train the ANN. To give the neural network an acceptable coefficient of correlation, the learning rate of the network was altered.

Genetic algorithm

Charles Darwin's "survival of the fittest" idea is the foundation of a genetic algorithm, which is used to address challenging biological process optimisation issues. Due to its effectiveness in resolving fitness functions that are discontinuous or non-differentiable, GA is becoming increasingly popular for its genuine optimisation techniques [39, 46]. The GA creates individual chromosomes at random which form the starting population and handle an optimisation problem [16, 18]. The principle behind evolution by natural selection is similar in that the chromosomes that evolved in later iterations (generations) had a greater fitness value as compared to their progenitors. The three genetic operators of crossover, mutation, and selection were used to create new generations [17, 41].

Using the process of selection, chromosomes with the highest fitness values were selected as breeding parents. In a process known as crossover, the GA chooses two parent solutions (based on their best fitness value) to create progeny that largely resembles its parents [19, 20]. To promote diversity in the population, the mutation is a procedure that is used. The process is carried out until a close to optimal solution is found or it satisfies one of the termination criteria.

Procedure for Hybrid ANN and GA

Step 1: As the initial population, create a population of chromosomes that consists of bit strings of randomly generated binary values.

Step 2: In order to determine which input variables will be chosen, decode chromosomes (bit strings).

Step 3: To predict the rate of differentiation, run a three-layered feedforward ANN model.

Step 4: Consider the ANN prediction accuracy of each chromosome as a gauge of its GA fitness.

Step 5: Determine whether the loop should be continued or terminated.

Step 6: Employing the tournament selection method, select which chromosomes should cross across.

Step 7: To define a linear combination of two chromosomes, use a crossover arithmetic operator.

Step 8: To add additional genes to the population, use the uniform mutation operator. Then, select a random slot number for the crossed-over chromosome and flip the binary value there.

Step 9: For the next generation, substitute old chromosomes with the two best offspring chromosomes.

Step 10: If the termination condition or stopping criterion of the genetic algorithm is not satisfied, the process is repeated from step 2.

Results

Optimisation of rate of differentiation through RSM

RSM approach can highlight the importance of optimising culture conditions in attaining higher rate of differentiation. Four variables were evaluated for their role in enhancing the callas differentiation and it was observed that four factors namely agar content, light exposure, culture temperature, and humidity were important in the callas differentiation. As shown in Table 2, which displays the un-coded values of independent variables, experimental, and RSM projected differentiation, the four major design parameters were further optimised.

Using the CCD based RSM analysis, the importance of the independent process parameters, namely, agar content, light exposure, culture temperature, and humidity,

Table 2 CCD based combinations used for ANN modelling and response from RSM data

Run	A: Agar concentration (Y1, %)	B: Light duration (Y2, h/d)	C: Culture temperature (Y3, °C)	D: Relative humidity (Y4, %)	(Actual value) Rate of differentiation (A, %)	Predicted Value (P, %)
1	0.9	16	20	80	77.23	83.25
2	0.9	16	36	80	77.15	81.65
3	1.1	13	28	70	79.01	89.15
4	0.5	10	36	60	77.09	78.63
5	0.5	16	20	80	76.98	79.48
6	0.7	13	12	70	76.02	72.96
7	0.9	10	20	80	77.92	81.52
8	0.9	10	36	80	76.42	80.59
9	0.9	10	20	60	78.93	81.45
10	0.7	13	28	70	84.75	79.58
11	0.7	13	28	50	76.03	72.89
12	0.9	10	36	60	78.56	80.63
13	0.9	16	36	60	76.74	76.52
14	0.7	13	28	90	74.56	84.56
15	0.7	7	28	70	78.42	72.89
16	0.7	13	28	70	84.99	81.24
17	0.5	16	36	60	78.32	84.56
18	0.7	13	44	70	76.99	83.56
19	0.5	10	36	80	75.28	79.52
20	0.7	13	28	70	84.53	71.84
21	0.3	13	28	70	78.64	86.56
22	0.7	13	28	70	84.87	78.65
23	0.9	16	20	60	75.95	78.26
24	0.5	16	20	60	76.8	90.56
25	0.7	19	28	70	78.91	74.15
26	0.7	13	28	70	84.15	79.06
27	0.5	10	20	60	77.34	83.85
28	0.7	13	28	70	84.58	63.96
29	0.5	16	36	80	78.59	72.45
30	0.5	10	20	80	76	82.96

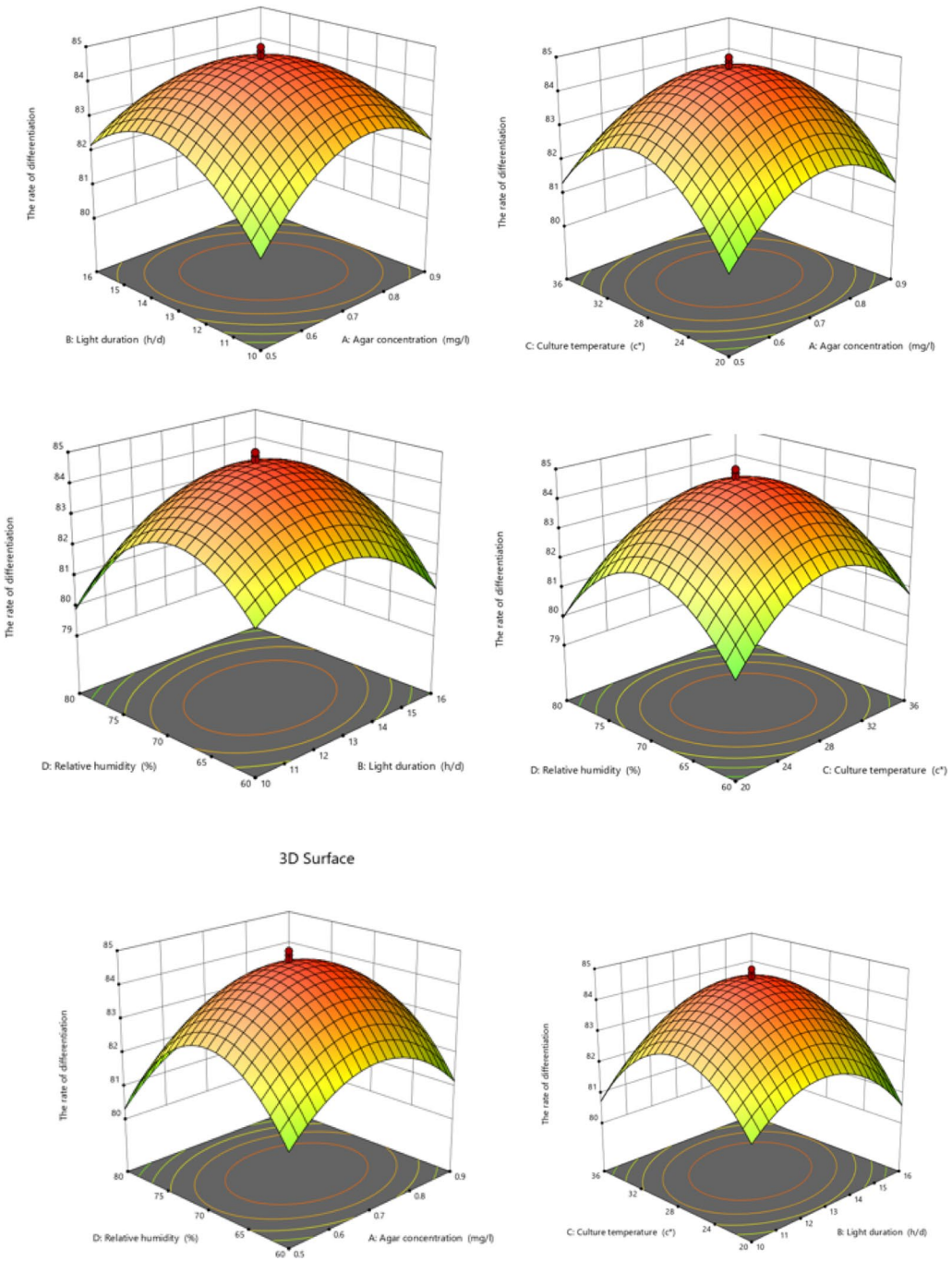

Fig. 3 Interactive plots for different combinations of selected Factors

were examined on culture differentiation. Figure 3 shows the interaction upshots (3D response surface) of different combinations of two selected parameters on culture differentiation. The outcome with respect to interaction effects for all chosen combinations on the development of culture differentiation exhibited an increasing trend up to an optimal level, then, it showed a decline in response except the optimal point. This optimal value can be statistically determined by solving the model suggested by RSM. The Eq. (2) depicts the correlation between clean

Table 3 The statistical response from ANOVA analysis for the developed model on differentiation

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	297.79	14	21.27	247.99	< 0.0001	Significant
A-Agar concentration	0.4374	1	0.4374	5.10	0.0393	
B-Light duration	0.0600	1	0.0600	0.6995	0.4161	
C-Culture temperature	0.3601	1	0.3601	4.20	0.0584	
D-Relative humidity	2.10	1	2.10	24.49	0.0002	
AB	5.93	1	5.93	69.13	< 0.0001	
AC	0.6889	1	0.6889	8.03	0.0126	
AD	0.0961	1	0.0961	1.12	0.3066	
BC	2.79	1	2.79	32.52	< 0.0001	
BD	4.45	1	4.45	51.91	< 0.0001	
CD	0.3540	1	0.3540	4.13	0.0603	
A^2	59.62	1	59.62	695.14	< 0.0001	
B^2	62.90	1	62.90	733.37	< 0.0001	
C^2	115.76	1	115.76	1349.63	< 0.0001	
D^2	152.36	1	152.36	1776.35	< 0.0001	
Residual	1.29	15	0.0858			
Lack of Fit	0.8434	10	0.0843	0.9516	0.5594	Not significant
Pure Error	0.4431	5	0.0886			
Cor Total	299.08	29				

culture and chosen parameters. Statistical analysis using the ANOVA has been provided in the Table 3 for the model that correlates with culture differentiation. The second order regression equation developed by RSM that provides the rate of differentiation is given in Eq. (2):

$$\begin{aligned}
&\text{Rate of differentiation (\%)}\\
&= -92.74 + 66.38(\text{Agar concentration})\\
&\quad + 3.38(\text{Light Duration})\\
&\quad + 1.80\,(\text{Culture temperature})\\
&\quad + 3.066(\text{ Relative Humidity})\\
&\quad -1.01(\text{Agar concentration} * \text{Light duration})\\
&= 0.129(\text{Agar}\\
&\quad \text{concentration} * \text{Culture temperature})\\
&\quad + -0.038(\text{Agar concentration} * \text{Relative humidity})\\
&\quad + 0.017(\text{Light duration} * \text{Culture temperature})\\
&\quad + 0.017(\text{Light duration} * \text{Culture temperature})\\
&= 0.001(\text{Culture temperature} * \text{Relative humidity})\\
&\quad -36.85\left(\text{Agar concentration}^2\right)\\
&\quad - 0.16\left(\text{Light duration}^2\right)\\
&\quad - 0.032\left(\text{Culture temperature}^2\right)\\
&= 0.023\left(\text{Relative humidity}^2\right)
\end{aligned} \tag{2}$$

Here, RMSE and R^2 were evaluated for testing the significance of the developed model using Eq. (3) and (4)

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N}(O_i - P_i)^2}{N}} \tag{3}$$

$$R^2 = \frac{\sum_{i=1}^{N}\left(O_i - O_i'\right)\left(P_i - P_i'\right)}{\sqrt{\sum_{i=1}^{N}\left(O_i - O_i'\right)^2 \sum_{i=1}^{N}\left(P_i - P_i'\right)^2}} \tag{4}$$

where O_i and P stand for the observed and predicted quantities, respectively; O_i and P'^i stand for the average observed and predicted amounts across N samples. Finally, the model's R^2 number, which measures its significance, was 98.65%.

Optimisation of induced differentiation by ANN-GA

An ANN with four input neurons, multiple hidden neurons, and one output neuron makes up the back-propagation algorithm. As depectited in Fig. 4, the modelling the reliance of differentiation on independent variables was done using the "Tansig" transfer function.

It was noted that the ANN model produced reliable forecasts. The data were trained in 100 epoch with a root mean square error of 0.0249 and an R^2 value of 0.99849 (shown in Figs). `

According to the findings, ANN-based training exhibits a higher connection with experimentally produced differentiation than does training that solely employs RSM regression model ($R^2 = 0.98$). The fitness of this trained data was assessed using the GA tool's fitness evaluation

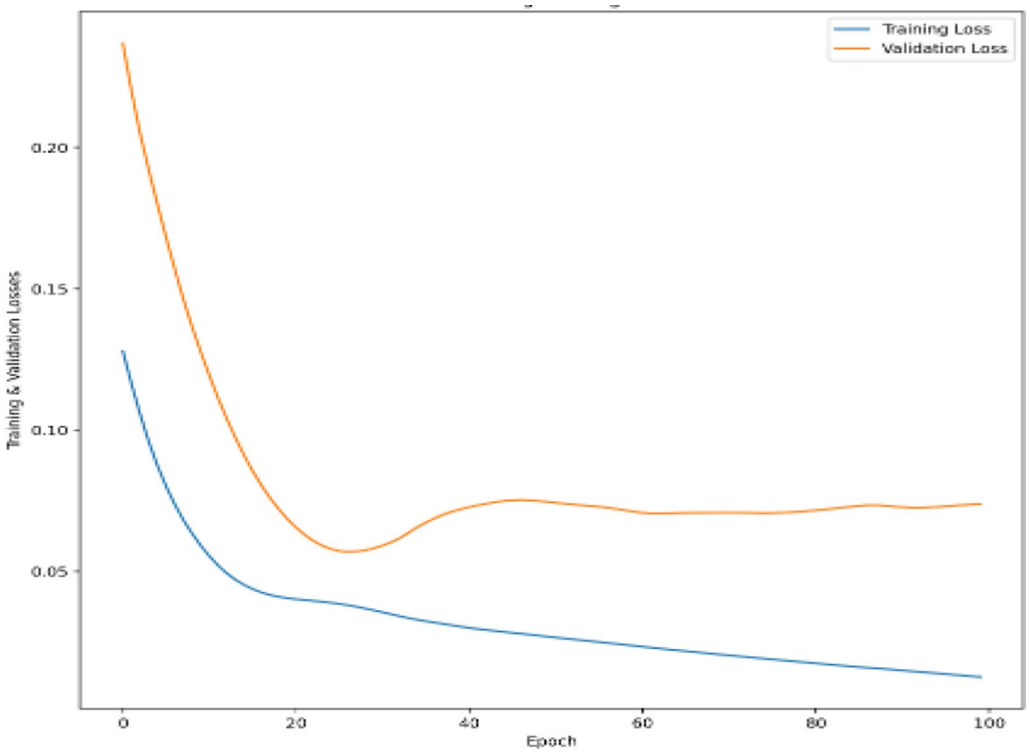

Fig. 4 Training loss vs validation loss

feature. The uniform cross-over rate of 0.8, mutation rate of 0.1, and population size of 10 were the factors selected for the GA optimisation. The results of the improvement using.

GA are shown in Fig. 5.

Optimum culture conditions for differentiation of gesho generated by the ANN-GA

The fitness of algorithm value over 45 generations came close to reaching the maximum anticipated differentiation rate of 93.87%, which could be attained under the following culture conditions: 0.8% agar concentration, 12 h/day light cycle, 28 °C culture temperature, and 75% relative humidity (Fig. 6).

Optimal culture conditions validation

The optimal in vitro culture conditions determined by the GA were confirmed by an in vitro plant tissue culture experiment. The experiment's three replicates' average differentiation rate was 92.01%, just 1.86% below the predicted value, demonstrating the viability and dependability of the culture conditions produced by the genetic algorithm.

The ideal culture conditions for differentiation in gesho, as determined by the response surface approach of the CCD design method, were 0.8% agar concentration, 12 h of daylight per day, 28 °C for the culture temperature, and 75% humidity. With an R^2 of 0.9951, the differentiation rate (predicted value) was 93.87%. According to Table 3, the real rate of differentiation under these anticipated circumstances was 87.62%, which was 2.83% less than the anticipated estimation. These findings show that in this experiment, the neural network method is fitter than the response surface method (Table 4, and 5).

The prediction accuracy of the genetic algorithm was evaluated by measuring the relative error between the ANN-GA predicted data and the actual experimental data, which was calculated using the formula below.

$$\mathrm{E}(\%) = \frac{P' - P}{P} \text{ x } 100 \tag{5}$$

where P is the real differentiation rate as determined by tissue culture experiments, and P' is the differentiation rate predicted by GA. Differentiation of gesho callus in optimised In vitro culture conditions are shown in Fig. 7

Discussion

Biological systems have non-deterministic, non-linear developmental patterns that are mainly controlled by genetic and environmental factors. These two vital components, which resemble plants, cells, or tissues that

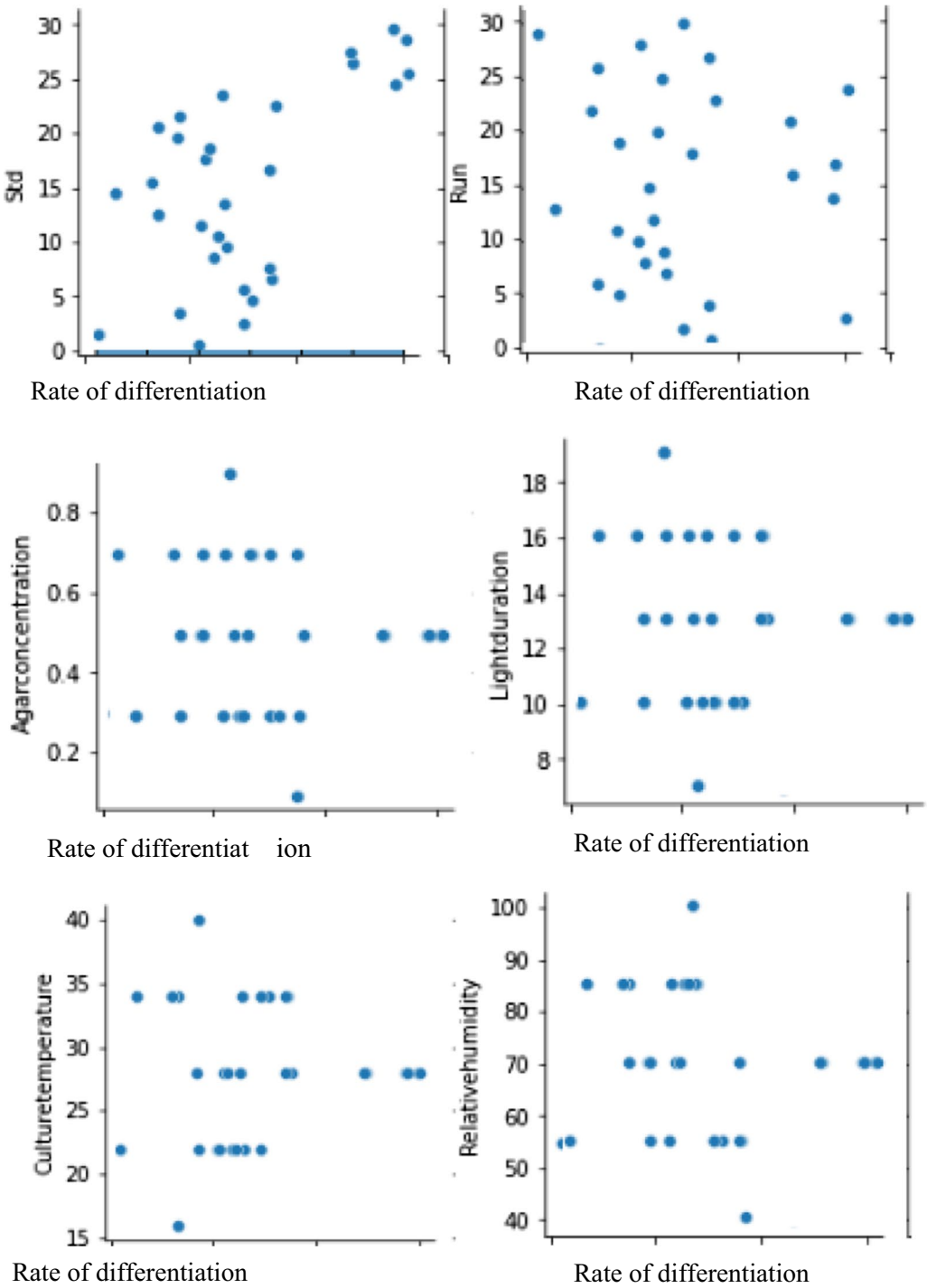

Fig. 5 Effect of rate of differentiation in different concentrations

are cultivated in vitro under aseptic and regulated environmental circumstances, have significant internal and exterior inconsistencies that result in unique biological growth patterns. In order to alleviate two crucial restrictions, time and cost, during tissue culture, there is a critical need for modelling systems that may effectively drive in vitro growth kinetics while satisfying the thermodynamic limitations of the culture settings. A common type of ANN utilised in micropropagation studies is

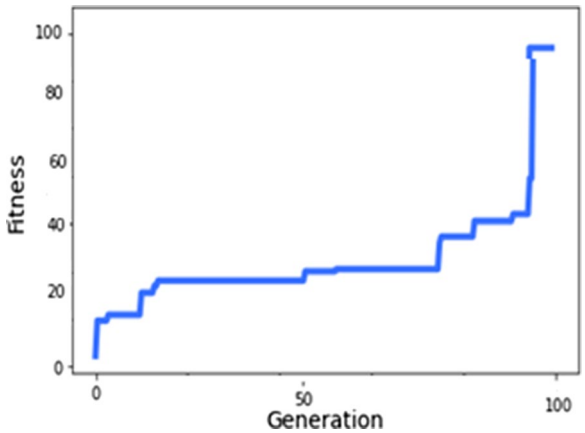

Fig. 6 Fitness plot for the performance of ANN-GA

Table 4 ANN-GA optimal Solutions

Replications	ANN-GA predicted value by ANN-GA(%)	Actual value (%)
1	93.87	91.82
2		93.78
3		90.45
Mean		92.01
Error (%)		1.86

Table 5 ANN-GA Optimal solutions with RSM value

Replications	Predicted value by RSM (%)	Actual value (%)
1	87.62	82.85
2		86.71
3		84.81
Mean		84.79
Error (%)		2.83

the MLP model, which has three basic layers: input, output, and one or more hidden layers [23, 40].

Recently, several aspects of plant science, including in vitro propagation, have been evaluated using machine learning, one of the most potent computational methodologies, crop improvement [13, 42] plant stress modelling [5, 45] plant distribution [35] recognition of plant diseases [37, 47] and precision agriculture [43]. Additionally, the accuracy of ANNs has recently been acknowledged for modelling, prediction, and optimisation of a variety of in vitro culture experiments, such as the aseptic procedures, in vitro shoot proliferation [2], germination of seeds [1], caulogenesis [14, 48], anther culture [31, 51], somatic embryogenesis, and secondary metabolites production [16, 40]. Modelled the impacts of light and sucrose as well as explored the formulation of culture media optimisation, prognostication and optimisation of development of the cells in controlled environment, direct shoot organogenesis, in vitro rooting, and somatic embryogenesis. Both the degree of medium solidification and the type of closure used in culture tubes have a significant impact on the ability of adventitious shoots to regenerate in plant explants, as well as the water content of the in vitro developed shoots. This makes the combination of ANNs with multi-objective optimisation algorithms a precise and trustworthy methodology for in vitro culture prediction and optimisation. The ANFIS-NSGAII model was used in a scientific study to gain a useful understanding of how different levels of 2,4-D, BAP, sucrose, fructose, and glucose as well as light affect chrysanthemum somatic embryogenesis and to gain new insights into how to improve chrysanthemum embryogenesis conditions [19, 20]. The primary element affecting photomorphogenesis and having a significant impact on laboratory protocol repeatability is light especially the amount and quality [4, 27].

The ANN-GA model was utilised in the current work to gain a valuable understanding of how varied levels of culture conditions like agar concentration, relative humidity, light duration, and culture temperature affect the callus differentiation in the medicinal plant gesho for gaining new perceptions into how to increase gesho embryonic conditions. According to a study conducted by Yun et al. [50], the biomass of soybean adventitious roots increased as a result of fluorescent light irradiation, and significant perturbations in the metabolism was also noticed. Particularly, soybean adventitious roots grown under fluorescent light irradiation accumulated more health-beneficial secondary metabolites than those grown under a dark condition, including soyasaponin (3.4-fold), isoflavones (3.9-fold), and coumestrol derivatives (1.3-fold). This was due to increased photosynthesis, which was shown by increased levels of glucose.

In this research, the RSM and ANN model was employed to predict and optimise the culture conditions for gesho differentiation in vitro techniques. The model showed a high coefficient of determination between observed and projected values during both the training and testing phases, indicating its effectiveness in evaluating and predicting culture conditions. The validation experiments further confirmed the expected outcomes.

The influence of the light environment on the differentiation, development, and morphogenesis of plant cell, tissue, and organ cultures is well-known. By utilising mathematical modelling and neural network-based computing, this research provides a reliable and practical approach to understanding the complex processes of

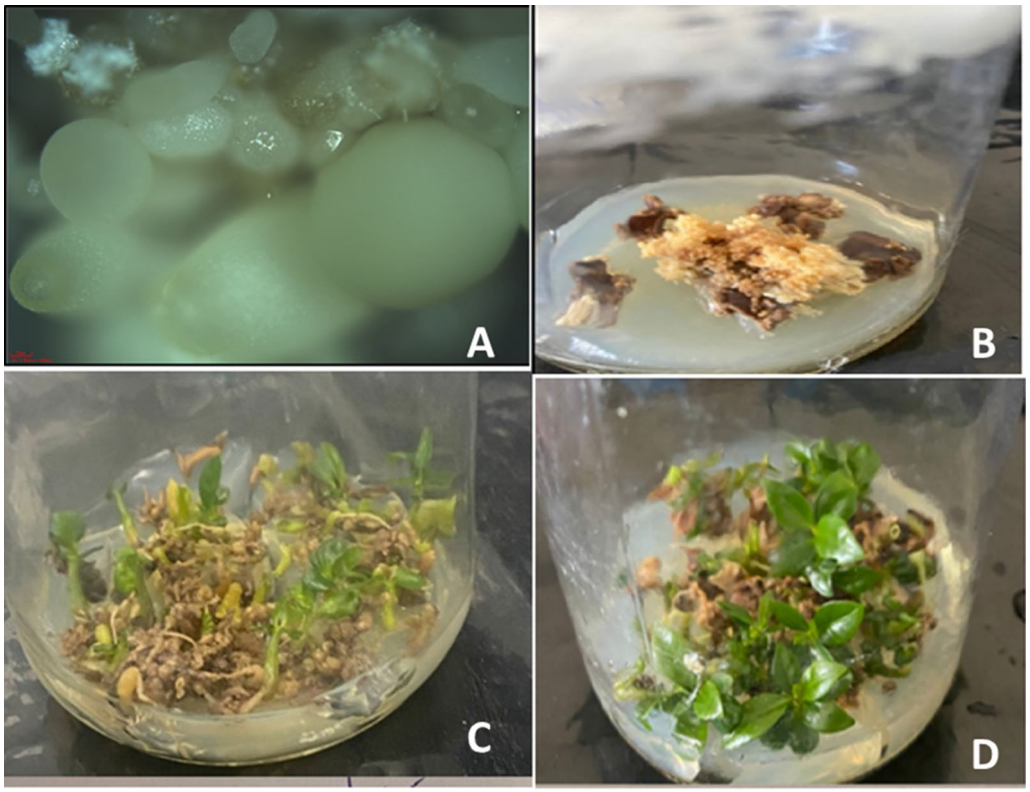

Fig. 7 Differentiation of gesho callus in optimised In vitro culture conditions (**A**). Somatic Embyos, (**B**). Gesho callus, (**C**). In vitro regenerated plantlets, (**D**). In vitro shooting

growth and development in both wild and in vitro environments. These findings highlight the potential of these modelling techniques in enhancing our understanding of biological systems and optimising their in vitro regeneration conditions. This could be a simple task that only needs access to knowledge and little effort. It has been found that ANN-based modelling techniques are more flexible, effective, and adaptable in handling the nonlinear interactions commonly observed in cell culture procedures. The method also has the notable benefit of not needing any prior understanding of how input and output signals are organised or correlated. Despite the fact that this field of study still needs a lot more attention to address a number of unresolved issues, the current research shows how to use artificial neural networks' to accurately feign, all the more so under culture different conditions, the strategies of large-scale cultivation systems for a variety of desirable plant species.

Abbreviations

RSM	Response Surface Methodology
MLP	Multilayer Perceptron
GA	Genetic Algorithm
RMSE	Root Mean Sum of Square
ANFIS-NSGAII	Adaptive Neuro-Fuzzy Inference System-Non-dominated Sorting Genetic Algorithm-II
CCD	Centre Composite design
ANOVA	Analysis of Variance

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Author contributions
HP and VPS conceived and designed the experiments. SS and SB reviewed the literature and formatted the manuscript. HS and VV involved in revision. MD performed the RSM modeling, MD and AY validated the optimised parameters through in vitro plant tissue culture experiments. All authors contributed to prepare the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
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Declarations

Competing interests
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