

# Computing artificial neural network and genetic algorithm for the feature optimization of basal salts and cytokinin-auxin for in vitro organogenesis of royal purple (*Cotinus coggygia* Scop)

Muhammad Aasim<sup>a,\*</sup>, Ayşe Ayhan<sup>b</sup>, Ramazan Katırcı<sup>c</sup>, Alpaslan Şevket Acar<sup>a,d</sup>,  
Seýid Amjad Ali<sup>e</sup>

<sup>a</sup> Department of Plant Protection, Faculty of Agricultural Sciences and Technologies, Sivas University of Science and Technology, Sivas, Türkiye

<sup>b</sup> Department of Biotechnology, Faculty of Science, Mersin University, Mersin, Türkiye

<sup>c</sup> Department of Metallurgical and Materials Engineering, Faculty of Engineering and Natural Sciences, Sivas University of Science and Technology, Sivas, Türkiye

<sup>d</sup> Ada Biotechnology, Kahramanmaraş, Türkiye

<sup>e</sup> Department of Information Systems and Technologies, Bilkent University, Ankara, Türkiye

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## ABSTRACT

This study presents the in vitro regeneration protocol for Royal purple [(*Cotinus coggygia* Scop. (syn.: *Rhus cotinus* L.)) from nodal segment explants followed by optimizing the input variable combinations with the aid of PyTorch ANN and Genetic Algorithm (GA). The Murashige and Skoog (MS) culture medium yielded relatively higher regeneration frequency (91.52 %) and shoot count (1.96) as compared to woody plant medium (WPM), which yielded 84.58 % regeneration and shoot count (1.61) per explant. The supplementation of plant growth regulators (PGRs) + MS medium yielded 80.0–100.0 % shoot regeneration and 1.48–3.25 shoot counts compared to 60.0–100.0 % shoot regeneration and 1.00–2.37 shoots from the combination of PGRs + WPM. In order to predict the shoot count and regeneration with the aid of a mathematical model, the machine learning algorithms of Multilayer Perceptron (MLP), Support Vector Regression (SVR), Extreme Gradient Boosting (XGB), and Random Forest (RF) models were utilized. The highest  $R^2$  values for both output variables were acquired using MLP model in PyTorch platform. The  $R^2$  scores for regeneration and shoot counting were recorded as 0.69 and 0.71 respectively. NSGA-II algorithm revealed the 1.25 mg/L BAP (6-Benzylaminopurine), 0.02 mg/L NAA (Naphthalene acetic acid), and 0.03 mg/L IBA (Indole butyric acid) in WPM medium as an optimum combination for 100 % regeneration. On the other hand, the algorithm suggested multiple combination in MS medium for maximum shoot counting.

## 1. Introduction

Royal purple [(*Cotinus coggygia* Scop. (syn.: *Rhus cotinus* L.)) is a perennial, shrub-like tree that belongs to the Anacardiaceae family (Matić et al., 2016), usually found in Southern Europe, Asia, the Himalayan regions (Ivanova et al., 2005), the Mediterranean region, Thrace and central Anatolian region of Türkiye (Kaymaz, 2018). The plant exhibits wide adaptation to different soil conditions and resistance against different abiotic stresses and can grow up to 5–12 m high (Shaboyan et al., 2021). The leaves of 'Royal Purple' change their color from green to dark wine purple subjected to different environmental factors such as temperature, light, and soil content (Oren-Shamir and

Levi-Nissim, 1997). The leaves turn into different colors like bright yellow, orange, and reddish-purple hues in autumn, hence making it an eye-catching plant preferred in winter landscapes. The plant can also be used as an ornamental (Rovină et al., 2010) and medicinal plant against different diseases and disorders (Erta et al., 2022). The propagation of *C. coggygia* plant has been done by traditional methods like seed or vegetative propagation along with in vitro micropropagation (Jacygrad et al., 2012).

Ornamental plants are playing a major role in establishing a strong bond between humans and nature, and continuous efforts have been made to propagate plants efficiently to meet local and international demand. The rapid increase in urbanization also enforces to increase in

\* Corresponding author.

E-mail address: [mshazim@gmail.com](mailto:mshazim@gmail.com) (M. Aasim).

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**Table 1**  
Impact of plant growth regulators (PGRs) and basal medium on in vitro shoot regeneration of *C. cogggyria*.

Medium	BAP (mg/L)	NAA (mg/L)	IBA (mg/L)	Mean Regeneration ( <i>p</i> 0.000)	StDev	95 % CI	Mean Shoot count ( <i>p</i> 0.009)	StDev	95 % CI
MS0	0	0	0	86.67AB	11.547	(74.18; 99.15)	1.72BC	0.465	(1.28; 2.15)
MS1	1.0	0	0	100.0A	0.000	(87.5; 112.5)	2.00BC	0.400	(1.56; 2.44)
MS2	2.0	0	0	80.00AB	0.000	(67.51; 92.49)	3.25A	0.750	(2.81; 3.69)
MS3	1.0	0.10	0	100.0A	0.000	(87.5; 112.5)	2.13BC	0.462	(1.70; 2.57)
MS4	1.0	0	0.10	80.00AB	0.000	(67.51; 92.49)	2.00BC	0.250	(1.56; 2.44)
MS5	2.0	0.10	0	93.33A	11.547	(80.85; 105.82)	1.77BC	0.635	(1.33; 2.20)
MS6	2.0	0	0.10	93.33A	11.547	(80.85; 105.82)	1.52BC	0.284	(1.08; 1.95)
MS7	1.0	0.10	0.10	93.33A	11.547	(80.85; 105.82)	1.67BC	0.306	(1.23; 2.10)
MS8	2.0	0.10	0.10	93.33A	32.146	(50.8; 75.8)	1.48BC	0.671	(1.05; 1.92)
MS9	1.0	0.05	0.05	93.33A	5.774	(34.18; 59.15)	1.55BC	0.397	(1.11; 1.99)
MS10	2.0	0.05	0.05	100A	0.000	(37.51; 62.49)	2.47AB	0.306	(2.03; 2.90)
WPM0	0	0	0	80.00AB	0.000	(67.51; 92.49)	1.00C	0.000	(0.56; 1.44)
WPM1	1.0	0	0	100.0A	11.547	(87.5; 112.5)	1.67BC	0.115	(1.23; 2.10)
WPM2	2.0	0	0	60.00B	0.000	(47.51; 72.49)	1.33BC	0.335	(0.90; 1.77)
WPM3	1.0	0.10	0	100.0A	0.000	(87.5; 112.5)	2.00BC	0.400	(1.56; 2.44)
WPM4	1.0	0	0.10	93.33A	11.547	(80.85; 105.82)	2.37AB	0.153	(1.93; 2.80)
WPM5	2.0	0.10	0	80.00AB	0.000	(67.51; 92.49)	1.42BC	0.289	(0.98; 1.85)
WPM6	2.0	0	0.10	80.00AB	20.000	(67.5; 92.5)	1.18C	0.166	(0.74; 1.61)
WPM7	1.0	0.10	0.10	73.33AB	11.547	(60.85; 85.82)	1.36BC	0.128	(0.92; 1.80)
WPM8	2.0	0.10	0.10	93.33A	11.547	(80.85; 105.82)	1.95BC	0.328	(1.51; 2.39)
WPM9	1.0	0.05	0.05	80.00AB	0.000	(67.51; 92.49)	1.58BC	0.144	(1.15; 2.02)
WPM10	2.0	0.05	0.05	93.33A	11.547	(80.85; 105.82)	1.85BC	0.132	(1.41; 2.29)

\*\*The means with different alphabets are statistically different at *p*0.01.

the use of ornamental plants in the cities. Ornamental and horticultural trade in the world exceeds 8 billion dollars (Prakash, 2007). In Türkiye, the market of ornamental plants has also developed considerably in the last 40 years and these plants are produced in 55 provinces by using both traditional and biotechnological approaches like plant tissue culture (Nunes et al., 2018). The advantage of using in vitro techniques includes the production at a large scale with uniform and disease-free true-to-type plants. However, the woody plants are generally more recalcitrant due to multiple factors like low regeneration efficiency, plant acclimatization, and establishing the repeatable protocol (Sahari Moghadam et al., 2022). To date, a large number of ornamental woody plants have been propagated through the successful establishment of in vitro regeneration protocol (Adibi Baladeh and Kaviani, 2021; Gaidamashvili and Benelli, 2021; Sahari Moghadam et al., 2022). However, the success of in vitro regeneration protocol is dependent on the manipulation of different input variables considering the target output variables (Adibi Baladeh and Kaviani, 2021).

In recent years, the use of ANN and ML based models are prevailing for validation, prediction and optimization of different input variables in plant tissue culture for different crops. In these studies, researchers employed either single or hybrid models for different targeted output variables of economic crops. Some of the examples include the use of ANN/ML, the use of GRNN model for in vitro rooting of *Passiflora caerulea* (Jafari et al., 2022), and shoot proliferation of wallflower with the aid of MLP-NSGAI (Fakhrzad et al., 2022). The general overview of these studies revealed the different targets like in vitro sterilization, germination, somatic embryogenesis, callogenesis, shoot proliferation, secondary metabolites, and rooting. On the other hand, the impact of different input variables like H<sub>2</sub>O<sub>2</sub>, nutrient composition, plant growth regulators, and culture conditions have been investigated by ANN/ML models.

In the present work, PyTorch ANN and Genetic Algorithm (GA) were employed together to find the optimal solution. The ANN algorithm within PyTorch has been implemented to predict the output based on the input parameters. PyTorch has commonly been employed in other scientific fields. The classification of images acquired from the chrome coated panels using neural network and machine learning algorithms has been documented (Katurci et al., 2021). Besides, similar methodology it was also employed to estimate the thickness and Ni % ratio of the ZnNi alloy coating (Katurci et al., 2021) and predict the throwing power of the Cr(III) electroplating bath (Katurci and Takçi, 2021). Machine

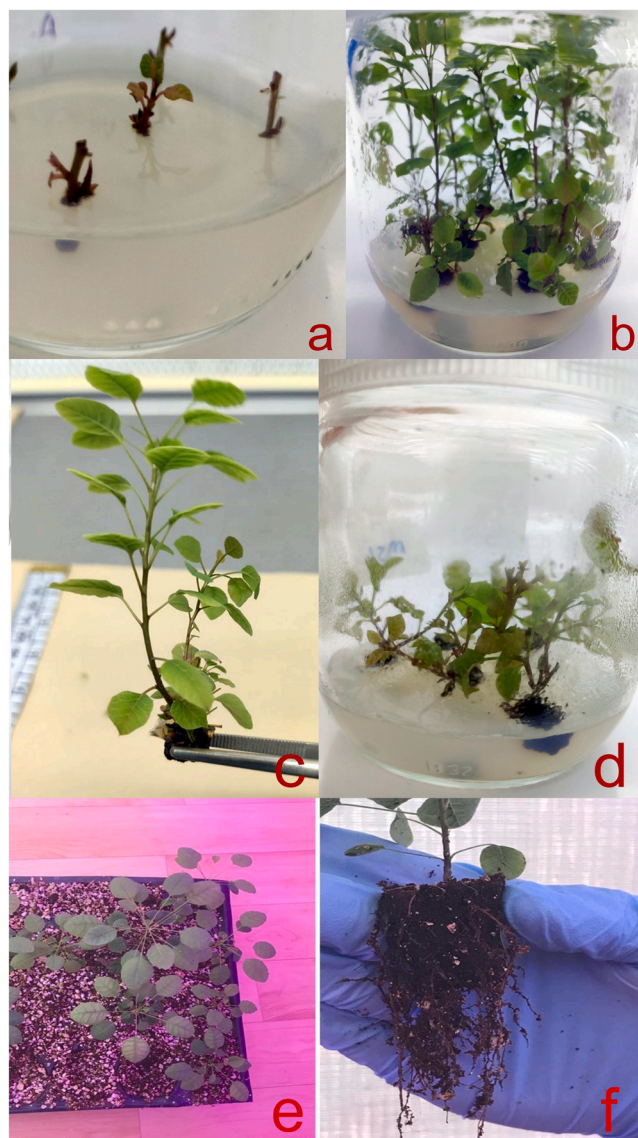
Learning (ML) algorithms — Random Forest (RF), Extreme Gradient Boosting (XGB), Multilayer Perceptron (MLP), and Support Vector Regressor (SVR) (Gardner and Dorling, 1998; Chen and Guestrin, 2016; Rigatti, 2017; Schulz et al., 2018; Speiser et al., 2019; Zhang and O'Donnell, 2020) were utilized to compare the results with PyTorch ANN. The performance of the algorithms was evaluated using the R<sup>2</sup> and MSE scores. The trained ANN model has been used as the fitness function in NSGA-II. The optimal solution has been detected for shoot count and regeneration when PyTorch ANN and NSGA-II. algorithms are used together.

## 2. Materials and methods

### 2.1. In vitro regeneration

The plant material of *C. cogggyria* was procured from Ada Biyoteknoloji, Kahramanmaraş, Türkiye. The one-year-old and approximately 50 cm tall plants were selected for in vitro regeneration. Nodal segment explants were taken directly from plants and subjected to a surface sterilization process. The explants were washed under tap water for 15–20 min followed by cleaning with Tween-20 and tap water. Subsequently, explants were treated with 70 % ethanol for 20–30 s followed by stirring in the sterilization mixture comprised of 1.25 % commercial bleaching (NaOCl - w/v) and 1–2 drops of Tween-20 for 20 min. The explants were cleaned with distilled water three times for 5 min each followed by drying on the sterilized filter papers and cultured on basal medium enriched with either MS (Murashige and Skoog, 1962) or WPM (McCown, 1981). The culture medium was also enriched with different concentrations of BAP (0–2 mg/L), NAA (0–0.10 mg/L), and IBA (0–0.10 mg/L) in different combinations (Table 1) for four weeks (Fig. 1a-d). The rooting medium was prepared using two different concentrations of MS (2.2 and 4.4 mg/L), enriched with different concentrations of Indole butyric acid (IBA) and Naphthalene acetic acid (NAA) singly and in combinations (Table 2). The rooted plantlets were acclimatized in the pots containing mixture of torf: perlite: cocopeat (1:1:1) for 1 months under greenhouse conditions (Figure 1ef).

The basal medium was prepared by using MS and WPM basal medium, enriched with 3.0 % sugar (commercial) and gelled with 0.7 % agar. The pH of the medium used in this study was maintained at 5.8 by adding either 1 N NaOH or 1 N HCl. The culture mediums were sterilized at 1.2 ATM (atmospheric pressure) and at 121 °C for 20 min. The



**Fig. 1.** In vitro shoot regeneration of *C. coggygia* from nodal segment explants (a) nodal segments (b,c) multiple shoot regeneration from MS medium, and (d) WPM medium, (e) rooting and hardening, (f) acclimatized plant.

culture medium (approximately 60 ML) were poured into glass vials covered with white lids. The culture vials were placed in the growth room equipped with 16/8 light photoperiod using LEDs lights at 3000 Lux and temperature was maintained at  $24 \pm 1$  °C. The data regarding shoot regeneration frequency (%) and shoot count were computed after 4 weeks of culture (Fig. 1 a–d). Shoot regeneration frequency was taken by considering the number of explants with shoots divided by total number of explants. The shoot count was taken was counting the total number of shoots divided by number of explants with shoots. The data regarding rooting frequency was taken after four week of culture. Oneway ANOVA (Analysis of Variance) was conducted using Minitab 20.4 program, and box plots were constructed for the graphical presentation of the data. Whereas, data regarding rooting frequency was tabulated with the help of SPSS program.

## 2.2. ANN modeling

The ANN model was generated by using the PyTorch interface. The ANN layers are made up of neurons, and the architecture of the ANN model that was used to train the dataset is provided in Fig. 2. The

**Table 2**  
In vitro rooting frequency of *C. coggygia*.

Rooting Medium	MS (mg/L)	IBA (mg/L)	NAA (mg/L)	Rooting Frequency (%)
R0	4.4	0	0	6.666BC
R1	4.4	0.5	0	0.00C
R2	4.4	1.0	0	6.666BC
R3	4.4	2.0	0	20.666ABC
R4	4.4	0	0.5	6.666BC
R5	4.4	0	1.0	0.00C
R6	4.4	0	2.0	26.666ABC
R7	4.4	0.5	0.5	13.333ABC
R8	4.4	1.0	1.0	40.000ABC
R9	4.4	2.0	2.0	40.000ABC
R10	2.2	0	0	20.000ABC
R11	2.2	0.5	0	13.333ABC
R12	2.2	1.0	0	26.666ABC
R13	2.2	2.0	0	46.666ABC
R14	2.2	0	0.5	26.666ABC
R15	2.2	0	1.0	60.000AB
R16	2.2	0	2.0	46.666ABC
R17	2.2	0.5	0.5	40.000ABC
R18	2.2	1.0	1.0	60.000AB
R19	2.2	2.0	2.0	66.666A

\*The means with different alphabets are statistically different at  $p < 0.05$ .

neurons in the input layer collect data from each variable and multiply them with selected weights. The data in the neurons are then transferred to the hidden layer. In this layer, the activation function is utilized and the updated data is transferred to the final output layer which composes the output data. In this step, the predicted data is compared to the actual data and the deviation is computed. If the deviation is not small enough, the weights are updated and a new cycle initiates (Rastegaripour et al., 2019). This procedure is repeated until the deviation is sufficient. The Leaky ReLU activation function was used in all layers.

The flowchart depicting the optimization steps is presented in Fig. 3. The dataset consisted of 66 samples and the two basal mediums (MS and WPM) were used to grow the plants. Each experiment was repeated 3 times and the standard deviation was computed. The dataset was trained with PyTorch ANN and other ML algorithms — Random Forest (RF), Extreme Gradient Boosting (XGBoost), Multilayer Perceptron (MLP), and Support Vector Regressor (SVR). The highest  $R^2$  and the lowest MSE were acquired with PyTorch ANN algorithm, so this algorithm was used to predict the output of objective function in the Non-dominated Sorting Genetic Algorithm (NSGA-II) (Deb et al., 2002). Two objective functions were employed in the NSGA-II algorithm. The functions acquired from the PyTorch ANN algorithm for both output variables and their respective standard deviation were used as the objective function.

To predict the shoot count and regeneration of the plant of *C. coggygia* in vitro medium. MLP, SVR, GP, XGB, and RF algorithms were utilized. The performance of the model was assessed via  $R^2$  and MSE (Kirtis et al., 2022) performance metrics and their equations are presented in Eqs. (1)–(2).

$$R^2 = 1 - \frac{\sum_{i=1}^n (Y_i - \hat{Y}_i)^2}{\sum_{i=1}^n (Y_i - \bar{Y})^2} \quad (1)$$

$$MSE = \frac{1}{n} \sum_{i=1}^n (Y_i - \hat{Y}_i)^2 \quad (2)$$

$Y_i$  indicates the actual value,  $\hat{Y}_i$  represents the predicted values,  $\bar{Y}$  depicts the mean of actual values, and  $n$  is the number of samples.

## 3. Results and discussion

In this work, two different basal media and different PGRs types and concentrations were employed to determine the best composition for in vitro regeneration of *C. coggygia*. Furthermore, the hybrid ANN-GA model was employed to optimize the best combination of input

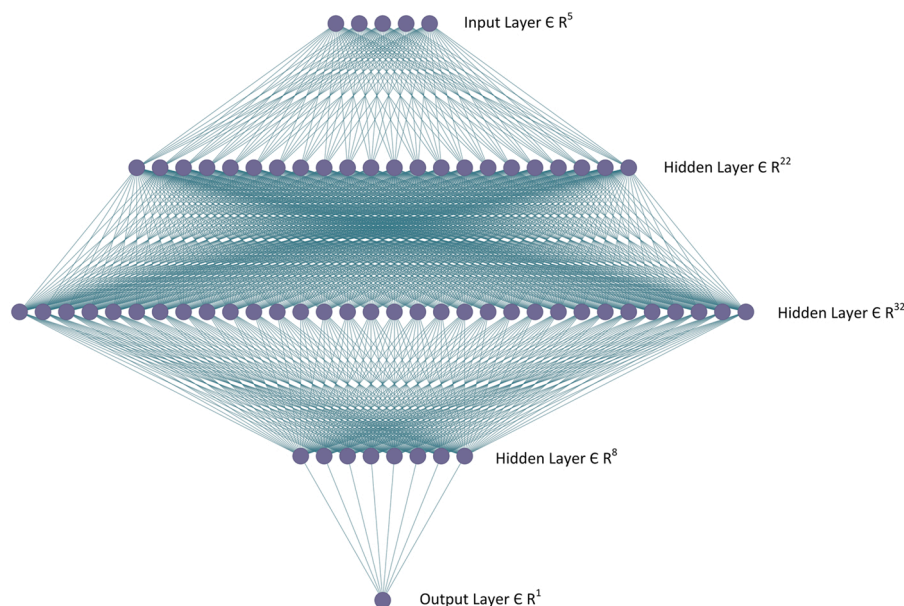


Fig. 2. Artificial Neural Network architecture used in PyTorch.

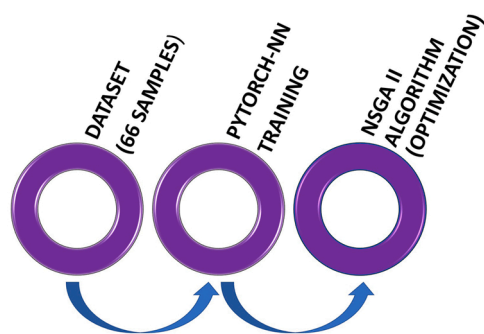


Fig. 3. The flowchart of optimization process.

variables. Optimization of economically important ornamental plants via plant tissue culture technique is becoming more prevalent due to advantages like the production of true-to-type plants over traditional propagation systems (Kaviani et al., 2022). In vitro regeneration system of woody plants is comparatively more difficult due to their recalcitrant nature (Dong et al., 2017) and required manipulation of variable input factors like basal medium, plant growth regulators, and culture conditions (Pyati, 2019).

Selection of proper basal medium for inducing multiple shoot induction of woody plants is established and showed the need for variable basal medium to be mainly dependent on plant type. The statistically significant impact of using different basal mediums was observed and recorded as  $p0.031$  for regeneration frequency and  $p0.012$  for shoot counts (Fig. 4a,b; S. Table 1). Supplementation of MS medium was more productive and yielded more regeneration frequency (91.52 %) and average shoot count (1.96) compared to WPM medium (84.58 % regeneration and 1.61 average shoot count). The better performance of the MS medium compared to the WPM medium also confirmed the findings in *Pistacia lentiscus*. Comparison of three different basal mediums (MS, QL, WPM) revealed the better performance of MS over other basal mediums (Yıldırım et al., 2018). Likewise, superior response of MS medium over other mediums have been documented for other woody plants like *Albizia lebbek* (Perveen et al., 2013), hazelnut (Daryani et al., 2016), black turmeric (Haida et al., 2022), orchids like *Aerides ringens* (Pyati, 2019) and *Cymbidium alofolium* (Rahman and Mahbubur, 2020), *Pyracantha angustifolia* (Kaviani et al., 2022) and.

The optimization of proper PGRs type and concentration is crucial and varies with species type (Adibi Baladeh and Kaviani, 2021). The statistically significant impact of PGRs type and concentration was also observed on both outputs (Fig. 4c,d; S. Table 2) and was recorded more than in the control group. In vitro shoot regeneration and shoot counts ranged between 70.0 % and 100.0 % and 1.35–2.29 shoots respectively. The maximum regeneration frequency (100 %) was computed from MS medium supplemented with 1.0 mg/L BAP  $\times$  0.10 mg/L NAA and 2.0 mg/L BAP  $\times$  0.10 mg/L NAA. Quite the opposite, minimum regeneration frequency (70.0 %) with maximum shoot counts (2.29) was attributed to the combination of 1.0 mg/L BAP  $\times$  0.10 mg/L IBA. Supplementation of both NAA and IBA exerted a negative impact on both output variables. It was evident from the results that either BAP concentration used alone or with either NAA or IBA exerted a positive impact on both shoot regeneration and shoot count. The positive impact of IBA on in vitro regeneration of plants from the Rosaceae family has been documented (Zare Khafri et al., 2021). A comparison of both auxins revealed the better efficiency of NAA compared to IBA with BAP. The results on black turmeric demonstrated an insignificant impact on shoot regeneration frequency. Whereas a significant impact on shoot count using different IBA and NAA concentrations with BAP (Haida et al., 2022). Supplementation of both auxins at the same time exerted a negative impact irrespective of their doses. The variable impact of PGRs on shoot proliferation is supposed to be due to the variable endogenous phytohormone contents in different plant species (Kucharska et al., 2020).

An investigation of the interaction of BM  $\times$  PGRs discerned a statistically significant but variable impact on both regenerations ( $p0.000$ ) and shoot count ( $p0.000$ ) variables. The combination of PGRs with MS medium yielded 80.0–100.0 % shoot regeneration frequency as compared to 60.0–100.0 % from the combination of WPM + PGRs. On the other hand, shoot counts ranged between 1.48 and 3.25 shoots (MS  $\times$  PGRs) and 1.00–2.37 shoots (WPM + PGRs). The maximum shoot count (3.25 shoot) from MS enriched medium was computed from 2.0 mg/L BAP containing medium followed by 2.47 shoots from MS medium enriched with 2.0 mg/L BAP + 0.05 mg/L NAA + 0.05 mg/L IBA PGRs. However, maximum shoot counts of 2.37 were recorded from WPM medium enriched with 1.0 mg/L BAP + 0.10 mg/L IBA followed by 1.95 shoot from the combination of 2.0 mg/L BAP + 0.10 mg/L NAA + 0.10 mg/L IBA (Table 1). It is evident from the results that MS basal medium is more productive compared to WPM basal medium. It is

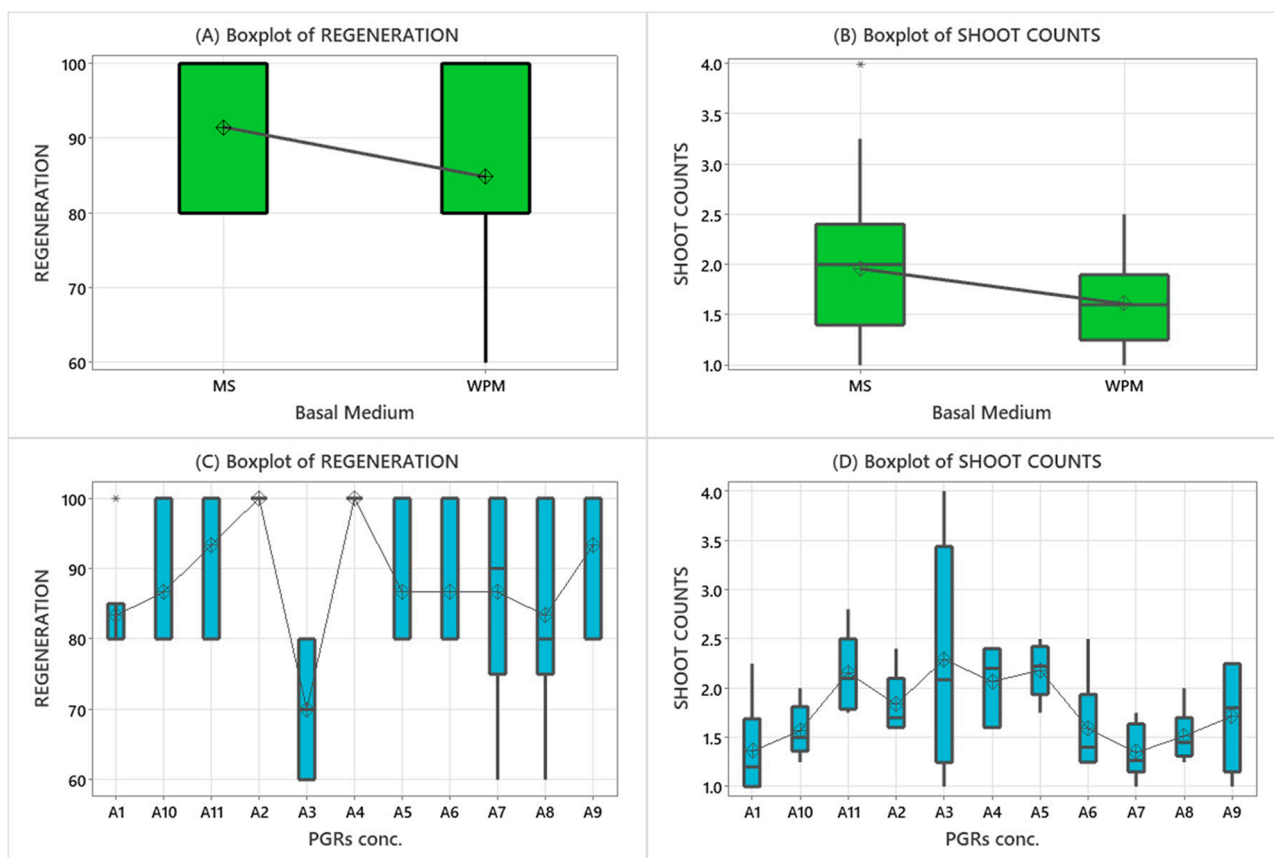


Fig. 4. Boxplot analysis of in vitro regeneration of *C. coggygia* (a-b) impact of basal medium, and (c-d) impact of different PGRs combinations.

apparent from the results that MS medium with high BAP concentration led to higher shoot proliferation (Jacygrad et al., 2012), but with less regeneration capacity. The studies on different woody plants revealed the variable required dose of BAP for shoot proliferation and generally ranged between 0.1 and 2.0 mg/L (Vujović et al., 2020). It was also interesting to see the better performance of BAP+NAA+IBA using WPM medium as compared to MS medium. The synergistic impact of cytokinin-auxin combination is considered to promote cell division and elongation under in vitro conditions and lead to more shoot proliferation (Kudikala et al., 2020; Farooq et al., 2021; Sultana et al., 2022). It is also well established that the cytokinin-auxin combination regulates the endogenous plant hormones, and nutrient uptake from the basal medium followed by translocation and regulating the metabolic processes (Gajula et al., 2022). The optimization of input variables is considered a mainstay of developing in vitro regeneration protocols for commercial ornamental plants. Thereafter, the optimization process using ANN-GA model was performed to divulge the best combination.

The optimization of entire plant regeneration is greatly hampered by the in vitro rooting of woody fruit plants, followed by acclimatization. In order to promote rooting, culture medium with auxin type and concentration alongwith basal salt are typically applied to the rooting medium (Ahmed, 2022). In vitro regenerated shoots cultured on the rooting medium resulted in rooting frequency of 0.00–66.67 % (Table 2). The maximum rooting frequency of 66.67 % was recorded from the medium enriched with 2.2 mg/L MS+ 2.0 mg/L IBA+ 2.0 mg/L NAA. Results further revealed that equal concentration of both IBA and NAA generated relatively more rooting frequency compared to provided individually, and also exhibited the elevated rooting frequency with their increase in concentration (Metivier et al., 2007). The MS concentration in the culture medium was more effective as supplementation of 2.2 mg/L generated more rooting frequency compared to 4.4 mg/L MS irrespective of concentration of IBA and NAA. The rooted plantlets

Table 3

Performance metrics for the validation of models.

	Shoot count		Regeneration	
	R <sup>2</sup>	MSE	R <sup>2</sup>	MSE
MLP	0.36	0.20	0.38	98.6
SVR	0.15	0.27	-0.24	196.3
XGB	0.40	0.20	0.29	111.1
RF	0.42	0.19	0.37	100.3

(Fig. 1e) were successfully acclimatized in the pots filled with torf and perlite under greenhouse conditions. The successful adaptation under greenhouse conditions is significant step for the adaptation of woody plants (Fira and Clapa, 2011; Ahmed, 2022), and findings will have a considerable economic impact on plant commercial propagation of *C. coggygia*.

### 3.1. Machine learning application

Table 2 indicates the R<sup>2</sup> and MSE values of the models for shoot count and regeneration. RF model showed the best performance for the prediction of the shoot count because it has the highest R<sup>2</sup> and the lowest MSE values. For the regeneration, the best model turned out to be MLP. The highest R<sup>2</sup> values were 0.42 and 0.38 for shoot count and regeneration, respectively. As the R<sup>2</sup> values of RF and MLP are very close to each other, they can be used interchangeably. The R<sup>2</sup> scores could not be increased even if the hyperparameter optimization was performed. Therefore, the other models were used to increase the scores, and the impact of data type is discussed below in detail.

The PyTorch NN library was utilized to increase the achievement of the model. The performance scores of the model are presented in Table 3. The R<sup>2</sup> scores of shoot count and regeneration were recorded as

MS	WPM	BAP	NAA	IBA	sc-real	sc-pred	sc-real-means
1	0	0	0	0	1.40	1.72	
1	0	0	0	0	2.25	1.72	
1	0	0	0	0	1.50	1.72	1.72
1	0	1	0	0	2.00	2.00	
1	0	1	0	0	2.40	2.00	
1	0	1	0	0	2.60	2.00	2.00
1	0	2	0	0	3.25	3.25	
1	0	2	0	0	4.00	3.25	
1	0	2	0	0	2.50	3.25	3.25
1	0	1	0.1	0	2.40	2.13	
1	0	1	0.1	0	2.40	2.13	
1	0	1	0.1	0	1.60	2.13	2.13
1	0	1	0	0.1	2.00	2.00	
1	0	1	0	0.1	2.25	2.00	
1	0	1	0	0.1	1.75	2.00	2.00

Fig. 5. Partial dataset used to generate the model.

Table 4  
The metrics of the dataset trained by PyTorch.

	Raw dataset		Averaged dataset	
	R <sup>2</sup>	MSE	R <sup>2</sup>	MSE
Shoot Counting	0.71	0.09	0.99	7.7e-05
Regeneration	0.69	48.48	0.98	2.02

0.71 and 0.69 respectively. These scores are pretty good when compared to the other models. The scores were not increased further despite employing other additional hyperparameters.

The reason for this was investigated on the dataset. Some of the datasets used to generate the model are presented in Fig. 5. The last three columns indicate the experimental (sc-real), predicted (sc-pred) and the means of experimental outputs (sc-real-means) respectively. As seen in Fig. 5, sc-pred values are very close to the means of sc-real values. The numbers in the sc-real-means column are the means of the numbers in the red cycle in sc-real column in Fig. 3. As seen in sc-pred column, the same prediction is made for the same input. However, the experimental output of each is different. When the R<sup>2</sup> values are computed with Eq. (1) the R<sup>2</sup> values become low. But when the R<sup>2</sup> values are computed using the mean of the actual values, the R<sup>2</sup> values increase abruptly to 0.99. The R<sup>2</sup> value drops with increasing the distance of the experimental values from the mean of the real values for the same input. The same situation is valid for regeneration. This is a handicap for repeated experiments. To overcome this problem, it is suggested that the mean and standard deviations of the experimental results should be used together for training and optimization. After this stage, the suggested method for the repeated experiments was utilized for the training and optimization. The edited dataset is presented in the supplementary material. The results acquired from the edited dataset are provided in the column of ‘‘Averaged dataset’’ in Table 4. The two models acquired using the averaged values and standard deviation for shoot counting and regeneration were employed as the objective function in the optimization process.

#### 4. Optimization of input variables

The optimization problem is generally defined as follows.

$$\text{Minimize : } f_m(x) \quad m = 1, \dots, M$$

$$\text{s.t. } g_j(x) \leq 0 \quad j = 1, \dots, J$$

$$h_k(x) = 0 \quad k = 1, \dots, K$$

$$x_i^L \leq x_i \leq x_i^U \quad x \in \Omega$$

where  $x_i$  depicts the  $i^{\text{th}}$  variable to be optimized,  $x_i^L$  and  $x_i^U$  shows the lower and upper bounds.  $f_m(x)$ ,  $g_j(x)$  and  $h_k(x)$  indicate the  $m^{\text{th}}$  objective function,  $j^{\text{th}}$  inequality constraint and  $k^{\text{th}}$  equality constraint respectively. In our study, we utilized two objective functions for each output (shoot count and regeneration). The models acquired from the machine learning algorithm were used as the objective function. The averaged value of shoot counting was maximized and its standard deviation was minimized in NSGA-II algorithm. The problem was generated as follows.

$$\min f_m(x) \quad \text{model generated via machine learning}$$

$$\text{s.t. } g_j(x) \max 100 \text{ for regeneration. No constraint for shoot count}$$

$$h_k(x) \text{ no constraint}$$

$$x_L = [0, 0, 0, 0, 0]$$

$$x_U = [1, 1, 2, 0.1, 0.1]$$

The optimum medium to acquire the maximum shoot count and regeneration was investigated with this model. The GA algorithm is an adaptive heuristic search algorithm based on an evolutionary method derived from biological evolution by Charles Darwin’s theory. A population is generated from individuals. Each individual is made up of genes, which are the parameters of the input. Chromosomes are formed by combining genes. In the first stage, a population, a group of chromosomes, is randomly generated (Kramer, 2017). The objective function

**Table 5**  
The solution set for the maximum shoot counting.

MS	WPM	BAP	NAA	IBA	SC-Avr	SS
1	0	0.04	0.00	0.10	3.9	0.36
1	0	0.10	0.00	0.10	3.8	0.36
1	0	0.17	0.01	0.10	3.6	0.35
1	0	0.20	0.01	0.10	3.6	0.35
1	0	0.26	0.00	0.10	3.5	0.34
1	0	0.28	0.00	0.10	3.5	0.34
1	0	0.33	0.00	0.10	3.4	0.32
1	0	0.33	0.00	0.10	3.4	0.32
1	0	0.39	0.00	0.10	3.3	0.30
0	1	0.15	0.00	0.10	1.6	0.03
0	1	0.11	0.00	0.10	1.5	0.01
0	1	0.12	0.00	0.06	1.5	0.00

**Table 6**  
The solution set for the regeneration.

MS	WPM	BAP	NAA	IBA	REG	SS
0	1	1.25	0.02	0.03	100	0.00

computes the scores of the chromosomes and ascertains how fit they are. Two chromosomes are decided based on their objective function scores for reproduction. In the stage of reproduction of the new generation, new chromosomes are generated by the operations of crossover and mutation. The crossover process transfers the information between the parents to create offspring. In the mutation step, the genes created by the crossover process are modified (Srinivas and Deb, 1994). GA is an algorithm developed for a single objective. NSGA algorithm, which can generate diverse solutions and improve performance, was proposed in the early 1990 s to implement a sorted hierarchy of solutions within the generation of a genetic algorithm. The NSGA-II algorithm, based on NSGA algorithm, for a multi-objective problem was proposed (Deb et al., 2002). The algorithm was generated to overcome the limitations of the NSGA algorithm (Srinivas and Deb, 1994; Deb et al., 2002; Blank and Deb, 2020). The algorithm, cited over 4700 times, has shown high

success (Deb et al., 2002). The number of mutation and crossover processes is crucial because they have an impact on the optimization performance. In this study, the crossover and mutation rates were set to 0.5 and 0.2 respectively. Besides, the population size and the maximum iterations were set to 10, and 10 respectively.

Multi-objective Optimization in Python (pymoo) framework (Blank and Deb, 2020) was utilized to design and solve the problem. Table 5 indicates the solution set for the maximum shoot count and the minimum standard deviation as suggested by the NSGA-II algorithm. For high shoot count, MS medium should be preferred because, in WPM medium, the highest shoot count achieved is 1.6, whereas, in MS medium, it is 3.9. As for regeneration, just one medium was suggested by the algorithm. The optimization results are presented in Table 6. For high regeneration, WPM medium is favorable. This study showed us that it is not possible to achieve both the highest shoot counting and the highest regeneration through the same medium. But if the effects and interactions of the chemicals in WPM and MS mediums on the shoot count and the regeneration are investigated, it may be possible to develop the new medium imparting the highest regeneration and the highest shoot count together.

Fig. 6 shows the correlation between the inputs and the outputs. IBA has the most positive impact on the shoot counting. The effects of BAP alone and BAP + NAA on shoot count are close to each other. While MS medium has an increasing effect on shoot count, WPM medium demonstrated a decreasing effect. Standard deviation decreased in the order of IBA > NAA > BAP. The use of heat map in plant tissue culture studies is important to find out the correlation between the inputs and output variables. The previous studies reported for Cannabis (Aasim et al., 2022b), chickpea (Kirtis et al., 2022), and common bean (Aasim et al., 2022a) also revealed the use of heat map to present the correlation between the inputs and output variables.

**5. Conclusion**

The optimization of input variables for optimizing plant tissue protocol is highly significant for commercial propagation of economically important ornamental plants. Application of ANN and ML based models



Fig. 6. Heatmap of predicted shoot counting and inputs.

predicted the results moderately. However, subjecting data set to Pytorch resulted in significantly enhanced metrics scores. Optimization of in vitro protocol with the aid of employing ANN- NSGA-II algorithms yielded the multiple input combinations for maximizing the shoot counts for both basal mediums. However, hybrid ANN-NSGA-II algorithm extracted only single combination of input variable for yielding maximum regeneration by using WPM basal medium. The results can be used for optimizing the in vitro regeneration protocols of other economic plants for commercial propagation.

### CRedit authorship contribution statement

**Muhammad Aasim:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Supervision, Project administration. **Ayşe Ayhan:** Investigation, Data curation. **Ramazan Katurcı:** Software, Writing – original draft. **Alpaslan Şevket Acar:** Investigation, Data curation. **Seyid Amjad Ali:** Formal analysis, Writing – review & editing, Visualization.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data Availability

Data will be made available on request.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.indcrop.2023.116718](https://doi.org/10.1016/j.indcrop.2023.116718).

### References

- Aasim, M., Katirci, R., Baloch, F., et al., 2022a. Innovation in the breeding of common bean through a combined approach of in vitro regeneration and machine learning algorithms. *Front. Genet.* 13.
- Aasim, M., Katurcı, R., Akgur, O., et al., 2022b. Machine learning (ML) algorithms and artificial neural network for optimizing in vitro germination and growth indices of industrial hemp (*Cannabis sativa* L.). *Ind. Crops Prod.* 181, 114801.
- Adibi Baladeh, D., Kaviani, B., 2021. Micropropagation of medlar (*Mespilus germanica* L.), A Mediterranean Fruit Tree. *Int. J. Fruit Sci.* 21, 242–254.
- Ahmed, M.E.A.E., 2022. In vitro propagation and improving accumulation of coumarin in *Lycium barbarum*, a rare plant in the flora of Egypt. *Bull. Natl. Res. Cent.* 46, 220.
- Blank, J., Deb, K., 2020. Pymoo: Multi-objective optimization in python. *IEEE Access* 8, 89497–89509.
- Chen T., Guestrin C., 2016. XGBoost. In: *Proceedings of the 22nd ACM SIGKDD International Conference on Knowledge Discovery and Data Mining*. ACM, New York, NY, USA, pp 785–794.
- Daryani, P., Zare, N., Chamani, E., et al., 2016. Evaluation of the effects of different basal medium and plant growth regulators on in vitro growth of hazelnut. *J. Hortic. Sci.* 30.
- Deb, K., Pratap, A., Agarwal, S., Meyarivan, T., 2002. A fast and elitist multiobjective genetic algorithm: NSGA-II. *IEEE Trans. Evol. Comput.* 6, 182–197.
- Dong, C., Li, X., Xi, Y., Cheng, Z.-M., 2017. Micropropagation of *Pyracantha coccinea*. *HortScience* 52, 271–273.
- Ertar, B., Okuyan, B., Ali, Ş.E.N., et al., 2022. The effect of *Cotinus coggygia* L. ethanol extract in the treatment of burn wounds. *J. Res. Pharm.* 26.
- Fakhrzad, F., Jowkar, A., Hosseinzadeh, J., 2022. Mathematical modeling and optimizing the in vitro shoot proliferation of wallflower using multilayer perceptron non-dominated sorting genetic algorithm-II (MLP-NSGAI). *PLoS One* 17, e0273009.
- Farooq, I., Qadri, Z.A., Rather, Z.A., et al., 2021. Optimization of an improved, efficient and rapid in vitro micropropagation protocol for *Petunia hybrida* Vilm. Cv. "Bravo. *Saudi J. Biol. Sci.* 28, 3701–3709.
- Fira, A., Clapa, D., 2011. Results regarding in vitro proliferation in Goji (*Lycium barbarum*). *Bull. UASVM Hortic.* 68, 503.
- Gaidamashvili, M., Benelli, C., 2021. Threatened woody plants of Georgia and micropropagation as a tool for in vitro conservation. *Agronomy* 11, 1082.
- Gajula, H., Kumar, V., Vijendra, P.D., et al., 2022. In vitro regeneration of *Psoralea corylifolia* Linn.: influence of polyamines during in vitro shoot development. *Vitr Cell Dev. Biol.* 58, 103–113.
- Gardner, M.W., Dorling, S.R., 1998. Artificial neural networks (the multilayer perceptron)-a review of applications in the atmospheric sciences. *Atmos. Environ.* 32, 2627–2636.
- Haida, Z., Sinniah, U.R., Nakasha, J.J., Hakiman, M., 2022. Shoot induction, multiplication, rooting and acclimatization of black turmeric (*Curcuma caesia* Roxb.): an important and endangered Curcuma Species. *Horticulturae* 8, 740.
- Ivanova, D., Gerova, D., Chervenkov, T., Yankova, T., 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *J. Ethnopharmacol.* 96, 145–150.
- Jacygrad, E., Ilczuk, A., Mikos, M., Jagiello-Kubiec, K., 2012. Effect of medium type and plant growth regulators on the in vitro shoot proliferation of *Cotinus coggygia* Scop. *Royal Purple*. *Acta Sci. Pol. Hortorum Cultus* 11, 143–151.
- Jafari, M., Daneshvar, M.H., Jafari, S., Hesami, M., 2022. Machine learning-assisted in vitro rooting optimization in *Passiflora caerulea*. *Forests* 13, 2020.
- Katirci, R., Takçı, H., 2021. Makine Öğrenmesi Metotları Kullanarak Krom III Kaplama Banyosunun Örtme Gücünün Tahmin Edilmesi. *Firat Univ. J. Eng.* 33.
- Katirci, R., Aktas, H., Zontul, M., 2021. The prediction of the ZnNi thickness and Ni % of ZnNi alloy electroplating using a machine learning method. *Trans. Inst. Met Finish* 99, 162–168. <https://doi.org/10.1080/00202967.2021.1898183>.
- Katurcı, R., Yılmaz, E.K., Kaynar, O., Zontul, M., 2021. Automated evaluation of Cr-III coated parts using Mask RCNN and ML methods. *Surf. Coat. Technol.* 422, 127571. <https://doi.org/10.1016/j.surfcoat.2021.127571>.
- Kaviani, B., Deltalab, B., Kulus, D., et al., 2022. In vitro propagation of *Pyracantha angustifolia* (Franch.) CK Schneid. *Horticulturae* 8, 964.
- Kaymaz M.B., 2018. Yanık yarası üzerine *Cotinus coggygia* (duman ağacı) yaprak ekstresi ve fenitoinin etkileri.
- Kirtis, A., Aasim, M., Katurcı, R., 2022. Application of artificial neural network and machine learning algorithms for modeling the in vitro regeneration of chickpea (*Cicer arietinum* L.). *Plant Cell Tissue Organ Cult.* 1–12.
- Kramer O., 2017. Genetic algorithms. In: *Genetic algorithm essentials*. Springer, pp 11–19.
- Kucharska, D., Orlikowska, T., Maciorowski, R., et al., 2020. Application of meta-Topolin for improving micropropagation of gooseberry (*Ribes grossularia*). *Sci. Hortic.* 272, 109529.
- Kudikala, H., Jogam, P., Sirikonda, A., et al., 2020. In vitro micropropagation and genetic fidelity studies using SCOT and ISSR primers in *Annona reticulata* L.: an important medicinal plant. *Vegetos* 33, 446–457.
- Matić, S., Stanić, S., Mihailović, M., Bogojević, D., 2016. *Cotinus coggygia* Scop.: an overview of its chemical constituents, pharmacological and toxicological potential. *Saudi J. Biol. Sci.* 23, 452–461.
- McCown, B.H., 1981. Woody Plant Medium (WPM)-a mineral nutrient formulation for microculture for woody plant species. *Hort. Sci.* 16, 453.
- Metivier, P.S.R., Yeung, E.C., Patel, K.R., Thorpe, T.A., 2007. In vitro rooting of microshoots of *Cotinus coggygia* Mill, a woody ornamental plant. *Vitr Cell Dev. Biol.* 43, 119–123.
- Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant* 15, 473–497.
- Nunes, S., Sousa, D., Pereira, V.T., et al., 2018. Efficient protocol for in vitro mass micropropagation of slash pine. *Vitr Cell Dev. Biol.* 54, 175–183.
- Oren-Shamir, M., Levi-Nissim, A., 1997. Temperature effects on the leaf pigmentation of *Cotinus coggygia* 'Royal Purple'. *J. Hortic. Sci.* 72, 425–432.
- Perveen, S., Anis, M., Aref, I.M., 2013. Resource communication. In vitro plant regeneration of *Albizia lebbek* (L.) Benth. from seed explants. *For. Syst.* 22, 241–248.
- Prakash J., 2007. Micropropagation of ornamental perennials: progress and problems. In: *III International Symposium on Acclimatization and Establishment of Micropropagated Plants 812*. pp 289–294.
- Pyati, A.N., 2019. In vitro seed germination, protocorm formation and plantlet regeneration in *Aerides ringens* Fisher. *Plant Tissue Cult. Biotechnol.* 29, 49–62.
- Rastegaripour, F., Saboni, M.S., Shojaei, S., Tavassoli, A., 2019. Simultaneous management of water and wastewater using ant and artificial neural network (ANN) algorithms. *Int. J. Environ. Sci. Technol.* 16, 5835–5856.
- Rigatti, S.J., 2017. Random forest. *J. Insur. Med.* 47, 31–39.
- Rovina, E.A., Călinescu, M., Plopa, C., Isac, V., 2010. In vitro regeneration capacity of the ornamental varieties related to the cultural media. *J. Hortic. For. Biotechnol.* 14, 13–18.
- Sahari Moghadam, A., Kaviani, B., Mohammadi Torkashvand, A., et al., 2022. Micropropagation of English yew, an ornamental-medicinal tree. *J. Ornament. Plants* 12, 91–99.
- Schulz, E., Speekenbrink, M., Krause, A., 2018. A tutorial on Gaussian process regression: Modelling, exploring, and exploiting functions. *J. Math. Psychol.* 85, 1–16.
- Shaboyan, N.K., Moghrovyan, A.V., Dumanyan, K.H., et al., 2021. Phytochemical analysis and antioxidant activity of *Cotinus coggygia* Scop. from Armenian Flora. *Pharmacogn. J.* 13.
- Speiser, J.L., Miller, M.E., Tooze, J., Ip, E., 2019. A comparison of random forest variable selection methods for classification prediction modeling. *Expert Syst. Appl.* 134, 93–101.
- Srinivas, N., Deb, K., 1994. Multiobjective optimization using nondominated sorting in genetic algorithms. *Evol. Comput.* 2, 221–248.

- Sultana, K.W., Das, S., Chandra, I., Roy, A., 2022. Efficient micropropagation of *Thunbergia coccinea* Wall. and genetic homogeneity assessment through RAPD and ISSR markers. *Sci. Rep.* 12, 1–11.
- Vujović, T., Jevremović, D., Marjanović, T., Glišić, I., 2020. In vitro propagation and medium-term conservation of autochthonous plum cultivar 'Crvena Ranka'. *Acta Agric. Serbica* 25, 141–147.
- Yıldırım H., Çalar N., Onay A., 2018. An effective protocol for in vitro germination and seedling development of lentisk (*Pistacia lentiscus* L.).
- Zare Khafri, A., Solouki, M., Zarghami, R., et al., 2021. In vitro propagation of three Iranian apricot cultivars. *Vitr Cell Dev. Biol.* 57, 102–117.
- Zhang, F., O'Donnell, L.J., 2020. Support vector regression. In: *Machine Learning*. Elsevier, pp. 123–140.