

Modeling and optimizing *in vitro* seed germination of industrial hemp (*Cannabis sativa* L.)

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ABSTRACT

In vitro seed germination of cannabis as the first physiological stage in the plant life cycle is not only important for studying factors affecting cultivation conditions but also crucial for obtaining juvenile tissue as a potential explant for different *in vitro* procedures. On the other hand, *in vitro* seed germination is a multi-variable biological process that can be influenced by genetic (genotype) and physical factors (medium composition and environmental conditions). Therefore, a powerful mathematical methodology such as artificial neural networks (ANNs) is well suited to analyze the data and optimize the conditions this complex system. The current study was aimed to evaluate the effect of different types and concentrations of carbohydrate sources (sucrose and glucose) as well as different strengths of DKW (Driver and Kuniyaki Walnut) and mMS (Murashige and Skoog Medium, Van der Salm modification) media on seed germination indices as well as morphological features of *in vitro*-grown cannabis seedlings by using three ANNs including multilayer perceptron (MLP), radial basis function (RBF), and generalized regression neural network (GRNN). The GRNN model displayed higher predictive accuracy ($r^2 > 0.70$) in both training and testing sets for all germination indices and morphological traits in comparison to RBF or MLP. Moreover, non-dominated sorting genetic algorithm-II (NSGA-II) was subjected to the GRNN to find the optimal type and level of media and carbohydrate source for obtaining the best seed germination indices (germination rate and mean germination time). According to the optimization process, 0.43 strength mMS medium supplemented with 2.3 % sucrose would result in the best outcomes. This result showed that a moderate level of salts existing in culture media (0.43 strength of mMS medium) supplemented with a moderate level of sucrose (2.3 %) can improve *in vitro* seed germination of hemp. The results of a validation experiment revealed that there was a negligible difference between the experimental data and the optimized result. Therefore, GRNN-NSGA-II provided an accurate prediction of seed germination and can likely be employed to optimize different factors involved in *in vitro* culture of this multi-purpose crop.

1. Introduction

Hemp (*Cannabis sativa* L.) is cultivated in many parts of the world for oil, fiber, seeds and its medicinal values (Kovalchuk et al., 2020; Salami et al., 2020). There is an exponential increase in demanding the products of cannabis all over the world, including a multi-billion dollar market for non-psychoactive cannabinoids that can be obtained from industrial hemp (Monthony et al., 2021). However, studies on this multipurpose crop are lacking and biotechnological methods are in their infancy due to the long history of prohibition (Hesami et al., 2020c). Plant tissue culture is an important agricultural biotechnology that provides the production of crops with improved food, fuel, fiber, and feed

characteristics (Chandran et al., 2020; Krasteva et al., 2021). The advantages of tissue culture are numerous and include: (1) a reduced space requirement owing to the small size of explant used as starting material; (2) production of multiples of plants without the need for any pollinators; (3) cultivation of plants under sterile conditions therefore limiting the transmission of pests, diseases, and pathogens; (4) highly space and time-efficient production and storage of mature plants impervious to weather and without the need for soil (Chadipiralla et al., 2020; El-Sherif, 2018).

Applying and developing biotechnological tools, such as plant tissue culture, to cannabis will help tackle some species-specific bottlenecks to improve productivity, develop disease resistance, studying functional

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genomics, and genetic manipulation (Hurgobin et al., 2020). To study *in vitro* functional genomics or genetic manipulation and secondary metabolite production via callus culture, juvenile tissues and organs such as different parts of *in vitro*-growing seedling have been recommended as a potential explant material (Deguchi et al., 2020; Ioannidis et al., 2020; Sorokin et al., 2020). Thus, efficient seed germination protocol to provide *in vitro*-grown seedlings can play a conspicuous role in successful cannabis genetic manipulation and regeneration. Moreover, seed germination can be considered as the first and most important physiological stage in the plant life cycle (Hu et al., 2018). Therefore, successful seed germination is not only important for studying factors affecting cultivation conditions, but also crucial for obtaining juvenile tissue as a potential explant for different *in vitro* procedures (e.g. gene transformation, callus culture, secondary metabolite and biofuel production) and for screening stress-tolerant genotypes (Hu et al., 2018; Sorokin et al., 2021). In an *ex vitro* study, Hu et al. (2018) investigated the effect of different concentrations of various salts (NaCl, Na₂CO₃, Na₂SO₄, and NaHCO₃) on seed germination of two hemp cultivars and reported that a low level of salts improved seed germination rate, resulted in longer hypocotyls and radicles in comparison with a high concentration of salts. They also showed that the quality of seedlings was more sensitive to salt concentrations than seed germination rate and that seed germination indices of hemp could be different with the type and concentration of salts. These results show the importance of studying the type and concentration of salts on seed germination, but to the best of our knowledge, no comprehensive studies exist concerning *in vitro* cannabis seed germination where salts are typically included in the culture medium.

In vitro seed germination is a multi-variable biological process (Genze et al., 2020) that can be influenced by genetic (genotype) and physical factors such as type and concentrations of plant growth regulators, carbon sources, gelling agent, medium composition, disinfection procedure, as well as light, pH, and temperature (Fig. 1) (Hesami and Jones, 2020; Roni et al., 2018). Among these factors, medium

composition (e.g., type and concentration of salts and vitamins), carbohydrate sources, and their interaction play a pivotal role. Previous studies showed that the high concentration of media (full strength medium) can inhibit seed germination in many species (Bozdemir et al., 2018; Huh et al., 2016; Lin et al., 2017). Also, different studies showed that each plant needs specific medium composition (salts and vitamins) and carbohydrate source for optimal germination (Roni et al., 2018; Stewart and Kane, 2010; Sumaryono et al., 2012). For instance, Knudson (1922) showed that orchid seed requires a specific culture medium supplemented with sucrose as a simple carbohydrate source for *in vitro* germination. In another study, Sumaryono et al. (2012) studied the effect of different strengths of MS medium along with different concentrations and types of carbohydrates for *in vitro* growth of *Metroxylon sagu* seedlings. They reported that half-strength MS medium along with 30 g/l sucrose resulted in the highest performance and growth of the seedlings. Generally, it has been suggested that the type and strength of media as well as carbohydrate sources should be adjusted for each plant (Roni et al., 2018; Stewart and Kane, 2010; Sumaryono et al., 2012). As a non-linear and complex biological process, conventional statistical approaches such as simple regression is not suitable for studying and analyzing *in vitro* seed germination due to the number of treatments that would be required (Niazian and Niedbala, 2020). New non-linear computational approaches have great potential to optimize the process with minimal treatments.

Machine learning algorithms have been successfully employed for predicting and optimizing various complex biological systems (Hesami et al., 2021; Niazian and Niedbala, 2020; Silva et al., 2019; Yoosefzadeh Najafabadi et al., 2021). Artificial neural networks (ANNs) can be considered as the most well-known models among different machine learning algorithms (Jafari and Shahsavari, 2020; Kusumo et al., 2017; Sebayang et al., 2017). The usefulness and reliability of ANNs in studying and predicting different steps of plant tissue culture such as *in vitro* sterilization, callogenesis, shoot proliferation, and *in vitro* production of secondary metabolites have been recently confirmed (Farhadi et al., 2020; Hesami and Jones, 2020; Salehi et al., 2020, 2021). Genetic algorithm (GA) is a well-known single objective optimization algorithm that has been previously been applied for optimizing different *in vitro* processes (Hesami and Jones, 2020; Salehi et al., 2020). However, as a single objective optimization algorithm, it can only find the optimal level of inputs for each individual target variable separately and cannot optimize the level of inputs for all target variables simultaneously (Bozorg-Haddad et al., 2016; Hesami et al., 2020b; Tanabe and Ishibuchi, 2020; Yoosefzadeh-Najafabadi et al., 2021). Therefore, a multi-objective optimization algorithm that can target different outputs simultaneously may be preferable (Hesami and Jones, 2020; Yilmaz, 2021). In multi-objective optimization algorithms, input variables are considered as multi-objective optimization problems, whereas the solutions recognize the best possible balance among various functions (target variables) (Tanabe and Ishibuchi, 2020; Yilmaz, 2021). Recently non-dominated sorting genetic algorithm-II (NSGA-II) was used for optimizing different *in vitro* culture systems such as *in vitro* sterilization (Hesami et al., 2019a), somatic embryogenesis (Hesami et al., 2020a), and shoot proliferation (Hesami et al., 2019b). Therefore, the hybridization of ANNs and multi-objective optimization algorithms can be considered as an accurate and reliable methodology for studying, predicting, and optimizing *in vitro* seed germination.

The measurement and assessment of morphological characteristics of *in vitro*-grown plantlets is a tedious and time-consuming process in *in vitro* studies, making protocol optimization a time consuming and expensive process (Niazian et al., 2019). New methods that incorporates machine vision approaches have the potential overcome those difficulties and streamline the process (Dutta Gupta and Pattanayak, 2017; Genze et al., 2020). Image processing is one of the most well-known machine vision methods and provides detailed information as well as greater reliability and accuracy than conventional counting and visual determination (Niazian et al., 2019). For instance, Genze et al. (2020)

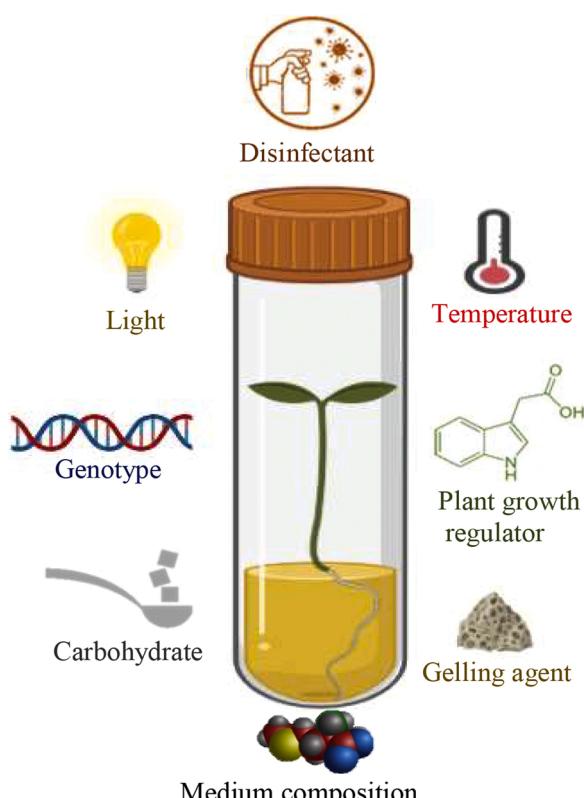


Fig. 1. The schematic view of factors affecting *in vitro* seed germination.

compared the conventional assessment method with hybrid image processing and ANN for studying *ex vitro* seed germination of three different crops including rye (*Secale cereale* L.), maize (*Zea mays* L.), and pearl millet (*Pennisetum glaucum* [L.] R. Br.). They reported that the machine learning-based method was robust and helped to speed up seed germination assessment. In another study, Colmer et al. (2020) suggested a hybrid image processing and ANN as a reliable method for phenotypic analysis of seed germination of *Brassica napus* L. varieties. Hence, image processing provides a robust tool for studying morphological features of *in vitro*-grown seedling.

The main objective of this study is to optimize the medium composition (type and concentration of media and carbohydrate sources) for *in vitro* seed germination of hemp. Therefore, the current study was performed to evaluate the effect of different types and concentrations of carbohydrate sources (sucrose and glucose) as well as different strengths of DKW (Driver and Kuniyuki, 1984) and mMS (Murashige and Skoog Medium, Van der Salm modification (Van der Salm et al., 1994)) on *in vitro* seed germination indices as well as morphological features of *in vitro*-grown seedlings through the image processing in hemp by using three well-known ANNs including multilayer perceptron (MLP), radial basis function (RBF), and generalized regression neural network (GRNN) in combination with NSGA-II. Also, performance of these models in modeling and forecasting *in vitro* hemp seed germination was compared and provides a foundation to optimize other *in vitro* methods.

2. Material and methods

2.1. Plant material, culture medium, and growth conditions

Industrial hemp seeds (*Cannabis sativa* cv. Finola) were used for this study (CSGA No.1 Certified seed, Lot #: 1908-18637-17-KKF-01). First, the seeds were washed for 15 min with running tap water. Afterward, the seeds were dipped for 60 s in 70 % ethanol and then washed with sterilized deionized water for 5 min under a laminar airflow cabinet. Once in the laminar flow hood and following the wash, seeds were sterilized using 12 % (v/v) commercial bleach for 12 min, followed by three, 5-minute deionized water rinses. Different (one-tenth, half, and full) strengths of DKW medium (D2470, PhytoTech Labs, Kansas, USA) supplemented with various levels of sucrose (2 % and 5 %) as well as different (one-tenth, half, and full) strengths of mMS medium (PhytoTech Labs, Kansas, USA) supplemented with various levels of glucose (2 % and 5 %) were used in this study. All media had 0.6 % agar (Thermo-Fisher Scientific, Waltham, MA) and the pH of the media was adjusted to 5.8 using 1 M HCl and NaOH before autoclaving for 20 min at 120 °C. For each treatment, 30 ml of media were poured into a Magenta GA7 vessel (Fisher Scientific, NJ, USA). The *in vitro* seed germination experiments were performed based on a completely randomized design (CRD) with a factorial arrangement with 14–16 replicates per treatment, and each replicate consisted of 4–5 seeds. All culture boxes were placed in the growth chamber at 25 ± 2 °C under 16-h photoperiod with 40 ± 5 μmol m⁻² s⁻¹ light intensity.

2.2. Germination indices

Hemp seeds were considered germinated seed upon emergence of the radicle and germination was recorded every 10 days for 40 days (4 sets of counting). Different seed germination indices were used for evaluating the *in vitro* germination of hemp seed as follow:

- 1) Germination index (GI) was computed based on the following equation.

$$\text{GI} = \left(\frac{\text{Number of germinated seeds}}{\text{days of first count}} \right) + \dots + \left(\frac{\text{Number of germinated seeds}}{\text{days of last count}} \right) \quad (1)$$

- 2) Mean germination time (MGT) was calculated based on the following equation.

$$\text{MGT} = \sum fd / f \quad (2)$$

where f is the number of germinated seeds on day d .

- 3) Germination rate (GR) was calculated using the following equation:

$$\text{GR} = \frac{G}{n} \times 100 \quad (3)$$

Where G is the number of germinated seeds by the end of the experiment and n equals the total number of cultivated seeds.

- 4) Timson germination index (TGI) is determined based on the following equation.

$$\text{TGI} = \sum \frac{G}{P} \quad (4)$$

where G is the percentage of germinated seeds per period, and P shows the germination period.

- 5) Vigor index (VI) was determined according to the following equation.

$$\text{VI} = G \times W \quad (5)$$

Where G is the number of germinated seeds at the end of the experiment, and W represents the weight of seedlings. The weights of the seedlings were weighed on day 40.

2.3. Morphological traits

At the end of the experiment (day 40) morphological traits including seedling fresh weight, leaf number, shoot length, and root length were assessed. To measure shoot length and root length, an image processing was used. The images of the *in vitro* grown seedlings were captured by iPhone Xs with the auto-focus feature from a vertical distance of 25 cm. The reliability of smartphones in capturing images to study plant morphological traits has been approved by different studies (Friedrichs et al., 2017; Mohan and Gupta, 2019; Vesali et al., 2017). The acquired images were processed with ImageJ 1.50i software (National Institute of Mental Health). The steps of the image processing using ImageJ software consisted of (i) image acquisition and set scale, (ii) red, green, and blue (RGB) color cropping filtering, (iii) creation of a grayscale image, (iv) development of a binary image, (v) remove noises from the binary image and filling holes, and (vi) measuring the image (shoot or root) length (Fig. 2a).

The obtained data were used for modeling, predicting, and optimizing seed germination indices and morphological traits of cannabis seedlings. The step-by-step procedure of this experiment has been summarized in Fig. 2. The Pearson correlation of morphological traits were estimated using *corplot* package (Wei et al., 2017) in R software version 3.6.1.

2.4. Modeling procedures

In the present study, MLP, GRNN, and RBF as three well-known ANNs were employed to model and predict *in vitro* cannabis seed germination. Before using the ANNs, Box-Cox transformation was employed to normalize the data. To detect outliers, principal component analysis (PCA) was used; however, no outlier was identified. In this study, the five-fold cross-validation approach with 10 repetitions were applied to evaluate the prediction accuracy of the tested ANNs.

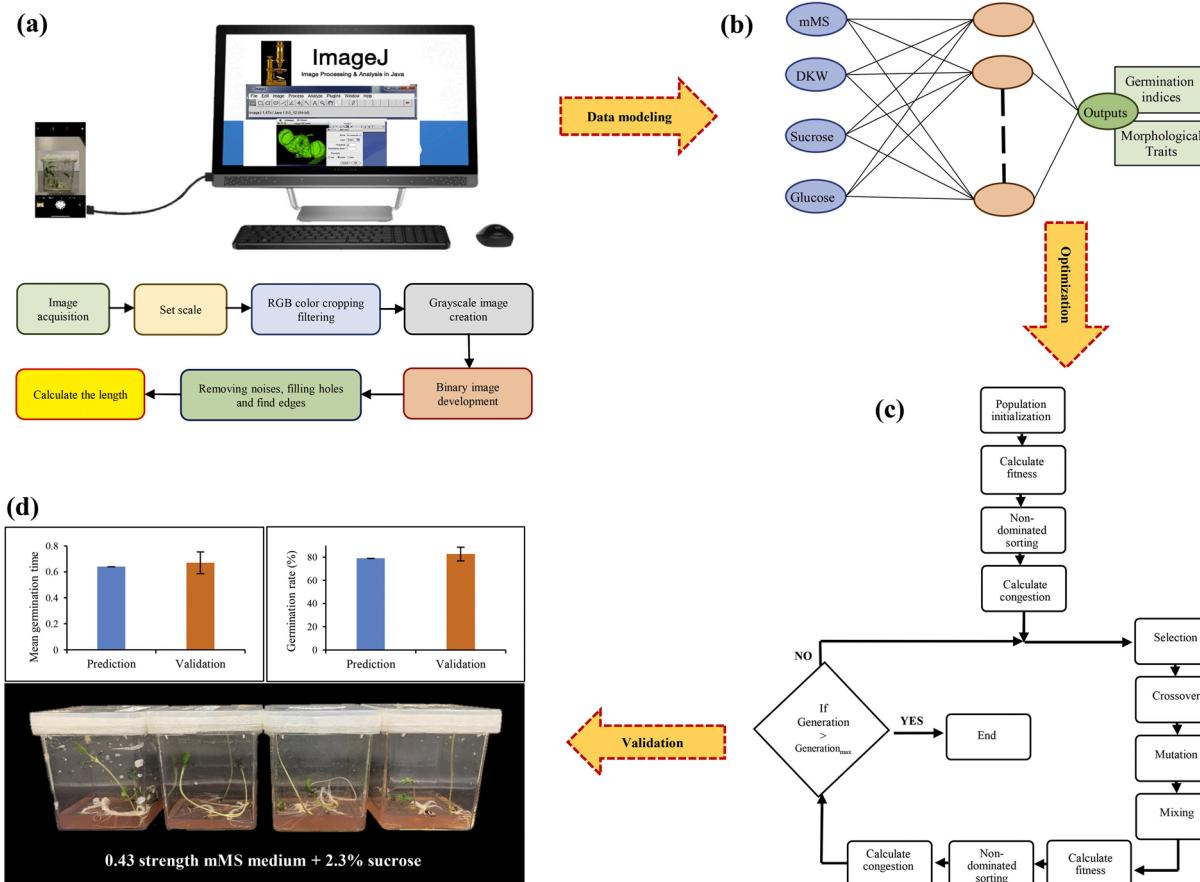


Fig. 2. The schematic view of the step-by-step procedure of this study including (a) image processing, (b) modeling seed germination indices and morphological traits based on four input variables including sucrose, glucose, Driver and Kuniyaki walnut (DKW) medium, and Murashige and Skoog medium Van der Salm modification (mMS) using artificial neural networks (ANNs), (c) optimization process via non-dominated sorting genetic algorithm-II (NSGA-II), and (d) experimental validation test.

Different strengths of DKW and mMS media and different levels of sucrose and glucose were selected as input variables, while seed germination indices (GI, MGT, GR, TGI, and VI) and morphological traits (seedling fresh weight, shoot length, leaf number, and root length) were considered as target (output) variables (Fig. 2b).

To evaluate and compare the efficiency and accuracy of the ANNs, r^2 (coefficient of determination), mean bias error (MBE), and root mean square error (RMSE) were employed.

2.4.1. Multilayer perceptron (MLP) model

The 3-layer backpropagation MLP is a distributed and parallel model that employs supervised learning for the training set that minimizes the error between the output and input variables through the following function:

$$\text{Error} = \frac{1}{n} \sum_{s=1}^n (y_s - \hat{y}_s)^2 \quad (6)$$

where n is the number of observations, y_s is the s th observed variable, and \hat{y}_s is the s th predicted variable.

To compute the \hat{y} in the MLP with p neurons in the hidden layer and k output variables, following equation is implemented:

$$\hat{y} = f \left[\sum_{j=1}^p w_j \cdot g \left(\sum_{i=1}^k w_{ji} x_i + w_{j0} \right) + w_o \right] \quad (7)$$

where x_i is the i th output variable, w_j shows the weighted input data into the j th hidden neuron, f shows activation function for the output neuron,

w_{ji} represents the weight of the direct relationship of input neuron i to the hidden neuron j , w_{j0} shows the bias for node j , w_0 equals the bias connected to the neuron of output, and g represents the activation function for the hidden neuron.

It is crucial to determine the number of neurons in each node and the number of hidden units because they play a pivotal role in the performance of MLP. In the current study, the optimal neuron number in the hidden layer was detected based on trial and error. Also, linear function (purelin) and hyperbolic tangent sigmoid function (tansig) were applied as the transfer functions of output layer and hidden layer, respectively. Moreover, bias and weights were adjusted by using a Levenberg-Marquardt algorithm.

2.4.2. Radial basis function (RBF) model

Radial basis function is a three-layer (an input, a hidden, and an output) ANN model. RBF as a basis of radial basis networks makes a network of artificial neurons. Euclidean distance between the neural center and the input in RBF provides activation function input for each neuron. If the input x as an n -dimensional vector connects to each hidden neuron, each output of the hidden neuron will only relate to the radial distance between the neural center and the x input vector. Then, the following function is used for computing the output of each neuron (h_j):

$$h_j = \phi \left(\| x - c_j \| \right) \quad j = 1, 2, 3, \dots, m \quad (8)$$

where $\| \cdot \|$ is Euclidean rule, ϕ shows activation function, c_j represents the j th hidden neuron center, and m equals the number of neurons.

The following equation represents the Gaussian function as the well-known activation function in RBF:

$$\phi_j(x) = \exp \left[-\frac{\|x - c_j\|^2}{2\sigma^2} \right] \quad (9)$$

Where $\phi_j(x)$ shows the reaction of the jth unit and σ equals the width of the Gaussian function. Afterward, the minimum mean square is used to obtain the connection between the weights of hidden and output layers. Finally, the kth output of the model is determined based on the following equation:

$$\hat{y}_k = \sum_{j=1}^m W_{jk} \phi_j(x) + w_0 \quad (10)$$

where \hat{y}_k shows the kth output variable, w_{jk} represents connecting weight between the kth output variable and the jth hidden neuron, and w_0 shows the bias.

2.4.3. Generalized regression neural network (GRNN) model

Generalized regression neural network as a standard regression method is another kind of radial basis network including the input layer, pattern layer, summation layer, and output layer. The input node completely enters the pattern node. The distance between stored pattern outputs is considered as the pattern layer output. The output of each pattern neuron is connected to S-summation and D-summation neurons in the summation layer. D-summation neuron computes the unweighted pattern neuron outputs. S-summation neuron sums the weighted pattern neuron outputs. Finally, the following equation is used for calculating the output:

$$\hat{y} = \frac{\sum_{i=1}^n y_i \exp \left(-\frac{D_i^2}{2\sigma^2} \right)}{\sum_{i=1}^n \exp \left(-\frac{D_i^2}{2\sigma^2} \right)} \quad (11)$$

$$D_i^2 = (x - x_i)^T (x - x_i) \quad (12)$$

where \hat{y} represents the average of all the weighted observed output data, σ equals width parameter, D_i^2 shows a scalar function which is based on any x_i and y_i observed data, and y_i represents the ith output variable.

2.5. Optimization process

The non-dominated sorting genetic algorithm (NSGA-II) introduced by Deb et al. (2002) is a multi-objective evolutionary optimization algorithm with a strong elitism strategy (Fig. 2c). The pseudocode of NSGA-II has been described in Fig. 7 (Matnei Filho and Vergilio, 2016).

NSGA-II was used to find the optimized levels of mMS and sucrose, mMS and glucose, DKW and sucrose, as well as DKW and glucose to maximize two most important germination indices including GR and MGT as fitness functions. The crossover rate, generation number, initial population, and mutation rate were respectively set to 0.7, 1000, 200, and 0.5 to obtain the best fitness. To choose the elite population for crossover, a roulette wheel selection approach was employed.

MATLAB (Matlab, 2010) software was used for writing ANNs and optimization algorithms codes.

2.6. Validation experiment

To confirm the efficiency and reliability of the developed model, the best predicted-optimized result was experimentally tested with 15 replicates, and each replicate containing five seeds.

3. Results

3.1. Effect of media and carbohydrate sources on in vitro seed germination

In the current study, the effect of different concentrations (2% and 5%) of two carbohydrate sources (sucrose and glucose) and different (one-tenth, half, and full) strengths of DKW and mMS media were investigated on germination indices and seedling morphological traits of cannabis. Based on our results (Table 1 and Fig. 3), various morphological responses were observed in different treatments. The highest root length (8.68 cm) and leaf number (6.67) were obtained from DKW + 2 % sucrose and 1/2 DKW + 5 % sucrose, respectively (Table 1). The maximum seedling fresh weight (0.37 g) and shoot length (13.99 cm) were observed on the half-strength mMS medium along with 5 % glucose (Table 1). Generally, seedling fresh weight and shoot length were decreased on both full-strength media (DKW and mMS) and this could be a result of the increased osmotic stress arising from higher basal salt concentrations in those media. Based on the correlation coefficient results (Fig. 4), all morphological traits were in a significant correlation with each other. The highest correlation was found between weight and shoot length and the lowest observed between Root length and Leaf number with the values of 0.62 and 0.25, respectively.

According to the results of the current study (Table 1), the maximum values for all germination indices including GI (1.27), MGT (0.67), GR (73.50 %), TGI (11.57), and VI (2.19) were observed in half-strength mMS supplemented with 2 % glucose. Generally, increasing the strength of both media (from half-strength to full-strength) and the level of carbohydrate sources resulted in decreasing the seed germination indices (Table 1).

3.2. Artificial neural networks evaluation

Data modeling by ANNs displays a reliable solution to provide comprehensive knowledge on *in vitro* seed germination of cannabis after training and testing from the experimental dataset. In the current study, three ANNs (RBF, MLP, and GRNN) were employed to predict seed germination indices and seedling morphological traits based on the various strengths of DKW and mMS media supplemented with different levels of sucrose and glucose. The GRNN model displayed higher predictive accuracy ($r^2 > 0.70$) in comparison to RBF or MLP in both training and testing sets for all germination indices and morphological traits including GI ($r^2 > 0.72$ for GRNN vs. $r^2 > 0.61$ for MLP or $r^2 > 0.67$ for RBF), MGT ($r^2 > 0.72$ for GRNN vs. $r^2 > 0.67$ for MLP or $r^2 > 0.62$ for RBF), GR ($r^2 > 0.71$ for GRNN vs. $r^2 > 0.64$ for MLP or $r^2 > 0.64$ for RBF), TGI ($r^2 > 0.73$ for GRNN vs. $r^2 > 0.69$ for MLP or $r^2 > 0.70$ for RBF), VI ($r^2 > 0.70$ for GRNN vs. $r^2 > 0.61$ for MLP or $r^2 > 0.64$ for RBF), seedling fresh weight ($r^2 > 0.71$ for GRNN vs. $r^2 > 0.64$ for MLP or $r^2 > 0.64$ for RBF), root length ($r^2 > 0.72$ for GRNN vs. $r^2 > 0.63$ for MLP or $r^2 > 0.66$ for RBF), shoot length ($r^2 > 0.73$ for GRNN vs. $r^2 > 0.64$ for MLP or $r^2 > 0.66$ for RBF), and leaf number ($r^2 > 0.73$ for GRNN vs. $r^2 > 0.63$ for MLP or $r^2 > 0.64$ for RBF) (Table 2). Also, the regression lines revealed a good fit correlation between experimental and predicted values for all the germination indices and morphological traits in both training (Fig. 5) and testing (Fig. 6) sets. In addition, model accuracy was evaluated by RMSE and MBE, which proved the better performance of GRNN in comparison with RBF and MLP (Table 2).

3.3. Optimization via non-dominated sorting genetic algorithm-II and validation experiment

NSGA-II as an evolutionary multi-objective optimization algorithm was subjected to the GRNN (the most accurate model in this study) in order to find the optimal type and level of media and carbohydrate sources for obtaining the best seed germination indices (GR and MGT). According to the optimization process (Table 3), 0.43 strength mMS

Table 1

The effect of different strengths of mMS and DKW media as well as various levels of sucrose and glucose on different seed germination indices and morphological traits in cannabis.

Strength media	Carbohydrate sources (%)	GI	MGT	GR (%)	TGI	VI	Seedling fresh weight (g)	Root length (cm)	Shoot length (cm)	Leaf number
DKW × Sucrose										
0.1	2	0.25 ± 0.165	0.14 ± 0.085	59.52 ± 32.499	8.27 ± 5.487	0.53 ± 0.601	0.31 ± 0.229	8.37 ± 5.096	9.37 ± 6.653	5.50 ± 3.568
0.1	5	0.25 ± 0.141	0.13 ± 0.070	54.17 ± 23.960	8.19 ± 4.698	0.37 ± 0.178	0.23 ± 0.091	7.24 ± 3.789	10.29 ± 3.670	4.38 ± 1.455
0.5	2	0.49 ± 0.194	0.26 ± 0.092	63.75 ± 19.621	9.89 ± 3.871	1.09 ± 0.733	0.34 ± 0.171	7.35 ± 5.003	12.89 ± 6.125	5.69 ± 2.414
0.5	5	0.31 ± 0.235	0.18 ± 0.127	47.41 ± 29.094	6.71 ± 4.517	0.54 ± 0.629	0.25 ± 0.163	5.78 ± 4.847	9.45 ± 5.963	6.67 ± 3.144
1	2	0.24 ± 0.103	0.13 ± 0.053	51.11 ± 21.331	7.87 ± 3.450	0.38 ± 0.217	0.25 ± 0.138	8.68 ± 6.156	8.95 ± 3.811	4.73 ± 3.058
1	5	0.17 ± 0.131	0.10 ± 0.064	45.83 ± 26.874	5.76 ± 4.357	0.17 ± 0.134	0.16 ± 0.120	5.56 ± 4.795	6.86 ± 4.487	3.75 ± 1.438
mMS × Glucose										
0.1	2	0.88 ± 0.315	0.48 ± 0.165	66.67 ± 22.722	9.81 ± 3.496	2.00 ± 1.413	0.32 ± 0.170	6.72 ± 2.761	13.47 ± 4.090	5.17 ± 2.517
0.1	5	0.84 ± 0.240	0.45 ± 0.125	63.89 ± 17.806	9.28 ± 2.667	1.78 ± 0.717	0.31 ± 0.096	6.54 ± 2.135	13.87 ± 3.568	5.17 ± 1.946
0.5	2	1.27 ± 0.581	0.67 ± 0.290	73.50 ± 11.958	11.57 ± 3.105	2.19 ± 1.388	0.26 ± 0.108	5.87 ± 2.174	13.45 ± 4.036	6.17 ± 2.480
0.5	5	0.54 ± 0.232	0.30 ± 0.112	67.96 ± 24.971	10.36 ± 4.688	1.37 ± 0.850	0.37 ± 0.203	8.20 ± 3.679	13.99 ± 3.065	4.92 ± 1.975
1	2	1.17 ± 0.533	0.65 ± 0.249	62.90 ± 13.218	8.99 ± 3.563	1.72 ± 0.712	0.21 ± 0.082	6.96 ± 2.926	11.33 ± 3.601	5.86 ± 2.035
1	5	0.80 ± 0.334	0.44 ± 0.179	59.26 ± 23.371	8.94 ± 3.712	1.27 ± 0.862	0.26 ± 0.137	5.63 ± 2.401	10.66 ± 3.960	4.00 ± 1.651

Values in each column represent means ± SD.

DKW: Driver and Kuniyaki walnut medium; GI: Germination index; MGT: Mean germination time; GR: Germination rate; mMS: Murashige and Skoog medium Van der Salm modification; TGI: Timson germination index; VI: Vigor index.

medium supplemented with 2.3 % sucrose would result in the best results with 78.93 % GR and 0.64 MGT. The results of the validation experiment revealed that there was a negligible difference between the experimental data and the optimized result (Fig. 2d).

4. Discussion

Successful *in vitro* cannabis seed germination is not only important for obtaining juvenile tissue as a potential explant for different *in vitro* procedures (e. g. gene transformation, callus culture, secondary metabolite and biofuel production), but also crucial for screening stress-tolerant genotypes (Deguchi et al., 2020; Hu et al., 2018; Sorokin et al., 2021). Therefore, it is necessary to study and optimize factors affecting *in vitro* seed germination of cannabis such as medium compositions including macronutrients, micronutrients, vitamins, plant growth regulators, amino acids, and carbohydrate sources (Boonsnongcheep and Pongkitwittoon, 2020; Hesami and Jones, 2020). During *in vitro* seed germination, seeds are cultured on artificial media containing essential nutrients, amino acids, vitamins, and carbohydrate required for the successful germination and growth of the seedlings (Hesami et al., 2018; Utami and Hariyanto, 2019). Consequentially, the success of *in vitro* seed germination is significantly affected by culture media composition (Kim et al., 2019). The carbohydrate type and concentration during *in vitro* seed germination not only influences growth and development, but also seed germination indices such as germination percentage (Bozdemir et al., 2018; Roni et al., 2018). Several studies have previously demonstrated that different species and plant tissues need a specific carbohydrate source in different stages of plant tissue culture (Hesami et al., 2019c; Roni et al., 2018; Yaseen et al., 2013). Moreover, the effect of type and strength of the basal medium as a source of nutrients and vitamins on *in vitro* seed germination leads to good outcomes for seedling growth and development without any physiological disorders (Hesami et al., 2018; Kim et al., 2019; Roni et al., 2018). Therefore, the type and concentration of different elements in the media (e. g.

macronutrients, micronutrients, vitamins, carbohydrate) should be selected and optimized based on species and *in vitro* culture procedure (Hesami and Jones, 2020; Lin et al., 2017). The current study demonstrated the impact of type and concentration of carbohydrate sources (sucrose and glucose) on *in vitro* cannabis seed germination using different strengths of mMS and DKW media.

However, optimizing *in vitro* seed germination as a nonlinear, multivariable, and complex system is a highly tedious, expensive, and time-consuming process. (Hesami and Jones, 2020; Lin et al., 2017) Thus, there is a serious need for the application of new computational approaches such as machine learning algorithms to analyze and optimize this type of system more efficiently using fewer treatments (Nianzian and Niedbala, 2020; Silva et al., 2019). Recently, the reliability and accuracy of different types of ANNs such as RBF, MLP, and GRNN have been studied in different stages of plant tissue culture (Hesami and Jones, 2020). For instance, Zhang et al. (2020) used MLP for modeling and predicting organogenic callus production based on four input variables (agar concentration, humidity, light time, and culture temperature), and reported that MLP was able to accurately model and predict the system ($r^2 > 0.96$). In another study, MLP was employed to model and predict *in vitro* root formation in grapevine based on the concentration of indole-3-butryic acid (IBA) and Exposure time to IBA (Gago et al., 2010). They reported that r^2 of developed model for modeling roots number was 78.53. In another study, Hesami et al. (2019b) employed RBF for modeling and predicting *in vitro* shoot proliferation in chrysanthemum based on four input variables including 6-benzylaminopurine (BAP), IBA, phloroglucinol, and sucrose. They showed that RBF was able to accurately model and predict shoot proliferation ($r^2 > 0.98$). Recently, Salehi et al. (2021) used GRNN for modeling and predicting *in vitro* paclitaxel biosynthesis in hazel based on four inputs including culture filtrate levels, cell extract time, elicitor adding day, and cell harvesting time, and reported that r^2 of developed GRNN for modeling paclitaxel biosynthesis was over 0.88. However, uncertainties regarding machine learning outcomes represents one of the main problems with



Fig. 3. *In vitro* grown seedling of cannabis in different treatments including (a) one-tenth strength of Driver and Kuniyaki walnut (DKW) medium along with 5 % sucrose, (b) full strength of DKW medium along with 5 % sucrose, (c) one-tenth strength of Murashige and Skoog medium Van der Salm modification (mMS) along with 2 % glucose, (d) half strength of DKW medium along with 2 % sucrose, (e) full strength of mMS medium along with 5 % glucose, (f) one-tenth strength of mMS medium along with 5 % glucose, (g) one-tenth strength of DKW medium along with 2 % sucrose, (h) full strength of DKW medium along with 2 % sucrose, (i) full strength of mMS medium along with 2 % glucose, (j) half strength of DKW medium along with 5 % sucrose, (k) half strength of mMS medium along with 2 % glucose, and (l) half strength of mMS medium along with 2 % glucose.

machine learning algorithms (Yang et al., 2020). There are three main sources of uncertainty in machine learning studies including data quality, the sample of data collected from the domain, and model fit (Saltzman and Yung, 2018). To avoid uncertainties, several studies have suggested using different machine learning algorithms and validating the predicted results in the lab (Englert et al., 2019; Saltzman and Yung, 2018; Yang et al., 2020). Therefore, in the current study, different ANNs were used and the results of the most accurate model were tested in a validation experiment.

According to our results, GRNN had better performance than RBF and MLP for modeling and predicting germination indices and morphological traits of cannabis. Although there are no studies in the plant tissue culture area to compare these models, several studies in other fields of agriculture reported that GRNN had better accuracy than other ANNs such as MLP and RBF. In line with our results, Jafari and Shahsavari (2020) compared the efficiency of different ANNs for modeling and forecasting morphological responses of lime under water deficiency and reported that GRNN had the highest degree of accuracy in comparison with MLP and RBF. Also, Hosseini-Moghari and Araghinejad (2015) demonstrated that GRNN had better performance than MLP and RBF in predicting drought indices and drought classes.

Moreover, we linked the GRNN models to NSGA-II in order to optimize morphological traits and germination indices. The efficiency of

genetic algorithms, both GA and NSGA-II, have been previously confirmed in different plant tissue culture processes (Hesami and Jones, 2020). In the current study, GR and MGT as the two most important germination indices were selected as target variables for optimizing medium composition through the hybrid GRNN-NSGA-II. The results of the optimization process accurately predicted that 0.43 strength mMS medium along with 2.3 % sucrose would result in the maximum seed germination outcomes. This result showed that the moderate level of sucrose (2.3 %) in 0.43 strength mMS medium improved the seed germination percentage compared with the higher levels of sucrose in more or lower strength mMS medium. Similar results have been found in other species, where moderate levels of sucrose and basal salts were found to be ideal (Huh et al., 2016; Johnson et al., 2011; Roni et al., 2018). For instance, Roni et al. (2018) reported that a moderate level of sucrose (3 %) and half-strength MS medium resulted in the maximum seed germination percentage in *Eustoma grandiflorum*. In line with our result, Hu et al. (2018) previously showed that *ex vitro* seed germination of hemp is inhibited by high concentrations of salts. Generally, it seems that seed germination in hemp in both *ex vitro* and *in vitro* conditions is sensitive to a high level of salts, yet moderate levels are useful to provide the necessary nutrients. Therefore, a moderate level of salts existing in culture media (0.43 strength of the medium) can improve *in vitro* seed germination of hemp.

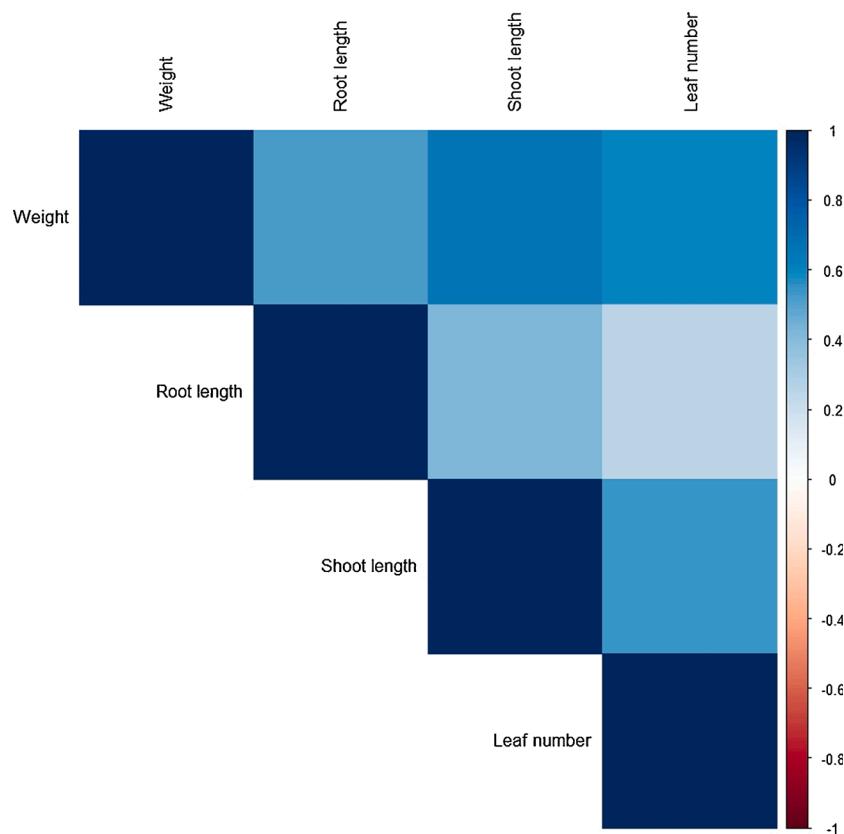


Fig. 4. Heat map obtained for studied morphological traits of *in vitro* grown seedling of cannabis.

Table 2

Performance criteria of artificial neural networks (ANNs) for different seed germination indices and morphological traits in cannabis.

Model	Processing set	Performance criteria	GI	MGT	GR	TGI	VI	Seedling fresh weight	Root length	Shoot length	Leaf number
MLP	Training	r^2	0.59	0.67	0.67	0.69	0.67	0.66	0.67	0.67	0.68
		RMSE	0.306	0.129	11.714	4.103	0.803	0.169	1.103	1.401	1.401
		MBE	-0.076	-0.014	2.104	0.706	-0.031	0.072	-0.602	0.302	0.568
	Testing	r^2	0.61	0.68	0.64	0.69	0.61	0.64	0.63	0.64	0.63
		RMSE	0.201	0.145	11.123	3.883	0.798	0.161	1.321	0.824	1.735
		MBE	-0.044	-0.006	2.197	-0.176	0.057	-0.043	0.112	0.651	0.203
RBF	Training	r^2	0.74	0.69	0.68	0.70	0.69	0.66	0.69	0.69	0.68
		RMSE	0.306	0.123	9.806	3.933	0.730	0.157	1.200	1.321	1.331
		MBE	0.083	0.004	1.133	0.706	-0.041	0.036	-0.425	0.224	0.399
	Testing	r^2	0.67	0.62	0.64	0.72	0.64	0.64	0.64	0.66	0.64
		RMSE	0.214	0.145	11.008	3.883	0.798	0.158	1.321	0.714	1.668
		MBE	0.048	0.001	2.197	-0.151	0.044	0.051	0.106	0.643	-0.182
GRNN	Training	r^2	0.79	0.76	0.74	0.74	0.71	0.71	0.74	0.73	0.74
		RMSE	0.276	0.123	9.403	3.818	0.701	0.138	1.039	0.956	1.148
		MBE	-0.076	0.001	0.849	-0.265	-0.001	-0.003	-0.004	0.053	-0.011
	Testing	r^2	0.72	0.72	0.71	0.73	0.70	0.71	0.72	0.73	0.73
		RMSE	0.201	0.135	10.210	3.701	0.683	0.157	1.862	0.632	1.575
		MBE	-0.044	0.001	-0.074	-0.139	0.029	0.008	0.021	0.248	-0.102

MLP: Multilayer perceptron; RBF: Radial basis function; GRNN: Generalized regression neural network; r^2 : Coefficient of determination; RMSE: Root mean square error; MBE: Mean bias error; GI: Germination index; MGT: Mean germination time; GR: Germination rate; TGI: Timson germination index; VI: Vigor index.

The results of the validation experiment showed that there was no considerable difference between predicted optimized results and experimental results for seed germination in *C. sativa* cv. 'Finola', demonstrating the accuracy of this algorithm. In contrast with *in vitro* seed germination in cannabis, *in vitro* propagation and regeneration methods have been well studied and the challenges surrounding recalcitrance of the species and replicability of many methods has been reviewed extensively (Monthony et al., 2021). Recently, Page et al. (2021) assessed the effect of various concentrations of different plant growth regulators as well as basal media types for shoot growth and explant multiplication in cannabis. Their study highlighted the necessity

of media optimization for maximizing shoot proliferation and minimizing physiological defects. Therefore, the developed models in the current study can be employed for predicting and optimizing other *in vitro* culture procedures in cannabis.

5. Conclusion

In vitro seed germination is a complex biological process affecting by various factors such as type and concentration of macronutrients, micronutrients, vitamins, gelling agent, carbohydrate, temperature, and light. Data modeling by ANNs can display a reliable solution to provide

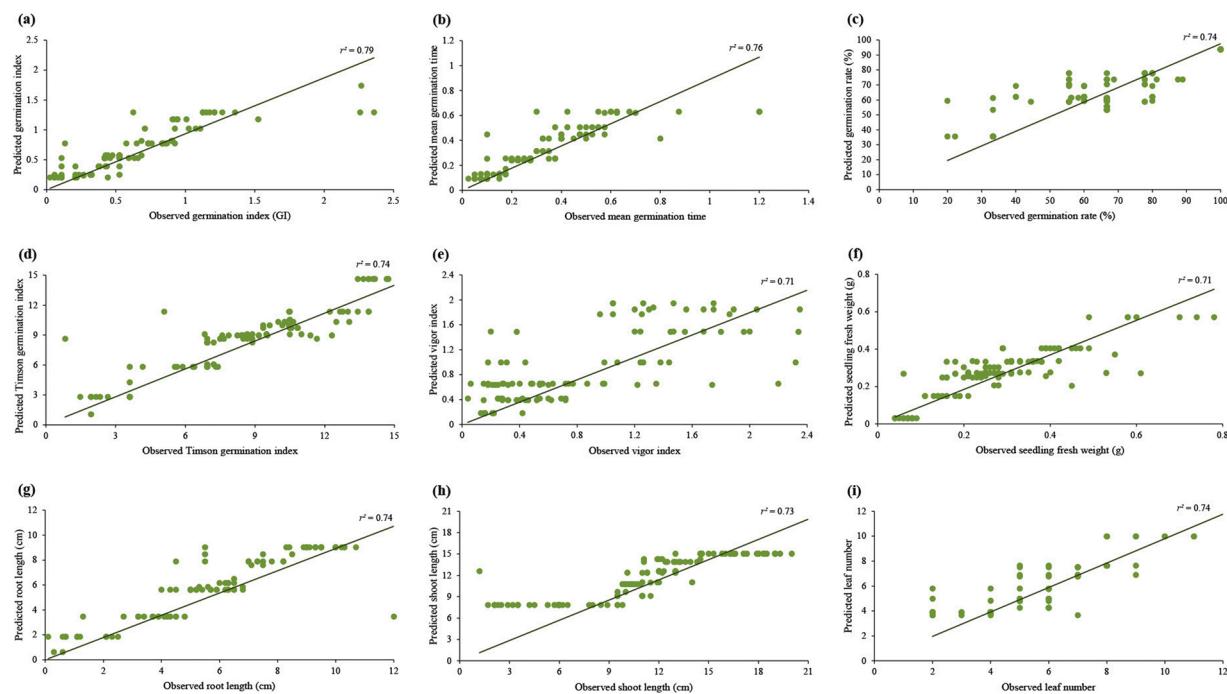


Fig. 5. Scatter plot of observed data against predicted values of (a) germination index, (b) mean germination time, (c) germination rate, (e) Timson germination index, (f) seedling fresh weight, (g) root length, (h) shoot length, and (i) leaf number in *in vitro* seed germination of cannabis using generalized regression neural network (GRNN) in training process.

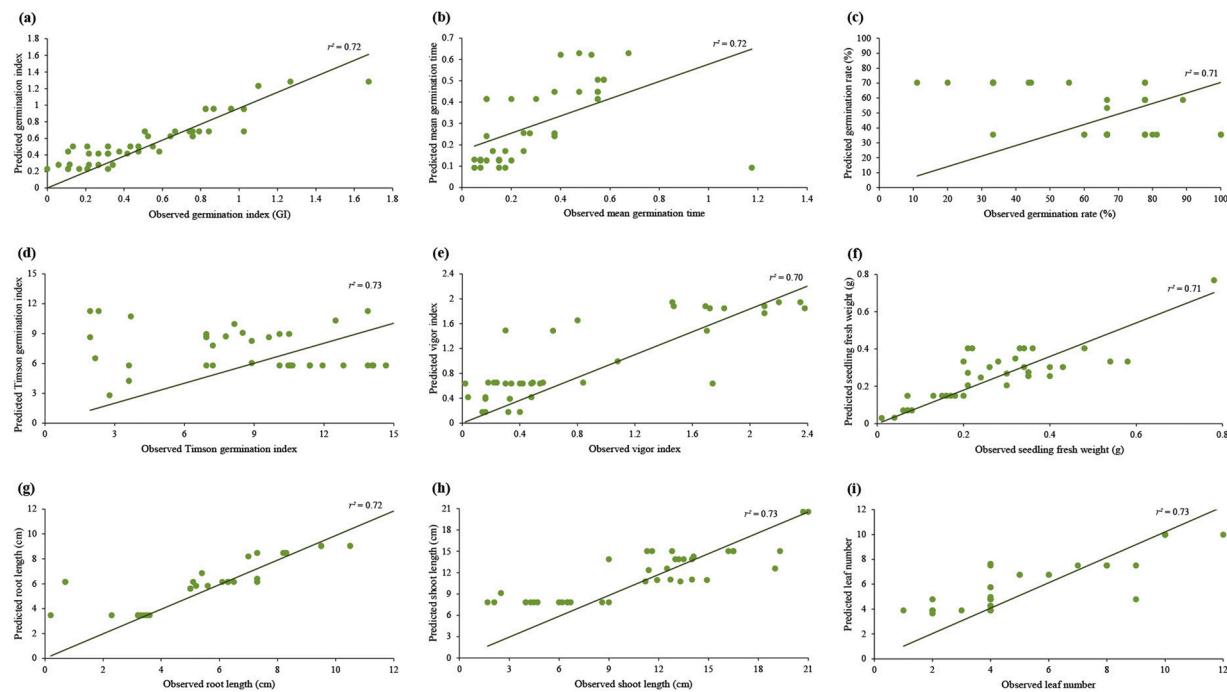


Fig. 6. Scatter plot of observed data against predicted values of (a) germination index, (b) mean germination time, (c) germination rate, (e) Timson germination index, (f) seedling fresh weight, (g) root length, (h) shoot length, and (i) leaf number in *in vitro* seed germination of cannabis using generalized regression neural network (GRNN) in testing process.

comprehensive knowledge on *in vitro* seed germination. According to the results of the current study, GRNN has better accuracy than other studied ANNs. Also, the hybrid GRNN and NSGA-II as a multi-objective optimization algorithm could precisely predict and optimize *in vitro* seed germination of industrial hemp cultivar 'Finola' and future studies should assess the suitability of this model and the optimized media in

germination of other commercial drug-type and fiber-type *C. sativa*. The results of the current study show that the high seed germination rate of hemp can be achieved from the moderate level (0.43 strength) of mMS medium supplemented with a moderate level of sucrose (2.3%). We suggest that the potential of this treatment investigate for seed germination of other cannabis genotypes, which is even more important due to

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Input:  $N'$ ,  $g$ ,  $f_k(X)$ ,  $N'$  members evolved  $g$  generations to solve  $f_k(X)$ 

Initialize Population  $P'$ ;

Generate random population – size  $N'$ ;

Evaluate objective values;

Assign rank (level) based on Pareto – sort;

Generate child population;

Roulette wheel selection;

Recombination and mutation;

for  $i=1$  to  $g$  do

    for each parent and child in population do

        Assign rank (level) based on Pareto – sort;

        Determine crowding distance;

        Loop (inside) by adding solutions to next generation starting from the first front until  $N'$  individuals;

    end

    Select points on the lower front with high crowding distance;

    Create next generation;

    Roulette wheel selection;

    Recombination and mutation;

end

```

Fig. 7. The pseudocode of non-dominated sorting genetic algorithm (NSGA-II).

Table 3

The results of optimization process via non-dominated sorting genetic algorithm-II (NSGA-II).

Optimal solution	Prediction	
	GR (%)	MGT
0.48 strength DKW Medium + 2.7 % Sucrose	61.35	0.59
0.52 strength DKW Medium + 3.8 % Glucose	71.39	0.64
0.43 strength mMS Medium + 2.3 % Sucrose	78.93	0.64
0.47 strength mMS Medium + 3.2 % Glucose	74.07	0.62

DKW: Driver and Kuniyaki walnut medium; GR: Germination rate; MGT: Mean germination time; mMS: Murashige and Skoog medium Van der Salm modification.

the high cost of drug type cannabis. Furthermore, future studies can use this treatment as a basal medium for studying other factors affecting *in vitro* seed germination. While the current model was developed for predicting and optimizing *in vitro* seed germination media, we also suggest that its adaptation in other *in vitro* culture procedures could present a forward-thinking aid to predicting and optimizing media compositions for the micropropagation and regeneration of mature stage 2 *in vitro* explants and consequently overcome many of the challenges faced currently in cannabis micropropagation. Therefore, GRNN-NSGA-II can be employed in future tissue culture studies to optimize different factors involved in *in vitro* culture of cannabis and other species.

Author contributions

All authors have equal contribution in this study; and read and approved the manuscript.

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Data availability

All processed data are available without restriction upon inquiry.

Compliance with ethical standards

This work does not involve any human participation nor live animals performed by any of the listed authors.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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References

- Boonsnongcheep, P., Pongkitwitoon, B., 2020. Factors affecting micropropagation of *Cannabis sativa* L.: a review. Pharm. Sci. Asia 47, 21–29. <https://doi.org/10.29090/psa.2020.01.019.0030>.
- Bozdemir, H., Cig, A., Türkoglu, N., 2018. Effects of different concentrations of carbohydrate forms on *Orchis sancta* L. propagation in vitro. Appl. Ecol. Environ. Res. 16, 4849–4864. https://doi.org/10.1566/aer/1604_48494864.
- Bozorg-Haddad, O., Azarnivand, A., Hosseini-Moghari, S.-M., Loaiciga Hugo, A., 2016. Development of a comparative multiple criteria framework for ranking pareto optimal solutions of a multiobjective reservoir operation problem. J. Irrig. Drain Eng. 142, 04016019 [https://doi.org/10.1061/\(ASCE\)IR.1943-4774.0001028](https://doi.org/10.1061/(ASCE)IR.1943-4774.0001028).
- Chadipiralla, K., Gayathri, P., Rajani, V., Reddy, P.V.B., 2020. Plant tissue culture and crop improvement. In: Roychowdhury, R., Choudhury, S., Hasanuzzaman, M., Srivastava, S. (Eds.), Sustainable Agriculture in the Era of Climate Change. Springer, Cham, pp. 391–412.
- Chandran, H., Meena, M., Barupal, T., Sharma, K., 2020. Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. Biotechnol. Rep. 26, e00450 <https://doi.org/10.1016/j.btre.2020.e00450>.
- Colmer, J., O'Neill, C.M., Wells, R., Bostrom, A., Reynolds, D., Websdale, D., Shiraliagi, G., Lu, W., Lou, Q., Le Cornu, T., Ball, J., Renema, J., Flores Andaluz, G., Benjamins, R., Penfield, S., Zhou, J., 2020. SeedGerm: a cost-effective phenotyping platform for automated seed imaging and machine-learning based phenotypic analysis of crop seed germination. New Phytol. 228, 778–793. <https://doi.org/10.1111/nph.16736>.

- Deb, K., Pratap, A., Agarwal, S., Meyarivan, T., 2002. A fast and elitist multiobjective genetic algorithm: NSGA-II. *EEE Trans. Evol. Comput.* 6, 182–197. <https://doi.org/10.1109/4235.996017>.
- Deguchi, M., Bogush, D., Weeden, H., Spuhler, Z., Potlakayala, S., Kondo, T., Zhang, Z.J., Rudrabhatla, S., 2020. Establishment and optimization of a hemp (*Cannabis sativa* L.) agroinfiltration system for gene expression and silencing studies. *Sci. Rep.* 10, 3504 <https://doi.org/10.1038/s41598-020-60323-9>.
- Driver, J.A., Kuniyuki, A.H., 1984. In vitro propagation of Paradox walnut rootstock. *HortScience* 19, 507–509.
- Dutta Gupta, S., Pattanayak, A.K., 2017. Intelligent image analysis (IIA) using artificial neural network (ANN) for non-invasive estimation of chlorophyll content in micropaginated plants of potato. *In Vitro Cell. Dev. Biol. Plant* 53, 520–526. <https://doi.org/10.1007/s11627-017-9825-6>.
- El-Sherif, N.A., 2018. Impact of plant tissue culture on agricultural sustainability. *Sustainability of Agricultural Environment in Egypt: Part II*. Springer, pp. 93–107.
- Englert, C., Galler, P., Harris, P., Spannousky, M., 2019. Machine learning uncertainties with adversarial neural networks. *Eur. Phys. J. C* 79, 4. <https://doi.org/10.1140/epjc/s10052-018-6511-8>.
- Farhadi, S., Salehi, M., Moieni, A., Safaei, N., Sabet, M.S., 2020. Modeling of paclitaxel biosynthesis elicitation in *Corylus avellana* cell culture using adaptive neuro-fuzzy inference system-genetic algorithm (ANFIS-GA) and multiple regression methods. *PLoS One* 15, e0237478. <https://doi.org/10.1371/journal.pone.0237478>.
- Friedrichs, A., Busch, J.A., Van der Woerd, H.J., Zieliński, O., 2017. SmartFluo: A method and affordable adapter to measure chlorophyll fluorescence with smartphones. *Sensors* 17, 678. <https://doi.org/10.3390/s17040678>.
- Gago, J., Landín, M., Gallego, P.P., 2010. Artificial neural networks modeling the in vitro rhizogenesis and acclimatization of *Vitis vinifera* L. *J. Plant Physiol.* 167, 1226–1231. <https://doi.org/10.1016/j.jplph.2010.04.008>.
- Genz, N., Bharti, R., Grieb, M., Schultheiss, S.J., Grimm, D.G., 2020. Accurate machine learning-based germination detection, prediction and quality assessment of three grain crops. *Plant Methods* 16, 157. <https://doi.org/10.1186/s13007-020-00699-x>.
- Hesami, M., Jones, A.M.P., 2020. Application of artificial intelligence models and optimization algorithms in plant cell and tissue culture. *Appl. Microbiol. Biotechnol.* 104, 9449–9485. <https://doi.org/10.1007/s0253-020-10888-2>.
- Hesami, M., Daneshvar, M.H., Yoosefzadeh-Najafabadi, M., 2018. Establishment of a protocol for in vitro seed germination and callus formation of *Ficus religiosa* L., an important medicinal plant. *Jundishapur J. Nat. Pharm. Prod.* 13, e62682 <https://doi.org/10.5812/jjnp.62682>.
- Hesami, M., Naderi, R., Tohidfar, M., 2019a. Modeling and optimizing in vitro sterilization of Chrysanthemum via multilayer perceptron-non-dominated sorting genetic Algorithm-II (MLP-NSGAI). *Front. Plant Sci.* 10, 282. <https://doi.org/10.3389/fpls.2019.00282>.
- Hesami, M., Naderi, R., Tohidfar, M., 2019b. Modeling and optimizing medium composition for shoot regeneration of Chrysanthemum via radial basis function-non-dominated sorting genetic Algorithm-II (RBF-NSGAI). *Sci. Rep.* 9, 18237 <https://doi.org/10.1038/s41589-019-54257-0>.
- Hesami, M., Naderi, R., Tohidfar, M., Yoosefzadeh-Najafabadi, M., 2019c. Application of adaptive neuro-fuzzy inference system-non-dominated sorting genetic Algorithm-II (ANFIS-NSGAI) for modeling and optimizing somatic embryogenesis of Chrysanthemum. *Front. Plant Sci.* 10 <https://doi.org/10.3389/fpls.2019.00869>.
- Hesami, M., Naderi, R., Tohidfar, M., 2020a. Introducing a hybrid artificial intelligence method for high-throughput modeling and optimizing plant tissue culture processes: the establishment of a new embryogenesis medium for chrysanthemum, as a case study. *Appl. Microbiol. Biotechnol.* 104, 10249–10263. <https://doi.org/10.1007/s0253-020-10978-1>.
- Hesami, M., Naderi, R., Tohidfar, M., Yoosefzadeh-Najafabadi, M., 2020b. Development of support vector machine-based model and comparative analysis with artificial neural network for modeling the plant tissue culture procedures: effect of plant growth regulators on somatic embryogenesis of chrysanthemum, as a case study. *Plant Methods* 16, 112. <https://doi.org/10.1186/s13007-020-00655-9>.
- Hesami, M., Pepe, M., Alizadeh, M., Rakei, A., Baiton, A., Jones, A.M.P., 2020c. Recent advances in cannabis biotechnology. *Ind. Crops Prod.* 158, 113026 <https://doi.org/10.1016/j.indcrop.2020.113026>.
- Hesami, M., Yoosefzadeh Najafabadi, M., Adamek, K., Torkamaneh, D., Jones, A.M.P., 2021. Synergizing off-target predictions for in silico insights of CENH3 knockout in cannabis through CRISPR/Cas. *Molecules* 26, 2053. <https://doi.org/10.3390/molecules26072053>.
- Hosseini-Moghari, S.M., Araghinejad, S., 2015. Monthly and seasonal drought forecasting using statistical neural networks. *Environ. Earth Sci.* 74, 397–412. <https://doi.org/10.1007/s12665-015-4047-x>.
- Hu, H., Liu, H., Liu, F., 2018. Seed germination of hemp (*Cannabis sativa* L.) cultivars responds differently to the stress of salt type and concentration. *Ind. Crops Prod.* 123, 254–261. <https://doi.org/10.1016/j.indcrop.2018.06.089>.
- Huh, Y.S., Lee, J.K., Nam, S.Y., Hong, E.Y., Paek, K.Y., Son, S.W., 2016. Effects of altering medium strength and sucrose concentration on in vitro germination and seedling growth of *Cypripedium macranthos* Sw. *J. Plant Biotechnol.* 43, 132–137. <https://doi.org/10.5010/JPB.2016.43.1.132>.
- Hurgobin, B., Tamiru-Oli, M., Welling, M.T., Doblin, M.S., Bacic, A., Whelan, J., Lewsey, M.G., 2020. Recent advances in *Cannabis sativa* genomics research. *New Phytol.* <https://doi.org/10.1111/nph.17140>.
- Ioannidis, K., Dadiotis, E., Mitsis, V., Melliou, E., Magiatis, P., 2020. Biotechnological approaches on two high CBD and CBG *Cannabis sativa* L. (Cannabaceae) varieties: in vitro regeneration and phytochemical consistency evaluation of micropaginated plants using quantitative 1H-NMR. *Molecules* 25, 5928. <https://doi.org/10.3390/molecules25245928>.
- Jafari, M., Shahsavari, A., 2020. The application of artificial neural networks in modeling and predicting the effects of melatonin on morphological responses of citrus to drought stress. *PLoS One* 15, e0240427. <https://doi.org/10.1371/journal.pone.0240427>.
- Johnson, T.R., Kane, M.E., Pérez, H.E., 2011. Examining the interaction of light, nutrients and carbohydrates on seed germination and early seedling development of *Bletia purpurea* (Orchidaceae). *Plant Growth Regul.* 63, 89–99. <https://doi.org/10.1007/s10725-010-9516-3>.
- Kim, D.H., Kang, K.W., Enkhtaivan, G., Jan, U., Sivanesan, I., 2019. Impact of activated charcoal, culture medium strength and thidiazuron on non-symbiotic in vitro seed germination of *Pecteilis radiata* (Thunb.). *Raf. S. Afr. J. Bot.* 124, 144–150. <https://doi.org/10.1016/j.sajb.2019.04.015>.
- Knudson, L., 1922. Nonsymbiotic germination of orchid seeds. *Bot. Gaz.* 73, 1–25. <https://doi.org/10.1086/332956>.
- Kovalchuk, I., Pellino, M., Rigault, P., van Velzen, R., Ebersbach, J., Ashnest, J., Mau, M., Schranz, M., Alcorn, J., Laprairie, R., 2020. The genomics of cannabis and its close relatives. *Annu. Rev. Plant Biol.* 71, 713–739. <https://doi.org/10.1146/annurev-arplant-081519-040203>.
- Krasteva, G., Georgiev, V., Pavlov, A., 2021. Recent applications of plant cell culture technology in cosmetics and foods. *Eng. Life Sci.* 21, 68–76. <https://doi.org/10.1002/elsc.202000078>.
- Kusumo, F., Silitonga, A.S., Masjuki, H.H., Ong, H.C., Siswantoro, J., Mahlia, T.M.I., 2017. Optimization of transesterification process for *Ceiba pentandra* oil: a comparative study between kernel-based extreme learning machine and artificial neural networks. *Energy* 134, 24–34. <https://doi.org/10.1016/j.energy.2017.05.196>.
- Lin, Y., Wang, Y., Iqbal, A., Shi, P., Li, J., Yang, Y., Lei, X., 2017. Optimization of culture medium and temperature for the in vitro germination of oil palm pollen. *Sci. Hortic.* 220, 134–138. <https://doi.org/10.1016/j.scienta.2017.03.040>.
- Matnei Filho, R.A., Vergilio, S.R., 2016. A multi-objective test data generation approach for mutation testing of feature models. *J. Softw. Eng. Res. Dev.* 4, 4. <https://doi.org/10.1186/s40411-016-0030-9>.
- Mohan, P.J., Gupta, S.D., 2019. Intelligent image analysis for retrieval of leaf chlorophyll content of rice from digital images of smartphone under natural light. *Photosynthetica* 57, 388–398. <https://doi.org/10.32615/ps.2019.046>.
- Monthony, A.S., Page, S.R.G., Hesami, M., Jones, A.M.P., 2021. The past, present and future of *Cannabis sativa* tissue culture. *Plants* 10, 185. <https://doi.org/10.3390/plants10010185>.
- Niazian, M., Niedbala, G., 2020. Machine learning for plant breeding and biotechnology. *Agriculture* 10, 436. <https://doi.org/10.3390/agriculture10100436>.
- Niazian, M., Sharifiapanahi, M.E., Abdipour, M., Oroojlooo, M., 2019. Modeling callus induction and regeneration in an anther culture of tomato (*Lycopersicon esculentum* L.) using image processing and artificial neural network method. *Protoplasma* 256, 1317–1332. <https://doi.org/10.1007/s00709-019-01379-x>.
- Page, S.R.G., Monthony, A.S., Jones, A.M.P., 2021. DKW basal salts improve micropropagation and calllogenesis compared to MS basal salts in multiple commercial cultivars of *Cannabis sativa*. *Botany* 99, 269–279. <https://doi.org/10.1139/cjb-2020-0179>.
- Roni, M.Z.K., Islam, M.S., Shimasaki, K., 2018. In vitro seed germination and tracking the seedling growth of eustoma. *N. Z. J. Crop Hortic. Sci.* 46, 224–242. <https://doi.org/10.1080/01140671.2017.1391300>.
- Salami, S.A., Martinelli, F., Giovino, A., Bachari, A., Arad, N., Mantri, N., 2020. It is our turn to get cannabis high: put cannabinoids in food and health baskets. *Molecules* 25, 4036. <https://doi.org/10.3390/molecules25184036>.
- Salehi, M., Farhadi, S., Moieni, A., Safaei, N., Ahmadi, H., 2020. Mathematical modeling of growth and paclitaxel biosynthesis in *Corylus avellana* cell culture responding to fungal elicitors using multilayer perceptron-genetic algorithm. *Front. Plant Sci.* 11, 1148. <https://doi.org/10.3389/fpls.2020.01148>.
- Salehi, M., Farhadi, S., Moieni, A., Safaei, N., Hesami, M., 2021. A hybrid model based on general regression neural network and fruit fly optimization algorithm for forecasting and optimizing paclitaxel biosynthesis in *Corylus avellana* cell culture. *Plant Methods* 17, 13. <https://doi.org/10.1186/s13007-021-00714-9>.
- Saltzman, B., Yung, J., 2018. A machine learning approach to identifying different types of uncertainty. *Econ. Lett.* 171, 58–62. <https://doi.org/10.1016/j.econlet.2018.07.003>.
- Sebayang, A.H., Masjuki, H.H., Ong, H.C., Dhama, S., Silitonga, A.S., Kusumo, F., Milano, J., 2017. Optimization of bioethanol production from sorghum grains using artificial neural networks integrated with ant colony. *Ind. Crops Prod.* 97, 146–155. <https://doi.org/10.1016/j.indcrop.2016.11.064>.
- Silva, J.C.F., Teixeira, R.M., Silva, F.F., Brommonschenkel, S.H., Fontes, E.P.B., 2019. Machine learning approaches and their current application in plant molecular biology: a systematic review. *Plant Sci.* 284, 37–47. <https://doi.org/10.1016/j.plantsci.2019.03.020>.
- Sorokin, A., Yadav, N.S., Gaudet, D., Kovalchuk, I., 2020. Transient expression of the β-glucuronidase gene in *Cannabis sativa* varieties. *Plant Signal. Behav.* 15, 1780037 <https://doi.org/10.1080/15592324.2020.1780037>.
- Sorokin, A., Yadav, N.S., Gaudet, D., Kovalchuk, I., 2021. Development and standardization of rapid and efficient seed germination protocol for *Cannabis sativa*. *Bioprotocol* 11, e3875. <https://doi.org/10.21769/BioProtoc.3875>.
- Stewart, S.L., Kane, M.E., 2010. Effects of carbohydrate source on the in vitro asymbiotic seed germination of the terrestrial orchid *Habenaria macroceratitis*. *J. Plant Nutr.* 33, 1155–1165. <https://doi.org/10.1080/01904161003763757>.
- Sumaryono, Muslihatin, W., Ratnadewi, D., 2012. Effect of carbohydrate source on growth and performance of in vitro sago palm (*Metroxylon sagu* Rottb.) plantlets. *HAYATI J. Biosci.* 19, 88–92. <https://doi.org/10.4308/hjb.19.2.88>.

- Tanabe, R., Ishibuchi, H., 2020. An easy-to-use real-world multi-objective optimization problem suite. *Appl. Soft Comput.* 89, 106078 <https://doi.org/10.1016/j.asoc.2020.106078>.
- Utami, E.S.W., Hariyanto, S., 2019. In vitro seed germination and seedling development of a rare indonesian native orchid *Phalaenopsis amboinensis* J.J.Sm. *Scientifica* 2019, 8105138. <https://doi.org/10.1155/2019/8105138>.
- Van der Salm, T.P.M., Van der Toorn, C.J.G., Hänsch ten Cate, C.H., Dubois, L.A.M., De Vries, D.P., Dons, H.J.M., 1994. Importance of the iron chelate formula for micropropagation of *Rosa hybrida* L. 'Moneyway'. *Plant Cell Tiss. Org. Cult.* 37, 73–77. <https://doi.org/10.1007/BF00048120>.
- Vesali, F., Omid, M., Mobli, H., Kaleita, A., 2017. Feasibility of using smart phones to estimate chlorophyll content in corn plants. *Photosynthetica* 55, 603–610. <https://doi.org/10.1007/s11099-016-0677-9>.
- Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., Zemla, J., 2017. Package 'corrplot'. *Statistician* 56, e24.
- Yang, F., Wanik, D.W., Cerrai, D., Bhuiyan, M.A., Anagnostou, E.N., 2020. Quantifying uncertainty in machine learning-based power outage prediction model training: a tool for sustainable storm restoration. *Sustainability* 12, 1525. <https://doi.org/10.3390/su12041525>.
- Yaseen, M., Ahmad, T., Sablok, G., Standardi, A., Hafiz, I.A., 2013. Review: role of carbon sources for in vitro plant growth and development. *Mol. Biol. Rep.* 40, 2837–2849. <https://doi.org/10.1007/s11033-012-2299-z>.
- Yilmaz, V., 2021. A Non-Dominated Sorting Genetic Algorithm-II-based approach to optimize the spectral and spatial quality of component substitution-based pansharpened images. *Concurr. Comp.-Pract. E* 33, e6030. <https://doi.org/10.1002/cpe.6030>.
- Yoosefzadeh Najafabadi, M., Earl, H.J., Tulpan, D., Sulik, J., Eskandari, M., 2021. Application of machine learning algorithms in plant breeding: predicting yield from hyperspectral reflectance in soybean. *Front. Plant Sci.* 11, 624273 <https://doi.org/10.3389/fpls.2020.624273>.
- Yoosefzadeh-Najafabadi, M., Tulpan, D., Eskandari, M., 2021. Application of machine learning and genetic optimization algorithms for modeling and optimizing soybean yield using its component traits. *PLoS One* 16, e0250665. <https://doi.org/10.1371/journal.pone.0250665>.
- Zhang, Q., Deng, D., Dai, W., Li, J., Jin, X., 2020. Optimization of culture conditions for differentiation of melon based on artificial neural network and genetic algorithm. *Sci. Rep.* 10, 3524 <https://doi.org/10.1038/s41598-020-60278-x>.