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1 **Comparative analysis of machine learning and evolutionary**
2 **optimization algorithms for precision tissue culture of *Cannabis sativa*:**
3 **Prediction and validation of *in vitro* shoot growth and development**
4 **based on the optimization of light and carbohydrate sources**

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12 **Keywords: artificial neural networks, biogeography-based optimization, genetic algorithm,**
13 **interior search algorithm, *in vitro* culture, neuro-fuzzy logic, symbiotic organisms search**

14 **Abstract**

15 Micropropagation techniques offer opportunity to proliferate, maintain, and study dynamic plant
16 responses in highly controlled environments without confounding external influences, forming the
17 basis for many biotechnological applications. With medicinal and recreational interests for *Cannabis*
18 *sativa* L. growing, research related to the optimization of *in vitro* practices is needed to improve
19 current methods while boosting our understanding of the underlying physiological processes.
20 Unfortunately, due to the exorbitantly large array of factors influencing tissue culture, existing
21 approaches to optimize *in vitro* methods are tedious and time-consuming. Therefore, there is great
22 potential to use new computational methodologies for analysing data to develop improved protocols
23 more efficiently. Here, we first tested the effects of light qualities using assorted combinations of
24 Red, Blue, Far Red, and White spanning 0-100 $\mu\text{mol}/\text{m}^2/\text{s}$ in combination with sucrose
25 concentrations ranging from 1-6 % (w/v), totaling 66 treatments, on *in vitro* shoot growth, root
26 development, number of nodes, shoot emergence, and canopy surface area. Collected data were then
27 assessed using multilayer perceptron (MLP), generalized regression neural network (GRNN), and
28 adaptive neuro-fuzzy inference system (ANFIS) to model and predict *in vitro Cannabis* growth and
29 development. Based on the results, GRNN had better performance than MLP or ANFIS and was
30 consequently selected to link different optimization algorithms (genetic algorithm, biogeography-
31 based optimization, interior search algorithm, and symbiotic organisms search) for prediction of
32 optimal light levels (quality/intensity) and sucrose concentration for various applications. Predictions
33 of *in vitro* conditions to refine growth responses were subsequently tested in a validation experiment
34 and data showed no significant differences between predicted optimized values and observed data.
35 Thus, this study demonstrates the potential of machine learning and optimization algorithms to
36 predict the most favourable light combinations and sucrose levels to elicit specific developmental
37 responses. Based on these, recommendations of light and carbohydrate levels to promote specific
38 developmental outcomes for *in vitro Cannabis* are suggested. Ultimately, this work showcases the
39 importance of light quality and carbohydrate supply in directing plant development as well as the
40 power of machine learning approaches to investigate complex interactions in plant tissue culture.

41 Introduction

42 The multifaceted value of *Cannabis sativa* L. (cannabis) as a quality fiber, seed oil, and therapeutic
43 crop have been recognized for millennia (Hesami et al., 2020; Sandler et al., 2019). Over the past two
44 decades, interest relating to its medicinal applications have largely been emphasized due to the
45 discovery of over 500 unique secondary metabolites (ElSohly and Gul, 2014). Of these compounds,
46 there are more than 100 cannabinoids that contribute to cannabis' pharmacological properties
47 (Fathordoobady et al., 2019). Medicinal use can relieve symptoms associated with glaucoma, nausea,
48 irritability, epilepsy, chronic pain, etc. (Barrus et al., 2017), showing potential to revolutionize the
49 pharmaceutical industry, and technologies related to extraction and administration of bioactive
50 compounds (Fathordoobady et al., 2019; Vita et al., 2020). Due to the important industrial
51 implications of drug-type cannabis, it is imperative to establish methods for the production of high
52 quality biomass with consistent secondary metabolite profiles, which is achievable in part through
53 micropropagation (Chandra et al., 2020).

54 Since many nations have adopted the more liberal view of cannabis, it's since gained higher
55 economic status as an industrial crop, and additional secondary products such as extract derivatives
56 are expected to further amplify economic expansion (Moher et al., 2020). The need to maintain
57 product consistency, while supporting innovation and development (Burgel et al., 2020) requires a
58 better understanding of the physiological responses of cannabis to external stimuli. Research
59 initiatives are needed to optimize current production strategies, enhancing our recognition of, and the
60 precision at which we can invoke specific physiological responses to fit an assortment of industrial
61 applications. Micropropagation offers unique opportunities to produce and maintain extensive
62 populations of genetically uniform plantlets in time and cost-effective systems (Nathiya et al., 2013).
63 Tissue culture techniques can be applied to examine essential plant responses to external stimuli in
64 highly controlled environments under axenic conditions for biotechnological (Shukla et al., 2017),
65 conservation (Ayuso et al., 2019) and various -omics related technologies (Andre et al., 2016). These
66 approaches can be re-applied to suit the needs of the emerging cannabis industry.

67 Until recently, cannabis micropropagation has largely been an underground effort with few peer
68 reviewed studies. This lack of insight concerning *in vitro* cannabis techniques has limited
69 biotechnological utility of this crop (Smýkalová et al., 2019). While the current cannabis boom has
70 led to the emergence of numerous *in vitro* protocols (Galán-Ávila et al., 2020; Lata et al., 2016;
71 Wróbel et al., 2020), a robust and efficient protocol has yet to be fully developed. Several intrinsic
72 (e.g., genotype, type, and age of explant) and extrinsic (e.g., basal salt medium, vitamins, plant
73 growth regulators (PGRs), gelling agents, carbohydrate source, additional additives, temperature, and
74 light) factors (Fig. 1) influence *in vitro* shoot growth and development and contribute to challenges in
75 reproducibility (Hesami and Jones, 2020). Most previous studies in cannabis have investigated the
76 effect of basal media, along with different types and concentrations of PGRs for shoot growth and
77 regeneration (Chaohua et al., 2016; Lata et al., 2016; Movahedi and Torabi, 2015). Clonal line
78 proliferation using apical and nodal explants on medium with reduced PGRs has been demonstrated
79 as an effective approach for non-medicinal cannabis, while reducing the amount of emergent genetic
80 variability (Wróbel et al., 2020). A cannabis micropropagation approach truly optimized for cross-
81 cultivar maintenance and proliferation should allow formative physiological development on a
82 pathway to photoautotrophic competence, coaxed through abiotic conditioning in the absence of
83 PGRs. Though certain *in vitro* propagation, embryogenesis, and regeneration procedures rely on
84 PGRs, many beneficial physiological characteristics can be induced or enhanced by appropriately
85 adjusting light quality, quantity, and carbohydrate supply. *In vitro* shoot growth and development
86 may be achieved or enhanced by manipulating light and sugar in the absence of PGRs.

87 Though micropropagation protocols show promise to advance certain aspects of the cannabis
88 industry, there remain issues with conventional *in vitro* systems. Photosynthetically incompetent
89 organs, and fragile roots are phenotypic traits commonly observed in cultures (Jha and Bansal, 2012).
90 Anatomical variations tend to be culture-induced, emerging with high humidity, elevated PGR
91 concentrations, low light intensity, and high substrate water potential, causing physiological disorders
92 such as hyperhydricity (Majada et al., 2000). These abiotic factors, along with limited culture CO₂
93 availability, and potential for ethylene accumulation frequently impede photosynthetic responses
94 (Nguyen et al., 2001), complicating the *ex vitro* transfer of specimens (Nathiya et al., 2013). Due to
95 such limitations, new cultures must be supplied alternative carbon sources to maintain metabolic
96 activity in otherwise daunting, closed environments (Eckstein et al., 2012). Sugar occurrence allows
97 continuous plantlet development under low irradiance (Cioć et al., 2018), commonly used *in vitro*. A
98 standardized addition of 3% (w/v) sucrose to the micropropagation media helps to counteract short-
99 term, negative environmental impacts by providing substitute carbohydrates to elicit photo-
100 mixotrophic metabolism (Gago et al., 2014). *In vitro* sucrose levels impact plantlet physiology by
101 regulating genes relating to primary and secondary metabolic function (Yang et al., 2012). While
102 supplemental carbon supply is a necessity for early-stage explants, developing plantlets can build
103 sucrose dependence (Lembrechts et al., 2015), further limiting idealized physiological function and
104 subsequent *ex vitro* re-localization. Conversely, previous work has also demonstrated that sugar can
105 have positive effects on plantlet development under different environmental conditions *in vitro*
106 (Eckstein et al., 2012; Kozai et al., 1987; Roh and Choi, 2004).

107 Although the occurrence of sucrose often activates photomixotrophic metabolic responses, light
108 nevertheless bears high influence over *in vitro* success (Miler et al., 2019). Sugar and light signal
109 essential metabolic processes which govern the condition of cultured plantlets (Eckstein et al., 2012).
110 Though low light intensity *in vitro* hampers photosynthetic efficiency, overly high intensities can
111 limit synthesis of photo-absorptive pigments and damage certain components of the photosynthetic
112 apparatus (Cioć et al., 2018). Since high light levels throughout different culture stages can be
113 stressful to developing plantlets, substitute carbon sources can help elicit photo-protective responses,
114 indicating a possible sugar/light signalling pathway for photo-protection (Eckstein et al., 2012). Thus,
115 photosynthetic limitation *in vitro* could largely be more related sub-optimal abiotic conditions in the
116 presence of exogenous sugar, rather than the impact of the sugar itself (Arigita et al., 2002).
117 Chloroplast localization (Eckstein et al., 2012), leaf area index (Snowden et al., 2016), and leaf
118 thickness are influenced by changes in light quantity and quality. Proper development of these traits
119 can increase photoabsorption saturation point (Macedo et al., 2011), enhancing plantlet fitness.
120 Sustainable adjustment of the abiotic conditions combined with exogenous sugar can improve
121 protective and repair responses (Eckstein et al., 2012; Tichá et al., 1998), allowing plantlets to more
122 effectively sequester and utilize otherwise excessive and damaging photo-irradiation. Preliminary
123 work conducted by our lab points in this direction in the case on micropropagated cannabis.
124 Modifying abiotic factors and their interactions with sugar-related dynamics, is sometimes
125 overlooked in micropropagation (Eckstein et al., 2012). Thus, research surrounding the potential to
126 improve tissue culture protocols by optimizing abiotic influence and sugar-related dynamics should
127 be thoroughly pursued.

128 The use of light emitting diodes (LEDs) for plant tissue culture allows strategic manipulation of light
129 quality and intensity, impacting biomass and secondary metabolite accumulation of various species
130 (Cioć et al., 2018; Manivannan et al., 2015; Ucar et al., 2016). Cool fluorescent lights have been
131 popular for conventional micropropagation systems (Fanga et al., 2011) due to relatively low energy
132 consumption, heat dissipation, and cost. However, they deliver light at wavelengths outside of the
133 photoabsorptive range and lack control over spectral quality, which limits its power over

134 physiological conditioning (Bello-Bello et al., 2016). There exists an established dogma that blue
135 light (B) heavily influences chloroplast development, chlorophyll production, and stomata
136 functionality, while red light (R) influences carbohydrate localization, and various anatomical
137 processes such as leaf expansion (Hung et al., 2016; Ucar et al., 2016). Various combinations of
138 these wavelengths can mutually and individually persuade shoot and root elongation (Ramírez-
139 Mosqueda et al., 2017). LED technologies hold significant potential in the pursuit of plant growth in
140 controlled environments, including plant tissue culture (Fontana et al., 2019). Control over spectral
141 composition with LEDs allows wavelength emission that match photoreceptor action spectra to more
142 directly trigger morphogenic responses (Li et al., 2010), while limiting heat dissipation, and energy
143 consumption (Zhao et al., 2020). Photomorphogenic responses are primarily prompted by light
144 quality through phytochrome reception of R and far-red light (Fr), and cryptochrome absorption of B
145 (Miler and Zalewska, 2006), which largely shape plant development and physiology (Legris et al.,
146 2019).

147 Despite the apparent simplicity of light quality and intensity, it is a complex factor comprised of
148 nearly infinite potential mixtures which interact with other factors such as sucrose levels to influence
149 *in vitro* shoot growth and development as a nonlinear, multifactorial, and complex process. The
150 establishment and optimization of *in vitro* culture protocols have been principal challenges for many
151 tissue culture researchers. Historically, micropropagation systems have been developed through serial
152 manipulation and optimization of single factors, individually. Conventional statistical methods such
153 as simple regression and ANOVA have typically been recommended for small databases with limited
154 dimensions, and are therefore inappropriate for analyzing data derived from complex and non-linear
155 processes such as light quality (Hesami et al., 2021b; Yoosefzadeh-Najafabadi et al., 2021a). The
156 high probability of overfitting is one of the main disadvantages of using conventional statistical
157 methods (Jafari and Shahsavari, 2020; Yoosefzadeh-Najafabadi et al., 2021b). Using conventional
158 statistical methods, some of the puzzle pieces of *in vitro* practices have been sequentially assembled.
159 However, many factors in tissue culture systems remain unoptimized. To overcome such setbacks,
160 different factors can be simultaneously optimized through precision *in vitro* culture techniques using
161 machine learning methods (Fig. 2). In recent years, machine learning algorithms such as artificial
162 neural networks (ANNs) and neuro-fuzzy logic have been successfully applied for modeling and
163 predicting various *in vitro* culture systems such as shoot growth and development, callogenesis,
164 somatic embryogenesis, androgenesis, secondary metabolite production, and rhizogenesis (Hesami
165 and Jones, 2020; Niazian and Niedbała, 2020). However, in most plant tissue culture studies,
166 individual models were employed, and the efficiency of different machine learning algorithms has
167 not been compared (Hesami et al., 2021c).

168 There exist two general groups of optimization methods. Classical optimization algorithms include
169 dynamic programming (DP), linear programming (LP), stochastic dynamic programming (SDP)
170 which have limitations restricting their flexibility and efficiency. For instance, LP requires objective
171 function and constraint to be linear, which is not ideal for plant tissue culture. Conversely,
172 evolutionary optimization algorithms are considered more powerful mathematic methods for solving
173 complex, multidimensional problems such as designating optimal factors for micropropagation with
174 high accuracy and pace (Hesami and Jones, 2020). Although there are different types of evolutionary
175 optimization algorithms, the genetic algorithm (GA) has been applied to the vast majority of plant
176 tissue culture optimization studies relating to shoot proliferation, secondary metabolite production,
177 and somatic embryogenesis. Despite the advantages that GA imparts over classical methods,
178 premature convergence can sometimes lead to failure in obtaining a fully optimized solution
179 (Hosseini-Moghari et al., 2015). To overcome this, new evolutionary optimization algorithms,
180 including biogeography-based optimization (BBO), interior search algorithm (ISA), and symbiotic

181 organisms search (SOS) have been developed. These approaches have been evaluated in different
182 fields of study (Bozorg-Haddad et al., 2016; Hosseini-Moghari et al., 2015; Mokhtari Fard et al.,
183 2012; Moravej and Hosseini-Moghari, 2016), and are expected to be superior in optimizing plant
184 tissue culture protocols.

185 The current study tests the combined effects of B (400-500nm), R (600-700nm), Fr (700-800nm),
186 and White (W) (400-700nm) (Figure 3, Figure 9) light at different intensities, and carbohydrate
187 concentrations on shoot length, root length, number of nodes, number of shoots, and canopy surface
188 area. Data collected were assessed using machine learning and evolutionary optimization algorithms
189 to predict and optimize these factors for cannabis maintenance and proliferation *in vitro*. Predictions
190 were then tested in a validation experiment to identify the best optimization algorithm for *in vitro*
191 plant applications. Ultimately, the research presented will facilitate development of current practices
192 for maintenance, proliferation, and acclimation of micropropagated cannabis, boosting our
193 understanding of the dynamics between light and sugar-related plantlet responses, while identifying
194 superior predictive analytic practices to guide future experimentation.

195

196 **1 Materials and Methods**

197 **1.1 Plant material and experimental design**

198 In this study, the effects of different light qualities, intensities and sucrose concentrations were
199 evaluated for shoot growth, canopy surface area, and additional growth parameters, using the
200 medicinal strain of cannabis “UP-802” supplied by Hexo, Brantford, ON. To this end, four plantlets
201 per treatments were cultured in single Magenta boxes, allowing one experimental unit per treatment.
202 Stock UP-802 specimens were maintained in cultures supplemented with 3% (w/v) sucrose,
203 maintained under 16-hr photoperiod with 75% R, 12.5% B and 12.5% W LEDs at 50 $\mu\text{mol}/\text{m}^2/\text{s}$.
204 Both stock and experimental plantlets were grown at approximately 26°C. Non-experimental media
205 components included 0.53% (w/v) DKW with vitamins, 0.10% (w/v) plant preservation mixture, and
206 0.60% (w/v) agar. Media pH was adjusted to 5.7 prior to agar addition, sterilization, and use.
207 Chemicals were obtained from PhytoTech Labs.

208 To test the multivariable influences of sugar and light quality (intensity and spectrum) on *in vitro*
209 cannabis development, plantlets were grown for 6-weeks with alternative sucrose concentrations, in
210 compartmentalized light treatments. Programmable LED lights were used to provide light, allowing
211 different combinations of B (400-500nm), R (600-700nm), Fr (700-800nm), and W (400-700nm)
212 (Figure 3, Figure 9) light at specific intensities between 0-100 $\mu\text{mol}/\text{m}^2/\text{s}$. The FinMax
213 (bigfin.github.io/Prismatic) programmable LED lighting system was developed in-house to empower
214 photobiology research with precise lighting treatments (Figure 3). The intensity of the 9
215 independently dimmable channels were programmed and calibrated at plant height using a
216 spectrometer (Li-Cor LI-180).

217 At the end of each experiment, shoot length was measured by selecting the longest shoot and
218 measuring from the root-shoot junction to apical meristem. Similarly, root length was measured from
219 the root-shoot junction to root tip of the longest root. Number of nodes was collected by counting
220 nodes on longest shoots. Shoot number was determined by counting emergent stems. Canopy surface
221 areas were obtained by dissecting leaves and processing through ImageJ. All raw data were collected
222 and processed using ImageJ software (Rueden et al., 2017).

223 For the preliminary experiment, apical explants were collected from stock UP-802 cultures, and sub-
224 cultured to Magenta boxes with experimental media containing 1, 3 or 6% (w/v) sucrose. One culture
225 of each sucrose concentration was randomly assigned to one of 22 different light treatments listed in
226 Table 1, where they remained for 6-weeks. Data on shoot length, root length, number of shoots,
227 number of nodes, and canopy surface area that was collected from 264 plantlets, as presented in
228 Table 1, and processed with ImageJ software (Rueden et al., 2017). Raw experimental datasets were
229 then analyzed using machine learning algorithms to build an appropriate model for cannabis shoot
230 growth and development.

231 1.2 Modeling procedure

232 Three well-known machine learning algorithms, MLP, GRNN, and ANFIS, were applied to model
233 and predict *in vitro* shoot growth and development of cannabis using the collected dataset. Box-Cox
234 transformation was employed to normalize the data before using the machine learning algorithms.
235 Principal component analysis (PCA) was applied to detect outliers, but no outliers were identified. In
236 this study, the five-fold cross-validation approach, with 10 repetitions was applied to evaluate the
237 prediction accuracy of the tested machine learning algorithms.

238 Different light qualities (B, R, W, and Fr) at various intensities and different levels of Sucrose were
239 selected as input variables, while shoot length, root length, number of nodes, number of shoots, and
240 canopy surface area were considered as target (output) variables (Fig. 4a).

241 To evaluate and compare the efficiency and accuracy of the machine learning algorithms, R^2
242 (coefficient of determination), mean bias error (MBE), and root mean square error (RMSE) were
243 employed based on the following equations:

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

244

$$RMSE = \sqrt{\left(\sum_{i=1}^n (y_i - \hat{y}_i)^2 \right) / n} \quad (2)$$

245

$$MBE = 1/n \sum_{i=1}^n (y_i - \hat{y}_i) \quad (3)$$

246

247 Where y_i is the value of prediction, n is the number of data, and \hat{y}_i is value of observation.

248 2.2.1 Multi-Layer Perceptron (MLP)

249 MLP belongs to the ANNs which is inspired by the neural structure of the human brain. A neuron in
250 the human neural network receives impulses by using a number of dendrites from other neurons.
251 Based on the received impulses, a neuron through its single axon may send a signal to other neurons.
252 Like the human neural network, ANNs contain nodes, each of which receives a number of input
253 variables and produce a single target variable, where the target variable is a relatively simple function
254 of the input variables (Fig. 4b).

255 The 3-layer backpropagation MLP is a parallel and distributed algorithm that uses supervised
256 learning for the training subset. The following equation is employed to minimize the error between
257 the input and target variables:

$$258 \text{Error} = \frac{1}{n} \sum_{s=1}^n (y_s - \hat{y}_s)^2 \quad (4)$$

258

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

261 To determine the \hat{y} in the model k output variables and with p neurons in the hidden layer, following
262 function is employed:

$$263 \hat{y} = f \left[\sum_{j=1}^p w_j \cdot g \left(\sum_{i=1}^k w_{ji} x_i + w_{j0} \right) + w_o \right] \quad (5)$$

263

264 where w_j represents the weighted input data into the j^{th} neuron of the hidden layer, w_o equals the bias
265 connected to the neuron of output, w_{ji} represents the weight of the direct relationship of input neuron
266 i to the hidden neuron j , x_i is the i^{th} target variable, f represents activation function for the target
267 neuron, w_{j0} shows the bias for node j^{th} , and g shows the activation function for the hidden neuron.

268 Since the number of hidden units and the number of neurons in each node play an important role in
269 the efficiency of MLP, they should be determined. In the present investigation, trial and error-based
270 approach was used to detect the optimal neuron number in the hidden layer. Also, linear function
271 (purelin) as the transfer functions of output layer and hyperbolic tangent sigmoid function (tansig) as
272 the transfer functions of hidden layer were applied. Moreover, A Levenberg-Marquardt algorithm
273 was employed for adjusting bias and weights.

274 2.2.2 Generalized Regression Neural Network (GRNN)

275 The GRNN as another kind of ANNs consists of four layers (Fig. 4c). The node in input layer
276 completely enters the node in pattern layer. The output of each neuron in pattern layer is connected to
277 the summation neurons. The unweighted pattern neuron outputs are determined by D-summation

278 neuron, while the weighted pattern neuron outputs are computed by S-summation neuron. Finally, the
 279 following equation is employed to determine the output:

$$\hat{y} = \frac{\sum_{i=1}^n y_i \exp\left(-\frac{D_i^2}{2\sigma^2}\right)}{\sum_{i=1}^n \exp\left(-\frac{D_i^2}{2\sigma^2}\right)} \quad (6)$$

280

$$D_i^2 = (x - x_i)^T (x - x_i) \quad (7)$$

281

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

285 2.2.3 Adaptive Neuro-Fuzzy Inference System (ANFIS)

286 ANFIS developed by Jang (1993) is one of the most well-known neuro-fuzzy logic systems. The
 287 overall ANFIS model with two Takagi and Sugeno type if-then rules can be defined as follow:

$$\begin{aligned} \text{Rule I: if } x \text{ is } A_1 \text{ and } y \text{ is } B_1 \text{ then } f_1 &= p_1x + q_1y + r_1 \\ \text{Rule II: if } x \text{ is } A_2 \text{ and } y \text{ is } B_2 \text{ then } f_2 &= p_2x + q_2y + r_2 \end{aligned} \quad (8)$$

288

289 Where x and y are input variables; f_1 and f_2 are the outputs within the fuzzy area determined by the
 290 fuzzy rule; $A_1, A_2, B_1,$ and B_2 are the fuzzy sets; $p_1, p_2, q_1, q_2, r_1,$ and r_2 are the design parameters that
 291 are specified during the training set. The ANFIS model is built of five layers (Fig. 4d) as follow:

292 Layer 1 (adaptive or input layer): Every adaptive (input) node i in layer 1 defines a square node with
 293 a node function:

$$O_i^1 = \mu_{A_i}(x) \quad (9)$$

294 Where O_i^1 is the fuzzy membership grade, x is the input of adaptive node i , and μ_{A_i} is Gaussian
 295 membership function which is deremined as follow:

$$\mu_{A_i}(x) = \exp\left[-\left(\frac{x - c_i}{a_i}\right)^2\right] \quad (10)$$

296 where a_i and c_i are premise parameters.

297 Layer 2 (rule layer): Every role node in layer 2 can be considered as a circle node labeled \square where
 298 the output is the result of all incoming inputs.

$$w_i = \mu_{A_i}(x) \times \mu_{B_i}(x) \quad i = 1, 2 \quad (11)$$

299 Each node output displays the rule's firing strength.

300 Layer 3 (average layer): Each node as a fixed node in layer 3 is labeled N . The i^{th} node determines
301 the ratio of the firing strength of i^{th} rule to the total rules' firing strengths. The outputs of layer 3
302 (normalized firing strengths) are calculated as follow:

$$\bar{w}_i = \frac{w_i}{w_1 + w_2} \quad i = 1, 2 \quad (12)$$

303 Where \bar{w}_i is output of this layer.

304 Layer 4 (consequent layer): Nodes in layer 4 are called consequent nodes. The following equation is
305 used to calculate the output of this layer.

$$O_1^4 = \bar{w}_i f_i = \bar{w}_i(p_i x + q_i y + r_i) \quad (13)$$

306 Where p_i , q_i , and r_i are parameter sets and \bar{w}_i is output of layer 3.

307 Layer 5 (output layer): There is only one single fixed node labeled S in this layer. The final output
308 (O_i^5) of the model is calculated based on the following equation:

$$O_i^5 = \sum_{i=1}^2 \bar{w}_i f_i \quad (14)$$

309 In the current study, the Gaussian membership function (between 3 and 5 membership functions for
310 different variables) was considered based on a trial and error approach. The number of epochs to train
311 the models was also set to 10. Moreover, the least-squares method and backpropagation algorithm
312 were applied to adjust the consequent and premise parameters, respectively.

313 1.3 Sensitivity analysis

314 Sensitivity analysis was performed to assess the degree of importance of various forms of light (B, R,
315 W, and Fr) and exogenous carbohydrates on shoot length, root length, number of nodes, number of
316 shoots, and canopy surface area by determining the variable sensitivity ratio (VSR). VSR can be
317 defined as the ratio of variable sensitivity error (VSE) to the RMSE of the developed model. A
318 greater VSR shows a higher degree of importance.

319 1.4 Optimization procedure

320 In the current study, four different single-objective evolutionary optimization algorithms including
321 BBO, ISA, SOS, and GA were separately employed to find optimal levels of input variables
322 (Sucrose, B, R, W, and Fr) for maximizing each fitness function (shoot length, root length, number of
323 nodes, number of shoots, and canopy surface area). Generally, evolutionary optimization algorithms
324 consist of five main steps including creating an initial population, fitness computation, selection,

325 creating a new generation, and displaying the best solution (Fig. 4e). The details of each algorithm
326 have been presented below.

327 **2.4.1 Biogeography-Based Optimization (BBO)**

328 The term "Biogeography" refers to the study of ecosystems and the geographical distribution of
329 species. BBO introduced by Simon (2008) is based on biogeographic concepts such as migration,
330 evolution, adaptation, and extinction of organisms among habitats. In theory, appropriate regions for
331 living organism's settlement are defined by the habitat suitability index (HSI) that depends on several
332 factors such as precipitation, temperature, area, and vegetative cover which are known as suitability
333 index variables (SIVs). Indeed, HIS as a dependent variable is determined by SIVs as independent
334 variables. Therefore, more living organisms can be accommodated in habitats with higher values of
335 HIS and vice versa, lower HSI values support fewer organisms. Subsequently, a stronger tendency
336 for living organisms to emigrate from the habitat to find new places with lower population density
337 and more suitable conditions can be seen by increasing the number of species in a habitat.

338 The highest λ can be seen when there are no species in the habitat. The λ decreases by increasing the
339 number of species in the habitat and, finally, the λ becomes zero when the habitat capacity is
340 completed (the maximum number of species in the habitat equals S_{max}). On the other hand, the μ
341 enhances by increasing the number of species in the habitat until the habitat becomes empty. Hence,
342 the equilibrium number of species in the habitat can be seen when λ equals μ . Generally, λ and μ can
343 be determined based on the following equations:

$$\mu = E \times \left(\frac{S}{S_{max}} \right) \quad (15)$$

344

$$\lambda = I \times \left(1 - \frac{S}{S_{max}} \right) \quad (16)$$

345

346 Where S is the number of species, I shows the maximum rate of immigration, and E is the maximum
347 rate of emigration.

348 In the BBO method, habitat and SIVs play the role of solution and the decision variables,
349 respectively. Therefore, the HSI can be considered as the objective function in this optimization
350 algorithm. If there is a particular graph with $E=I$ for each solution, HSI has a direct relationship with
351 S , in which case HSI values can be used instead of S . The step-by-step procedure of the ISA method
352 has been presented in Figure 5.

353 With a specific probability P_{mod} , different solutions can help each other for improvement. If the S_i is
354 selected as an improvement, the λ is employed to adjust its SIVs. Subsequently, the μ relevant to
355 other solutions is applied to choose the improved solution. The SIVs of the S_i solution are then used
356 for randomly replacing SIVs from selected solutions. The suitable values of μ can be arbitrarily
357 considered by using an arithmetic progression, with the common difference of successive members
358 equal to $1/(\text{population size} - 1)$, between 0 and 1. After calculation of μ , λ can be determined as
359 $\lambda=1-\mu$.

360 For lack of elitism, all solutions should be modified at all steps. However, modifying the amount of
361 any solution is conversely related to its HSI. A roulette wheel is used for choosing the modifier
362 solution which is based on a probability proportional to the μ . Transferring SIVs, as an inferior
363 strategy, from one solution to another solution restricts the search choices within the decision space.
364 Therefore, the following equation has been recommended for replacing SIVs:

$$SIV_{i,m}^{new} = SIV_{i,m} + \alpha (SIV_{j,m} + SIV_{i,m}) \quad (17)$$

365

366 Where $SIV_{i,k}^{new}$ equals m^{th} modified SIV of the i^{th} solution, α is a parameter between 0 and 1, which is
367 determined by the user, $SIV_{j,m}$ is m^{th} SIV of the j^{th} solution, and $SIV_{i,m}$ equals m^{th} SIV of the i^{th}
368 solution.

369 Severe catastrophes such as natural hazards, the spreading of infectious diseases, and other
370 catastrophes can quickly change the HSI of a habitat. These unfavorable conditions act like mutations
371 in GA.

372 **2.4.2 Interior Search Algorithm (ISA)**

373 The ISA method introduced by Gandomi, (2014) is based on the concepts of interior design and
374 decoration using mirrors, such that, several mirrors can be used to create a more decorative
375 environment. To meet decoration project goals, it is necessary to satisfy the desires of the clients'
376 desires using available resources. The interior design commences with centering bounded elements to
377 create a more appealing interior vista based on client approval. The ISA method is inspired by this
378 repetitive process to solve optimization problems. With this algorithm, an element can only be moved
379 to a position allowing a more decorative view (better fitness) while satisfying customer resource and
380 need demands (constraints).

381 The most important step of interior design is positioning the mirrors by the fittest and most striking
382 elements to highlighting their attractiveness. Generally, the elements are classified in two ways (i) the
383 composition category, which is applied for composition optimization, and (ii) the mirror category,
384 which is employed for mirror search. Therefore, the ISA method can be explained as follow.

385 1) Create the position of elements between upper bound (UB) and lower bound (LB) randomly and
386 determine their fitness value.

387 2) Discover the element with minimum objective function in minimization problem (the fittest
388 element) in j^{th} iteration.

389 3) Apply a random variable $r1$ (ranging between 0 and 1 for each element) and α as a threshold value
390 (α is also a value between 0 and 1) to divide other elements, except the fittest element, into mirror
391 category and composition category. Elements with $\alpha \leq r1$ go to the composition category;
392 otherwise, they go to the mirror category. Since α is the only parameter of the ISA method, it is
393 necessary to carefully tune α for obtaining balance between diversification and intensification.

394 In the current study, a linear equation from 0.1 to 0.9 was used for determining the value of α during
395 optimization iterations, meaning the α value modifies as iteration goes up towards its maximum
396 number. This method provides a parametric optimization algorithm in which the algorithm can
397 automatically adjust its parameter. As the iteration approaches the highest iteration number, the α

398 value reaches 0.9. Subsequently, the optimization procedure slowly shifts to mirror search to promote
399 exploitation at the end of repetitions.

400 4) For the fittest element, it is beneficial to lightly change positions using the random walk for a local
401 search around the fittest element. The following equation can be used for calculating the fittest
402 element.

$$x_{gb}^j = x_{gb}^{j-1} + rn \times \lambda \quad (18)$$

403 Where, x_{gb}^j is the fittest element, λ is scale factor = $0.01(UB - LB)$, and rn presents vector of
404 normally distributed random numbers.

405 5) For the composition category, each element in this category is randomly displaced. The following
406 equation is used for determining the changes in UB and LB:

$$x_i^j = LB^j + (UB^j - LB^j) \times r2 \quad (19)$$

407

408 Where x_i^j shows i^{th} element in the j^{th} iteration, UB^j and LB^j represent upper and lower bounds of the
409 class in j^{th} iteration, respectively, and $r2$ is random value between 0 and 1.

410 6) For the mirror category, a mirror is randomly placed between the fittest element and each
411 composition element. The following equation is applied for calculating the position of a mirror for
412 the i^{th} element of the j^{th} iteration:

$$x_{m,i}^j = r3 x_i^{j-1} + (1 - r3) \times x_{gb}^j \quad (20)$$

413

414 Where $x_{m,i}^j$ equals the position of a mirror for the i^{th} element of the j^{th} iteration, and $r3$ is a random
415 value between 0 and 1.

416 The virtual location of the element (the image of the element in the mirror) depends on the position
417 of the mirror and is calculated based on the following equation:

$$x_i^j = 2x_{m,i}^j - x_i^{j-1} \quad (21)$$

418

419 7) The virtual elements and fitness values of the new positions of the elements should be determined.
420 The positions should be updated if their fitnesses are improved. It can be calculated based on the
421 following equation (for a minimization problem):

$$x_i^j = \begin{cases} x_i^j f(x_i^j) < f(x_i^{j-1}) \\ x_i^{j-1} & \text{otherwise} \end{cases} \quad (22)$$

422

423 8) If any of the termination criteria are not satisfied, the steps should be repeated from step 2. The
424 step-by-step procedure of the ISA method has been presented in Figure 6.

425 **2.4.3 Symbiotic organisms search (SOS) algorithm**

426 The SOS introduced by Cheng and Prayogo (2014) can be considered a nature-inspired optimization
427 method. The SOS algorithm simulates three various interactions of symbioses amongst species of an
428 ecosystem. Much like the majority of evolutionary optimization algorithms, SOS creates an
429 ecosystem as an initial population plus particular operators through an iterative method to find a
430 near-optimal solution among candidate organisms as possible solutions within the promising space of
431 a search area. However, the SOS method does not reproduce offspring. Step-by-step SOS procedure
432 methods are presented in Figure 7.

433 After defining the maximal number of iterations and the number of species, the initial ecosystem is
434 specified by generating a uniform random number between the upper and lower values of ecosystem
435 size and a design variable (D) number. After that, X_{best} as the best current solution should be
436 determined. In a process, named mutualism, two randomly chosen species along with X_{best} participate
437 in a dialectic relationship that is profitable for both. New candidate solutions are generated based on
438 the following equations:

$$x_{i_{new}} = x_i + \mathit{rand}(0,1) \times (x_{best} - MV \times BF_1) \quad (23)$$

439

$$x_{j_{new}} = x_j + \mathit{rand}(0,1) \times (x_{best} - MV \times BF_2) \quad (24)$$

440 Where $\mathit{rand}(0,1)$ shows a vector of random numbers, and the mutual vector (MV) equals the average
441 value of x_j and x_i which enables the organisms to be updated concurrently rather than separately. In a
442 mutualistic symbiosis between two species within nature, one species might gain a great advantage
443 while the other receives no significant profit. This is presented by BF_1 and BF_2 , which are randomly
444 specified as either 1 or 2 ($BF_i = \mathit{Rand}(\mathit{rand}(0,1) + 1)$; $i = 1$ and 2) to display the level of profits
445 obtained from the relationship.

446 In the next step, the entire population is updated. Subsequently, the old candidate solutions x_j and x_i
447 are compared with the new ones. More fit organisms are chosen as new solutions for the next
448 iteration. The selections and comparisons start and end with the counter 1 and the counter equal to
449 the *population size* (n_{pop}), respectively. For each i , the solution j is randomly chosen within the new
450 population. Afterward, fitter organisms take part in the next step which is named commensalism. In
451 commensalism, although one organism gains profits, the other remains neutral. Similar to the
452 previous step, x_j is randomly chosen from the population to interact with x_i . While x_i attempts to get
453 profits from the engagement, x_j remains unaffected. If the new fitness value shows better
454 performance than the previous one, the following equation is employed for updating x_i :

$$x_{inew} = x_i + rand(0,1) \times (x_{best} - x_j) \quad (25)$$

455

456 In the third step, which is named parasitism, the mutation operator of the SOS is required. In this
457 step, x_j and x_i are the artificial host and parasite, respectively. In parasitism, one organism receives
458 profits while the other is harmed. The sign of the parasite vector (PV) is that it competes with other
459 randomly chosen dimensions instead of its parent with a series between upper and lower bounds. In
460 this step, an initial parasite vector is produced by multiplying organism x_j . Some of the decision
461 variables from the parasite vector are randomly changed to recognize the parasite vector from x_j . A
462 random number should be produced in the range of [1, decision variable number] to describe the total
463 number of changed variables. A uniform random number is produced for each dimension to achieve
464 the position of the changed variables. Finally, a uniform distribution within the search area is needed
465 for changing the variables and providing a parasite vector for the parasitism step. If the parasite
466 vector displays better performance than x_j it becomes part of the population, whereas if x_j is not
467 outperformed the parasite vector, PV eliminates from the population. The parasite vector is produced
468 by changing x_j in random dimensions with random numbers rather than making small modifications
469 in x_j . If the current x_j and parasite vector are not the last member of the population, the SOS returns to
470 the mutualism step that chosen X_{best} until obtaining a specified stopping criterion.

471 **2.4.4 Genetic algorithm (GA)**

472 The GA, introduced by Holland (1992) is based on the Darwinian concepts of genetics and natural
473 selection. Before applying the GA, some parameters such as crossover fraction, selection method,
474 mutation rate, etc... should be specified. Subsequently, a set of possible answers are generated. The
475 GA considers a set of chromosomes containing genes as an initial population. The genes represent
476 the number of problem dimensions. During the optimization process, the genetic operators (e.g.,
477 Roulette Wheel and Tournament Selection) of the mutation and crossovers improve these genes.

478 Based on the competence of the chromosomes' corresponding objective function, genes are selected
479 to transfer to the next generation. The crossover operator replaces a number of genes from two
480 chromosomes with each other. Moreover, the mutation operator changes some genes randomly. The
481 elitism parameter is used to improve the chance of choosing the best chromosomes, then increase the
482 convergence of the algorithm. When creating each new generation, three operators (i.e., crossover,
483 selection, and mutation) regulate the optimization process in a way that the generated chromosomes
484 improve the objective function value at each repetition until the optimization process will be
485 completed by satisfying one of the termination criteria. The step-by-step procedure of the GA method
486 has been presented in Figure 8.

487 **1.5 Validation experiment**

488 To evaluate the efficiency and reliability of the hybrid GRNN-evolutionary optimization algorithms,
489 the predicted-optimized treatments obtained from evolutionary optimization algorithms (GA, ISA,
490 SOS, and BBO) were separately evaluated in the lab as the validation experiment (Figure 9). The
491 validation experiment was performed based on a completely randomized design with 4 replications.
492 Effectiveness of optimized treatments were assessed by comparing error bars, representing standard
493 error of means, as presented in Figure 4g,h.

494 **2 Results**

495 2.1 Effects of light and carbohydrate sources on cannabis shoot growth and development

496 While this experiment was designed specifically for machine learning applications and standard
497 statistical comparisons cannot be made, a wide range of responses were observed through the
498 different treatments applied (Table 1). For instance, the greatest shoot length was acquired from 25
499 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 3 % Sucrose (154.68 ± 51.228 mm), while shoot length was most
500 stunted when grown with 100 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 1 % Sucrose (27.70 ± 2.311 mm). Greatest root length
501 was achieved with 33.33 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 33.33 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 33.33 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 3 % Sucrose
502 (477.10 ± 287.094 mm), though no roots emerged from 100 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 1 % Sucrose, or 50
503 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 50 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 1 % Sucrose specimens, and lowest root lengths were observed
504 from those of the 100 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 1 % Sucrose (4.36 ± 4.363 mm) treatment. Plantlets developing
505 the most nodes came from 33.33 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 33.33 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 33.33 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 3 %
506 Sucrose (11.50 ± 2.901), while the fewest nodes were observed in 100 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 1 % Sucrose
507 (5.75 ± 0.479) treated plantlets. The largest canopy surface area was attained by plantlets grown
508 under 50 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 50 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 3 % Sucrose (13061.97 ± 10839.642 mm²), whereas
509 smallest canopy was observed in 100 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 1 % Sucrose (493.01 ± 111.615 mm²), 100
510 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 1 % Sucrose (519.06 ± 182.411 mm²). Results for the preliminary experiment are
511 outlined in Table 1. Treatments consisted of single experimental units with 4 biological replicates
512 each, which satisfied the models used, with high accuracy.

513 Based on our observations (Table 1), a general trend is observed for appreciable shoot length when
514 sucrose concentration is 3% (w/v), irradiance levels are in range of 50-100 $\mu\text{mol}/\text{m}^2/\text{s}$, and when W is
515 included in multi-spectral treatments. These treatments allowed long shoot length that developed
516 between 32.44 ± 7.036 - 154.68 ± 51.228 mm. Additionally, there was a broad tendency for multi-
517 spectral treatments with 75-100 $\mu\text{mol}/\text{m}^2/\text{s}$ that included R and 3% (w/v) sucrose to develop large
518 canopy surface areas, which ranged from 2483.71 ± 627.011 - 13061.97 ± 10839.642 mm² (Table 1).

519 Of the 66 treatments tested, 50 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 50 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 1 % Sucrose noticeably
520 accumulated phenolic compounds in the media, which was not observed in any other treatment.
521 Additionally, 12 cultures produced plantlets with floral organs despite being grown under a long day
522 photoperiod. Cultures included 25 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 1 % Sucrose, 25 $\mu\text{mol}/\text{m}^2/\text{s}$ R +
523 75 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 1 % Sucrose, 25 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 75 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 3 % Sucrose, 33.33 $\mu\text{mol}/\text{m}^2/\text{s}$
524 R + 33.33 + 33.33 Fr + 1 % Sucrose, 33.33 R + 33.33 B + 33.33 Fr + 3 % Sucrose, 25 $\mu\text{mol}/\text{m}^2/\text{s}$ R
525 + 75 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 6 % Sucrose, 25 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 25
526 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 6 % Sucrose, 25 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 6 % Sucrose, 100 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 6
527 % Sucrose, 50 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 6 % Sucrose, 25 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 6 % Sucrose, 50
528 $\mu\text{mol}/\text{m}^2/\text{s}$ + 6 % Sucrose, 50 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 50 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 3 % Sucrose, 50 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 50
529 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 3 % Sucrose.

530 50 $\mu\text{mol}/\text{m}^2/\text{s}$ B treatments, for the most part, showed higher values relating to developmental
531 features than 100 $\mu\text{mol}/\text{m}^2/\text{s}$ B (Table 1). This is likely due to malfunctioning 100 $\mu\text{mol}/\text{m}^2/\text{s}$ B
532 lights, which were repaired within a few days. However, we nonetheless attribute the delayed
533 development of 100 $\mu\text{mol}/\text{m}^2/\text{s}$ B treatments to the brief period of light malfunction.

534 2.2 Data modeling through MLP, GRNN, and ANFIS

535 Machine learning algorithms including MLP, GRNN, and ANFIS were employed to model and
536 predict cannabis shoot growth and development traits (shoot length, root length, number of nodes,

537 number of shoots, and canopy surface area) as target variables based on five input variables (Sucrose,
538 B, R, W, and Fr). R^2 , RMSE, and MBE were used to assess the prediction performance of the
539 developed machine learning algorithms (Table 2). The GRNN model presented higher R^2 as one of
540 the most important performance indices in comparison to MLP or ANFIS in both training and testing
541 processes for all shoot growth and development traits including shoot length ($R^2 > 0.96$ for GRNN vs.
542 $R^2 > 0.58$ for ANFIS or $R^2 > 0.95$ for MLP), root length ($R^2 > 0.91$ for GRNN vs. $R^2 > 0.58$ for
543 ANFIS or $R^2 > 0.89$ for MLP), number of nodes ($R^2 > 0.74$ for GRNN vs. $R^2 > 0.54$ for ANFIS or R^2
544 > 0.39 for MLP), number of shoots ($R^2 > 0.71$ for GRNN vs. $R^2 > 0.50$ for ANFIS or $R^2 > 0.42$ for
545 MLP), and canopy surface area ($R^2 > 0.94$ for GRNN vs. $R^2 > 0.64$ for ANFIS or $R^2 > 0.92$ for MLP)
546 (Table 2). Also, higher RMSE and MBE for GRNN in comparison to MLP and ANFIS for all studied
547 traits indicated that the assessed results were highly accurate and correlated, showing the good
548 performance of the developed GRNN models (Table 2). Moreover, the regression lines displayed a
549 good fit correlation between experimental and predicted data for all the shoot growth and
550 development traits in both training and testing processes (Fig. 10).

551 **2.3 Determining the importance of each input on cannabis shoot growth and development**

552 To determine the importance of each input variable on the objective function (studied parameter
553 including shoot length, root length, number of nodes, number of shoots, and canopy surface area)
554 sensitivity analysis was performed by calculating VSR. The results showed that both shoot length
555 and node number were more sensitive to Sucrose followed by B, Fr, R, and W, while root length was
556 more sensitive to Sucrose followed by R, Fr, W, and B light (Table 3). Also, the results demonstrated
557 more sensitivity of shoot number to Sucrose followed by B, red, Fr, and W color (Table 3).
558 Moreover, Sucrose > R > B > W > Fr were ranked for canopy surface area (Table 3).

559 **2.4 Optimization process via GA, SOS, ISA, and BBO**

560 In the present study, four different evolutionary optimization algorithms including BBO, ISA, SOS,
561 and GA were separately used to determine the optimal level of Sucrose, B, R, W, and Fr for
562 maximizing each fitness function (shoot length, root length, number of nodes, number of shoots, and
563 canopy surface area). Although all optimization algorithms predicted the same best fitness function
564 value, they found a different optimal level of inputs for each fitness function (Table 4). For instance,
565 the maximum shoot length (160.78 mm) would be achieved from 15.412 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 9.412
566 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 15.997 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 43.271 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 3.142 % Sucrose based on the BBO,
567 4.460 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 19.051 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 27.337 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 39.472 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr light +
568 3.157 % Sucrose based on the SOS, 0.439 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 18.494 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 36.234 $\mu\text{mol}/\text{m}^2/\text{s}$ W
569 + 33.122 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 3.319 % Sucrose based on the ISA, or 2.937 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 0.270
570 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 13.036 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 30.605 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr light + 3.505 % Sucrose based on the GA
571 (Table 4). Also, 52.563 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 84.052 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 26.262 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 22.456
572 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr light + 3.809 % Sucrose based on the BBO, 54.688 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 95.974 $\mu\text{mol}/\text{m}^2/\text{s}$ R
573 + 30.099 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 24.543 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr light + 3.664 % Sucrose based on the SOS, 44.889
574 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 99.642 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 49.994 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 24.674 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr light + 3.285%
575 Sucrose based on the ISA, or 37.646 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 83.928 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 17.507 $\mu\text{mol}/\text{m}^2/\text{s}$ W +
576 1.811 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 3.083 % Sucrose based on the GA would result in the highest canopy surface
577 area (7168.05 mm^2) (Table 4).

578 **2.5 Determining the reliability of the developed models**

579 The optimized-predicted results from each evolutionary optimization algorithm for shoot length and
580 canopy surface area as fitness functions were experimentally tested in a validation experiment to

581 evaluate the reliability of the developed models. Based on the validation experiment results, the
582 differences among evolutionary optimization algorithms (GA, ISA, BBO, and SOS) and optimized-
583 predicted results for both shoot length (Fig. 4f) and canopy surface area (Fig. 4g) were negligible,
584 which demonstrated the reliability of the developed models. However, the maximum shoot length
585 (206.76 ± 41.542 mm) (Fig. 4h) and canopy surface area (8193.49 ± 2102.624 mm²) (Fig. 4i) were
586 achieved from the GRNN-SOS, while GRNN-BBO resulted in the lowest shoot length ($181.83 \pm$
587 39.676 mm) and canopy surface area (5745.34 ± 919.848 mm²). Therefore, it seems that the SOS has
588 better performance than the other optimization algorithms.

589 3 Discussion

590 As with any *in vitro* culture system, many intrinsic (e.g., genotype, type and age of explant) and
591 extrinsic (e.g., basal salt medium, vitamins, PGRs, gelling agent, carbohydrate source, additives,
592 temperature, and light) factors influence *in vitro* shoot growth and development. Fortunately, due to
593 the highly controlled nature of plant tissue culture, most of these factors can be manipulated to
594 evaluate their impact on system optimization. Historically, micropropagation systems were refined
595 using traditional statistical models to sequentially manipulate and optimize single factors. This
596 approach often requires hundreds or even thousands of treatments to be tested, and even then
597 sequential optimization does not account for interactions and can miss the best combinations (García-
598 Pérez et al., 2020; Hameg et al., 2020). Due to the cost and time requirements, most species are
599 cultured in conditions optimized for other species with minor modifications and are not fully
600 optimized for any given application. In our study, we demonstrate that specific growth responses of
601 *in vitro* cannabis can be directed by manipulating abiotic factors such as light intensity, spectrum,
602 and exogenous carbon availability, and that machine learning approaches provide an effective
603 approach to optimize these factors for specific outcomes. It is possible that these modifications could
604 trigger developmental changes by regulating photosynthetic activity (Hdider and Desjardins, 1994),
605 or by regulating intrinsic concentrations of phytohormones (Premkumar et al., 2001). Additional
606 experiments must be completed to indicate the precise mechanisms by which dynamic physiological
607 responses occur. Ultimately, we clearly show that plant growth and development can be influenced
608 by light quality and sucrose levels in the absence of PGRs.

609 Efficient protocol development is a long-standing challenge in the field and more advanced statistical
610 models using surface response curves have been applied with some success (Niedz and Evens, 2016;
611 Niedz and Marutani-Hert, 2018; Pence et al., 2020). While these methods are more efficient than
612 sequential optimization and can account for interactions among factors, they are still limited in the
613 number of factors that can be included in a single experiment, require several assumptions to be met
614 that are often not possible to achieve, require significant numbers of treatments, and rely on relatively
615 simple interactions that can be compared using regression analyses. An alternative to address the
616 inherent complexity of plant tissue culture systems is to apply machine learning methodology. This
617 approach leverages modern computing power and developments in artificial intelligence to efficiently
618 recognize patterns in complex and disorderly datasets, typical of what is observed in plant tissue
619 culture (Hesami et al., 2021; Hesami and Jones, 2020). Machine learning algorithms can then be
620 combined with optimization algorithms to decipher complex interactions and predict theoretically
621 optimized combinations of factors for desired outcomes. The combination of machine learning and
622 optimization algorithms has the potential to overcome many of the challenges associated with
623 optimizing *in vitro* plant systems and enable development of more effective protocols using fewer
624 treatments. Ultimately, this approach can be used to change the face of plant tissue culture
625 advancements by enhancing the viability of optimization for specific species, or even individual
626 genotypes.

627 Here, ANNs (MLP and GRNN) and neuro-fuzzy logic (ANFIS) were employed and compared to
628 model and predict the effects of light quality and carbohydrate supply on growth and development of
629 *in vitro* cannabis plants. Based on our results, using the stated parameters, GRNN had better
630 performance than either MLP or ANFIS. Although there are no studies in plant tissue culture
631 comparing the predictive performances of neuro-fuzzy logic systems and ANNs, several studies in
632 other fields have demonstrated that GRNN often performs better than MLP or ANFIS. For instance,
633 Sridharan (2021) reported that the prediction accuracy of GRNN was better than MLP and ANFIS
634 for modeling and predicting global solar irradiance. Similar results were also reported by Ausati and
635 Amanollahi (2016) who showed GRNN performed better than ANFIS and MLP for modeling and
636 predicting air pollution.

637 In the present study, four evolutionary optimization algorithms (BBO, GA, ISA, and SOS) were
638 individually linked to the GRNN to determine optimal levels of Sucrose, B, R, W, and Fr for
639 maximizing each fitness function (shoot length, root length, number of nodes, number of shoots, and
640 canopy surface area). Based on mean standard errors reported in our results, there is no difference in
641 the predicted values of fitness functions among different optimization algorithms. Although the
642 results of the validation experiments showed that the differences in the performance of the
643 optimization algorithms were negligible, SOS led to the highest level of studied fitness functions. For
644 instance, GRNN-SOS showed that using the theoretical optimal combination of light quality and
645 sucrose levels, average shoot length and canopy surface area were 206.76 ± 41.542 mm and 8193.49
646 ± 2102.624 mm², respectively. Although no studies exist for using and comparing dissimilar
647 optimization algorithms for *in vitro* culture optimization, several studies previously showed that SOS
648 can be considered one of the most powerful of the evolutionary optimization approaches (Bozorg-
649 Haddad et al., 2016; Cheng and Prayogo, 2014). Bozorg-Haddad et al., (2016) compared GA and
650 SOS for optimization of reservoir operation. They run these two algorithms 10 times and reported
651 that there was no significant difference between the performances of GA and SOS, however, SOS
652 calculated higher fitness function values than GA in all 10 runs. Similar to this result, the results of
653 our validation experiment showed that SOS resulted in a higher value of fitness function in
654 comparison with other algorithms.

655 Based on the sensitivity analysis, sucrose was the most important factor for all traits studied (shoot
656 length, root length, shoot number, node number, and canopy surface area). This likely reflects the
657 mixotrophic nature of *in vitro* plants and limitations the sealed environment (depletion of CO₂, high
658 relative humidity, etc.) places upon their photosynthetic capacity (De La Viña et al., 1999; Nguyen et
659 al., 2001; Shin et al., 2013). Due to these limitations, supplemental sucrose appears to be critical to
660 support plant growth and development. It is likely that different results may be obtained if this
661 experiment were conducted using vented lids or forced air, which would improve potential
662 photosynthesis and increase the relative importance of light quality.

663 In our experiment, evolutionary optimization algorithms predicted that ~3 % sucrose would result in
664 the highest shoot growth and development. A plethora of previous studies have found 2–4 %
665 sucrose, in particular 3 % (w/v), to be optimal for various species and this has become a standard for
666 most micropropagation systems (reviewed by Yaseen et al., 2013). For instance, the results of
667 GRNN-SOS showed that 3.157 % sucrose would lead to the highest shoot length. Similar to our
668 results, Romano et al. (1995) and Baskaran and Jayabalan (2016) respectively studied different levels
669 of *in vitro* sucrose on shoot growth and development of *Quercus suber* L. and *Eclipta alba* (L.)
670 Hassk. They reported that 3% sucrose was optimal for maximizing shoot length *in vitro*. Although
671 the effect of sucrose concentration on cannabis micropropagation needs more attention, previous
672 reports generally use 3% (w/v) for shoot growth and development (reviewed by Hesami et al., 2021).

673 These results support the standard use of 3% sucrose for micropropagation, but more importantly
674 demonstrate the ability of machine learning techniques to optimize environmental factors in tissue
675 culture systems using a relatively small number of treatments.

676 Though sucrose is identified as the most important factor in plant growth and development in this
677 study, light intensity and spectrum also play important roles for *in vitro* morphogenic and
678 developmental processes (Batista et al., 2018). Different photoreceptors recognize the quality and
679 quantity of light (e.g., phytochromes absorb red and far-red, phototropins and cryptochromes absorb
680 blue light), and subsequently use this information to direct photomorphogenic functions (Li et al.,
681 2012; Parihar et al., 2016). Several studies have previously shown the impact of light quality and
682 quantity on different tissue culture systems for shoot growth and development (Hung et al., 2016),
683 somatic embryogenesis (Ferreira et al., 2017; Hesami et al., 2019), rhizogenesis (Gago et al., 2014),
684 and secondary metabolite production (Dutta Gupta and Karmakar, 2017; Silva et al., 2017).
685 However, each *in vitro* developmental stage requires a specific light condition (Batista et al., 2018).
686 Our sensitivity analysis showed that, among light treatments, B was the most important factor for
687 shoot length, shoot number, and node number, while R had the highest degree of importance on root
688 length and canopy surface area. The importance of B and R on *in vitro* shoot growth and
689 development has been previously confirmed in different plants such as *Myrtus communis* L. (Cioć et
690 al., 2018), *Plectranthus amboinicus* (Lour.) Spreng (Silva et al., 2017), *Pfaffia glomerata* (Spreng.)
691 Pedersen (Silva et al., 2020), *Achillea millefolium* L. (Alvarenga et al., 2015), and *Stevia rebaudiana*
692 Bertoni (Ramírez-Mosqueda et al., 2017).

693 Light intensity is another important parameter that should be optimized for each *in vitro* culture
694 stage. Through GRNN-SOS, the predicted optimal spectrum included 4.460 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 19.051
695 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 27.337 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 39.472 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr light + 3.157 % Sucrose to maximize
696 shoot length. In total, this provides about 50.8 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR plus 39.472 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr. In line
697 with our results, Silva et al. (2017) reported that light intensity below 51 $\mu\text{mol}/\text{m}^2/\text{s}$ resulted in the
698 highest shoot length in *P. amboinicus*. Similar results were also reported by Alvarenga et al (2015)
699 for *A. millefolium*. However, using GRNN-SOS to predict the optimal spectrum to maximize canopy
700 surface area, the conditions included 54.688 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 95.974 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 30.099 $\mu\text{mol}/\text{m}^2/\text{s}$
701 W + 24.543 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 3.664 % sucrose, for a total of 180.8 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR plus 24.5
702 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr. Alternatively, GRNN-BBO conditions included 52.563 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 84.052
703 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 26.262 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 22.465 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 3.809 % sucrose, totaling 162.9 PAR +
704 22.5 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr, which ultimately resulted in the lowest canopy surface areas. Here, the difference
705 between GRNN-SOS and GRNN-BBO relating to Fr fluence is 2.1 $\mu\text{mol}/\text{m}^2/\text{s}$, while the total
706 difference in PAR fluence is 17.9 $\mu\text{mol}/\text{m}^2/\text{s}$, 11.9 $\mu\text{mol}/\text{m}^2/\text{s}$ of which is the dissimilarity of R
707 intensity. This leads us to speculate that PAR intensity, specifically R, is an important factor
708 governing canopy development. This is confirmed with the sensitivity analysis ranking, as R is the
709 most important spectra for this growth parameter. Alternative wavelengths of light can be efficiently
710 absorbed at different depths within the leaf tissue. This can also be enhanced with increasing light
711 intensity. While certain wavelengths of green light can penetrate deeper into leaves, R and B can
712 effectively be absorbed toward the leaf surface (Zheng and Van Labeke, 2017), triggering
713 phytochrome and cryptochrome –mediated re-localization of phytohormones for photo-
714 morphogenesis (Miler and Zalewska, 2006). Although the optimal light intensity varies by species,
715 most micropropagation systems use light levels ranging from 40-80 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR (Miler et al.,
716 2019; Murphy and Adelberg, 2021; Nhut et al., 2003). However, some species perform better at
717 higher fluence rates, for example, *Actinidia deliciosa* (Gago et al., 2014), *Lippia gracilis* (Lazzarini et
718 al., 2018), and *Solanum tuberosum* (Kulchin et al., 2018). In general, cannabis is known to grow best
719 *in vivo* under higher light levels (Murphy and Adelberg, 2021; Wróbel et al., 2020), with yields

720 increasing linearly up to at least 1600 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR (Chandra et al., 2008; Lata et al., 2016;
721 Rodriguez-Morrison et al., 2021), depending on the culture system. As such, the prediction to use
722 such high light levels may reflect the nature of the species. Our validation experiment demonstrates
723 that cannabis responded to these high light levels as predicted.

724 Similarly, in our initial experiment, we observed higher intensities of R in combination with equal or
725 lower intensity of B or W to be beneficial to canopy development in the presence of 3 % Sucrose.
726 Unlike some other treatments, 50 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 50 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 3 % Sucrose, with the largest
727 canopy surface area, did not give largest averages in any additional growth parameters measured.
728 This is counterintuitive on the premise that canopy surface area is a metric of leaf size in addition to
729 leaf number. We might expect highest canopy surface area treatments to be mutually high in other
730 shoot growth parameters such as shoot length, number of nodes, or number of shoots. R significantly
731 impacts endogenous action of gibberellic acid which is involved in cell elongation, root inhibition,
732 and stimulating mitosis in meristematic cells (Manivannan et al., 2015) for replication. Gibberellic
733 acid action is known to trigger anisotropic responses for leaf expansion in monocots (Sprangers et al.,
734 2020; Xu et al., 2016), though R can impart different effects on leaf morphology for different plants
735 *in vitro*. B increased leaf thickness, leaf numbers and leaf areas compared to R, which reduced leaf
736 thickness and area in cultured *Alternanthera brasiliana* (Macedo et al., 2011). Similarly, B mutually
737 amplified leaf thickness and leaf area of *Ficus benjamina* (Zheng and Van Labeke, 2017), and
738 *Cucumis sativus in vivo*, as well as micropropagated *Solanum tuberosum* L. (Chen et al., 2020). B
739 also had a tendency to increase leaf area of *Cordyline australis* and *Sinningia speciosa in vivo* (Zheng
740 and Van Labeke, 2017). Since we observed opposite influences of B, we can speculate that
741 influences of this spectrum to be species-dependent. Wei et al. (2021) found that LED-treated hemp
742 plants produced smaller leaf areas than high pressure sodium treatments, though the LED treatments
743 with higher R:B at higher intensities produced larger leaf areas than treatments of lower R:B ratios at
744 higher or lower intensities. They also found leaf areas to bear a significantly positive correlation leaf
745 number, though no additional growth responses or treatments were significantly correlated with leaf
746 area (Wei et al., 2021). These results correspond more similarly with the data obtained in our study,
747 though it's difficult to imply for certain if *in vitro* medicinal cannabis responds to light quality and
748 intensity with the same general trend when influenced by sucrose in a sub-optimal gaseous
749 environment. It is also difficult to infer molecular mechanisms for such *in vitro* plant responses, since
750 they are beyond the scope of the presented study. Thus, subsequent experiments should be devised to
751 test molecular mechanisms of the growth parameters measured to further elucidate the molecular
752 devices contributing to the factors observed.

753 Our preliminary experiment also indicated shade avoidance-like responses observed when comparing
754 R + B + Fr + 3 % Sucrose treatments at different light intensities. Higher light intensity generally
755 produced shorter shoots with more nodes versus longer shoots with fewer nodes when irradiance was
756 lower. At higher light intensities, R + B + Fr + 6 % Sucrose specimens also averaged longer stems
757 with more nodes than at 1% Sucrose, though at low light intensity R + B + Fr + 1 % Sucrose grew
758 longer stems with more nodes than 6 % plants of the same light treatment. These observations imply
759 that that there is a complex interaction between sugar and light signaling whereby the impact of sugar
760 can allow plants to dynamically adjust to higher light intensities (Tichá et al., 1998), or impede
761 certain physiological responses when exogenous carbon is too high and abiotic factors are sub-
762 optimal (Roh and Choi, 2004). However, in all cases, greatest averages were achieved with 3 %
763 sucrose, which suggests that sugar concentrations above 4 % and below 2 % can have diminishing
764 returns on shoot development and number (Sivanesan and Park, 2015). This observation supports the
765 widespread practice of using 3% sucrose in plant tissue culture systems, and the results of our
766 sensitivity analysis. Gago et al. (2014) modeled 14 growth parameters of *in vitro* kiwifruit based on

767 Sucrose concentration and irradiance, using Neuro-fuzzy logic. They found an *in vitro* sucrose
768 concentration of 2.3 % or higher to be indispensable in achieving many of the optimal growth
769 parameters investigated, either independently or in interaction with light intensity. Dynamic
770 interactions between light and exogenous sugar are important for evoking many additional
771 physiological responses relating to light attenuation and metabolism (Gago et al., 2014; Roh and
772 Choi, 2004; Tichá et al., 1998).

773 Lalge et al. (2017) observed that taller cannabis clones developed with W compared to B + R LEDs
774 when grown in controlled climates. The optimal levels of R:Fr in promoting stem elongation has
775 been well documented (Ballaré and Pierik, 2017; Ma and Li, 2019; Trupkin et al., 2014). Though B
776 also impacts stem growth (Ma and Li, 2019; Magagnini et al., 2018; Snowden et al., 2016), it can
777 sometimes have the opposite influence of R:Fr, resulting in more compact phenotypes (Magagnini et
778 al., 2018). The optimized combinations R:Fr in addition to B could have ultimately impacted shoot
779 elongation of the treatments assessed (Cope and Bugbee, 2013). Emission of low B from warm W
780 LEDs can amplify stem elongation and leaf expansion, while high B from cold W LEDs can have the
781 opposite effect, resulting in more compact specimens. Results from our preliminary experiment
782 provide evidence that appropriate levels of R:Fr can greatly influence stem elongation to a greater
783 degree than B (Magagnini et al., 2018). The reduction of photosynthetically active radiation resulting
784 from shading limits the amount of R, B, and Fr received by the canopy, though the degree of R
785 reduction tends to be far greater than that of Fr (Xu et al., 2020). Hence, low irradiance and
786 wavelength perception work mutually to allow shoot elongation, perhaps in combination with the
787 influence of exogenous sucrose. In agreement with these principles, the predicted optimal conditions
788 for shoot elongation included low R:Fr ratios, including Fr intensities ranging from 30.6 – 43.3
789 $\mu\text{mol}/\text{m}^2/\text{s}$ and R between 0.3 – 19.1 $\mu\text{mol}/\text{m}^2/\text{s}$. Likewise, shoot elongation was maximized under
790 relatively low PAR light levels from 15.7 – 80.8 $\mu\text{mol}/\text{m}^2/\text{s}$, while canopy area and number of nodes
791 were predicted to be greater with low levels of Fr (1.8 – 24.7 $\mu\text{mol}/\text{m}^2/\text{s}$ and 8.8 – 19.9 $\mu\text{mol}/\text{m}^2/\text{s}$,
792 respectively) and higher PAR (139.1 – 194.5 $\mu\text{mol}/\text{m}^2/\text{s}$, and 150.3 – 203.8 $\mu\text{mol}/\text{m}^2/\text{s}$, respectively)
793 fluence rates. As with previous literature, it appears that *in vitro* cannabis plants produce longer
794 stems with fewer nodes and more narrow leaves when cultured at low light levels and higher amounts
795 of Fr. These results demonstrate that *in vitro* cannabis plants respond to light signals similar to what
796 would be expected *in vivo*. Further, the ability of machine learning and optimization algorithms to
797 make predictions that agree with the general body of literature further supports the ability to
798 recognize complex patterns using relatively quickly with few treatments. Thus, balances between
799 alternative light spectra, their intensities and exogenously supplied carbohydrates are critical factors
800 determining the outcome of many plantlet responses *in vitro*.

801 The effect of light quantity and quality studied together *in vitro* has been perused for many years in
802 micropropagation but have been hampered due to the limitations of lighting systems and difficulties
803 in proper replication (Kim et al., 2004; Miler et al., 2019; Tanaka et al., 1998). Many previous
804 experiments explored the influences of single or binary combinations light spectra and their
805 intensities on *in vitro* plantlet development (Lian et al., 2002; Manivannan et al., 2015; Shukla et al.,
806 2017). Our study enlists novel LED technology combined with machine learning and optimization
807 algorithms in an innovative system that assesses a vast assortment of sucrose concentrations and the
808 cumulative impact of four different light qualities at a wide array of intensities to devise precision
809 tissue culture protocols. Furthermore, for the first time, we suggest a superior machine learning and
810 optimization algorithm approach for future plant tissue culture studies. Additionally, the results of the
811 preliminary experiment exemplify that specific growth responses of *in vitro* cannabis can be directed
812 by manipulating abiotic factors such as light intensity and quality in addition to exogenous carbon
813 availability. This is further demonstrated by the results of the validation experiment. Such discoveries

814 have valuable implications for the development of cannabis tissue culture techniques in the absence
815 of PGRs.

816 **Conclusion**

817 This machine learning –assisted, multivariable micropropagation study has demonstrated that distinct
818 growth responses in cannabis can be shaped by changing the influences of sugar and light dynamics
819 in the absence of PGRs. The development of alternative protocols to guide plant growth toward
820 specific responses shows endless value for numerous *in vitro* applications. For instance, protocols to
821 induce long stems, large internodes, many nodes, or many stems could be implemented when
822 growing cultures for clonal propagation and sub-culturing, while cultures developing large root
823 masses and large canopies could very well be more suited for *ex vitro* transfer. In addition,
824 culmination of the protocols devised could be implemented, perhaps to trigger different
825 developmental responses during different growth phases. Finally, the results obtained from this
826 experiment allows us to recommend GRNN-SOS to be a more efficacious algorithm to study
827 dynamic plant responses to multivariable stimuli *in vitro* for development of new methods, and
828 optimization of current protocols. Rather than using traditional statistics to evaluate large datasets for
829 making optimization predictions for tissue culture applications, the use of effective machine learning
830 strategies for optimization of *in vitro* protocols should further be assessed as an alternative, or in
831 combination with traditional statistical approaches to allow precision tissue culture practices.

832 **Conflict of Interest**

833 The authors declare that the research was conducted in the absence of any commercial or financial
834 relationships that could be construed as a potential conflict of interest.

835 **Author Contributions**

836 MP, MH, and AMPJ conceived and designed the study, MP and MH conducted the experiments and
837 analysed the data, AMPJ administrated the project and acquired funding, MP, MH, FS, and AMPJ
838 wrote the manuscript. All authors approved the manuscript for publication.

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842 **Data Availability Statement**

843 All processed data are available within the manuscript.

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846 **Reference**

- 847 Al-Mayahi, A.M.W., 2016. Effect of red and blue light emitting diodes “CRB-LED” on *in vitro* organogenesis of date
848 palm (*Phoenix dactylifera* L.) cv. Alshakr. *World J. Microbiol. Biotechnol.* 32, 1–8. [https://doi.org/10.1007/s11274-](https://doi.org/10.1007/s11274-016-2120-6)
849 016-2120-6
- 850 Alvarenga, I.C.A., Pacheco, F.V., Silva, S.T., Bertolucci, S.K.V., Pinto, J.E.B.P., 2015. *In vitro* culture of *Achillea*

- 851 millefolium L.: quality and intensity of light on growth and production of volatiles. *Plant Cell. Tissue Organ Cult.*
852 122, 299–308. <https://doi.org/10.1007/s11240-015-0766-7>
- 853 Andre, C.M., Hausman, J.F., Guerriero, G., 2016. *Cannabis sativa*: The plant of the thousand and one molecules. *Front.*
854 *Plant Sci.* 7, 1–17. <https://doi.org/10.3389/fpls.2016.00019>
- 855 Arigita, L., González, A., Sánchez Tamés, R., 2002. Influence of CO₂ and sucrose on photosynthesis and transpiration of
856 *Actinidia deliciosa* explants cultured in vitro. *Physiol. Plant.* 115, 166–173. <https://doi.org/10.1034/j.1399-3054.2002.1150119.x>
857
- 858 Ausati, S., Amanollahi, J., 2016. Assessing the accuracy of ANFIS, EEMD-GRNN, PCR, and MLR models in predicting
859 PM_{2.5}. *Atmos. Environ.* 142, 465–474. <https://doi.org/10.1016/j.atmosenv.2016.08.007>
- 860 Ayuso, M., García-Pérez, P., Ramil-Rego, P., Gallego, P.P., Barreal, M.E., 2019. In vitro culture of the endangered plant
861 *Eryngium viviparum* as dual strategy for its ex situ conservation and source of bioactive compounds. *Plant Cell.*
862 *Tissue Organ Cult.* 138, 427–435. <https://doi.org/10.1007/s11240-019-01638-y>
- 863 Ballaré, C.L., Pierik, R., 2017. The shade-avoidance syndrome: Multiple signals and ecological consequences. *Plant Cell*
864 *Environ.* 40, 2530–2543. <https://doi.org/10.1111/pce.12914>
- 865 Barrus, D.G., Capogrossi, K.L., Cates, S.C., Gourdet, C.K., Peiper, N.C., Novak, S.P., Lefever, T.W., Wiley, J.L., 2017.
866 Tasty THC: Promises and Challenges of Cannabis Edibles. *Physiol. Behav.* 176, 139–148.
- 867 Baskaran, P., Jayabalan, N., 2016. Role of Basal Media, Carbon Sources and Growth Regulators in Micropropagation of
868 Two Valuable Medicinal Orchids of Bangladesh. *Int. J. Sci. Res.* 5, 1022–1026.
869 <https://doi.org/10.21275/v5i6.nov164303>
- 870 Batista, D.S., Felipe, S.H.S., Silva, T.D., de Castro, K.M., Mamedes-Rodrigues, T.C., Miranda, N.A., Ríos-Ríos, A.M.,
871 Faria, D.V., Fortini, E.A., Chagas, K., Torres-Silva, G., Xavier, A., Arencibia, A.D., Otoni, W.C., 2018. Light
872 quality in plant tissue culture: does it matter? *Vitr. Cell. Dev. Biol. - Plant* 54, 195–215.
873 <https://doi.org/10.1007/s11627-018-9902-5>
- 874 Bello-Bello, J.J., Martinez-Estrada, E., Caamal-Velazquez, J.H., Morales-Ramos, V., 2016. Effect of LED light quality
875 on in vitro shoot proliferation and growth of vanilla (*Vanilla planifolia* Andrews). *African J. Biotechnol.* 15, 272–
876 277. <https://doi.org/10.5897/ajb2015.14662>
- 877 Bozorg-Haddad, O.B., Hosseini-Moghari, S.-M., Loáiciga, H.A., 2016. Biogeography-Based Optimization Algorithm for
878 Optimal Operation of Reservoir Systems. *J. Water Resour. Plan. Manag.* 142, 04015034.
879 [https://doi.org/10.1061/\(asce\)wr.1943-5452.0000558](https://doi.org/10.1061/(asce)wr.1943-5452.0000558)
- 880 Burgel, L., Hartung, J., Schibano, D., Graeff-Hönninger, S., 2020. Impact of Different Phytohormones on Morphology,
881 Yield and Cannabinoid Content of *Cannabis sativa* L. *Plants* 9, 1–16.
- 882 Chandra, S., Lata, H., ElSohly, M.A., 2020. Propagation of *Cannabis* for Clinical Research: An Approach Towards a
883 Modern Herbal Medicinal Products Development. *Front. Plant Sci.* 11, 1–10.
884 <https://doi.org/10.3389/fpls.2020.00958>
- 885 Chandra, S., Lata, H., Khan, I.A., Elsohly, M.A., 2008. Photosynthetic response of *Cannabis sativa* L. to variations in
886 photosynthetic photon flux densities, temperature and CO₂ conditions. *Physiol. Mol. Biol. Plants* 14, 299–306.
887 <https://doi.org/10.1007/s12298-008-0027-x>
- 888 Chaohua, C., Gonggu, Z., Lining, Z., Chunsheng, G., Qing, T., Jianhua, C., Xinbo, G., Dingxiang, P., Jianguang, S.,
889 2016. A rapid shoot regeneration protocol from the cotyledons of hemp (*Cannabis sativa* L.). *Ind. Crops Prod.* 83,
890 61–65. <https://doi.org/10.1016/j.indcrop.2015.12.035>
- 891 CHEN, L. li, ZHANG, K., GONG, X. chen, WANG, H. ying, GAO, Y. hui, WANG, X. quan, ZENG, Z. hai, HU, Y. gao,
892 2020. Effects of different LEDs light spectrum on the growth, leaf anatomy, and chloroplast ultrastructure of potato
893 plantlets in vitro and minituber production after transplanting in the greenhouse. *J. Integr. Agric.* 19, 108–119.

- 894 [https://doi.org/10.1016/S2095-3119\(19\)62633-X](https://doi.org/10.1016/S2095-3119(19)62633-X)
- 895 Cheng, M.Y., Prayogo, D., 2014. Symbiotic Organisms Search: A new metaheuristic optimization algorithm. *Comput.*
896 *Struct.* 139, 98–112. <https://doi.org/10.1016/j.compstruc.2014.03.007>
- 897 Cioć, M., Szewczyk, A., Żupnik, M., Kalisz, A., Pawłowska, B., 2018. LED lighting affects plant growth, morphogenesis
898 and phytochemical contents of *Myrtus communis* L. in vitro. *Plant Cell. Tissue Organ Cult.* 132, 433–447.
899 <https://doi.org/10.1007/s11240-017-1340-2>
- 900 Cope, K.R., Bugbee, B., 2013. Spectral effects of three types of white light-emitting diodes on plant growth and
901 development: Absolute versus relative amounts of blue light. *HortScience* 48, 504–509.
902 <https://doi.org/10.21273/hortsci.48.4.504>
- 903 De La Viña, G., Pliego-Alfaro, F., Driscoll, S.P., Mitchell, V.J., Parry, M.A., Lawlor, D.W., 1999. Effects of CO₂ and
904 sugars on photosynthesis and composition of avocado leaves grown in vitro. *Plant Physiol. Biochem.* 37, 587–595.
905 [https://doi.org/10.1016/S0981-9428\(00\)80111-4](https://doi.org/10.1016/S0981-9428(00)80111-4)
- 906 Dutta Gupta, S., Karmakar, A., 2017. Machine vision based evaluation of impact of light emitting diodes (LEDs) on
907 shoot regeneration and the effect of spectral quality on phenolic content and antioxidant capacity in *Swertia chirata*.
908 *J. Photochem. Photobiol. B Biol.* 174, 162–172. <https://doi.org/10.1016/j.jphotobiol.2017.07.029>
- 909 Eckstein, A., Zie, P., Gabrys, H., 2012a. Sugar and Light Effects on the Condition of the Photosynthetic Apparatus of
910 *Arabidopsis thaliana* Cultured in vitro. *J. Plant Growth Regul.* 13, 90–101. <https://doi.org/10.1007/s00344-011-9222-z>
911
- 912 Eckstein, A., Zieba, P., Gabrys, H., 2012b. Sugar and Light Effects on the Condition of the Photosynthetic Apparatus of
913 *Arabidopsis thaliana* Cultured in vitro. *J. Plant Growth Regul.* 31, 90–101. <https://doi.org/10.1007/s00344-011-9222-z>
914
- 915 ElSohly, M.A., Gul, W., 2014. Constituents of *Cannabis Sativa*, in: *Handbook of Cannabis*. pp. 1–22.
916 <https://doi.org/10.1093/acprof>
- 917 Fanga, W., Lee, Y.I., Chen, C.C., Chang, M.Y., 2011. Development of LED lids for tissue culture lighting. *Acta Hortic.*
918 907, 397–402. <https://doi.org/10.17660/ActaHortic.2011.907.67>
- 919 Fathordoobady, F., Singh, A., Kitts, D.D., Pratap Singh, A., 2019. Hemp (*Cannabis Sativa* L.) Extract: Anti-Microbial
920 Properties, Methods of Extraction, and Potential Oral Delivery. *Food Rev. Int.* 35, 664–684.
921 <https://doi.org/10.1080/87559129.2019.1600539>
- 922 Ferreira, L.T., de Araújo Silva, M.M., Ulisses, C., Camara, T.R., Willadino, L., 2017. Using LED lighting in somatic
923 embryogenesis and micropropagation of an elite sugarcane variety and its effect on redox metabolism during
924 acclimatization. *Plant Cell. Tissue Organ Cult.* 128, 211–221. <https://doi.org/10.1007/s11240-016-1101-7>
- 925 Fontana, D.C., Becker, C.E., Pinheiro, M.V.M., Pretto, M.M., Dos Santos, J., Caron, B.O., Schmidt, D., 2019. Impact of
926 light quality on the physiological characteristics of capsicum chinense seeds. *Adv. Hortic. Sci.* 33, 235–243.
927 <https://doi.org/10.13128/ahs-22792>
- 928 Gago, J., Martínez-Núñez, L., Landín, M., Flexas, J., Gallego, P.P., 2014. Modeling the effects of light and sucrose on in
929 vitro propagated plants: A multiscale system analysis using artificial intelligence technology. *PLoS One* 9.
930 <https://doi.org/10.1371/journal.pone.0085989>
- 931 Gandomi, A.H., 2014. Interior search algorithm (ISA): A novel approach for global optimization. *ISA Trans.* 53, 1168–
932 1183. <https://doi.org/10.1016/j.isatra.2014.03.018>
- 933 García-Pérez, P., Lozano-Milo, E., Landin, M., Gallego, P.P., 2020. Machine Learning Unmasked Nutritional Imbalances
934 on the Medicinal Plant *Bryophyllum* sp. Cultured in vitro. *Front. Plant Sci.* 11, 1–14.
935 <https://doi.org/10.3389/fpls.2020.576177>

- 936 Hameg, R., Arteta, T.A., Landin, M., Gallego, P.P., Barreal, M.E., 2020. Modeling and Optimizing Culture Medium
937 Mineral Composition for in vitro Propagation of *Actinidia arguta*. *Front. Plant Sci.* 11.
938 <https://doi.org/10.3389/fpls.2020.554905>
- 939 Hdidier, C., Desjardins, Y., 1994. Effects of sucrose on photosynthesis and phosphoenolpyruvate carboxylase activity of
940 in vitro cultured strawberry plantlets. *Plant Cell. Tissue Organ Cult.* 36, 27–33.
941 <https://doi.org/10.1007/BF00048312>
- 942 Hesami, M., Baiton, A., Alizadeh, M., Pepe, M., Torkamaneh, D., Maxwell, A., Jones, P., 2021a. Advances and
943 Perspectives in Tissue Culture and Genetic Engineering of Cannabis.
- 944 Hesami, M., Jones, A.M.P., 2020. Application of artificial intelligence models and optimization algorithms in plant cell
945 and tissue culture. *Appl. Microbiol. Biotechnol.* 104, 9449–9485. <https://doi.org/10.1007/s00253-020-10888-2>
- 946 Hesami, M., Maxwell, A., Jones, P., Maxwell, A., Jones, P., 2021b. Modeling and optimizing callus growth and
947 development in *Cannabis sativa* using random forest and support vector machine in combination with a genetic
948 algorithm.
- 949 Hesami, M., Naderi, R., Tohidfar, M., Yoosefzadeh-Najafabadi, M., 2019. Application of adaptive neuro-fuzzy inference
950 system-non-dominated sorting genetic algorithm-II (ANFIS-NSGAI) for modeling and optimizing somatic
951 embryogenesis of chrysanthemum. *Front. Plant Sci.* 10, 1–12. <https://doi.org/10.3389/fpls.2019.00869>
- 952 Hesami, M., Pepe, M., Alizadeh, M., Rakei, A., Baiton, A., Phineas Jones, A.M., 2020. Recent advances in cannabis
953 biotechnology. *Ind. Crops Prod.* 158, 113026. <https://doi.org/10.1016/j.indcrop.2020.113026>
- 954 Hesami, M., Pepe, M., Monthony, A.S., Baiton, A., Phineas Jones, A.M., 2021c. Modeling and optimizing in vitro seed
955 germination of industrial hemp (*Cannabis sativa* L.). *Ind. Crops Prod.* 170, 1–12.
956 <https://doi.org/10.1016/j.indcrop.2021.113753>
- 957 Holland, J.H., 1992. *Genetic Algorithms* 267, 66–73.
- 958 Hosseini-Moghari, S.M., Morovati, R., Moghadas, M., Araghinejad, S., 2015. Optimum operation of reservoir using two
959 evolutionary algorithms: Imperialist competitive algorithm (ICA) and cuckoo optimization algorithm (COA). *Water*
960 *Resour. Manag.* 29, 3749–3769. <https://doi.org/10.1007/s11269-015-1027-6>
- 961 Hung, C.D., Hong, C.H., Kim, S.K., Lee, K.H., Park, J.Y., Nam, M.W., Choi, D.H., Lee, H.I., 2016. LED light for in
962 vitro and ex vitro efficient growth of economically important highbush blueberry (*Vaccinium corymbosum* L.).
963 *Acta Physiol. Plant.* 38. <https://doi.org/10.1007/s11738-016-2164-0>
- 964 Jafari, M., Shahsavari, A., 2020. The application of artificial neural networks in modeling and predicting the effects of
965 melatonin on morphological responses of citrus to drought stress. *PLoS One* 15, 1–17.
966 <https://doi.org/10.1371/journal.pone.0240427>
- 967 Jha, A., Bansal, Y.K., 2012. Induction of photoautotrophy in *Chlorophytum borivilianum* Sant . et Fernand , regenerated
968 in vitro 4, 26–31.
- 969 Kim, S.J., Hahn, E.J., Heo, J.W., Paek, K.Y., 2004. Effects of LEDs on net photosynthetic rate, growth and leaf stomata
970 of chrysanthemum plantlets in vitro. *Sci. Hortic. (Amsterdam)*. 101, 143–151.
971 <https://doi.org/10.1016/j.scienta.2003.10.003>
- 972 Kozai, T., Oki, H., Pujiwara, K., 1987. Effects of CO₂ Enrichment and Sucrose Concentration Under High Photon Fluxes
973 on Plantlet Growth of Carnation (*Dianthus caryophyllus* L.) in Tissue Culture During the Preparation Stage. *J.*
974 *Japanese Soc. Hortic. Sci.* 57, 279–288. <https://doi.org/10.2503/jjshs.57.279>
- 975 Kulchin, Y.N., Nakonechnaya, O.V., Gafitskaya, I.V., Grishchenko, O.V., Epifanova, T.Y., Orlovskaya, I.Y., Zhuravlev,
976 Y.N., Subbotin, E.P., 2018. Plant morphogenesis under different light intensity. *Defect Diffus. Forum* 386 DDF,
977 201–206. <https://doi.org/10.4028/www.scientific.net/DDF.386.201>

- 978 Lalge, A., Cerny, P., Trojan, V., Vyhnanek, T., 2017. The effects of red, blue and white light on the growth and
979 development of *Cannabis sativa* L. *Mendel Net* 2017 646–651.
- 980 Lata, H., Chandra, S., Techen, N., Khan, I.A., ElSohly, M.A., 2016. In vitro mass propagation of *Cannabis sativa* L.: A
981 protocol refinement using novel aromatic cytokinin meta-topolin and the assessment of eco-physiological,
982 biochemical and genetic fidelity of micropropagated plants. *J. Appl. Res. Med. Aromat. Plants* 3, 18–26.
983 <https://doi.org/10.1016/j.jarmap.2015.12.001>
- 984 Lazzarini, L.E.S., Bertolucci, S.K.V., Pacheco, F.V., dos Santos, J., Silva, S.T., de Carvalho, A.A., Pinto, J.E.B.P., 2018.
985 Quality and intensity of light affect *Lippia gracilis* Schauer plant growth and volatile compounds in vitro. *Plant*
986 *Cell. Tissue Organ Cult.* 135, 367–379. <https://doi.org/10.1007/s11240-018-1470-1>
- 987 Legris, M., Ince, Y.Ç., Fankhauser, C., 2019. Molecular mechanisms underlying phytochrome-controlled morphogenesis
988 in plants. *Nat. Commun.* 10. <https://doi.org/10.1038/s41467-019-13045-0>
- 989 Lembrechts, R., Verdoodt, V., De Proft, M.P., Ceusters, J., 2015. Influence of sucrose concentration on photosynthetic
990 performance of *Guzmania* “Hilda” in vitro. *Acta Hortic.* 1083, 403–408.
991 <https://doi.org/10.17660/ActaHortic.2015.1083.51>
- 992 Li, H., Xu, Z., Tang, C., 2010. Effect of light-emitting diodes on growth and morphogenesis of upland cotton (*Gossypium*
993 *hirsutum* L.) plantlets in vitro. *Plant Cell. Tissue Organ Cult.* 103, 155–163. <https://doi.org/10.1007/s11240-010-9763-z>
- 995 Li, L., Ljung, K., Breton, G., Schmitz, R.J., Pruneda-Paz, J., Cowing-Zitron, C., Cole, B.J., Ivans, L.J., Pedmale, U. V.,
996 Jung, H.S., Ecker, J.R., Kay, S.A., Chory, J., 2012. Linking photoreceptor excitation to changes in plant
997 architecture. *Genes Dev.* 26, 785–790. <https://doi.org/10.1101/gad.187849.112>
- 998 Lian, M.L., Murthy, H.N., Paek, K.Y., 2002. Effects of light emitting diodes (LEDs) on the in vitro induction and growth
999 of bulblets of *Lilium* oriental hybrid “Pesaro.” *Sci. Hortic. (Amsterdam)*. 94, 365–370.
1000 [https://doi.org/10.1016/S0304-4238\(01\)00385-5](https://doi.org/10.1016/S0304-4238(01)00385-5)
- 1001 Ma, L., Li, G., 2019. Auxin-dependent cell elongation during the shade avoidance response. *Front. Plant Sci.* 10, 1–8.
1002 <https://doi.org/10.3389/fpls.2019.00914>
- 1003 Macedo, A.F., Leal-Costa, M.V., Tavares, E.S., Lage, C.L.S., Esquibel, M.A., 2011. The effect of light quality on leaf
1004 production and development of in vitro-cultured plants of *Alternanthera brasiliana* Kuntze. *Environ. Exp. Bot.* 70,
1005 43–50. <https://doi.org/10.1016/j.envexpbot.2010.05.012>
- 1006 Magagnini, G., Grassi, G., Kotiranta, S., 2018. The Effect of Light Spectrum on the Morphology and Cannabinoid
1007 Content of *Cannabis sativa* L. *Med. Cannabis Cannabinoids* 1, 19–27. <https://doi.org/10.1159/000489030>
- 1008 Majada, J.P., Tadeo, F., Fal, M.A., Sánchez-Tamés, R., 2000. Impact of culture vessel ventilation on the anatomy and
1009 morphology of micropropagated carnation. *Plant Cell. Tissue Organ Cult.* 63, 207–214.
1010 <https://doi.org/10.1023/A:1010650131732>
- 1011 Manivannan, A., Soundararajan, P., Halimah, N., Ko, C.H., Jeong, B.R., 2015. Blue LED light enhances growth,
1012 phytochemical contents, and antioxidant enzyme activities of *Rehmannia glutinosa* cultured in vitro. *Hortic.*
1013 *Environ. Biotechnol.* 56, 105–113. <https://doi.org/10.1007/s13580-015-0114-1>
- 1014 Miler, N., Kulus, D., Woźny, A., Rymarz, D., Hajzer, M., Wierzbowski, K., Nelke, R., Szeffs, L., 2019. Application of
1015 wide-spectrum light-emitting diodes in micropropagation of popular ornamental plant species: a study on plant
1016 quality and cost reduction. *Vitr. Cell. Dev. Biol. - Plant* 55, 99–108. <https://doi.org/10.1007/s11627-018-9939-5>
- 1017 Miler, N., Zalewska, M., 2006. The influence of light colour on micropropagation of chrysanthemum. *Acta Hortic.* 725 I,
1018 347–350. <https://doi.org/10.17660/ActaHortic.2006.725.44>
- 1019 Moher, M., Jones, M., Zheng, Y., 2020. Photoperiodic Response of in vitro *Cannabis sativa* Plants. *Hort Sci.* 1689–1699.
1020 <https://doi.org/10.1017/CBO9781107415324.004>

- 1021 Mokhtari Fard, M., Noroozian, R., Molaei, S., 2012. Determining the optimal placement and capacity of DG in intelligent
1022 distribution networks under uncertainty demands by COA. 2012 2nd Iran. Conf. Smart Grids, ICSG 2012.
- 1023 Moravej, M., Hosseini-Moghari, S.M., 2016. Large Scale Reservoirs System Operation Optimization: the Interior Search
1024 Algorithm (ISA) Approach. *Water Resour. Manag.* 30, 3389–3407. <https://doi.org/10.1007/s11269-016-1358-y>
- 1025 Movahedi, M., Torabi, S., 2015. The effect of different concentrations of TDZ and BA on in vitro regeneration of Iranian
1026 cannabis (*Cannabis sativa*) using cotyledon and epicotyl explants. *J. Plant Mol. Breed.* 3, 20–27.
- 1027 Murphy, R., Adelberg, J., 2021. Physical factors increased quantity and quality of micropropagated shoots of *Cannabis*
1028 *sativa* L. in a repeated harvest system with ex vitro rooting. *Vitr. Cell. Dev. Biol. - Plant.*
1029 <https://doi.org/10.1007/s11627-021-10166-4>
- 1030 Nathiya, S., Pradeepa, D., Devasena, T., Senthil, K., 2013. Studies on the effect of sucrose, light and hormones on
1031 micropropagation and in vitro flowering of *Withania somnifera* var. Jawahar-20. *J. Anim. Plant Sci.* 23, 1391–
1032 1397.
- 1033 Nguyen, Q.T., Kozai, T., Heo, J., Xuan Thai, D., 2001. Photoautotrophic growth response of in vitro cultured coffee
1034 plantlets to ventilation methods and photosynthetic photon fluxes under carbon dioxide enriched condition. *Plant*
1035 *Cell. Tissue Organ Cult.* 66, 217–225. <https://doi.org/10.1023/A:1010662413486>
- 1036 Nhut, D.T., Takamura, T., Watanabe, H., Okamoto, K., Tanaka, M., 2003. Responses of strawberry plantlets cultured in
1037 vitro under superbright red and blue light-emitting diodes (LEDs). *Plant Cell. Tissue Organ Cult.* 73, 43–52.
1038 <https://doi.org/10.1023/A:1022638508007>
- 1039 Niazian, M., Niedbała, G., 2020. Machine learning for plant breeding and biotechnology. *Agric.* 10, 1–23.
1040 <https://doi.org/10.3390/agriculture10100436>
- 1041 Niedz, R.P., Evens, T.J., 2016. Design of experiments (DOE)—history, concepts, and relevance to in vitro culture. *Vitr.*
1042 *Cell. Dev. Biol. - Plant* 52, 547–562. <https://doi.org/10.1007/s11627-016-9786-1>
- 1043 Niedz, R.P., Marutani-Hert, M., 2018. A filter paper-based liquid culture system for citrus shoot organogenesis—a
1044 mixture-amount plant growth regulator experiment. *Vitr. Cell. Dev. Biol. - Plant* 54, 658–671.
1045 <https://doi.org/10.1007/s11627-018-9940-z>
- 1046 Parihar, P., Singh, R., Singh, S., Tripathi, D.K., Chauhan, D.K., Singh, V.P., Prasad, S.M., 2016. Photoreceptors mapping
1047 from past history till date. *J. Photochem. Photobiol. B Biol.* 162, 223–231.
1048 <https://doi.org/10.1016/j.jphotobiol.2016.06.020>
- 1049 Pence, V.C., Finke, L.R., Niedz, R.P., 2020. Evaluating a DOE screen to reduce hyperhydricity in the threatened plant,
1050 *Cycladenia humilis* var. *jonesii*. *Vitr. Cell. Dev. Biol. - Plant* 56, 215–229. <https://doi.org/10.1007/s11627-019-10038-y>
- 1052 Premkumar, A., Mercado, J.A., Quesada, M.A., 2001. Effects of in vitro tissue culture conditions and acclimatization on
1053 the contents of Rubisco, leaf soluble proteins, photosynthetic pigments, and C/N ratio. *J. Plant Physiol.* 158, 835–
1054 840. <https://doi.org/10.1078/0176-1617-00214>
- 1055 Ramírez-Mosqueda, M.A., Iglesias-Andreu, L.G., Bautista-Aguilar, J.R., 2017. The Effect of Light Quality on Growth
1056 and Development of In Vitro Plantlet of *Stevia rebaudiana* Bertoni. *Sugar Tech* 19, 331–336.
1057 <https://doi.org/10.1007/s12355-016-0459-5>
- 1058 Rodriguez-Morrison, V., Llewellyn, D., Zheng, Y., 2021. Cannabis Yield, Potency, and Leaf Photosynthesis Respond
1059 Differently to Increasing Light Levels in an Indoor Environment. *Front. Plant Sci.* 12, 1–16.
1060 <https://doi.org/10.3389/fpls.2021.646020>
- 1061 Roh, K.W., Choi, B.Y., 2004. Sucrose Regulates Growth and Activation of Rubisco in Tobacco Leaves In Vitro.
1062 *Biotechnol. Bioprocess Eng.* 229–235.

- 1063 Romano, A., Noronha, C., Martins-Loução, M.A., 1995. Role of carbohydrates in micropropagation of cork oak. *Plant*
1064 *Cell. Tissue Organ Cult.* 40, 159–167. <https://doi.org/10.1007/BF00037670>
- 1065 Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T., Eliceiri, K.W., 2017. ImageJ2:
1066 ImageJ for the next generation of scientific image data. *BMC Bioinformatics* 18, 1–26.
1067 <https://doi.org/10.1186/s12859-017-1934-z>
- 1068 Sandler, L.N., Beckerman, J.L., Whitford, F., Gibson, K.A., 2019. Cannabis as conundrum. *Crop Prot.* 117, 37–44.
1069 <https://doi.org/10.1016/j.cropro.2018.11.003>
- 1070 Shin, K.S., Park, S.Y., Paek, K.Y., 2013. Sugar metabolism, photosynthesis, and growth of in vitro plantlets of
1071 *Doritaenopsis* under controlled microenvironmental conditions. *Vitr. Cell. Dev. Biol. - Plant* 49, 445–454.
1072 <https://doi.org/10.1007/s11627-013-9524-x>
- 1073 Shukla, M.R., Singh, A.S., Piunno, K., Saxena, P.K., Jones, A.M.P., 2017. Application of 3D printing to prototype and
1074 develop novel plant tissue culture systems. *Plant Methods* 13, 1–10. <https://doi.org/10.1186/s13007-017-0156-8>
- 1075 Silva, S.T., Bertolucci, S.K.V., da Cunha, S.H.B., Lazzarini, L.E.S., Tavares, M.C., Pinto, J.E.B.P., 2017. Effect of light
1076 and natural ventilation systems on the growth parameters and carvacrol content in the in vitro cultures of
1077 *Plectranthus amboinicus* (Lour.) Spreng. *Plant Cell. Tissue Organ Cult.* 129, 501–510.
1078 <https://doi.org/10.1007/s11240-017-1195-6>
- 1079 Silva, T.D., Batista, D.S., Fortini, E.A., Castro, K.M. de, Felipe, S.H.S., Fernandes, A.M., Sousa, R.M. de J., Chagas, K.,
1080 Silva, J.V.S. da, Correia, L.N. de F., Farias, L.M., Leite, J.P.V., Rocha, D.I., Otoni, W.C., 2020. Blue and red light
1081 affects morphogenesis and 20-hydroxyecdisonone content of in vitro *Pfaffia glomerata* accessions. *J. Photochem.*
1082 *Photobiol. B Biol.* 203, 111761. <https://doi.org/10.1016/j.jphotobiol.2019.111761>
- 1083 Simon, D., 2008. Biogeography-based optimization. *IEEE Trans. Evol. Comput.* 12, 702–713.
1084 <https://doi.org/10.1109/TEVC.2008.919004>
- 1085 Sivanesan, I., Park, S.W., 2015. Optimizing factors affecting adventitious shoot regeneration, in vitro flowering and
1086 fruiting of *Withania somnifera* (L.) Dunal. *Ind. Crops Prod.* 76, 323–328.
1087 <https://doi.org/10.1016/j.indcrop.2015.05.014>
- 1088 Smýkalová, I., Vrbová, M., Cvečková, M., Plačková, L., Žukauskaitė, A., Zatloukal, M., Hrdlička, J., Plíhalová, L.,
1089 Doležal, K., Griga, M., 2019. The effects of novel synthetic cytokinin derivatives and endogenous cytokinins on the
1090 in vitro growth responses of hemp (*Cannabis sativa* L.) explants. *Plant Cell. Tissue Organ Cult.* 139, 381–394.
1091 <https://doi.org/10.1007/s11240-019-01693-5>
- 1092 Snowden, M.C., Cope, K.R., Bugbee, B., 2016. Sensitivity of seven diverse species to blue and green light: Interactions
1093 with photon flux. *PLoS One* 11, 1–33. <https://doi.org/10.1371/journal.pone.0163121>
- 1094 Sprangers, K., Thys, S., van Dusschoten, D., Beemster, G.T.S., 2020. Gibberellin Enhances the Anisotropy of Cell
1095 Expansion in the Growth Zone of the Maize Leaf. *Front. Plant Sci.* 11, 1–13.
1096 <https://doi.org/10.3389/fpls.2020.01163>
- 1097 Sridharan, M., 2021. Generalized Regression Neural Network Model Based Estimation of Global Solar Energy Using
1098 Meteorological Parameters. *Ann. Data Sci.* <https://doi.org/10.1007/s40745-020-00319-4>
- 1099 Tanaka, M., Takamura, T., Watanabe, H., Endo, M., Yanagi, T., Okamoto, K., 1998. In vitro growth of *Cymbidium*
1100 plantlets cultured under superbright red and blue light-emitting diodes (LEDs). *J. Hortic. Sci. Biotechnol.* 73, 39–
1101 44. <https://doi.org/10.1080/14620316.1998.11510941>
- 1102 Tichá, I., Cap, F., Pacovska, D., Hofman, P., Haisel, D., Caplova, V., Schafer, C., 1998. Culture on sugar medium
1103 enhances photosynthetic capacity and high light resistance of plantlets grown in vitro. *Physiol. Plant.* 102, 155–162.
- 1104 Trupkin, S.A., Legris, M., Buchovsky, A.S., Rivero, M.B.T., Casal, J.J., 2014. Phytochrome b nuclear bodies respond to
1105 the low red to far-red ratio and to the reduced irradiance of canopy shade in *Arabidopsis*. *Plant Physiol.* 165, 1698–

- 1106 1708. <https://doi.org/10.1104/pp.114.242438>
- 1107 UCAR, E., CAGLAYAN, N., TURGUT, K., 2016. The Effects of Various LED Light Wavelengths to the Physiological
1108 and Morphological Parameters of Stevia (*Stevia rebaudiana*) Bertoni. Not. Sci. Biol. 8, 354–359.
1109 <https://doi.org/10.15835/nsb839886>
- 1110 Vita, D. De, Madia, V.N., Tudino, V., Saccoliti, F., Leo, A. De, Messore, A., Roscilli, P., Botto, A., Santilli, G., Scipione,
1111 L., Costi, R., Santo, R. Di, Vita, D. De, Madia, V.N., Tudino, V., Saccoliti, F., Leo, A. De, Messore, A., Roscilli,
1112 P., Botto, A., Pindinello, I., Scipione, L., Costi, R., Di, R., Comparison, S., 2020. Comparison of different methods
1113 for the extraction of cannabinoids from cannabis. Nat. Prod. Res. 34, 2952–2958.
1114 <https://doi.org/10.1080/14786419.2019.1601194>
- 1115 Wei, X., Zhao, X., Long, S., Xiao, Q., Guo, Y., Qiu, C., Qiu, H., Wang, Y., 2021. Wavelengths of LED light affect the
1116 growth and cannabidiol content in Cannabis sativa L. Ind. Crops Prod. 165, 113433.
1117 <https://doi.org/10.1016/j.indcrop.2021.113433>
- 1118 Wróbel, T., Dreger, M., Wielgus, K., Słomski, R., 2020. Modified Nodal Cuttings and Shoot Tips Protocol for Rapid
1119 Regeneration of Cannabis sativa L. J. Nat. Fibers 00, 1–10. <https://doi.org/10.1080/15440478.2020.1748160>
- 1120 Xu, Q., Krishnan, S., Merewitz, E., Xu, J., Huang, B., 2016. Gibberellin-Regulation and Genetic Variations in Leaf
1121 Elongation for Tall Fescue in Association with Differential Gene Expression Controlling Cell Expansion. Sci. Rep.
1122 6, 1–12. <https://doi.org/10.1038/srep30258>
- 1123 Xu, Y., Wang, C., Zhang, R., Ma, C., Dong, S., Gong, Z., 2020. The relationship between internode elongation of
1124 soybean stems and spectral distribution of light in the canopy under different plant densities. Plant Prod. Sci. 00, 1–
1125 13. <https://doi.org/10.1080/1343943X.2020.1847666>
- 1126 Yang, S.Y., Hong, C.O., Lee, H., Park, S.Y., Park, B.G., Lee, K.W., 2012. Protective effect of extracts of Perilla
1127 frutescens treated with sucrose on tert-butyl hydroperoxide-induced oxidative hepatotoxicity in vitro and in vivo.
1128 Food Chem. 133, 337–343. <https://doi.org/10.1016/j.foodchem.2012.01.037>
- 1129 Yoosefzadeh-Najafabadi, M., Earl, H.J., Tulpan, D., Sulik, J., Eskandari, M., 2021a. Application of Machine Learning
1130 Algorithms in Plant Breeding: Predicting Yield From Hyperspectral Reflectance in Soybean. Front. Plant Sci. 11,
1131 1–14. <https://doi.org/10.3389/fpls.2020.624273>
- 1132 Yoosefzadeh-Najafabadi, M., Tulpan, D., Eskandari, M., 2021b. Application of machine learning and genetic
1133 optimization algorithms for modeling and optimizing soybean yield using its component traits. PLoS One 16, 1–18.
1134 <https://doi.org/10.1371/journal.pone.0250665>
- 1135 Zhao, J., Luci, T.T., Park, Y.G., Jeong, B.R., 2020. Light Quality Affects Growth and Physiology of Carpesium triste
1136 Maxim. Cultured In Vitro. Agriculture 258, 1–19.
- 1137 Zheng, L., Van Labeke, M.C., 2017. Long-term effects of red- and blue-light emitting diodes on leaf anatomy and
1138 photosynthetic efficiency of three ornamental pot plants. Front. Plant Sci. 8, 1–12.
1139 <https://doi.org/10.3389/fpls.2017.00917>

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Table 1. Effect of light and carbohydrate on *in vitro Cannabis* shoot growth and development.

Input variables					Output variables				
Blue ($\mu\text{mol}/\text{m}^2/\text{s}$)	Red ($\mu\text{mol}/\text{m}^2/\text{s}$)	White ($\mu\text{mol}/\text{m}^2/\text{s}$)	Far-red ($\mu\text{mol}/\text{m}^2/\text{s}$)	Sucrose (%)	Shoot length (mm)	Root length (mm)	Node number	Shoot number	Canopy surface area (mm^2)
25	0	25	0	1	38.77±8.101	108.87±10.097	8.50±0.645	1.00±0.000	2309.42±314.907
25	0	25	0	3	32.44±7.036	42.47±29.857	8.00±0.408	2.25±0.479	2028.24±598.380
25	0	25	0	6	63.26±16.667	117.06±20.197	8.00±0.408	2.25±0.479	1885.75±385.882
50	0	0	0	1	32.94±2.406	26.45±23.515	7.25±0.750	1.00±0.000	1848.11±214.644
50	0	0	0	3	44.35±20.174	24.56±15.198	7.25±1.601	1.50±0.500	1495.87±757.315
50	0	0	0	6	39.03±10.839	142.17±45.483	7.50±0.866	1.25±0.250	1589.48±578.975
50	0	50	0	1	31.20±5.443	151.40±35.982	8.25±0.629	1.00±0.000	1717.80±582.898
50	0	50	0	3	40.91±13.542	82.77±17.954	8.50±0.500	1.25±0.250	1802.86±390.860
50	0	50	0	6	53.83±13.807	112.46±26.505	9.00±0.707	1.75±0.479	1880.05±744.967
100	0	0	0	1	23.43±2.634	0.00±0.000	5.75±0.479	1.25±0.250	493.01±111.615
100	0	0	0	3	22.95±2.991	15.04±8.855	6.75±0.479	1.00±0.000	650.45±126.813
100	0	0	0	6	33.42±11.272	102.47±60.796	6.50±0.500	2.00±0.577	890.63±444.374
12.5	12.5	12.5	12.5	1	43.13±9.839	97.61±34.009	7.25±0.479	1.50±0.289	2442.35±506.213
12.5	12.5	12.5	12.5	3	59.60±10.319	89.45±31.042	7.75±0.854	1.75±0.250	3193.41±888.482
12.5	12.5	12.5	12.5	6	64.51±38.597	63.48±34.099	8.25±2.016	1.75±0.479	2594.11±1648.261
37.5	12.5	0	0	1	47.40±11.309	63.68±24.567	7.00±0.816	1.50±0.289	1519.41±345.197
37.5	12.5	0	0	3	66.39±16.880	136.61±28.052	8.00±1.080	2.25±0.629	2177.63±519.451
37.5	12.5	0	0	6	38.41±5.652	100.51±37.320	7.75±0.479	1.50±0.289	1698.48±448.503
16.69	16.69	0	16.69	1	106.24±35.988	127.38±40.798	8.00±0.707	1.25±0.250	3350.76±789.191
16.69	16.69	0	16.69	3	142.22±36.056	101.97±41.471	9.75±0.750	2.50±0.866	4355.61±1395.277
16.69	16.69	0	16.69	6	38.89±11.084	46.67±29.388	6.75±0.854	1.25±0.250	1360.77±155.798
25	25	0	0	1	57.72±14.566	149.92±35.873	8.75±1.181	1.00±0.000	3776.96±1017.968
25	25	0	0	3	56.83±32.880	105.16±44.817	7.50±0.645	1.75±0.250	1737.36±1056.285
25	25	0	0	6	61.38±9.666	38.72±23.529	8.25±0.946	2.00±0.408	1216.37±114.887
25	25	25	25	1	87.11±22.707	134.47±48.218	8.75±0.854	1.75±0.250	6340.05±1284.607
25	25	25	25	3	56.06±12.648	72.80±36.452	9.25±0.250	1.00±0.000	3117.44±887.353
25	25	25	25	6	59.93±24.137	146.00±54.432	7.00±0.707	1.25±0.250	1829.20±645.785
75	25	0	0	1	33.30±5.883	65.71±42.275	7.50±1.041	1.00±0.000	2560.02±620.724
75	25	0	0	3	103.74±44.839	112.05±16.975	10.50±2.021	2.00±0.408	3964.16±1336.336

Precision tissue culture of *Cannabis sativa*

75	25	0	0	6	41.43±1.379	29.16±13.225	7.50±0.645	1.25±0.250	813.38±188.339
33.33	33.33	0	33.33	1	36.42±6.816	150.89±51.445	8.75±0.854	1.25±0.250	2091.81±525.087
33.33	33.33	0	33.33	3	98.40±44.716	477.10±287.094	11.50±2.901	1.00±0.000	2483.71±627.011
33.33	33.33	0	33.33	6	85.94±16.989	147.32±7.069	9.25±0.854	1.75±0.250	7136.78±1770.492
12.5	37.5	0	0	1	49.05±14.862	120.84±70.678	7.75±0.854	1.25±0.250	3232.08±1237.421
12.5	37.5	0	0	3	32.11±3.359	10.84±10.841	7.00±0.000	2.00±0.408	2505.37±374.173
12.5	37.5	0	0	6	50.48±11.078	122.62±37.803	8.25±0.479	2.25±0.946	1992.30±318.378
0	50	0	0	1	77.72±11.483	97.43±36.702	8.25±0.479	1.25±0.250	2500.70±678.427
0	50	0	0	3	99.81±31.278	158.00±58.672	7.75±0.946	1.75±0.479	3148.87±1255.456
0	50	0	0	6	46.90±1.499	35.98±20.840	8.50±0.289	1.50±0.289	1383.03±349.575
50	50	0	0	1	29.51±6.815	77.13±45.344	8.50±0.866	1.25±0.250	2447.43±737.653
50	50	0	0	3	68.50±16.044	73.50±26.470	8.75±0.629	2.25±0.479	13061.97±10839.642
50	50	0	0	6	55.40±24.082	29.79±29.794	9.00±0.707	2.00±0.408	1963.27±1336.004
0	50	25	0	1	63.01±11.807	87.61±55.464	9.00±0.707	1.50±0.289	2763.95±630.766
0	50	25	0	3	130.47±48.757	152.19±40.475	9.75±1.181	1.25±0.250	6939.43±2672.142
0	50	25	0	6	91.80±61.557	132.78±92.911	9.50±1.555	1.75±0.750	3000.23±1620.640
0	50	50	0	1	55.39±6.538	47.25±33.256	8.50±0.866	1.00±0.000	3123.43±594.904
0	50	50	0	3	73.49±16.669	159.08±45.374	9.50±0.645	1.75±0.479	5721.65±2203.448
0	50	50	0	6	78.72±27.594	91.01±34.488	8.50±0.500	1.50±0.289	3337.97±1156.575
25	75	0	0	1	49.02±6.926	121.57±43.981	8.50±0.866	1.00±0.000	3843.37±1073.415
25	75	0	0	3	78.73±21.040	79.38±39.101	10.50±1.190	1.00±0.000	6154.32±1303.577
25	75	0	0	6	55.71±17.245	75.76±27.694	9.00±0.408	1.25±0.250	2682.36±913.655
0	100	0	0	1	48.72±17.838	152.42±43.433	8.00±0.816	1.25±0.250	1642.67±438.197
0	100	0	0	3	101.24±32.678	207.67±41.674	7.50±0.289	1.25±0.250	1529.03±407.505
0	100	0	0	6	84.14±37.295	143.37±84.434	8.25±0.854	2.00±0.408	915.09±717.054
0	0	25	25	1	76.46±34.634	160.01±49.307	7.75±0.750	1.25±0.250	2370.35±467.347
0	0	25	25	3	154.68±51.228	171.42±17.863	8.50±1.041	1.50±0.289	2707.43±652.476
0	0	25	25	6	86.78±29.794	86.57±38.613	8.00±0.408	1.25±0.250	2782.91±1022.655
0	0	50	0	1	39.50±5.238	128.04±12.026	8.50±0.289	1.25±0.250	2751.85±906.598
0	0	50	0	3	35.85±6.990	74.44±27.158	8.25±0.479	1.50±0.289	1706.28±611.107
0	0	50	0	6	82.77±43.133	72.39±41.802	8.00±1.000	1.50±0.289	2249.33±1412.720
0	0	50	50	1	38.61±6.648	0.00±0.000	8.75±0.479	1.00±0.000	2169.51±649.210

Precision tissue culture of *Cannabis sativa*

0	0	50	50	3	136.64±29.794	148.92±23.789	8.75±0.629	2.00±0.408	3411.27±345.452
0	0	50	50	6	36.78±0.374	4.40±4.396	8.00±0.408	1.25±0.250	859.96±78.081
0	0	100	0	1	27.70±2.311	4.36±4.363	7.25±0.479	1.50±0.289	519.06±182.411
0	0	100	0	3	39.88±3.684	9.05±5.546	8.00±0.707	1.00±0.000	1954.42±506.636
0	0	100	0	6	101.32±35.475	177.03±26.461	8.00±1.080	3.00±0.577	2593.13±526.681

Values in each column represent means ±Standard error.

Table 2. Performance indices of different machine learning algorithms (MLP, GRNN, and ANFIS) for modeling and predicting shoot length, root length, number of nodes, number of shoots, and canopy surface area of *Cannabis*.

Model	Performance index	Shoot length		Shoot number		Node number		Root length		Canopy surface area	
		Training	Testing	Training	Testing	Training	Testing	Training	Testing	Training	Testing
MLP	R ²	0.972	0.954	0.625	0.421	0.717	0.390	0.938	0.900	0.953	0.928
	RMSE	4.929	6.927	0.396	0.632	0.702	1.202	15.112	15.956	277.487	340.538
	MBE	-0.090	1.673	0.009	-0.001	0.017	0.260	0.001	2.259	30.952	25.016
GRNN	R ²	0.983	0.964	0.733	0.714	0.791	0.744	0.941	0.914	0.962	0.944
	RMSE	3.879	6.081	0.347	0.606	0.594	0.933	14.754	14.972	248.737	300.911
	MBE	0.001	1.540	0.001	0.012	-0.001	0.063	0.001	2.581	0.001	2.388
ANFIS	R ²	0.770	0.590	0.647	0.501	0.767	0.549	0.781	0.589	0.733	0.644
	RMSE	17.538	23.327	0.407	0.557	0.650	0.942	41.881	39.007	1282.011	1282.697
	MBE	-4.549	-5.508	0.006	-0.065	-0.003	0.037	5.962	8.546	-26.525	-32.037

ANFIS: adaptive neuro-fuzzy inference system; GRNN: generalized regression neural network; MBE: mean bias error; MLP: multi-layer perceptron; R²: coefficient of determination; RMSE: root mean square error.

Table 3. Importance degree of light (blue, red, white, and far-red) and carbohydrate sources on shoot length, root length, number of nodes, number of shoots, and canopy surface area of *Cannabis* through sensitivity analysis.

Trait	Item	Blue	Red	White	Far-red	Sucrose
Shoot length	VSR	3.005	1.957	1.647	2.141	5.191
	Rank	2	4	5	3	1
Root length	VSR	1.54	2.211	1.669	1.909	3.887
	Rank	5	2	4	3	1
Node number	VSR	1.379	1.257	1.065	1.288	1.597
	Rank	2	4	5	3	1
Shoot number	VSR	1.554	1.217	1.105	1.168	1.651
	Rank	2	3	5	4	1
Canopy surface area	VSR	1.662	2.616	1.657	1.622	3.693
	Rank	3	2	4	5	1

VSR: variable sensitivity ratio

Table 4. The results of optimization process via different evolutionary optimization algorithms (BBO, SOS, ISA, and GA).

Fitness function	Optimization algorithm	Optimal level of input variables				Sucrose (%)	Predicted fitness function value
		Blue ($\mu\text{mol}/\text{m}^2/\text{s}$)	Red ($\mu\text{mol}/\text{m}^2/\text{s}$)	White ($\mu\text{mol}/\text{m}^2/\text{s}$)	Far-red ($\mu\text{mol}/\text{m}^2/\text{s}$)		
Shoot length (mm)	BBO	15.412	9.412	15.997	43.271	3.142	160.78
	SOS	4.460	19.051	27.337	39.472	3.157	160.78
	ISA	0.439	18.494	36.234	33.122	3.319	160.78
	GA	2.937	0.270	13.036	30.605	3.505	160.78
Root length (mm)	BBO	5.756	87.381	31.523	19.343	3.504	262.21
	SOS	0.508	79.897	4.733	17.209	3.673	262.21
	ISA	3.779	81.519	25.386	13.321	3.634	262.21
	GA	5.797	98.198	36.208	1.237	3.507	262.21
Node number	BBO	62.998	92.238	48.520	8.830	3.709	12.25
	SOS	57.682	87.192	45.468	19.924	3.711	12.25
	ISA	46.845	90.135	20.969	16.824	3.220	12.25
	GA	50.960	88.355	11.000	11.673	3.709	12.25
Shoot number	BBO	16.581	36.686	0.592	19.995	2.909	3.75
	SOS	15.930	21.183	9.723	24.304	2.372	3.75
	ISA	21.336	29.584	0.332	17.387	2.174	3.75
	GA	25.303	25.471	0.262	18.125	3.160	3.75
Canopy surface area (mm^2)	BBO	52.563	84.052	26.262	22.456	3.809	7168.05
	SOS	54.688	95.974	30.099	24.543	3.664	7168.05
	ISA	44.889	99.642	49.994	24.674	3.285	7168.05
	GA	37.646	83.928	17.507	1.811	3.083	7168.05

BBO: biogeography-based optimization; GA: genetic algorithm; ISA: interior search algorithm; SOS: symbiotic organisms search.

1141 **Figure Captions**

1142 Figure 1. A schematic representation of factors influencing *in vitro* culture.

1143 Figure 2. A schematic representation of different classes of machine learning algorithms.

1144 Figure 3. Spectral analyses of light treatments from the initial experiment. Images indicate relative
1145 amounts of fluencies emitted per treatment. Light spectra presented were obtained using Li-Cor LI-
1146 180 spectrometer. Presented are (a) 25 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ W, (b) 50 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 50
1147 $\mu\text{mol}/\text{m}^2/\text{s}$ W, (c) 50 $\mu\text{mol}/\text{m}^2/\text{s}$ B, (d) 100 $\mu\text{mol}/\text{m}^2/\text{s}$ B, (e) 12.5 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 12.5 $\mu\text{mol}/\text{m}^2/\text{s}$ B +
1148 12.5 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 12.5 $\mu\text{mol}/\text{m}^2/\text{s}$ W, (f) 12.5 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 37.5 $\mu\text{mol}/\text{m}^2/\text{s}$ B, (g) 16.67
1149 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 16.67 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 16.67 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr, (h) 25 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ B + 25
1150 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ W, (i) 25 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ B, (j) 25 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 75
1151 $\mu\text{mol}/\text{m}^2/\text{s}$ B, (k) 25 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ W, (l) 33.33 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 33.33 $\mu\text{mol}/\text{m}^2/\text{s}$ B +
1152 33.33 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr, (m) 37.5 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 12.5 $\mu\text{mol}/\text{m}^2/\text{s}$ B, (n) 50 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 50 $\mu\text{mol}/\text{m}^2/\text{s}$
1153 B, (o) 50 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 50 $\mu\text{mol}/\text{m}^2/\text{s}$ W, (p) 50 $\mu\text{mol}/\text{m}^2/\text{s}$ R, (q) 75 $\mu\text{mol}/\text{m}^2/\text{s}$ R + 25 $\mu\text{mol}/\text{m}^2/\text{s}$
1154 B, (r) 100 $\mu\text{mol}/\text{m}^2/\text{s}$ R, (s) 25 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 25 $\mu\text{mol}/\text{m}^2/\text{s}$ Fr, (t) 50 $\mu\text{mol}/\text{m}^2/\text{s}$ W + 50 $\mu\text{mol}/\text{m}^2/\text{s}$
1155 Fr, (u) 50 $\mu\text{mol}/\text{m}^2/\text{s}$ W, and (v) 100 $\mu\text{mol}/\text{m}^2/\text{s}$ W.

1156 Figure 4. Step-by-step methodology of the current study, including (a) data obtained, (b-d) data
1157 modeling through multilayer perceptron (MLP), generalized regression neural networks (GRNN),
1158 and adaptive neuro-fuzzy inference system (ANFIS), respectively, (e) main steps of optimization
1159 process through different optimization algorithms, (f,g) results of the validation experiment for shoot
1160 growth and canopy surface area, respectively, and (h,i) shoot growth and canopy surface area
1161 obtained from symbiotic organisms search (SOS).

1162 Figure 5. A schematic representation of biogeography-based optimization (BBO) algorithm.

1163 Figure 6. A schematic representation of interior search algorithm (ISA) algorithm.

1164 Figure 7. A schematic representation of symbiotic organisms search (SOS) algorithm.

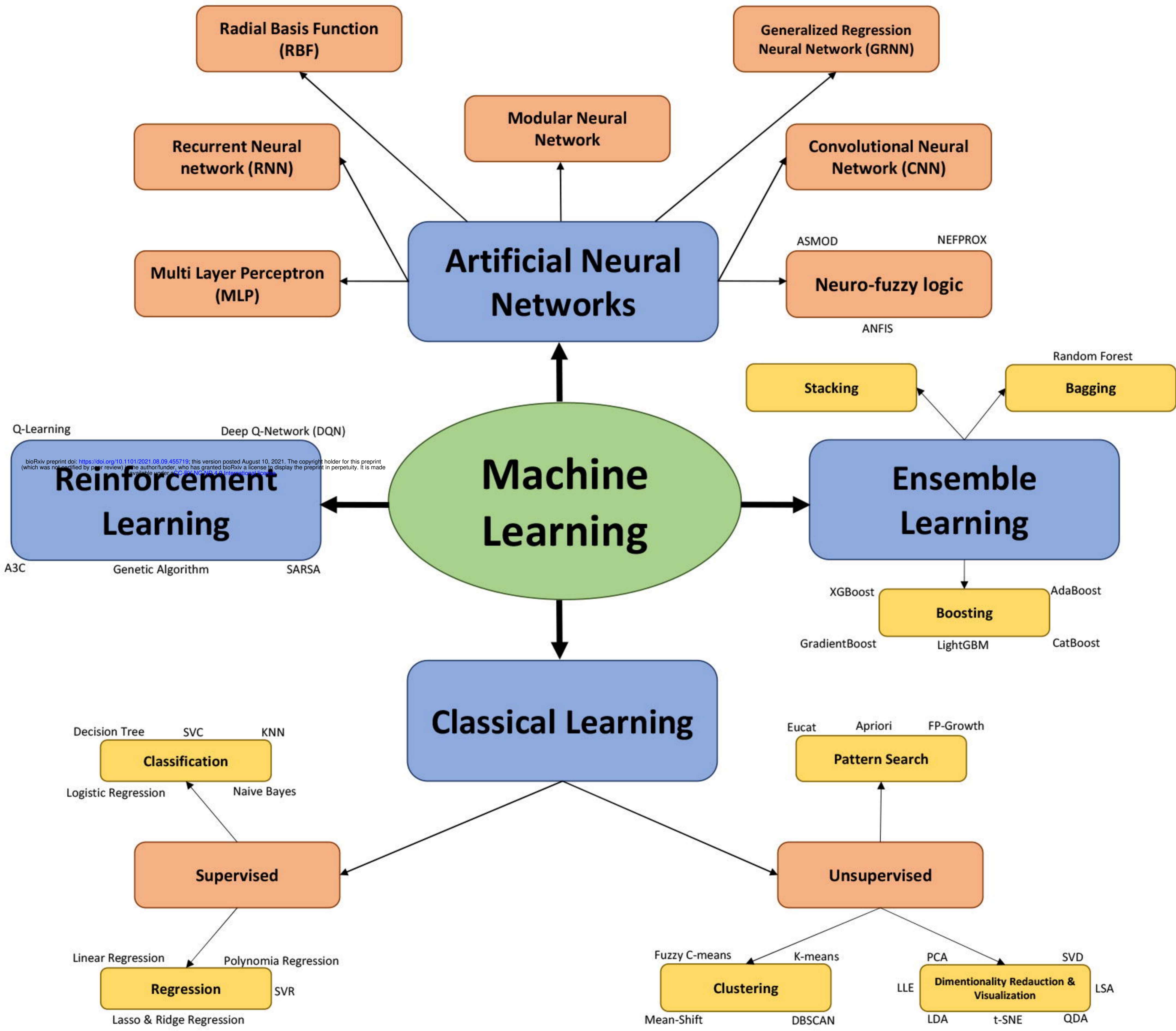
1165 Figure 8. A schematic representation of genetic algorithm (GA).

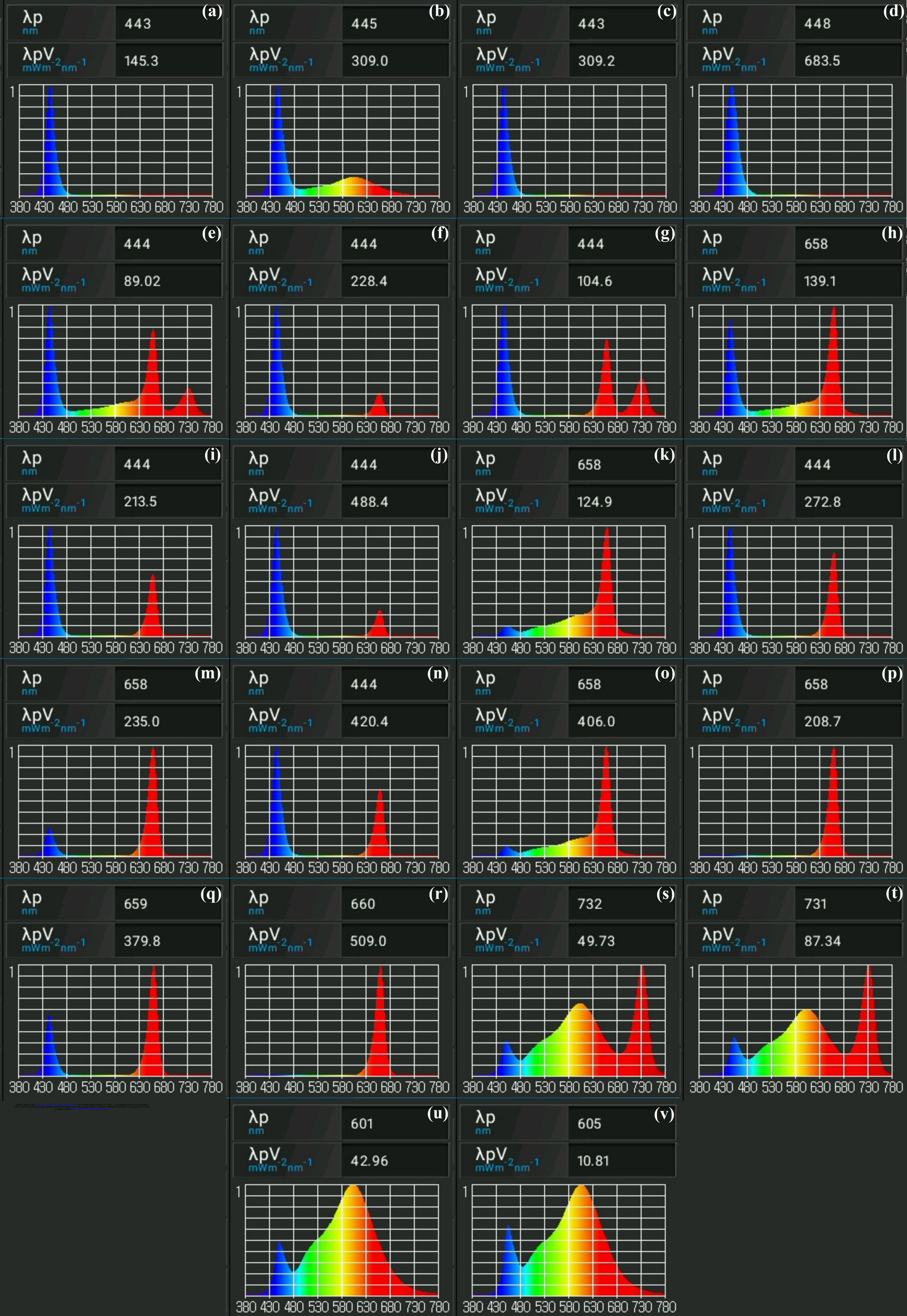
1166 Figure 9. Spectral analyses of light treatments from the validation experiment. Images demonstrate
1167 relative amounts of fluencies emitted per treatment. Light spectra presented were obtained using Li-
1168 Cor LI-180 spectrometer. Presented are optimized light treatments for (a) GA shoot length, (b) GA
1169 canopy surface area, (c) GA number of shoots, (d) GA number of nodes, (e) BBO canopy surface
1170 area, (f) BBO shoot length, (g) ISA shoot length, (h) ISA number of nodes, (i) ISA root length, (j)
1171 ISA number of shoots, (k) SOS shoot length, (l) ISA canopy surface area, (m) SOS root length, (n)
1172 BBO number of nodes, (o) SOS canopy surface area, (p) SOS number of nodes, (q) BBO number of
1173 shoots, and (r) SOS number of shoots.

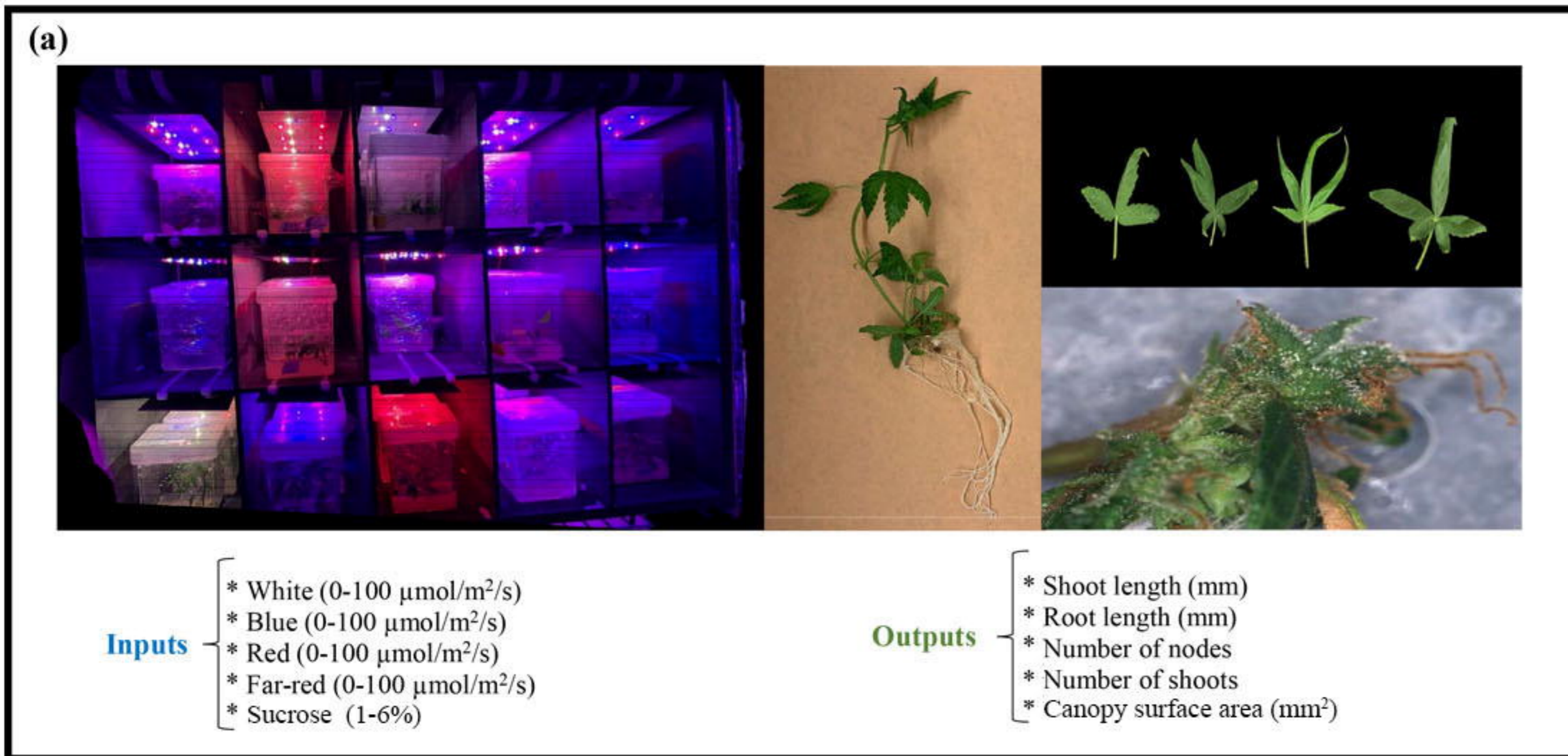
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1175 Figure 10. Scatter plot of experimental data versus predicted data of (a) shoot length, (b) root length,
1176 (c) node number, (d) shoot number, and (e) canopy surface area in *in vitro Cannabis* shoot growth
1177 and development, using generalized regression neural network (GRNN) in both training and testing
1178 subsets.

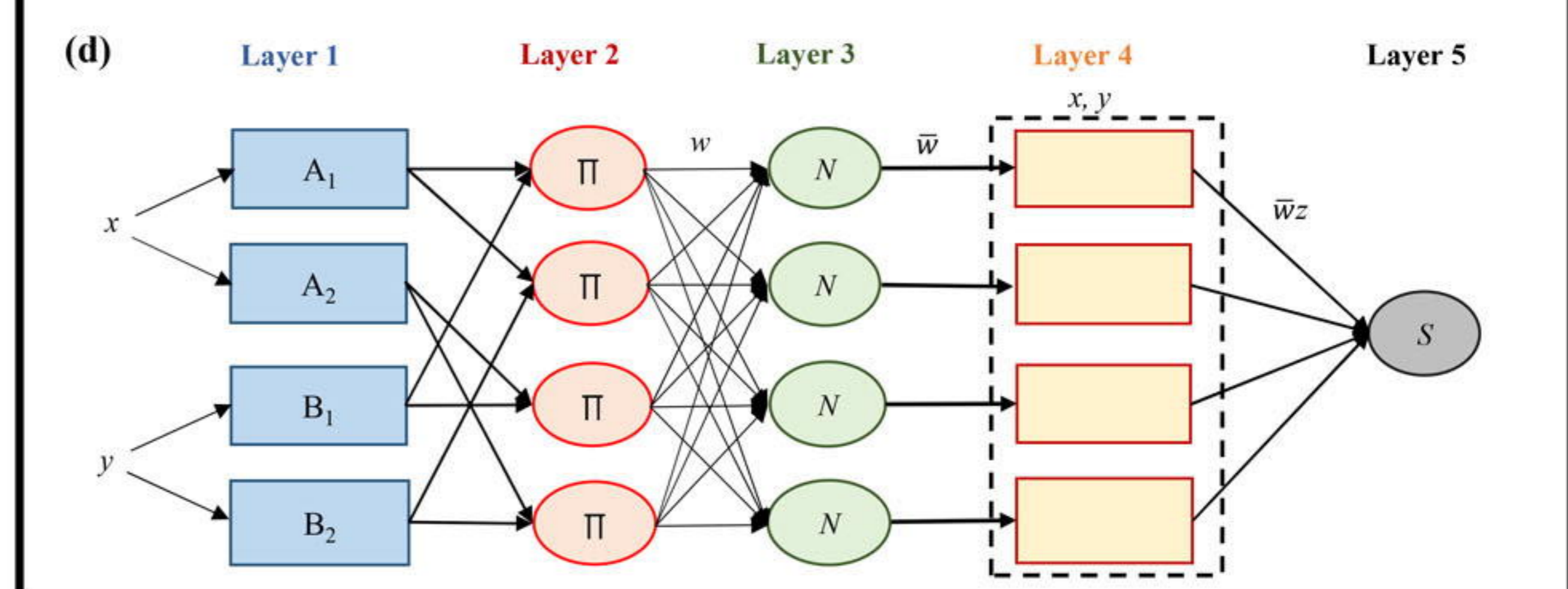
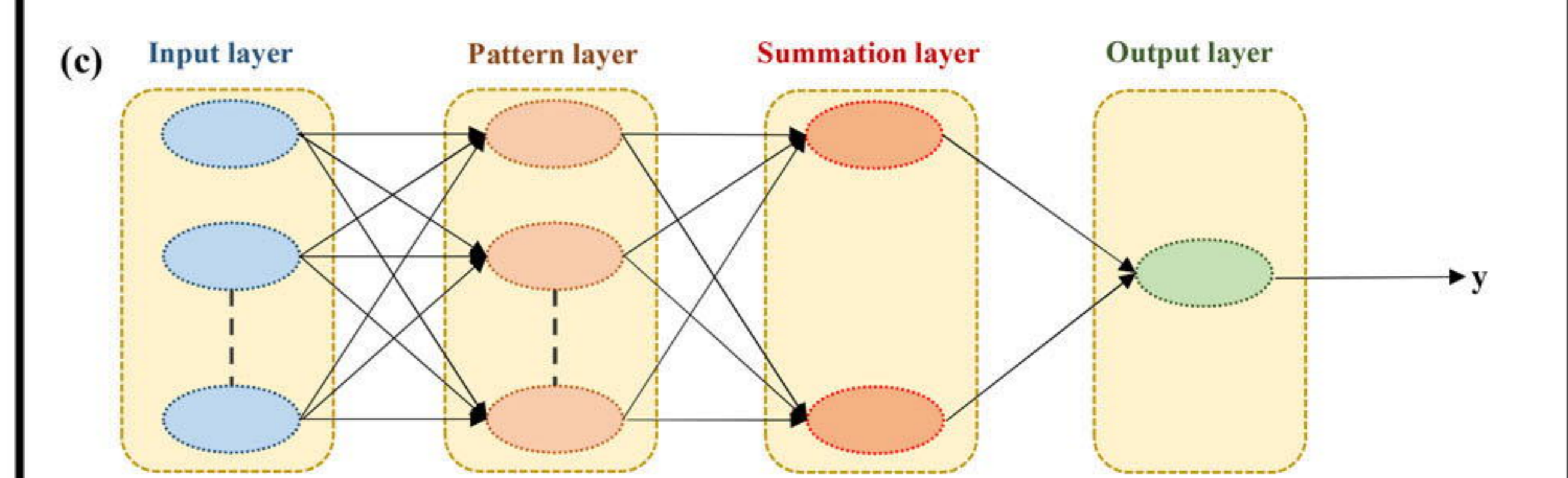
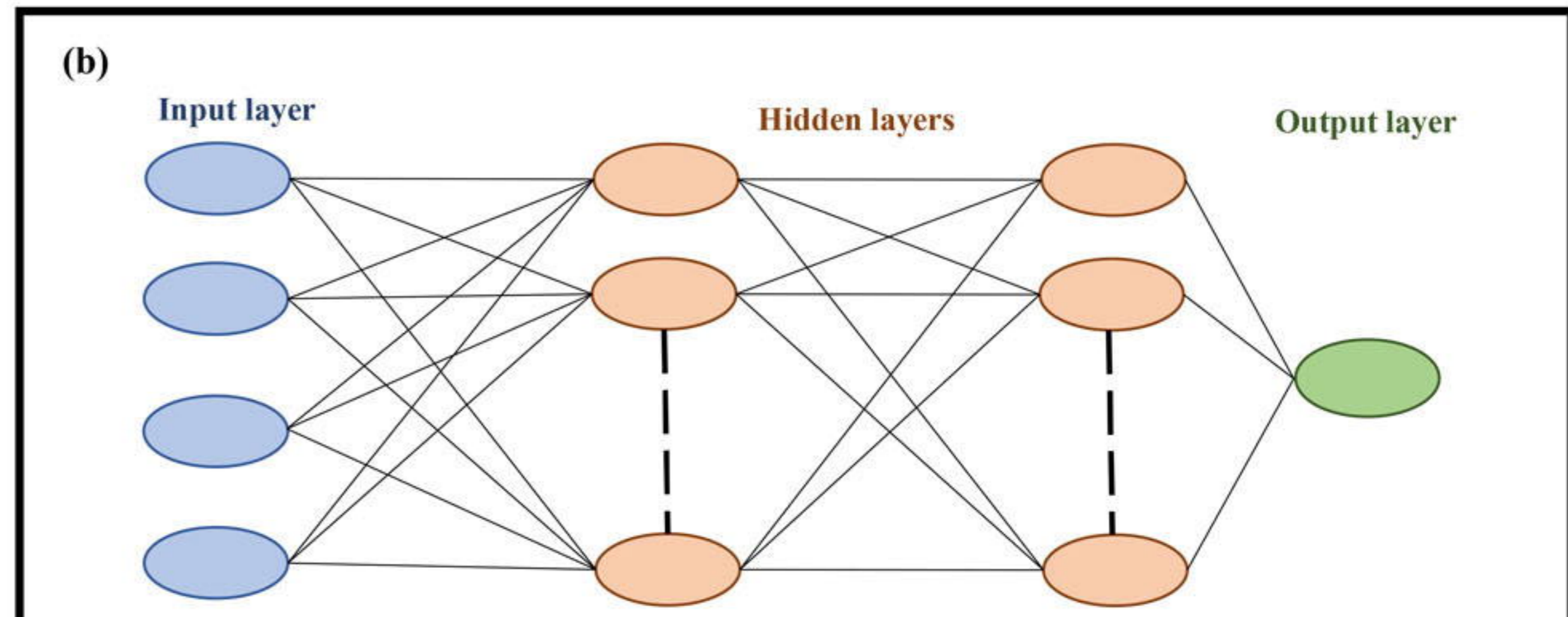




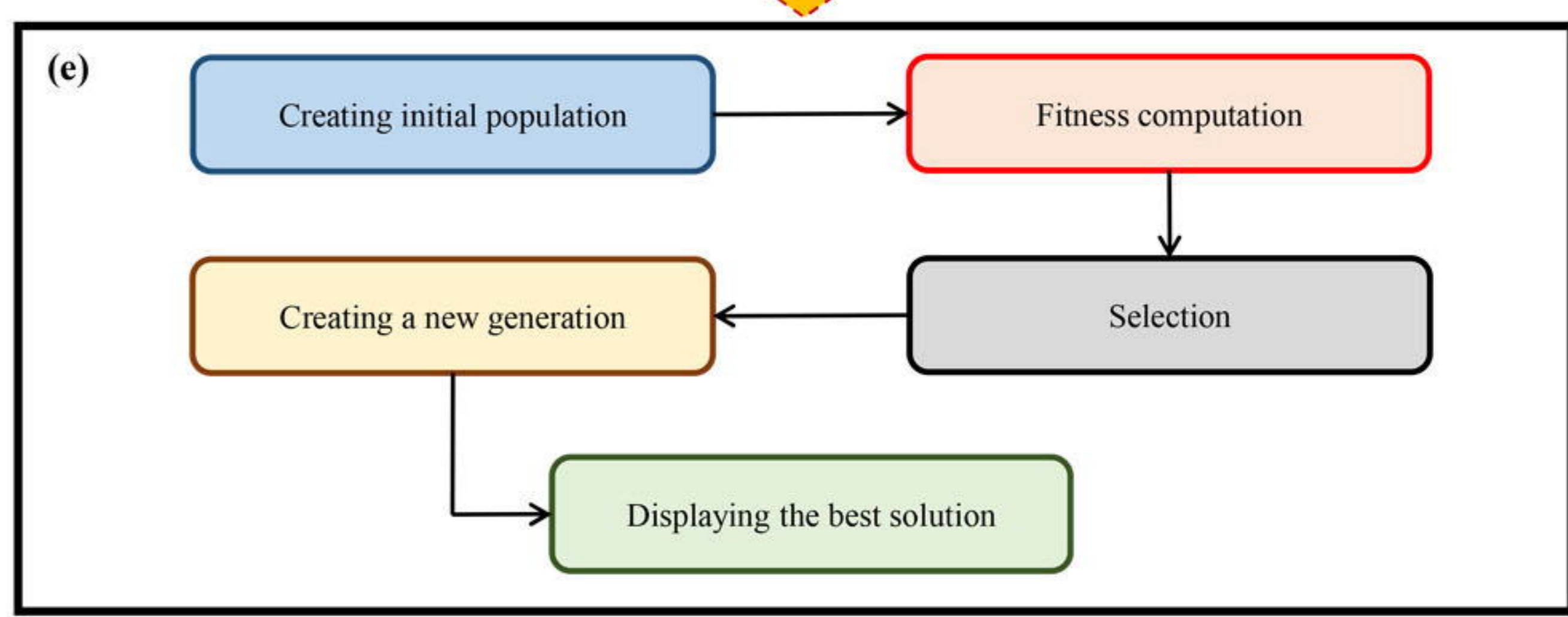




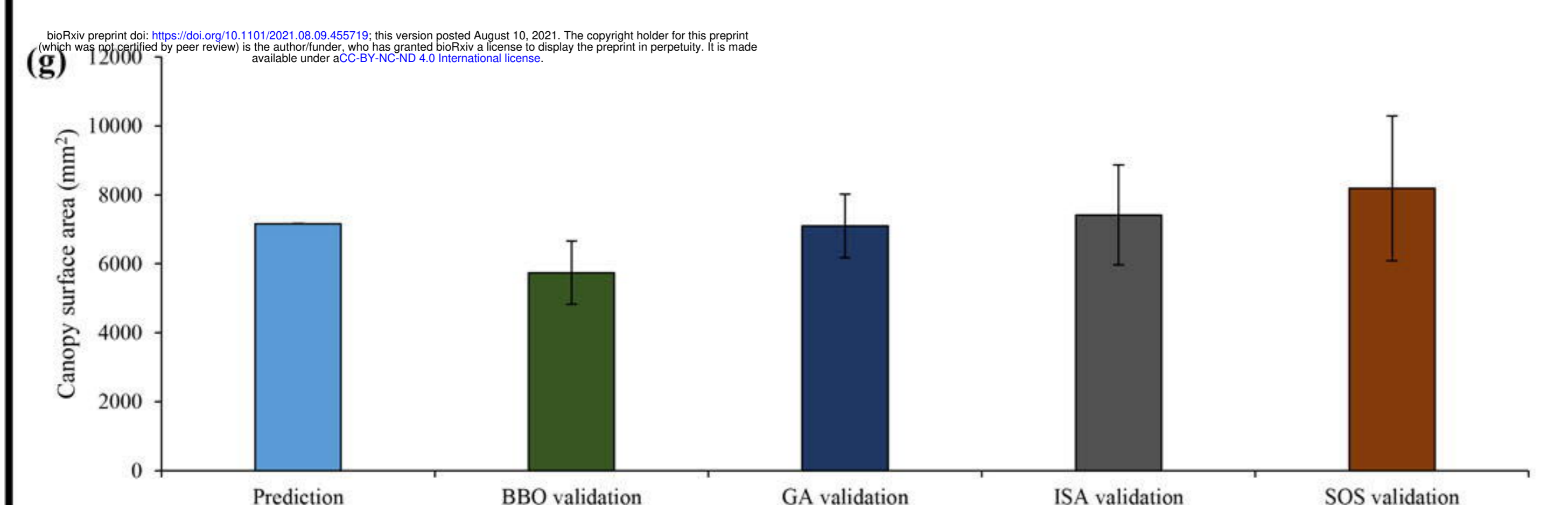
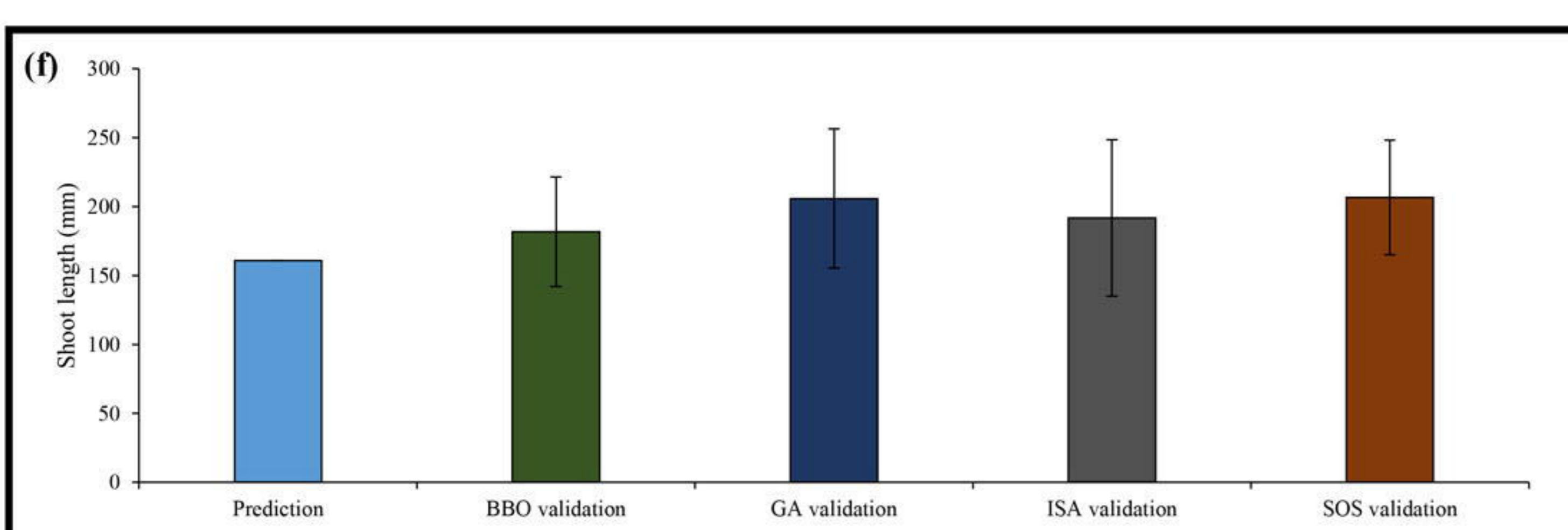
Modeling



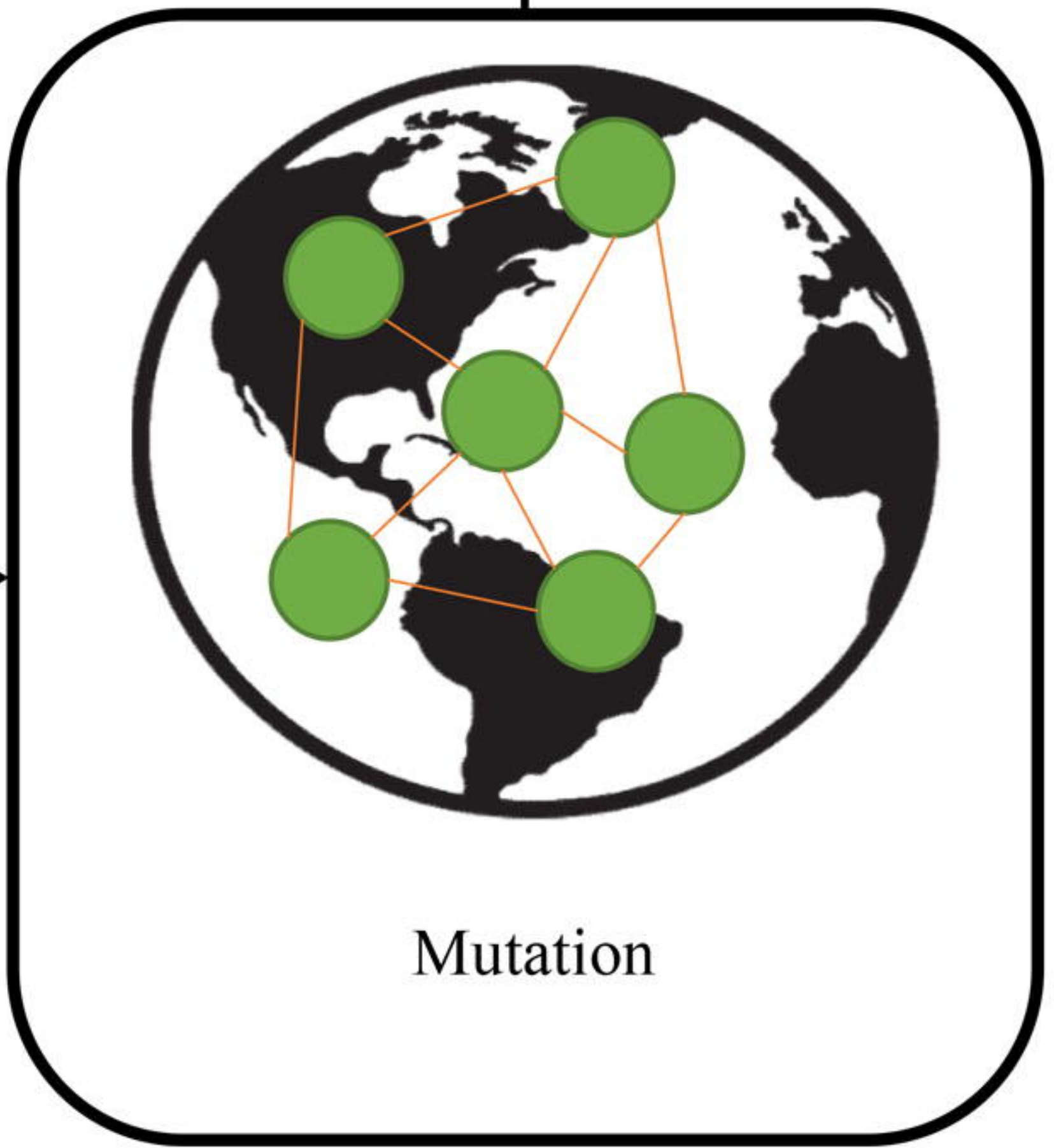
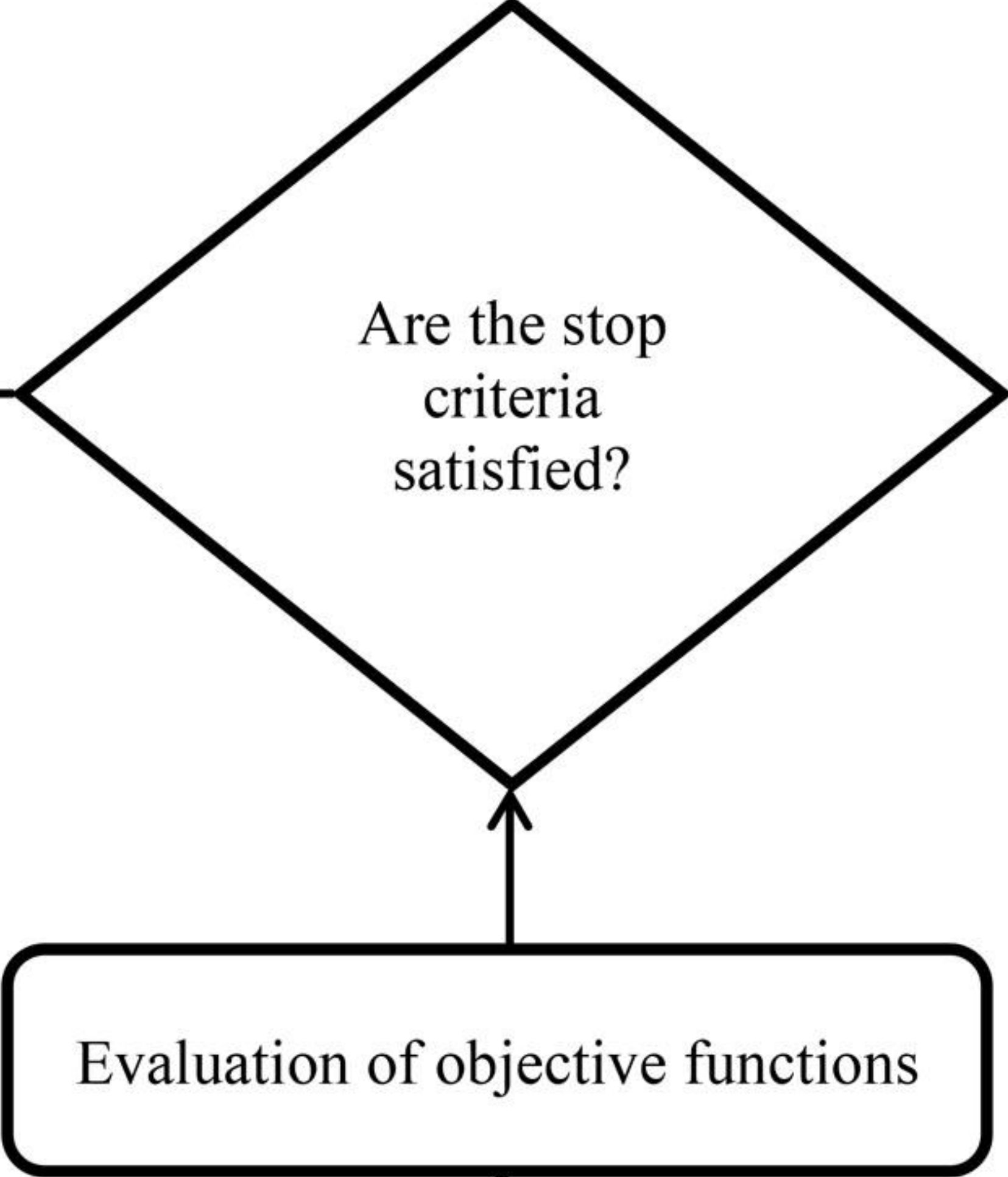
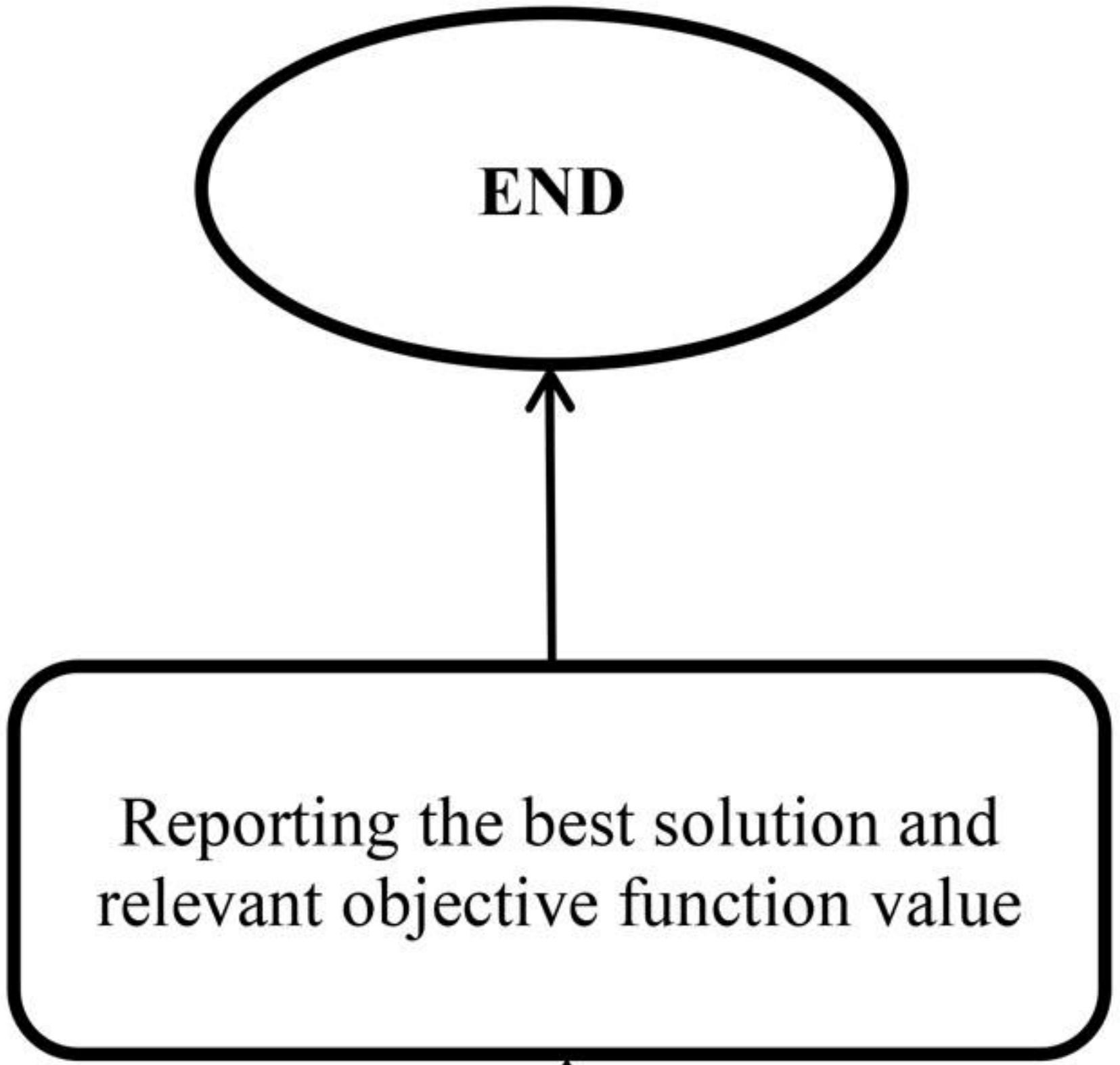
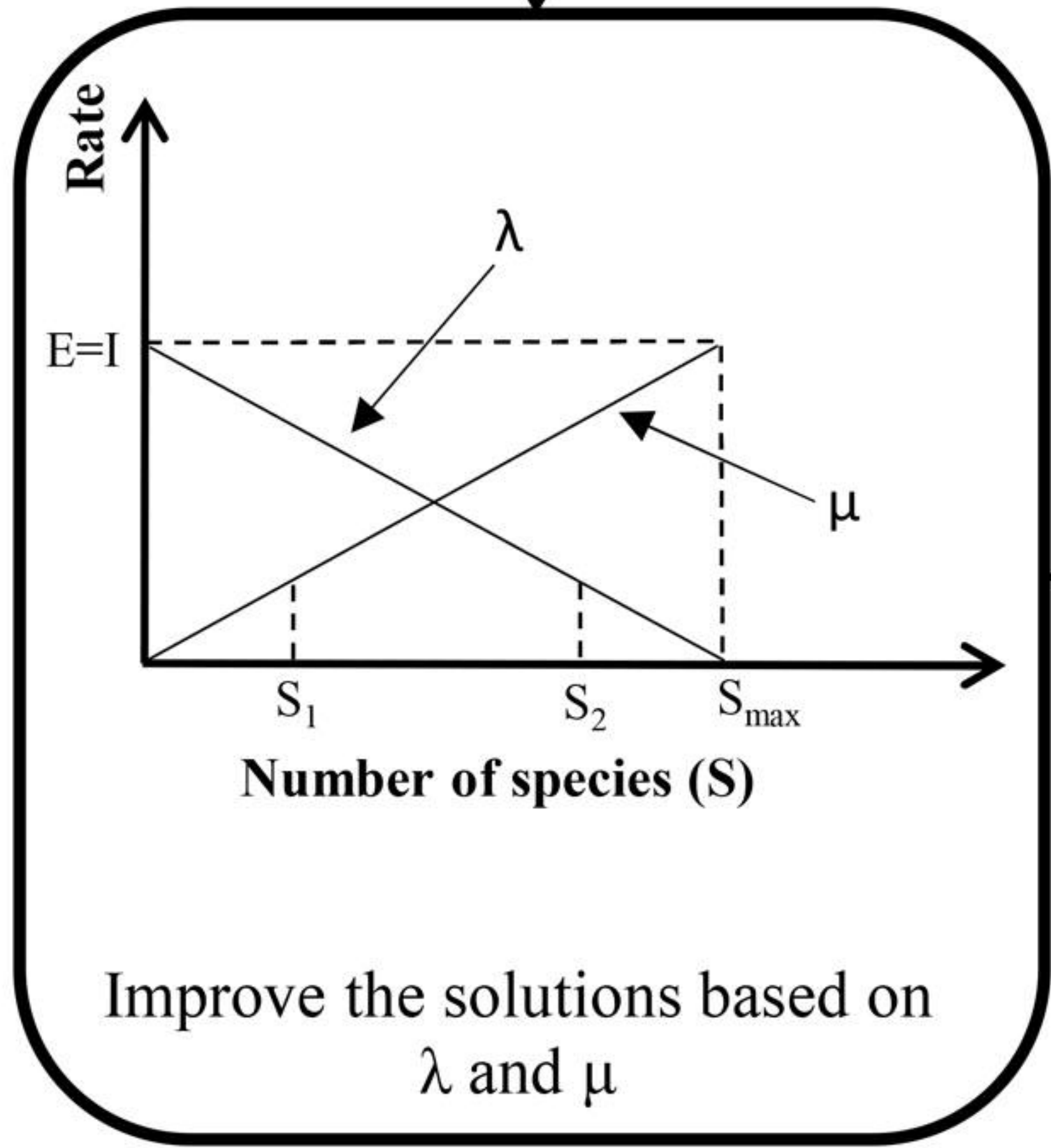
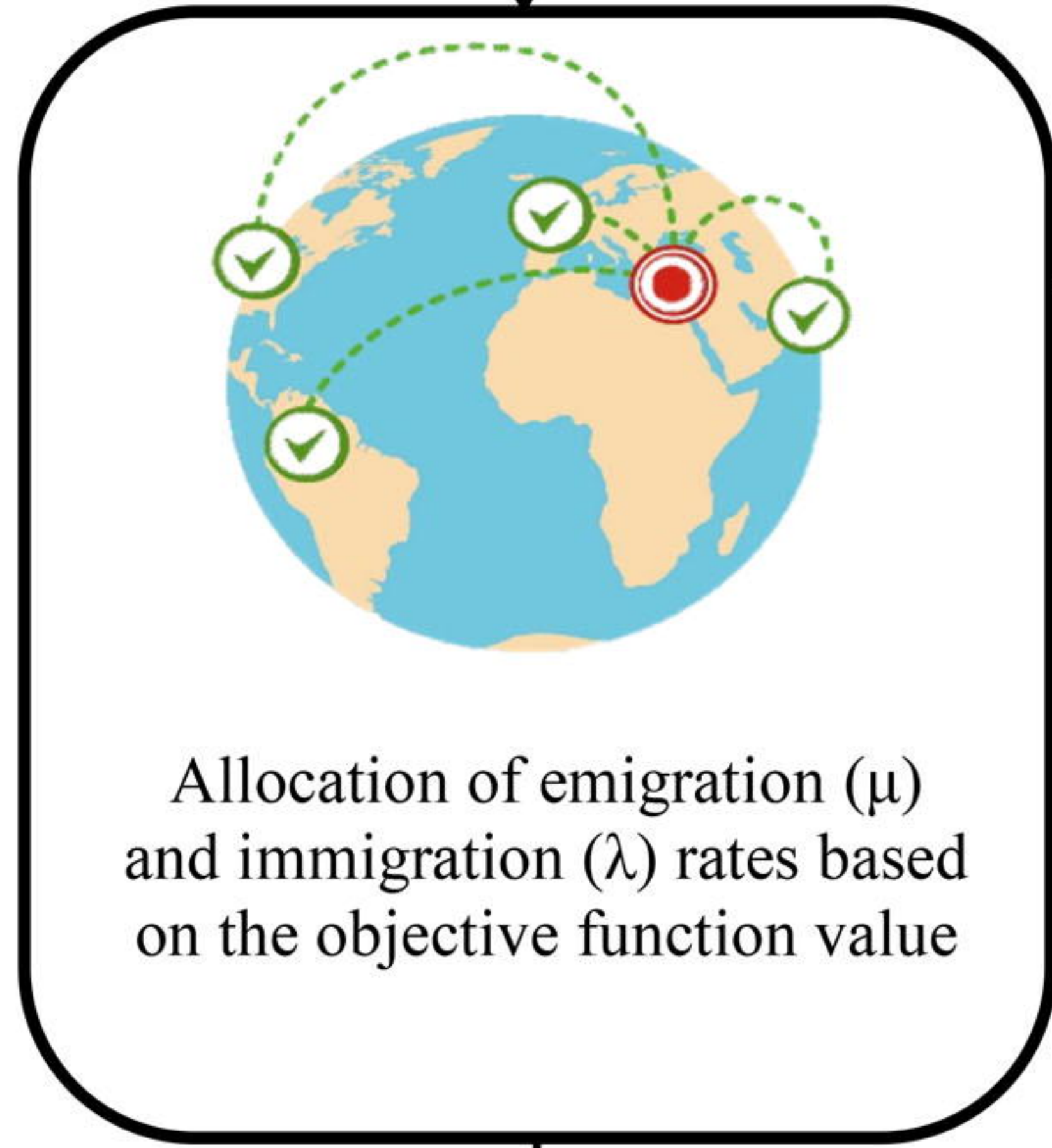
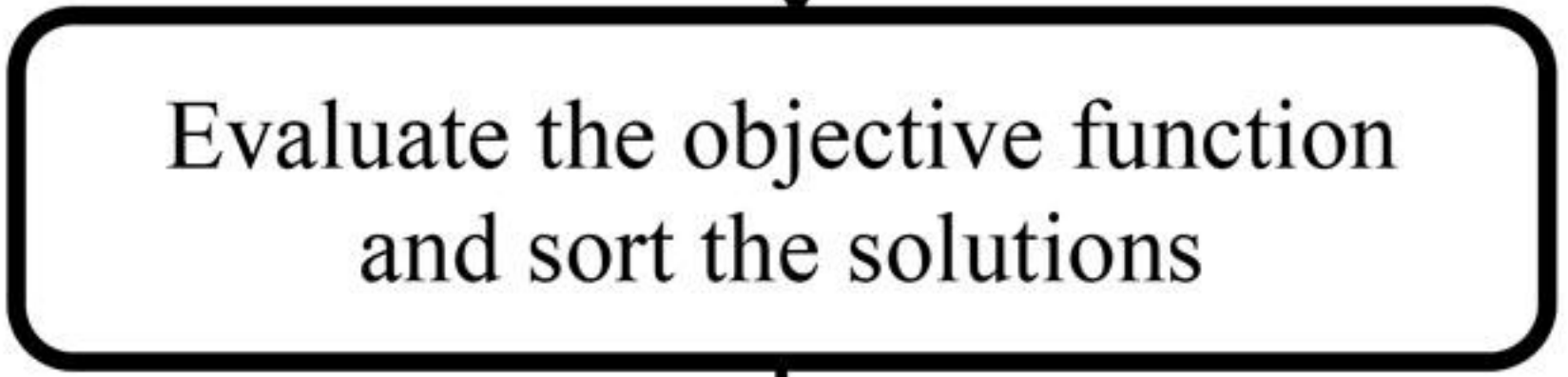
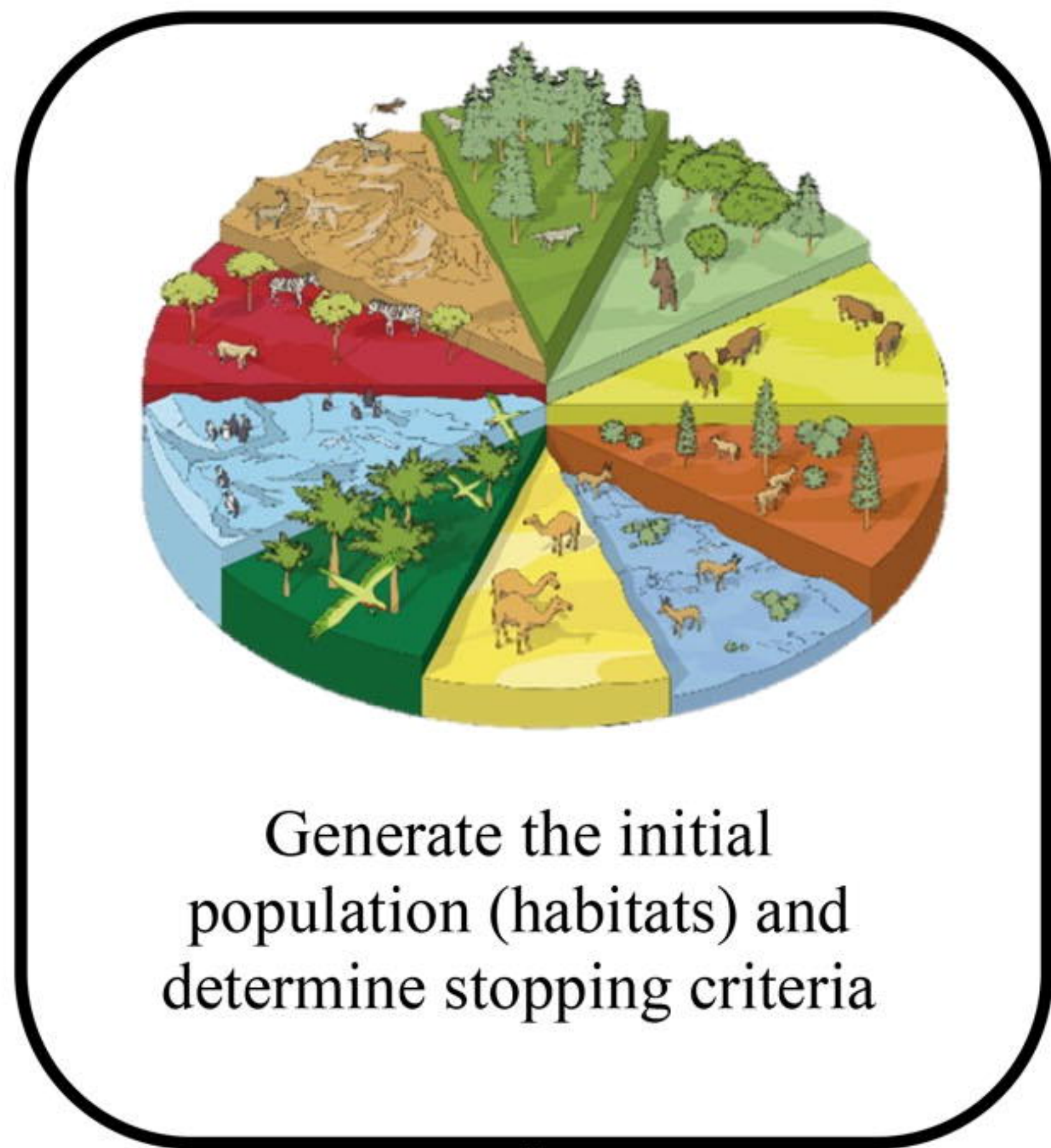
Optimizing



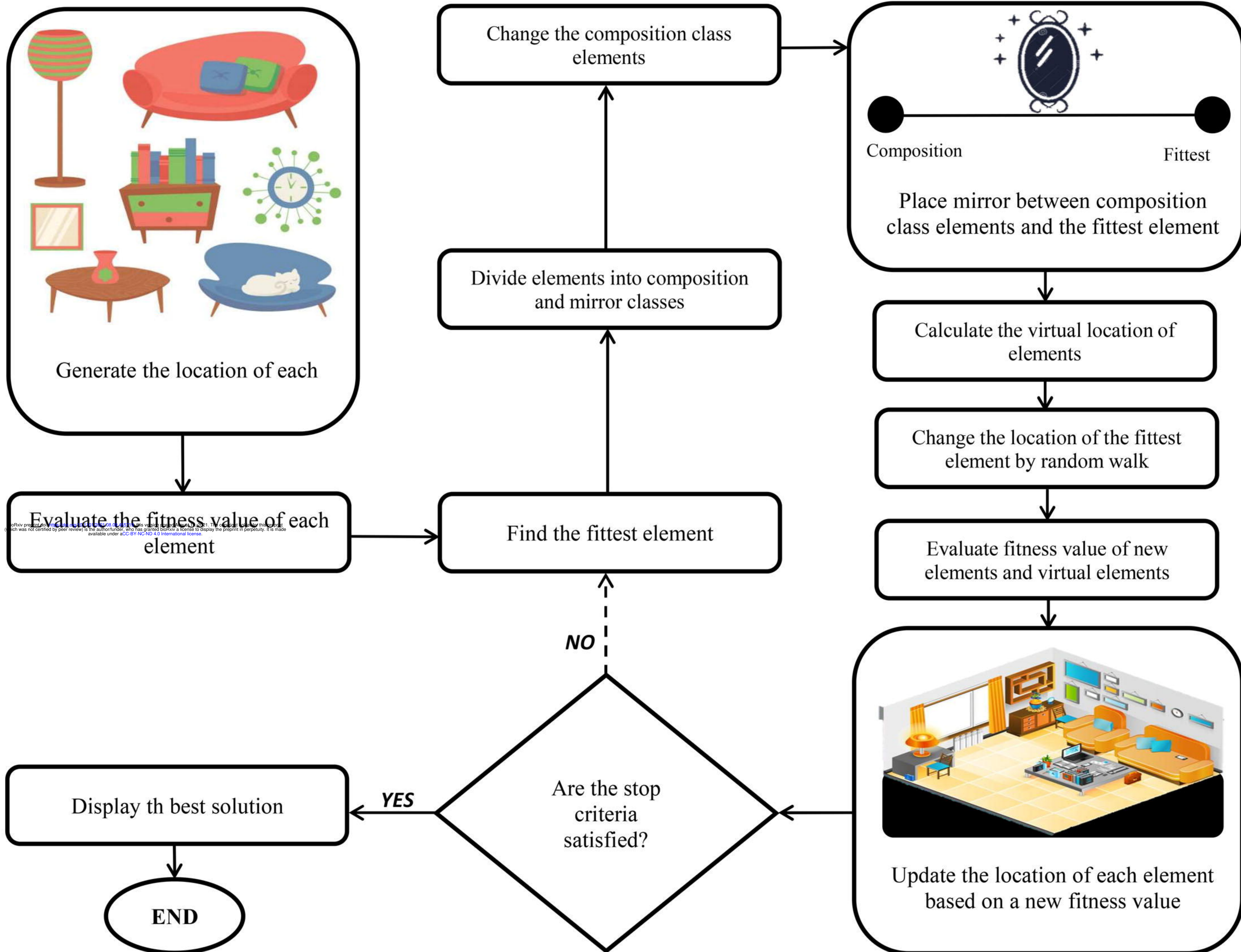
Validation

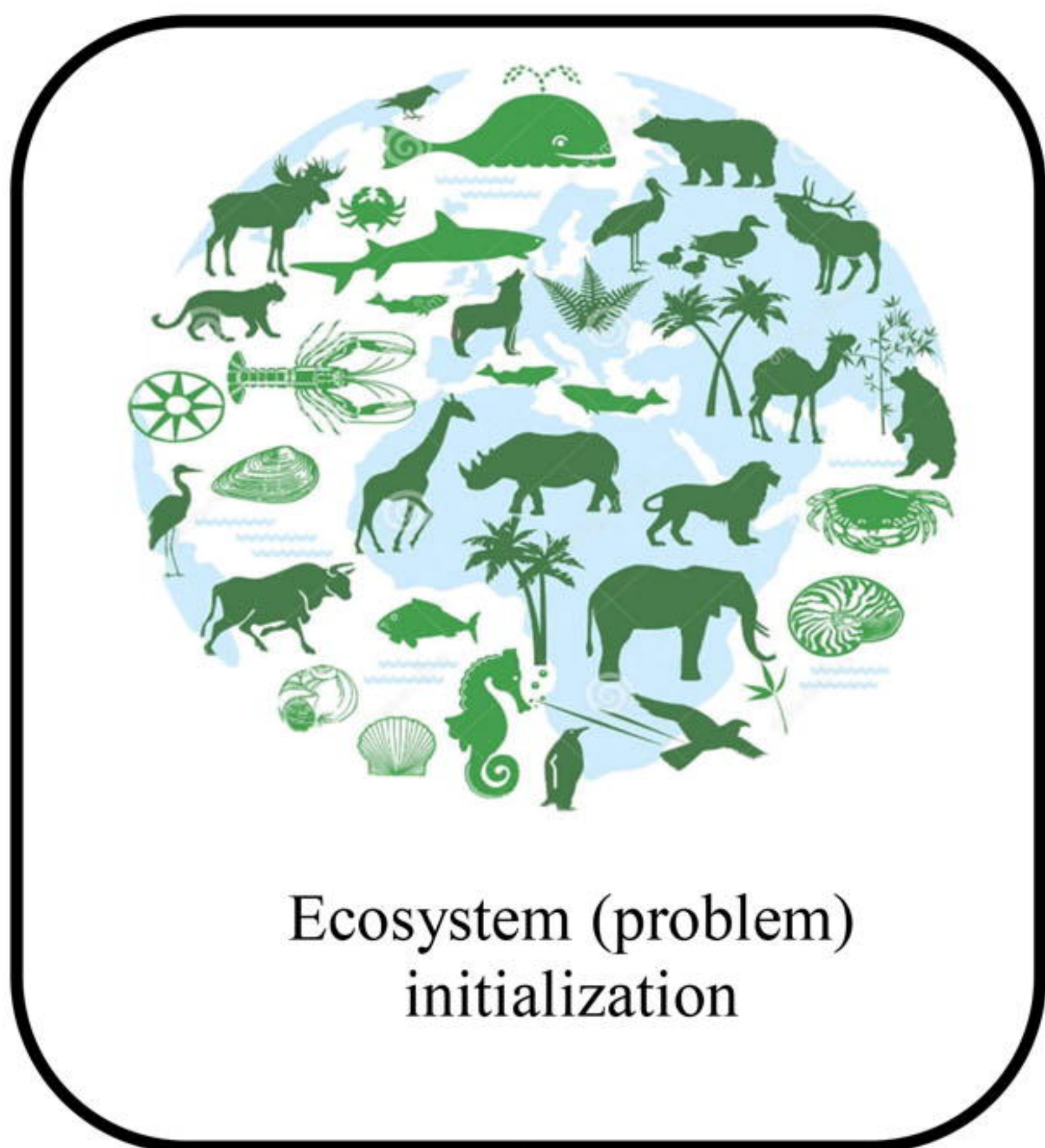


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$num_iter = 0$

$num_iter(i) = num_iter(i) + 1; i = 1$

Mutualism phase

- * Selection an organism (x_j) randomly; $x_i \neq x_j$
- * Obtaining *Mutual_Vector* along with benefit factor
- * Updating x_i and x_j with respect to their mutual relationship
- * Evaluating fitness value of the modified organisms
- * If updating is successful, accept the new x_i to replace the previous x_i
- * If updating is not successful, reject the new x_i and keep the previous x_i

Commensalism phase

- * Selection an organism (x_j) randomly; $x_i \neq x_j$
- * Updating x_i based on x_j
- * Evaluating fitness value of the new organism
- * If updating is successful, accept the new x_i to replace the previous x_i
- * If updating is not successful, reject the new x_i and keep the previous x_i

Parasitism phase

- * Selection an organism (x_j) randomly; $x_i \neq x_j$
- * Creating a *Parasite_Vector (PV)* based on x_j
- * Evaluating fitness value of the new organism
- * If *PV* is fitter than x_i , replace x_i with *PV*
- * If *PV* is fitter than x_j , eliminate *PV* and keep x_j

Display the best solution

END

Are the stop criteria satisfied?

YES

NO

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