ARTIFICIAL INTELLIGENCE-BASED MODELS FOR CALLUS PROPAGATION IN *RUTA CHALEPENSIS*

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Abstract:
Artificial intelligence, where machine learning is used, maybe a crucial tool for amending plant tissue culture. This method might potentially be used at different callus culture stages to research how quickly callus propagation occurs when it is exposed to various levels of plant growth hormone. Plant tissue culture is a field that enables culturing of various plants and parts of plants usually treated under a nutrient medium and in highly sterile conditions. Out of them callus culture is one of the very interesting arenas of plant biotechnology that encompasses many pivotal benefits. The study focuses on such callus enrichment using different hormones that thereby enhance its biological activities. The plant namely *Ruta chalepensis* was chosen upon wherein the callus growth was noticed. *Ruta chalepensis* has multiple medicinal activities like anti-cancer, anti-ulcer, anti-diabetic, and many more pharmacological properties that yield in treating and curing illness. The plant was examined characterized by MS medium and other hormones and its concentrations. auxin and cytokinin from 0.5mg to 2 mg(2,4-D, NAA, BAP, IAA). Increased concentration of 2,4-D (1.0 mg/L) alone in the MS medium showed profuse callus growth. Among the plant growth regulators that were studied separately, 2,4-D (1.0 mg/L) followed by NAA (0.5 mg/L) showed maximum callus initiation, hence further work was carried out in a combination of with the plant growth regulators for callus proliferation and accumulation to study the growth the pattern on a combination of hormones and fix the hormone concentrations for the mass propagation of callus from the explants and was noticed that 2,4-D (1.0 mg/L) + NAA (0.5 mg/L) showed profuse callus growth in comparison with the other hormonal concentrations treated which is (0.5 mg/L) until (2.0 mg/L). The hormone combinations were hence used to train multiple machine learning models. The models used in this analysis are Random Forest regressor, decision tree regressor, multilayer perceptron, and support vector regressor and the aim was to identify the model best suited for predicting callus formation using the hormonal combinations at various concentrations.

Key words: *Ruta chalepensis*, callus culture, hormone concentrations, 2,4-D, NAA, Artificial Intelligence, Model.

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1. Introduction

Plants are various in numbers and varieties not just in one area but throughout the world, there are certain plants that are cultivated and grown only in certain geographical locations which are due to climatic conditions, the kind of soil as well as many other parameters. Plants that could aid in many vital properties. It is seen that there are many systems of medicine ranging from allopathy, homeopathy, Siddha, and ayurveda and yet over the centuries it is noted that there are more advantageous properties seen over the usage of traditional system of medicine that is characterized through the usage of medicinal herbs or plants as a form of drug delivery aiding many hazardous disease conditions of the human body. There is many vital emphases that are been done in the usage of such traditional system of medicine through the usage of medicinal plants to the main reason as it justifies the fact of treating various issues of the human body through nil or mild side effects. These herbs are good form of pharmacological properties that have the major benefit of dealing with various spectrums of ailments in the human system. Many parts of the plant or the herb that ranges from the shoot, root, leaves, and flowers aid in particular aspects of specified functions that thereby help target ranges of diseases. In connection to the pharmaceuticals that are employed in other systems of medicine, there is unquestionably a significant area that is highlighted by the fruitfulness of such plants with pharmacological activity as it is generated naturally. There are various plants with qualities that are used for oil extractions, food flavoring, aroma, and many other herbal attributes that are important in the cosmetology industry. Out of all of these, a plant's ability to have medical properties is one of its most important trait. These medicinal plants contribute to many pharmacological values that help in curing infections and diseases, as nutritional values.. Plants that carry medicinal values, also called as medicinal plants are that aid in carrying pharmacological properties that could usually help in treating as well as curing illness [1]. One such of these medicinal plants that were being worked upon was *Ruta chalepensis,* commonly known as *fringed rue* and known as *Aruvada* in Tamil. This plant has been shown to carry many pharmacological properties such ranging from properties of anti-cancer, anti-diabetic, anti-inflammatory, analgesic, and many other tremendous characteristics. It is one of the most magnificent plants that have many important vital properties that could help in many areas to health and living. It is one of the very old ancient plants that have the commodity in herbal usage and remedies due to the presence of its pharmacological characteristics [2]. Through the practice of machine learning clubbed with plant tissue, there are many advantageous pointers noticed that could be reduced time to analyze results of callus proliferation rate, conservation of energy as well as highly cost-effective. The novelty of this study aims at analyzing the various plant growth regulators used for mass callus propagation and thereby predicting through machine learning and on which of the various hormone concentration could yield the best callus growth[3].

2. Materials and methods

Materials

2.1 Collection of explants and surface sterilization

The plant was procured fresh where in the leaf and the internode plant of *Ruta Chalepensis* was *been* collected from the hills of Nilgiris, Tamil Nadu. Once the collection or the procurement of the plant was done, it was made sure that the plants were kept sterile throughout until time and for obtaining the same, explants were thoroughly washed initially followed post which tween 20 was added, covered, and left under running tap water for about 30 minutes. Followed by which about 0.1% of sodium hypochlorite was added for about 20 minutes at 5-minute intervals and for a duration of about 5 minutes it was been washed in distilled water. Further, the sterilization process was taken place under aseptic conditions, in a Laminar airflow chamber. The next step was characterized where the explants were let to be treated with 0.5% mercuric chloride for 2 minutes and as the final step the explants were profusely washed for 5 minutes using sterile distilled water. The leaf and the internode were cut into sizes ranging from 0.5 cm – 1.0 cm and inoculated into an MS medium along with different hormonal concentrations.

2.2 Culture

For the process of induction of callus, MS medium were being used. Around six stocks of individual micro, macro, vitamins and iron were prepared and made to stock. For the preparation of media, FeSo4 and Na2EDTA were dissolved separately in 100 mL water. The iron stock was being procured and stored in a black bottle for the reason of preventing from photolysis of chemicals. Meso-inositol, auxin as well as cytokinin were made across every month. For the medium preparation, all the above six stock solution in the required levels of sterile distilled water. 0.1% meso-inositol (100 mg) and sucrose 3% (30 g/L) as well as required amount of plant growth regulators were being added to the medium and made the buffer with 1N HCl or 1N NaOH to adjust the medium pH to 5.6 prior to autoclaving.

The solidification of medium was done by adding 0.8% agar (800 mg/L). Sterile distilled water was used to make the final volume. The medium was poured into culture vials and autoclaved at 15 lb pressure for 15 min at 121ºC. Post the inoculation with the explants, all culture vials were kept under 16/8 h (light/dark) photoperiod at 25±2ºC.

2.3 *Callus induction & Initiation*

MS medium that was supplemented using various concentration dosages across the best of single hormone concentration was studied together as a combination, 2,4-D (0.5,1.9,1.5,2.0 mg) along with NAA (0.5,1.9,1.5,2.0 mg) was studied to see the initiation and induction of the callus. Multiple concentrations of dosages in the mentioned plant growth regulators were performed. The amount of callus induction was being calculated using the mentioned formulation Callus induction (%) = (No. of Plants produced/No. of Plants inoculated) X 100.

2.4 *Proposed Methodology*

Regression is a machine learning method used to explore correlations between independent features or characteristics and dependent variables or expected outcomes. This is a machine learning-based prediction model that uses algorithms to predict continuous outcomes[4].

2.4.1 Models Used:

Decision Tree Regressor: It is a tree-based model where the dataset is divided into smaller subsets. On receiving an input, the tree is traversed using a set of conditions or equations until reaching a leaf node that contains our expected result.

Random Forest Regressor: It is an ensemble learning-based supervised machine learning technique for regression. In order to produce predictions that are more accurate than those from a single model, the ensemble learning method integrates predictions from several machine learning algorithms.

Multi-Layer Perceptron: A multilayer perceptron is a deep learning-based technique that uses multilayered feedforward artificial neural network to predict the set of outputs after learning from the given set of inputs. They use multiple layers of input nodes connected as a directed graph between input and output layers. Backpropagation is characterized in usage of training the network and forward propagation is used to predict the outputs.

Support Vector Regressor (SVR): It is a supervised machine learning approach constructed on the concept of Support Vector Machine. It tries to find a hyperplane in an N-dimensional space that clearly classifies the data points. Radial Basis Function kernel-based SVR was used in our model[5].

2.4.2*Metrics used*

Root Mean Squared Error: The contrast between values predicted by a model or estimator and the values observed is commonly measured by the root-mean-square deviation, also known as the root-mean-square error.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} \|y_i - \hat{y}_i\|^2}{N}} \quad (1)$$

Mean Absolute Error: The error linking paired observations describing the same phenomenon is measured by this metric.

$$MAE = \frac{1}{n}\sum_{i=1}^{n} |y_i - \bar{y}| \quad (2)$$

R2 Score: R2 Score is characterised as a statisical measure which shows the proprotion of variance for a variable which is dependent that is explained by an independent variable or variables in a regression model.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \quad (3)$$

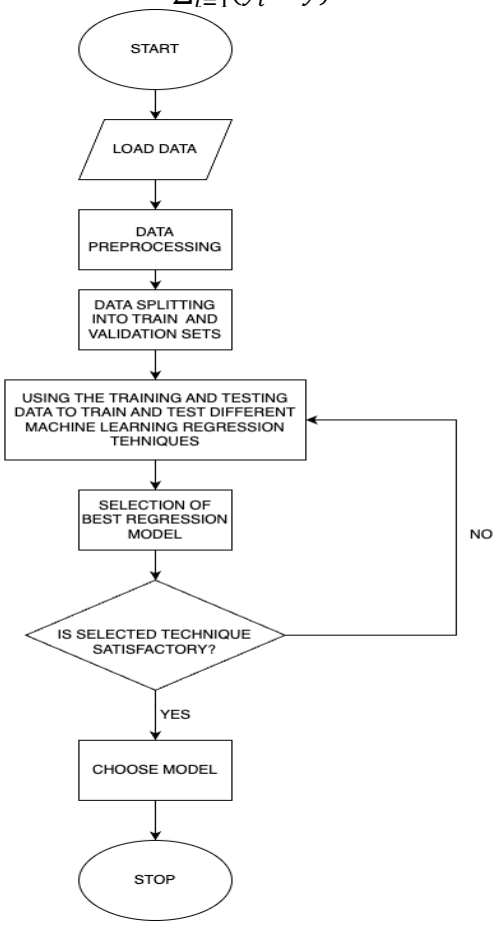

Figure 1: Proposed flow diagram of data procured models.

3. Results and discussion

3.1. Experimental results

Effect of hormones on callus initiation of *R. chalepensis*

In the present study, different hormones such as 2,4-D, NAA, BAP, IAA at concentrations of (0.5-2.0 mg/L) were used to evaluate the callus initiation of *R. chalepensis*. The results showed that compared to internode, the leaf explant showed higher callus initiation in 2,4-D, NAA, and BAP. The hormone IAA showed the least response on the callus initiation in both the internode and leaf explant of R. chalepensis. The maximum response rate of callus in the leaf explant was found to be 94.5 %, 81.26 %, and 73.33 % for 2,4-D, NAA, and BAP respectively. However, the maximum response rate of callus initiation in the internode explant did not exceed 68.33 % for 2,4-D and 75.83 % for NAA, and 73.16 % for BAP. For further studies, leaf explant and hormones (2,4-D and NAA) were chosen since the higher response of callus initiation was observed.

Dual hormone combinations and elicitors on callus initiation of *R. chalepensis*

From the initial analysis for the callus initiation, we choose 2,4-D and NAA for the combinatorial effect of callus induction. So, four concentrations of 2,4-D and NAA were used in combinations and the effect of elicitor L-phenylalanine and Riboflavin were also analyzed. The results showed that 2,4-D and NAA combinations of 1.0 mg/L and 0.5 mg/L respectively showed high callus initiation response of 95.13 % compared to other combinations. Further, elicitor

L-phenylalanine and Riboflavin treatment on 2,4-D (1.0 mg/L) and NAA (0.5 mg/L) showed the highest callus initiation of 97.06% in L-phenylalanine and 92.23 % in riboflavin was no compared to other hormone concentrations. Thus, 2,4-D (1.0 mg/L), and NAA (0.5 mg/L) were shortlisted as combinations since they showed better callus induction with lower concentrations.

3.2. Model Results:

The above regression models were trained and used for prediction and the following metrics were observed.

	MAE	RMSE	R2
Decision Tree Regressor	3.12	2.6	0.93
Random Forest Regressor	2.16	2.3	0.9943410003650968
Multi-Layer Perceptron	2.98	2.5	0.95
SVR	3.01	2.5	0.96

From the metric it is inferred that Random Forest Regressor is providing the best results with and RMSE 2.3. This could be as a good RMSE score taking into account the predicted value and the range between 7.4 to 94.7 keeping the mind the data content.

4. Conclusion

This study was performed in order to predict the callus formation of the plant species *Ruta Chalepensis* through the process of plant tissue culture. From the experimental results obtained it could be inferred that across different hormonal combinations studied 2,4-D (1.0 mg/L), and NAA (0.5 mg/L) showed maximum callus initiation, and hence machine learning models was performed in order to check various concentrations to check the callus formation. From the results of the above models, it is observed that Random Forest regressor is doing the best in predicting the results as it has a root mean square error value of 2.3 and a R2 score of 0.99. Considering the values in the data ranged from 7.4 to 94.7 the error can be considered nominal. Hence it could be understood that with this model we will be able to analyze the hormones at various combinations as well as different concentrations to predict the best callus formation which can be chosen for further studies.

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