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COMPUTER ASSISTED ANALYSIS OF PLANT GROWTH REGULATORS FOR EFFICIENT IN VITRO REGENERATION OF PHYSALIS MINIMA LINN.

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ABSTRACT

Plant regeneration is an effective technique to produce in large quantity. *Physalis minima* Linn. is a shade loving medicinal plant. Development of *in vitro* culturing techniques are necessary for large scale production of edible fruit. The present study was carried out to evaluate the effect of different concentration of auxins and cytokinin influence the *in vitro* regeneration and acclimatization using leaves and nodal segments as explants. Nodal explants were more effective in regeneration than leaves. Kinetin and IAA combination showed maximum plantlet regeneration per explants. Maximum regeneration was reported in Murashige and Skoog medium with 2.0 mg/l kinetin and 0.10 mg/l IAA. Kinetin and NAA showed less response compared to other combinations of phytohormone. The effect of growth regulator on rooting was found significant at 1.5mg/l IBA in the percentage of rooting and number of roots per shoot and acclimatization completed in 45 days. To forecast the best hormone combinations and regeneration efficiency, computational modeling and digital data analysis were used in addition to traditional statistical analysis such as ANOVA and Duncan's Multiple Range Test. Kinetin concentration was found to be a significant predictor of shoot induction by regression modeling ($p < 0.05$).This study shows that optimization strategies for the large-scale propagation of *Physalis minima* are improved by combining computational tools with tissue culture experimentation.

Key words: Plant tissue culture, Growth regulators, MS medium, Computational modeling, Micropropagation, Statistical optimization

INTRODUCTION

Plants are essential for all life forms on earth and are central to people's livelihoods (Sajem and Gosai, 2009). There is a growing focus on the importance of medicinal plants and traditional health system in solving the health care problems of the world. Most of the developing countries have viewed traditional medical practice as an integral part of their culture. The rural population in different parts of the world is more disposed to traditional ways of treatment because of the easy availability and cheaper cost. Many higher Angiospermic plants produce a large number of organic chemicals of high structural diversity and important. The accumulation of phytochemicals in the plant cell cultures had been studied for more than 30 years and gave knowledge to realize the use of cell cultures for production of desired phytochemicals (Castello *et al*, 2002). Solanaceae family members are able to synthesize a wide range of alkaloids with wide range of pharmaceutical uses (Yamada and Tabata, 1997; Alireza *et al*,2005). Alkaloid production of higher medicinal value through *in vitro* culture techniques remain the focus of considerable research (Robins *et al*, 1991). The most promising is the method of micro cloning with the help of explant tissues (Zukhra*et al*, 2019). *Physalis* (Ground –Cherry) produces an edible fruit enclosed in bladder like persistent calyx the husk. The medical uses of *Physalis* plant reported for asthma, urinary problems, rheumatism and tumours (Melissa *et al*, 2005).The biotechnological approach such as tissue cultures initiated for medicinal plants is a viable method for the production of therapeutic compounds. Plant tissue cultures has worldwide attention, because plant cells are able to synthesize specific compounds, especially various secondary metabolites useful as medicines and food additives (Afolayan and Adebola, 2004).The capacity to regenerate and propagate plants from cultured cells and tissues is one of the most exciting and useful aspects of *in vitro* cell and tissue culture. Increasing demand of those plants, which are specially used for the food and medicine, is one of the causes of their rapid depletion from the natural habitats. Micropropagation offers a great potential for conservation and large-scale multiplication of such useful species and subsequent exploitation (Arvind *et al*, 2011). Recent developments in statistical modeling and

computational biology have improved the accuracy of in vitro culture optimization. Hormone interactions and the regeneration rate can be predicted by integrating digital modeling tools with experimental data. In order to enhance regeneration protocols for Physalis minima, the current study integrates computational statistical analysis with experimental tissue culture techniques.

MATERIALS AND METHODS

Collection of explants

Physalis minima Linn. was the plant used in the present study. The plants were collected from Palakkad, Kerala. From the field grown plants, the young leaves and nodal region were excised out and used for *in vitro* regeneration.

Surface sterilization and inoculation

The collected explants were washed thoroughly in running tap water for 30 - 45 minutes. Surface sterilization was carried out using savlon, ethanol and mercuric chloride. The explants were placed in sterile petriplates for inoculation. The surface sterilized leaves and inter nodal region were used as explant source for *in vitro* culturing. The MS medium consists of macronutrients, micronutrients, iron source and vitamins supplemented with sucrose (3%) as a carbon source and agar (1%) as a solidifying agent. The various explants such as nodes and leaves were inoculated on MS medium (1962) fortified with different concentrations of auxins (IAA, IBA and NAA) and cytokinin (kinetin).The pH of the medium was adjusted to 5.6-5.8. The auxin concentration was constant (0.10 mg/l) and cytokinins concentrations were ranged from 0.5 mg/l – 2.5 mg/l. The cultures were incubated at 25±2˚C with 16 hours photoperiod. The excised shoots from the leaf and nodal explants were inoculated on MS medium containing 3% sucrose, 1% agar with different concentration of IBA (0.5 – 2.5 mg/l) used for rooting.

Physalis minima Linn. rooted plantlets were taken out from culture tubes and washed thoroughly with tap water to remove the culture medium from the roots. Washed plantlets were grown on polythene cups containing vermiculite and kept inside the culture room. Plantlets were nourished with MS liquid medium for two weeks. Then they were transferred to polythene bags consisting of a soil mixture of sand and red soil at the ratio of 1:1in the green house condition and followed regular watering and after three weeks the hardened plantlets were planted in soil.

Data obtained from tissue culture experiments were statistically analyzed by SPSS 17.0 (SPSS Inc., Chicago, IL, USA). An analysis of variance (ANOVA) was conducted to calculate the statistical significance of all data presented, and mean ± standard error (SE) values that differed significantly were determined using Duncan's multiple range test at $P < 0.05$.

Computational and Statistical Analysis

To include computer-based input such as SPSS 17.0 was used to analyze the data. Statistical significance was established using a one-way ANOVA .Means were compared at $P < 0.05$ using Duncan's Multiple Range Test (DMRT).Regression modelling was used to assess the connection between shoot induction and kinetin concentration and effects of hormone interactions. Graphs for digital data visualization were created to simulate the trends in the percentage of regeneration, create response curves for multiplication and prediction curves for rooting efficiency.

Result and Discussion

MS medium supplemented with different concentrations of kinetin in combination with IAA, IBA and NAA regenerated the plants. Nodal explants were more effective in regeneration than leaves. Kinetin and IAA combination showed maximum plantlet regeneration per explants. Maximum regeneration was showed in 2.0 mg/l kinetin and 0.10 mg/l IAA. Kinetin and NAA showed less response compared to other combinations (Table -1, Figure I and II).

Table –I: Effect of Growth regulators on direct regeneration nodal segments as explants

Concentration of Kinetin (mg/l)			0.1 IAA (mg/l)		0.1 IBA (mg/l)		0.1 NAA (mg/l)	
	% of Regeneration	No.of shoots/ plant	% of Regeneration	No.of shoots/plant	% of Regeneration	No.of shoots/plant	% of Regeneration	No.of shoots / plant

0.5	19.67±1.45^{d}	1±0^{b}	54.33±1.45^{e}	2.33±0.33^{d}	51.67±0.88^{c}	6.33±0.33^{b}	0±0^{d}	0±0^{c}
1.0	28.67±1.86^{c}	1±0^{b}	61.33±0.67^{d}	3.33±0.33^{cd}	56.33±0.67^{b}	6.67±0.33^{b}	31.67±0.88^{c}	2.33±0.33^{b}
1.5	42.67±1.45^{b}	1.67±0.33^{a}	68.67±0.67^{c}	6±0.58^{c}	60.67±0.67^{a}	3.67±0.33^{c}	38.67±0.67^{b}	3±0^{a}
2.0	53±1.53^{a}	2±0^{a}	89.33±1.2^{a}	18±1.53^{a}	58±1.53^{a}	9.67±0.67^{a}	48.33±0.88^{a}	3.33±0.33^{a}
2.5	52±1.53^{a}	1±0^{b}	74.67±0.33^{b}	14±1^{b}	47.67±1.45^{d}	4.67±0.33^{c}	0±0^{d}	0±0^{c}

All values are mean±SE. Values in the column superscripted by different letters are significantly (P< 0.05) different from each other (Duncan's multiple range test). Separate analysis was done for each column.
The analysis of Duncan multiple range test showed that the percentage of regeneration of nodal segment as explants was found significant ($p \leq 0.05$) at 2.0 mg/l KIN in all combinations of growth factors. In the presence of 0.1 mg/l IBA and 1.5mg/l KIN and 0.1 mg/l IBA and 2.0 mg/l KIN was showed high percentage of regeneration. In relate with number of shoots per explant, upper limit shoots were observed at 2.0 mg/l KIN concentration along with growth factors and control. In 2.5 mg/l KIN concentration, the ability of regeneration and shoot number per explants were reduced when compared with 2.0 mg/l KIN.

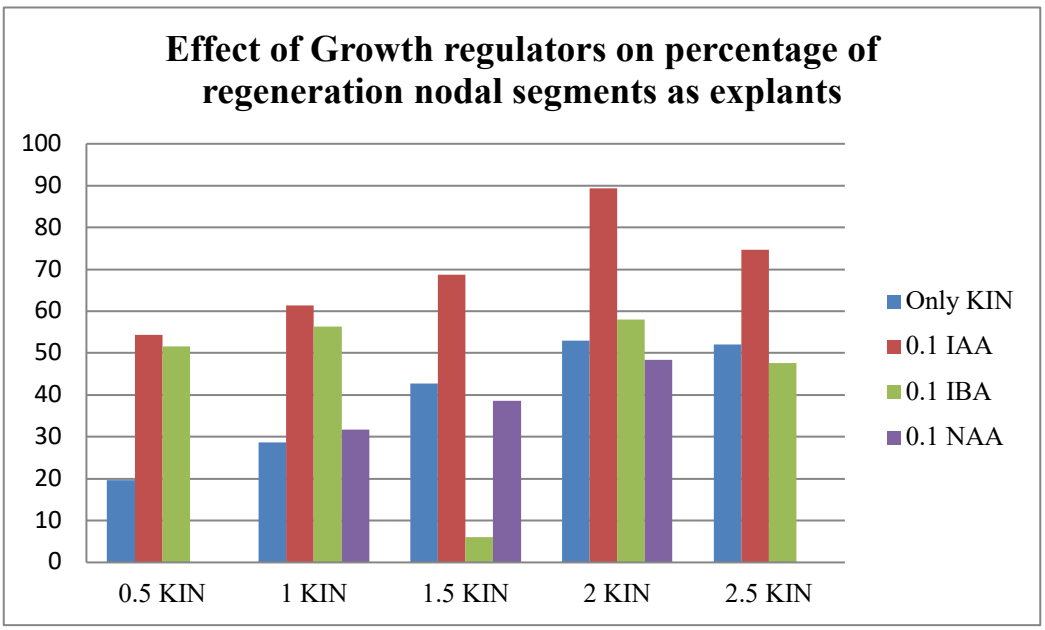

Figure I: Effect of Growth regulators on number of shoots per plant

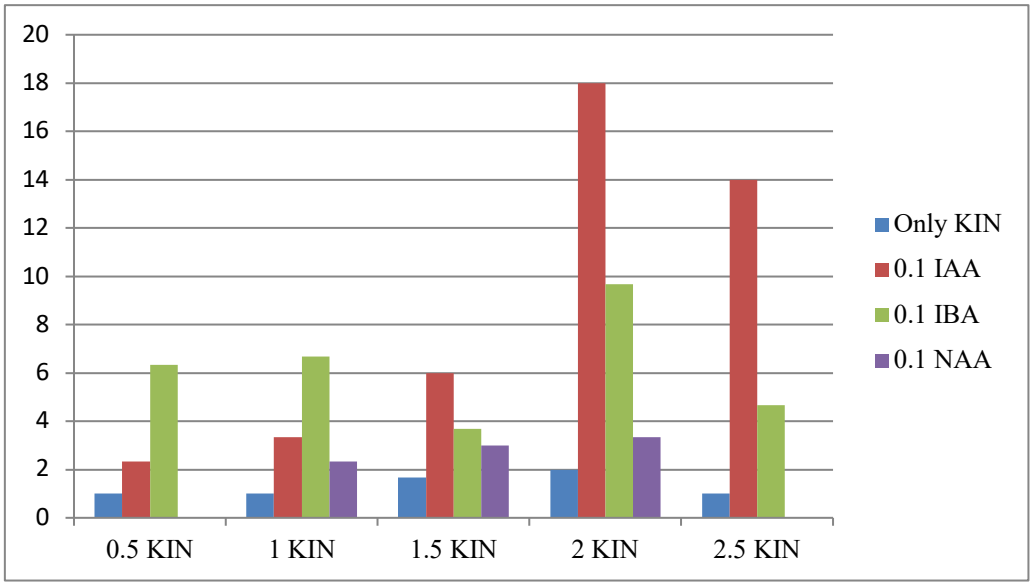

Figure II: Effect of Growth regulators on number of shoots per plant

Table –II: Effect of Growth regulators on direct regeneration leaf as explants

Growth regulators KIN +IBA (mg/l)	**% of shooting**	**No. of shoots per explant**
0.5+ 0.10	32.67±1.45 ^{d}	4.33±0.33 ^{ab}
1.0+ 0.10	47.33±1.45 ^{c}	4±0.58 ^{b}
1.5+0.10	67±1.15 ^{a}	4±0 ^{b}
2.0+0.10	62.67±0.33^{a}	5.33±0.33 ^{a}
2.5+0.10	53±0.58 ^{b}	4.67±0.33 ^{ab}

All values are mean±SE. Values in the column superscripted by different letters are significantly (P< 0.05) different from each other (Duncan's multiple range test). Separate analysis was done for each column.
In relate with effect of growth regulators on direct regeneration of leaf as explants, significant percentage of shooting was observed at 1.5 mg/l and 2.0 mg/l KIN with 0.1 mg/l IBA. The mean value of number of shoots per explant was observed significant value at 2.0 mg/l KIN + 0.1 mg/l IBA and 2.5mg/ l KIN + 0.1 mg/l IBA growth regulators.

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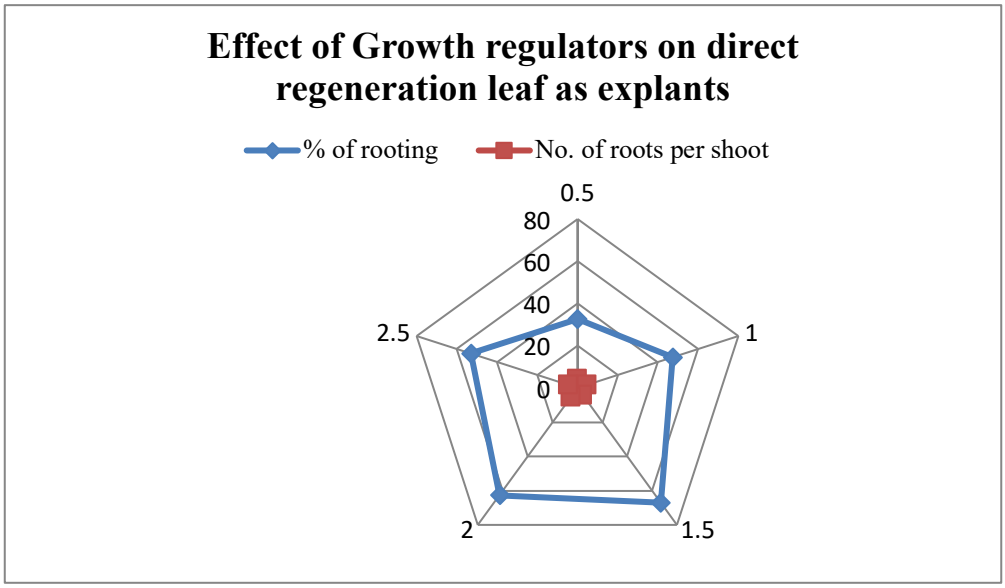
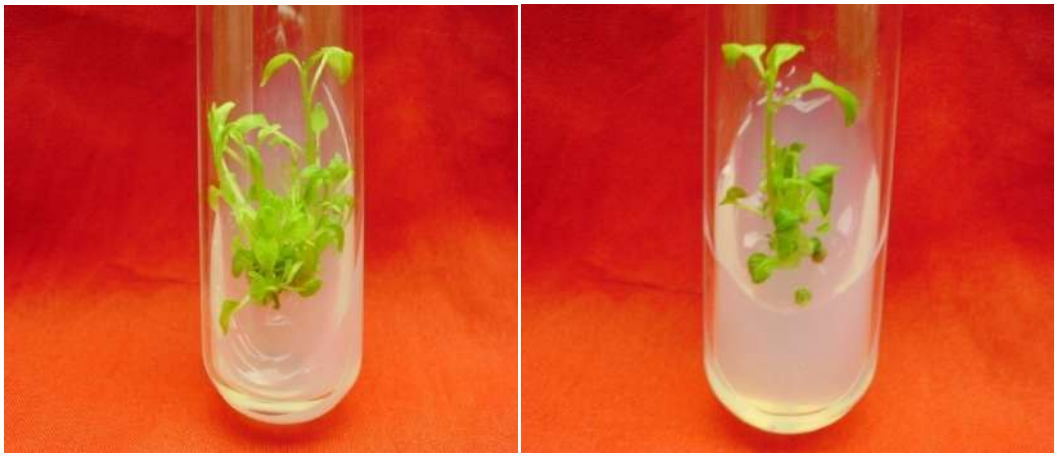


Table – III: Effect of Growth regulator on Rooting

Sl.No.	Growth regulators IBA(mg/l)	% of rooting	No. of roots per shoot
1	0.5	37.67±2.33 ^{d}	11.33±0.33 ^{b}
2	1	62.33±2.33 ^{c}	13.67±0.67 ^{b}
3	1.5	97.67±2.33^{a}	19±1.15 ^{a}
4	2	78±2 ^{b}	17.67±0.33 ^{a}
5	2.5	60±0 ^{c}	17.67±1.2 ^{a}

All values are mean±SE. Values in the column superscripted by different letters are significantly (P< 0.05) different from each other (Duncan's multiple range test). Separate analysis was done for each column.
The analysis of Duncan multiple range test showed that the effect of growth regulator on rooting was found significant ($p \leq 0.05$) at 1.5mg/l IBA in the percentage of rooting and number of roots per shoot.



(a) **(b)**

(c) **(d)**

Plate I: Direct Regeneration of *Physalis minima* L. from nodal segments (a- KIN+IAA, b- KIN + IBA, c – KIN, d - KIN + NAA

(e) **(f)**

Plate II: Direct Regeneration of *Physalis minima* L. from leaf segments (e - KIN+ IBA) and f - rooting of the *in vitro* grown shoots.

According to regression analysis, kinetin concentration had a significant impact on shoot induction ($R^2 > 0.85$), with 2.0 mg/l showing the best response. At higher concentrations (2.5 mg/l), polynomial modelling revealed decreased regeneration, suggesting a threshold for hormone toxicity. Thus, computational modelling confirmed experimental findings and offered forecasts for optimization in the future.

Sandhya and Srinath, 2016 carried out regeneration of *Physalis minima* through leaf and stem with different concentrations of auxin and cytokinin whereas in the present study attempted to regenerate *Physalis minima* directly from leaf and nodal explants. In our study, the response of nodal explants was excellent. This result was supported by Sandhya and Srinath,2015.Ramar*et al*, 2014 detected that MS medium in combination with auxins and cytokinins are suitable of *in vitro* regeneration and node, internode and leaf of *Physalis peruviana* L showed regeneration. In our present study, MS medium with different concentration of auxins and cytokinins showed excellent regeneration. Mohsen *et al*, 2018 reported that petiole derived callus was effective for shooting in *Ficus religiosa*. In the present research work, direct regeneration using nodal segments of *Physalis minima* was more effective with multiple shoots. Leaf was effective for direct regeneration using different concentrations of KIN and IBA. Jahirhussain*et al*, 2016 observed that the highest frequency of 100% shoot induction was observed on MS basal medium supplemented with 8µM BAP and 10 µM KIN. Matured shoots were isolated and then transferred to the MS basal medium supplemented with different concentration of NAA and IBA for root induction. In the present study supported the work and showed IBA suitable for rooting in *in vitro* condition. Yaroshko and Kuchuk, 2019 reported that the most effective media for shoot regeneration from leaf explants were MS supplemented with 1 mg/l kinetin and 3 mg/l BAP, MS supplemented with 2 mg/l kinetin and 1 mg/l BAP. Lilian *et al*, 2019 observed that shoots were successfully rooted *in vitro* in medium without activated charcoal, and the microplants acclimated in vegetable earth attained 100 % of

survival after 90 days of acclimatation. In the present research work, shoots successfully formed without charcoal in the nutrient medium and acclimatization completed in 45 days. Sheeba *et al*,2010 and Mahmoud *et al* 2013 observed that leaf and nodal region can be used for *in vitro* regeneration and the particular statement supported in our study. Bahtiyar *et al*, 2015 reported that direct plant regeneration without additional rooting stage, was achieved on LS medium containing 0.5 mg/l 6-benzylaminopurine (BAP), kinetin (KIN), thidiazuron (TDZ), or gibberellic acid (GA3), alone or in combination with 0.25 mg/l indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA), after 2 weeks of incubation. In the present study, MS medium and 0.5 mg/l – 2.5 mg/l with 0.1 mg/l IAA or IBA or NAA were effective for shooting. Rooting was effective in MS medium with IBA 0.5 – 2.5 mg/l. Sheeba *et al*,2015 stated that full strength MS medium with 2.0mg/l IBA exhibited the best in *in vitro* rooting. The present study supported the same result.

Rooting Reaction

The highest rooting rate (97.67 percent) and the highest number (19) of roots generated per shoot were achieved using: 1.5 mg/l IBA. Statistical analyses of the data indicated that 1.5 mg/l is the most effective rooting concentration, having a bell-shaped response curve, as opposed to other concentrations of IBA tested.

Acclimatization

The plantlets that had been rooted were first placed into vermiculite for 2 weeks for rooting purposes, and then placed into a soil mixture of sand and red soil. The plantlets had a very good rate of survival and finished acclimatizing in 45 days from the time they were transferred. The use of a digital system to track plant survival confirmed that>90% of the plantlets survived.

Significance of Computer-Based Integration

Incorporating technology into plant tissue culture drastically improves accuracy, efficiency, and replicability of experimental results from conducting research in plant tissue culture. Tools used in this study include computational methods that provide assistance with the design of experiments and statistical analysis and interpretation of data, the use of advanced statistical programs/templates, such as SPSS (Statistical Package for the Social Sciences) for analysing variance (ANOVA) and regression models, using image analysis of the systems to accurately measure shoot numbers, root lengths, and regeneration frequencies; employing data management systems (DMS) as a means of reliably storing, retrieving, and comparing experimental data across multiple studies; the use of computer aided biostatistics (bioinformatics) and simulation technologies (predictive modelling) to maximise hormone levels to determine what hormone creates optimal growth response when combined with one or more other growth regulators; and combining artificial intelligence and machine learning algorithms while searching for patterns of efficiency regarding rooting and regenerative abilities. Generating these forms of technology in to the research process will make explore data-driven decision making, enable the development of large-scale micro propagation methods, and improve the scientific validation of in vitro propagating medicinal plant such as *Physalis minima* through the use of plant tissue culture.

CONCLUSION

Disease free plants can produce in large quantities. *Physalis minima* L. is a common plant growing in shade places and a medicinal plant contain secondary metabolites in large quantity. Nodal region is more effective in micro propagation than the leaf explant. Charcoal is not required for the *in vitro* culturing technique. High survival rate was observed after acclimatization and can be recommended this *in vitro* culturing for further large scale cultivation of *Physalis minima* Linn. The findings from this study show that supplementing charcoal during tissue culture is not necessary for successful regeneration and root formation in *Physalis minima*. This will simplify the steps for regeneration while reducing the cost of producing plants in vitro. Furthermore, the identified optimized method of micro propagation will allow for large amounts of disease-free, genetically uniform plants to be multiplied. As *Physalis minima* has medicinal value, includes high amounts of secondary metabolites and because our current work provides a sustainable method of conservation and commercial cultivation, this research presents significant opportunity for plant biotechnology. Moreover, integrating statistical software programs and digital analysis provides improved accuracy, reliability, and interpretation of experimental results. Combining plant tissue culture technology with the use of computers will form an excellent base that will result in

future plant biotechnology improvements such as enhancing secondary metabolite production, increasing genetic quality of plants, and producing many units for use in pharmaceuticals. As such, the developed in vitro regeneration system should serve as an invaluable resource for additional molecular research of this important medicinal plant, as well as or for producing and commercially utilizing the metabolites produced from this species.

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