Annals of Bangladesh Agriculture
Journal homepage: bsmrau.edu.bd/aba

ORIGINAL ARTICLE

Efficient micropropagation protocol of holy basil (*Ocimum sanctum* L.) using nodal segments

Md. Abul Kalam Azad[1], Samia Jahan Purnota[1], Taufica Nusrat[1], Shah Mohammad Naimul Islam[1] and Md. Ashraful Haque[1*]

[1] Institute of Biotechnology and Genetic Engineering (IBGE) Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur 1706, Bangladesh

ARTICLE INFO.

Keywords:

in *vitro* propagation, plant growth regulators, nodal segments, acclimatization, *Ocimum sanctum*

Received : 10 July 2024
Revised : 22 October 2024
Accepted : 30 December 2024
Published : 9 January 2025

Citation:

Azad M. A. K., S. J. Purnota, T. Nusrat, S. M. N. Islam and M. A. Haque. 2024. Efficient micropropagation protocol of holy basil (*Ocimum sanctum* L.) using nodal segments. *Ann. Bangladesh Agric.* 28(2): 57-69

ABSTRACT

Holy Basil (*Ocimum sanctum* L.) is a valuable herb due to its medicinal, ornamental and culinary applications. The conventional propagation methods are unsuitable due to seed dormancy and slow growth. This study aimed to establish an optimized *in vitro* regeneration method using nodal segments obtained directly from mature plant to achieve rapid propagation of *O. sanctum*. The sterilized explants were placed on the Murashige and Skoog (MS) medium containing various concentrations of 6-Benzylaminopurine (BAP) and Kinetin (KIN) (0.5, 1.0, 1.5 and 2.0 mg/L) to promote shoot proliferation. Subsequently, indole butyric acid (IBA) and indole acetic acid (IAA) (0.5, 1.0, and 1.5 mg/L) were used on MS medium for the induction of roots. The results showed that the highest percentage of shoot response (92.7%), shoots per explant (6.5) and length of shoots (5.63cm) were observed with a concentration of 1.5 mg/L BAP, highlighting the efficiency of the *in vitro* culture method. The highest root induction (90.0%) and roots per explant (5) were achieved using 1.0 mg/L IBA. Hardening media were prepared using various combinations of garden soil, sand, and farmyard manure (FYM). The highest survival rate (90%) was observed in a combination of garden soil and sand in a 1:1 ratio. However, the *in vitro* culture method, as demonstrated in this study, is a highly efficient approach for the rapid propagation of *O. sanctum.*

[*]Corresponding Author: Institute of Biotechnology and Genetic Engineering (IBGE) Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur 1706, Bangladesh. Email: ashrafbiotech@bsmrau.edu.bd

https://doi.org/10.3329/aba.v28i2.84184
ISSN 1025-482X (Print)/2521-5477 (Online)

1. Introduction

Nature has given us a vast plant species growing around the world used to make a large number of drugs today (Manjudevi *et al.* 2022). Over 80% of the world's population relies substantially on traditional medicine for their fundamental medical needs (Bernardini *et al.* 2017). Traditional medicine practitioners serve a significant percentage of patients in nations like Bangladesh (90%) and Burma (85%), according to a World Health Organization survey (WHO, 2020). *O. sanctum* widely recognized species under the Lamiaceae family and known as holy basil or Tulsi (in Bangla) (Hanumanthaiah *et al.* 2020). It is considered as the most valuable plant due to its various medicinal qualities in root, leaves, flowers and seeds (Mandal *et al.* 2022). Native to India, it has been used as a traditional medicine for a long time due to its therapeutic properties. In Ayurvedic medicine, Tulsi (*O.sanctum*) is known as the "queen of herbs" and the "Elixir of Life" due to its anticancer properties (Hasan *et al.* 2023).

It has been found that *O. sanctum* plays an important role in pharmacological effects by producing secondary metabolites as part of a defense mechanism (Bhuvaneshwari *et al.* 2016; Jakovljevic *et al.* 2022; Kumari *et al.* 2022). Bioactive volatile compounds such as camphor, eugenol, beta-caryophyllene, alpha bisabolene, and beta bisabolene are known as essential oils present in the leaf extracts of *O. sanctum* that are responsible for antimicrobial properties (Piras *et al.* 2018; Yamani *et al.* 2016). It has antioxidant properties that neutralizes free radicals and secure human cell from oxidative damage (Chaudhary *et al.* 2020).

Seed propagation is the traditional method of this species which is not suitable due to its poor viability, low germination rate and, season dependence (Saha *et al.* 2014). Moreover, due to cross-pollination heterogenous populations developed that affect the chemical properties of this species (Singh & Sehgal, 1999)

In vitro culture techniques are the most efficient way for rapid multiplication of *Ocimum* species that produce genetically true-to-type progeny (Saha *et al.* 2012; Siddique and Anis, 2008). There are several *in vitro* studies have been conducted on this species, using inflorescence (Singh and Sehgal, 1999), shoot tip (Mohammad *et al.* 2016), axillary shoot buds (Pattnaik *et al.* 1996), and leaf (Mishra, 2015). Until now, there is a single study that has been conducted on this species used nodal explants derived from *in vitro* germinated seedlings, (Kayani *et al.* 2021), provided limited data compared to other herbaceous medicinal plants using nodal explants in their *in vitro* culture (Karim *et al.* 2013; David Raja and Arockiasamy, 2008; Rahman *et al.* 2021; Hassan *et al.* 2010). This study, focused on establishing a reliable and faster *in vitro* regeneration method for mass plantlet production of *O. sanctum* from nodal explants obtained directly from healthy, nursery-grown mother plants.

2. Materials and Methods

2.1 Collection of Explants and Sterilization

The young nodal explants were collected from one-year-old pot-grown Basil plants located in the nursery of Gazipur Agricultural University. The explants were rinsed under running tap water for 10 minutes to eliminate external dust. The nodal explants were cut (1.5-3) cm long and dipped into 70% ethanol for 30 seconds. Thereafter, the explants were immersed in 1.5% sodium hypochlorite solution for 2-3 minutes and rinsed 5–6 times with sterilized distilled water, ensuring thorough removal of any residual chemicals. Subsequently, 0.1% $HgCl_2$ used for 3 minutes after dipping explants into it, and rinsed 7-8 times with sterilized distilled water. The entire procedure was performed on a clean bench to avoid contamination.

2.2 Culture Media and Conditions

The MS basal medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and 8 g/L agar was used to initiate and proliferate shoots from explants. This medium was supplied by varying concentrations (mg/L) of 6-Benzylaminopurine (BAP) and Kinetin (Kin). The pH level of the culture medium containing different PGRs was calibrated to 5.7. Subsequently, the medium was heated using a microwave-oven for complete dissolution of the agar and poured into a test tube of dimensions 13x25 mm and tightly sealed with aluminum foil. Then, the cultures were subjected to autoclaving at 121°C and a pressure of 15 psi for 20 minutes. All the cultures were kept in a growth chamber under white fluorescent light at 33.73 µmol m^{-2} s^{-1} intensity for 16/8 hours (light/dark cycle) at 25±2°C, 55-60% humidity (Komakech *et al.* 2020)

.2.3 In vitro Shoot Induction

Nodal explants with a length of 1.5 to 3 cm were placed on medium prepared for shoot induction, containing BAP at concentrations of 0.5, 1.0, 1.5, and 2.0 mg/L, and Kin at concentrations of 0.5, 1.0, 1.5, and 2.0 mg/L. After four weeks, data were recorded on the percentage of shoot formation, the shoot count per explant, and the length of the shoots.

2.4 In vitro Root Formation

Shoots of 2 to 4 (cm) in length were placed onto MS medium (half-strength), which was prepared to produce roots supplied by IBA (.5, 1.0, 1.5 mg/L) and IAA (.5, 1.0, 1.5 mg/L). After four weeks of shoot inoculation, data were recorded on the percentage of root formation, the root count per explant, and the length of the roots.

2.5 Acclimatization of plantlets

Plantlets with roots were removed from the culture medium, and the agar substance was washed off under flowing water. The plantlets were subsequently planted into sterilized mixtures of garden soil, sand, and farmyard manure (FYM) in varying proportions within plastic cups and enclosed in transparent

polyethylene. Afterward, the transplanted plants covered by polythene were kept in controlled conditions for 28 days. After one week, pores were created on the polythene bag to ensure gaseous exchange and gradually reduce the relative humidity. Three weeks later, the polythene covers were removed, allowing the plantlets to transition to natural conditions (Sharma *et al.* 2023).

2.6 Data Analysis

To set up the experiments, a completely randomized block design was used, with three replicates, each containing 12 explants. The mean ± standard error (SE) values were presented, and one-way ANOVA was performed for statistical analysis. Duncan's multiple range test was used to examine the significance of the difference between means at $p \leq 0.05$. All statistical analyses were performed by R (4.4.2) version and Microsoft Office Excel 2020 program package.

3. Results and Discussion

3.1 Shoot Initiation

Nodal segments were utilized as initial explants, BAP and Kin in varying concentrations were added to MS medium for shoot initiation. The results showed that application of varied levels of BAP and Kin recorded a significant effect on shoot initiation (Table 1).

Both BAP and Kin significantly influenced shoot regeneration and growth parameters compared to the control, which showed no bud initiation or shoot development. BAP generally

Table 1. The effect of varying concentrations of BAP and Kin in MS medium on multiple shoot induction from nodal explants of Holy basil (*O. sanctum)* was studied after 4 weeks of culture

PGRs	Bud Initiation (Day)	Shoot Regeneration (%)	No. of shoots/ explant	Shoot length (cm)
Without PGR	0.00	0.00^{h}	0.00^{f}	0.00^{e}
BAP (mg/L)				
0.5	10^{th}	88.8 ± 0.61^{b}	4.4 ± 0.267^{b}	3.59 ± 0.138^{b}
1.0	9^{th}	84.7 ± 0.39^{c}	2.4 ± 0.221^{de}	3.40 ± 0.142^{b}
1.5	7^{th}	92.7 ± 0.68^{a}	6.5 ± 0.401^{a}	5.63 ± 0.208^{a}
2.0	7^{th}	78.5 ± 1.15^{d}	2.1 ± 0.233^{e}	2.88 ± 0.110^{c}
Kin (mg/L)				
0.5	12^{th}	64.5 ± 1.07^{g}	2.4 ± 0.163^{de}	1.63 ± 0.121^{d}
1.0	9^{th}	70 ± 0.74^{f}	3.3 ± 0.300^{c}	1.63 ± 0.096^{d}
1.5	9^{th}	76.5 ± 0.71^{de}	2.9 ± 0.277^{cde}	2.51 ± 0.127^{c}
2.0	7^{th}	75 ± 0.81^{e}	3.1 ± 0.277^{cd}	2.65 ± 0.095^{c}

Data expressed as mean±S.E. Means followed by the same letter (s) within a column do not differ significantly at $P \leq 0.05$ by DMRT.

proved more effective than Kin across all measured parameters. The optimal BAP concentration was 1.5 mg/L, which yielded the highest shoot regeneration percentage (92.7% ± 0.68), the greatest number of shoots per explant (6.5 ± 0.401), and the longest shoot length (5.63 ± 0.208 cm). Bud initiation was earliest (7 days) at both 1.5 mg/L and 2.0 mg/L BAP. Concentrations of BAP at 1.5 ppm generally resulted in reduced efficacy, although 0.5 mg/L BAP still showed strong regeneration (88.8% ± 0.61) and good shoot length (3.59 ± 0.138 cm). For Kin, the highest shoot regeneration percentage was (76.5% ± 0.71) at 1.5 ppm, while the maximum number of shoots per explant was (3.3 ± 0.300) at 1.0 mg/L. Shoot length peaked at (2.65 ± 0.095) cm with 2.0 mg/L Kin. Bud initiation with Kin treatments ranged from 7 days (2.0 mg/L) to 12 days (0.5 mg/L). Overall, Kin treatments consistently resulted in lower shoot regeneration percentages and fewer, shorter shoots compared to the optimal BAP concentrations. It was found that BAP also produced better results in the *in vitro* culture of *O. basilicum* (Siddique and Anis, 2008) and *O. Canum* (Saha *et al.* 2014). Several studies reported that BAP is the most effective plant growth regulator over Kin in tissue culture systems in a variety of medicinal plants (Rahman *et al.* 2021; David Raja and Arockiasamy, 2008).

The optimal concentration of 1.5 mg/L BAP achieving the highest shoot regeneration and proliferation suggests a balanced hormonal environment conducive to cell division and differentiation, leading to robust shoot

Table 2. The effects of varying concentrations of IAA and IBA in MS medium on root induction of Holy basil (*O. sanctum)* was studied after 4 weeks of culture

Plant Growth Regulators (mg/L)	Regeneration (%)	Number of Roots/Explant	Length of Root (cm)
Full MS (control)	00±0.00 ^{f}	00±0.00 ^{e}	00±0.00 ^{d}
½ MS + IBA			
0.5	78.1±0.95^{b}	3.6±0.33 ^{b}	2.93±0.11 ^{a}
1.0	90±1.79 ^{a}	5±0.21 ^{a}	2.92±0.20 ^{a}
1.5	78.4±0.88 ^{b}	3.2±0.24^{bc}	2.59±0.08 ^{ab}
½ MS + IAA			
0.5	69.3±1.22 ^{c}	2.7±0.3 ^{cd}	2.45±0.11^{bc}
1.0	63.3±1.42 ^{d}	3.2±0.13^{bc}	2.22±0.09 ^{c}
1.5	56.8±0.87 ^{e}	2±0.25 ^{d}	2.1±0.04 ^{c}

Data expressed as mean±S.E. Means followed by the same letter (s) within a column do not differ significantly at $P \leq 0.05$ by DMRT.

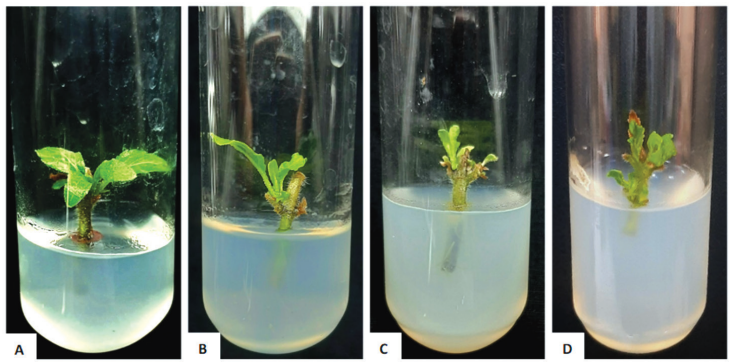

Plate 1. Influence of BAP and Kin on shoot induction of Holy basil (*O. sanctum)* after two weeks of culture (A) 1.5 mg/L BAP, (B) 1.0 mg/L BAP (C) 1.5 mg/L Kin (D) 1.0 mg/L Kin

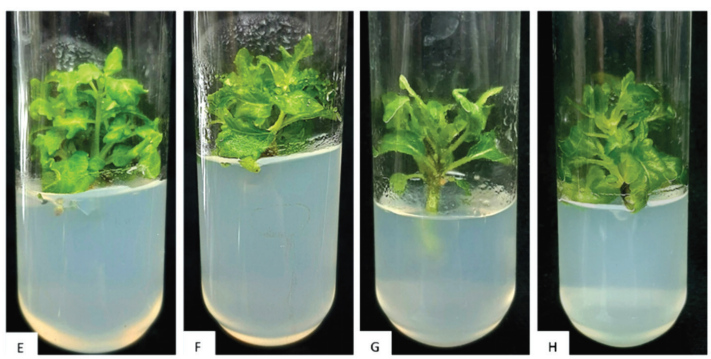

Plate 2. Influence of BAP and Kin on multiple shoot proliferation of Holy basil (*O. sanctum)* after four weeks of culture (E) 1.5 mg/L BAP, (F) 1.0 mg/L BAP (G) 1.5 mg/L Kin (H) 1.0 mg/L Kin

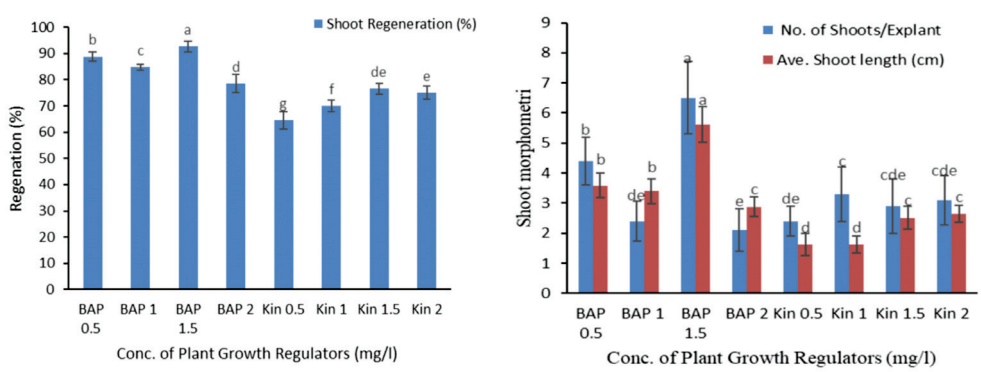

Fig. 1. The role of varying concentrations of BAP and Kin in MS medium on multiple shoot induction and shoot morphometry (i) Shoot regeneration percentage (ii) Shoot morphometry. Means followed by the same letter (s) do not differ significantly at P ≤ 0.05 by DMRT. Vertical bars representing the Standard Error (SE)

development. Concentrations below the optimum might provide insufficient stimuli, while higher concentrations (2.0 mg/L BAP) could lead to inhibitory effects, as evidenced by the decreased shoot regeneration and number of shoots. The earlier bud initiation observed with higher concentrations of both BAP and Kin (7 days) indicates that these PGRs effectively trigger the initial cellular processes required for meristematic activity. The complete lack of growth in the control group underscores the absolute requirement for exogenous cytokinin in the MS medium for shoot regeneration under the experimental conditions. This aligns with the fundamental principles of plant tissue culture, where PGRs are essential for manipulating plant growth and development *in vitro*.

3.2 In vitro Rooting

A half-strength MS medium supplemented with IBA and IAA at different concentrations was used for successful root induction. The influence of different concentrations of IBA and IAA on *in vitro* root regeneration is presented in Table 2.

The investigation into the effects of IBA and IAA on *in vitro* root regeneration revealed significant improvements over the control (Full MS medium), which exhibited no root regeneration, number of roots, or root length. Both auxins successfully induced rooting, but their efficacy varied with concentration and type. For IBA, the optimal concentration was 1.0 mg/L, achieving the highest root regeneration percentage (90% ± 1.79) and the maximum number of roots per explant (5 ± 0.21). While 0.5 mg/L IBA resulted in a slightly longer root length (2.93 ± 0.11 cm), the overall performance at 1.0 mg/L IBA was superior, demonstrating a balanced promotion of rooting parameters. Increasing IBA to 1.5 mg/L led to a decrease in regeneration percentage (78.4% ± 0.88) and number of roots (3.2 ± 0.24), suggesting a supra-optimal effect. In contrast, IAA generally showed lower efficacy compared to IBA, and its effectiveness for all parameters progressively decreased with increasing concentrations. The highest root regeneration with IAA was observed at 0.5 mg/L (69.3% ± 1.22), which also yielded the longest roots (2.45 ± 0.11 cm) and a moderate number of roots (2.7 ± 0.3). At 1.5 mg/L IAA, the regeneration percentage dropped to (56.8% ± 0.87), and the number of roots and length were the lowest among all tested auxin concentrations. The observed differences in efficacy between IBA and IAA are consistent with established principles in plant tissue culture, where IBA is often considered more stable and effective for root induction across a wider range of plant species due to its slower degradation and transport rates compared to IAA. The dose-dependent responses underscore the necessity of precise auxin concentration optimization for successful *in vitro* root regeneration, highlighting 1.0 mg/L IBA as the most promising treatment in this study for promoting

robust root development. It has been reported that low-strength MS medium produces higher rooting frequency in *O. kilimandscharicum* Guerk (Saha *et al.* 2010) and *O. canum* (Saha *et al.* 2014). A Half-strength MS medium was found to be more effective for root initiation in various medicinal plants, suggesting potential applications in the micropropagation of these species.

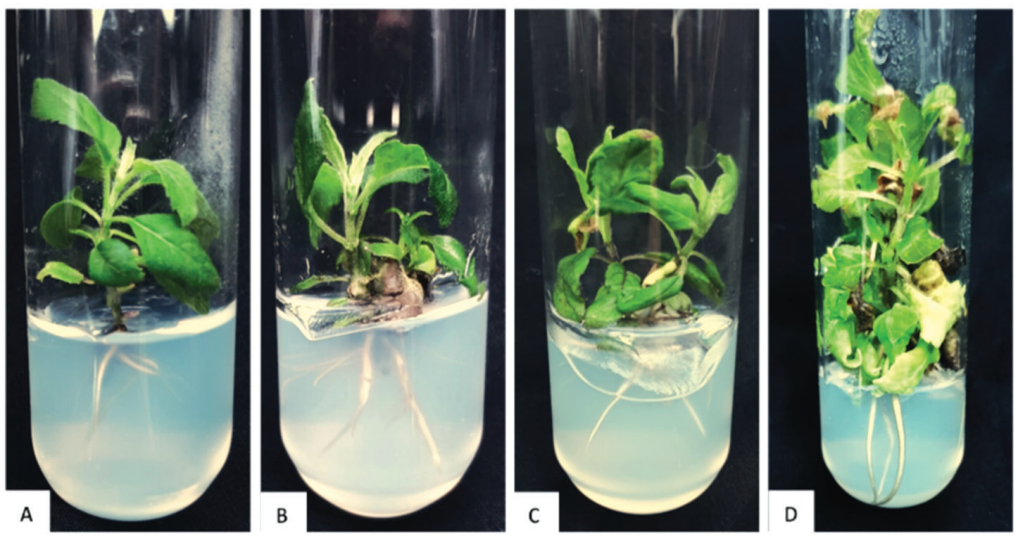

Plate 3. The influence of different concentrations of BAP and KIN on *in vitro* root regeneration of Holy basil *(O. sanctum)* after four weeks of culture (A) 0.5 mg/L IAA, (B) 0.5 mg/L IBA (C) 1.0 mg/L IAA (D) 1.0 mg/L IBA

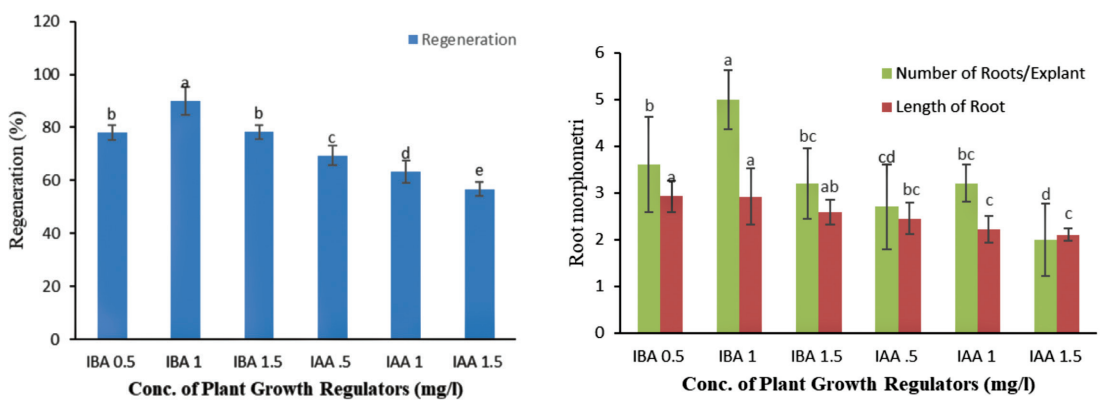

Fig. 2. The role of varying concentrations of IBA and IAA in MS medium on root induction of *O. sanctum* was studied after 4 weeks of culture (i) root regeneration percentage (ii) root morphometry. Means followed by the same letter (s) do not differ significantly at $P \leq 0.05$ by DMRT. Vertical bars representing the Standard Error (SE)

3.3 Acclimatization

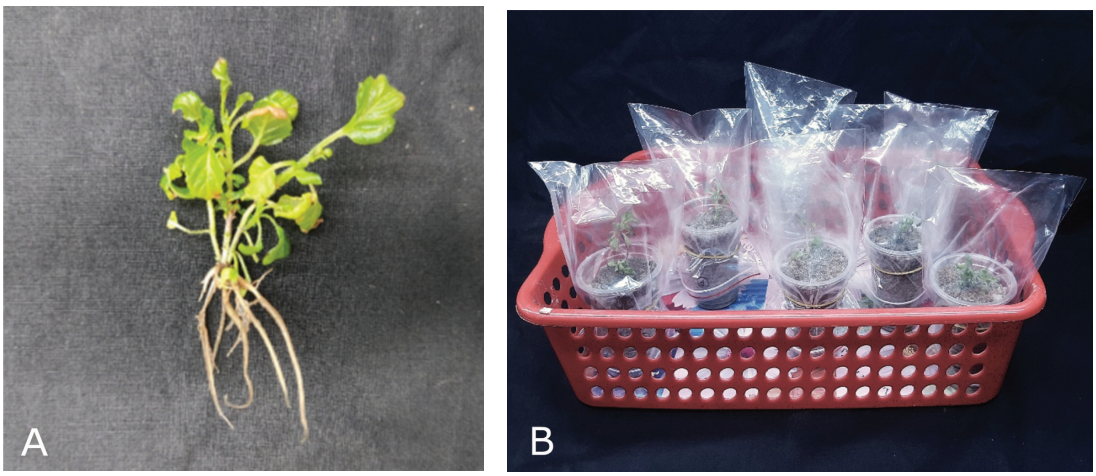

Plate 4. (A) *In vitro* rooted plantlet of Holy basil (*O. sanctum)* after 4 weeks of culture (B) Acclimatized plantlets of Holy basil (*O. sanctum)* undergoing *ex vitro* transition within a growth chamber."

Table 3. Effects of different hardening media on the survival rate of *in vitro* rooted plantlets of Holy basil (*O. sanctum*) under the green house conditions

Initial selected plants	Hardening media	Ratio	Number of survival plants	Survival (%)
20	Garden soil	100%	15	75
20	Sand	100%	12	60
20	FYM	100%	11	55
20	Garden soil + Sand	50% + 50%	18	90
20	Garden soil + FYM	50%+ 50%	16	71

Initially, 100 plantlets were selected and transplanted for hardening. Garden soil, sand, and FYM were used alone and in combination as hardening media in different ratios, as shown in Table 3. Among the five tested media compositions, the combination of garden soil and sand (50:50) showed the highest survival rate at 90%, with 18 out of 20 plants successfully acclimatized. This was followed by the garden soil and FYM (50:50) mixture, which exhibited a 71% survival rate, with 16 plants surviving. In contrast, the individual media types showed relatively lower survival percentages. Plants acclimatized in 100% garden soil showed a survival rate of 75%, whereas 100% sand and 100% FYM resulted in significantly lower survival rates of 60% and 55%, respectively.

The superior performance of the garden soil and sand mixture could be attributed to improved aeration, moderate water-holding capacity, and a balanced physical structure, which

likely facilitated better root establishment and reduced transplant shock. Sand improves drainage and reduces compaction, while garden soil provides essential nutrients and microbial support, making their combination ideal for hardening (Garg & Bahadur, 2023) Acclimatized plants looked healthy, and phenotypic variation was absent.

4. Conclusions

This study revealed the effectiveness of cytokinin (BAP, Kin) and Auxin (IAA, IBA) in promoting regeneration, growth, and development of *O. sanctum* under a controlled environment with nodal explants. The study identified the optimal concentration for shoot regeneration as 1.5 mg/L BAP, and the highest root formation was achieved with 1.0 mg/L IBA in the medium. Furthermore, the study found that a combination of garden soil and sand yields the best results for acclimatization. These findings can be applied to successfully multiply Holy basil (*O. sanctum*), thereby contributing to the advancement of plant regeneration techniques.

5. Author's contribution:

M.A.K. A. and **S.J. P.** conceived and designed the experiments, performed the practical work, and analyzed the data. **T. N.** edited final draft and developed manuscript. **S.M.N.I.** and **M.A.H.** assisted with data visualization and manuscript evaluation. All authors contributed to the critical review and editing of the manuscript.

6. Conflict of interest:

There is no conflict of interest among the authors.

7. References

Bernardini, S., A. Tiezzi, V. L. Masci and E Ovidi. 2017. Natural products for human health: an historical overview of the drug discovery approaches. *Nat. Prod. Res.* 32(16): 1926–1950. https://doi.org/10.1080/14786419.2017.1356838.

Bhuvaneshwari, K., A. Gokulanathan, M. Jayanthi, V. Govindasamy, L. Milella, S. Lee, D. C. Yang and S. Girija. 2016. Can Ocimum basilicum L. and Ocimum tenuiflorum L. in vitro culture be a potential source of secondary metabolites? *Food Chem.* 194: 55–60. https://doi.org/10.1016/j.foodchem.2015.07.136.

Chaudhary, A., S. Sharma, A. Mittal, S. Gupta and A. Dua. 2020. Phytochemical and antioxidant profiling of Ocimum sanctum. *J. Food Sci. Technol.* 57(10): 3852–3863. https://doi.org/10.1007/s13197-020-04417-2.

David, R. H and D. I. Arockiasamy. 2008. In vitro Propagation of Mentha viridis L. from Nodal and Shoot tip Explants. *Plant Tissue Cult. & Biotech* (Vol. 18, Issue 1). https://doi.org/https://doi.org/10.3329/ptcb.v18i1.3243.

Garg, K and V. Bahadur. 2023. Influence of Potting Media and GA3 on Seed Germination and Seedling Growth

of Guava (Psidium guajava) cv. Allahabad Safeda under Shade Net Condition. *Int. J. Plant Soil Sci.* 35(16): 393–403. https://doi.org/10.9734/ijpss/2023/v35i163166.

Hanumanthaiah, P, H. Panari, A. Chebte, A. Haile and G. Belachew. 2020. Tulsi (Ocimum sanctum) – a myriad medicinal plant, secrets behind the innumerable benefits. *DOAJ*. https://doi.org/10.48347/imist.prsm/ajmap-v6i1.20401.

Hasan, M. R., B. S. Alotaibi, Z. M. Althafar, A. H. Mujamammi and J. Jameela. 2023. An Update on the Therapeutic Anticancer Potential of Ocimum sanctum L.: "Elixir of Life." *Molecules*. 28(3): 1193. https://doi.org/10.3390/molecules28031193.

Hassan, A. K. M. S., R. Sultana, M. A. A. Jahan and R. Khatun. 2010. *In Vitro Mass Propagation of Mimosa pudica L., Using Shoot Tip and Nodal Explants*. *Bangladesh J. Sci. Ind. Res.* DOI:10.3329/bjsir.v45i2.5704.

Jakovljević, D., M. Stanković, M. Warchoł and E. Skrzypek. 2022. Basil (Ocimum L.) cell and organ culture for the secondary metabolites production: a review. *Plant Cell Tissue Organ Cult.* (Vol. 149: Issues 1–2, Pp. 61-79). Springer Science and Business Media B.V. https://doi.org/10.1007/s11240-022-02286-5.

Karim, M., R. Jannat, M. Rahman and M. Haque. 2013. Micropropagation of Stevia Plant from Nodal Segments. *Prog. Agric.* 19(2): 21-26. https://doi.org/10.3329/pa.v19i2.16914.

Kayani, H. A., M. Raziq, S. K. Kazmi, S. Naz and S. Khan. 2021. Efficient Protocol for in vitro Regeneration of Ocimum sanctum using Nodal Segments as Explants. *Pak. j. Sci. Ind. Res. Ser. B: Biol. Sci.* 64B(1): 13–17. DOI:10.52763/PJSIR.BIOL.SCI.64.1.2021.13.17.

Komakech, R., Y. Kim, W. J. Kim, F. Omujal, S. Yang, B. C. Moon, D. Okello, E. Rahmat, G. N. Kyeyune, M. G. Matsabisa and Y. Kang. 2020. A Micropropagation Protocol for the Endangered Medicinal Tree Prunus africana (Hook f.) Kalkman: Genetic Fidelity and Physiological Parameter Assessment. *Front. Plant Sci. 11*. https://doi.org/10.3389/fpls.2020.548003.

Kumari, K., S. Kumar, N. Prashant, A. K. Jha and N. Kumar. 2022. Biotechnological intervention in genetic improvement and regulation of secondary metabolites production in Ocimum sanctum L. *Ind. Crops Prod.* 187: 115329. https://doi.org/10.1016/j.indcrop.2022.115329.

Mandal, A. K., M. Poudel, N. P. Neupane and A. Verma. 2022. Phytochemistry, Pharmacology, and Applications of Ocimum sanctum (Tulsi). In *Springer eBooks* (Pp. 135–174). https://doi.org/10.1007/978-981-16-4959-2_4.

Manjudevi, M., R. Thirugnanasampandan, B. Vishnupriya and M. Gogul Ramnath. 2022. In vitro propagation of Ocimum sanctum L., Ocimum canum Sims., and Ocimum tenuiflorum L., and evaluation of antioxidant, MMP-9 down regulation of eugenol and camphor. *S. Afr. J. Bot.* 151: 208-217. https://doi.org/10.1016/j.sajb.2022.01.006.

Mishra, T. 2015. Protocol Establishment for Multiplication and Regeneration of "Holy Basil" (Ocimum sanctum Linn). An Important Medicinal plant with High Religious Value in India.*J. Med. Plants Stud.* 3(4): 16–19.

Mohammad, A. H. M. J., H. S. Imdadul, M. MS, A, N. M. R. B. R, A. B. Nilufa, R. I. Md and N. Md. 2016. In vitro regeneration of a common medicinal plant, Ocimum sanctum L. for mass propagation.*Afr. J. Biotechnol.* 15(24): 1269-1275. https://doi.org/10.5897/ajb2015.15200.

Murashige, T and Skoog F. 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant.* 15(3): 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.

Pattnaik, S., P. K. Chand and G. C. Phillips. 1996. In vitro propagation of the medicinal herbs Ocimum americanum L. syn. O. canum Sims. (hoary basil) and Ocimum sanctum L. (holy basil). *Plant Cell Rep.* (Vol. 15). https://doi.org/10.1007/BF00233154.

Piras, A., M. J. Gonçalves, J. Alves, D. Falconieri, S. Porcedda, A. Maxia and L. Salgueiro. 2018. Ocimum tenuiflorum L. and Ocimum basilicum L., two spices of Lamiaceae family with bioactive essential oils.*Ind. Crops Prod.* 113: 89–97. https://doi.org/10.1016/j.indcrop.2018.01.024.

Rahman, M. S., Nandi N. C and B. Goswami. 2021. In vitro regeneration of Mirabilis jalapa L. In *Bangladesh J. Sci. Ind. Res* (Vol. 56, Issue 1). https://doi.org/10.3329/bjsir.v56i1.52692.

Saha, S., T. Dey and P. Ghosh. 2010. Micropropagation of Ocimum kilimandscharicum guerke (Labiatae). *Acta Biol. Cracov. Ser. Bot.* 52(2): 50–58. https://doi.org/10.2478/v10182-010-0023-7.

Saha, S., A. Kader, C. Sengupta and P. Ghosh. 2012. In Vitro Propagation of Ocimum gratissimum L. (Lamiaceae) and Its Evaluation of Genetic Fidelity Using RAPD Marker.*Am. J. Plant Sci.* 03(01): 64–74. https://doi.org/10.4236/ajps.2012.31006.

Saha, S., S. Roy, C. Sengupta and P. Ghosh. 2014. Micropropagation and analysis of genetic stability in regenerated plantlets of Ocimum canum Sims. *Indian J. Plant Physiol*. 19(2): 174–183. https://doi.org/10.1007/s40502-014-0093-8.

Sharma, N., N. Kumar, J. James, S. Kalia and S. Joshi. 2023. Strategies for successful acclimatization and hardening of in vitro regenerated plants: Challenges and innovations in micropropagation techniques. *Plant Sci. Today*. 10: 90-7. https://doi.org/10.14719/pst.2376.

Siddique, I and M. Anis. 2008. An improved plant regeneration system and ex vitro acclimatization of Ocimum basilicum L. *Acta Physiol. Plant.* 30(4): 493–499. https://doi.org/10.1007/s11738-008-0146-6.

Singh, N. K and C. B. Sehgal. 1999. Micropropagation of "Holy Basil" (Ocimum sanctum Linn.) from young inflorescences of mature plants. In *Plant Growth Regul.* (Vol. 29). DOI https://doi.org/10.1023/A:1006201631824.

World Health Organization. 2020. Traditional medicine in the WHO South-East Asia Region: review of progress 2014–2019. *Traditional medicine in the WHO South-East Asia Region: review of progress 2014–2019*.

Yamani, H. A., E. C. Pang, N. Mantri and M. A. Deighton. 2016. Antimicrobial activity of Tulsi (Ocimum tenuiflorum) essential oil and their major constituents against three species of bacteria.Front. Microbiol. *7*(MAY): https://doi.org/10.3389/fmicb.2016.00681.