World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Effects of plant growth regulators on virus-free plantlet regeneration from *Abroma augustum* (L.) seeds

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World Journal of Biology Pharmacy and Health Sciences, 2024, 19(03), 176–183

Publication history: Received on 30 July 2024; revised on 08 September 2024; accepted on 11 September 2024

Article DOI: https://doi.org/10.30574/wjbphs.2024.19.3.0617

Abstract

Abroma augusta is a popular folk therapeutic plant in Bangladesh, rightly called Devil's cotton in English. The present study was performed to evaluate the *in vitro* multiple shoot and root generation of *Abroma augustum* plants. *In vitro* seed germination, shoot proliferation and root induction were performed following previous methods. The highest percentage of seed germination was 90±0.0% after seven days of inoculation, after which the plants were sterilized with 0.1% NaOCl for 2 min. The longest shoot length was 7.6±0.6 cm at the MS+2+0.3 mg/L BAP+NAA concentration. The greatest number of roots per shoot was 12.0±1.0 cm, and the greatest length of roots was 6.8±1.6 cm in MS media supplemented with 1.0 mg/L IBA. Both the shoots and roots were vigorous and sufficient in length and number. The present study established an efficient *in vitro* micropopagation process for *Abroma augustum* plantlet production through seeds. The findings of the present research will be helpful for rapidly regenerating disease-free plantlets.

Keywords: *Abroma augustum*; Seeds; *In vitro* regeneration; Shoot proliferation; Root proliferation

1. Introduction

Abroma augustum (L.) L.f. is an evergreen medicinal shrub belonging to the Malvaceae family and is commonly known as Ulat kambal in Bengali (Roy and Khan 2020). The plant is distributed in the Asian subcontinent, India, and is rarely found in Bangladesh (Jena et al. 2023; Miah et al. 2020). In other parts of the world, the trend of using medicinal plants for research has also been recognized. Several compounds, such as secondary metabolites and alkaloids, including triterpenes, steroids, benzohydrofurans megastigmanes and their glycosides, (Gupta et al. 2011) are present in this medicinal plant. Different types of disease, such as diabetes, leucorrhea, scabies, gonorrhea, cough, headache, stomachache, dermatitis, and uterine disorders, are treated with several parts of this plant (Al-bari et al. 2006). At present, human life is severely threatened by infections caused by pathogenic microorganisms, which are becoming crucial causes of mortality and morbidity worldwide, especially in developing countries where immunocompromised patients are found (Rahmatullah et al. 2010).

Abroma augustum plants are a very rich source of compounds, antimicrobial and cytotoxic agents in leaves (Saikot et al. 2012) and bark extracts (Miah et al. 2020). It can be an eminent naturalistic source for new drug establishment (Sunitha et al. 2018; Karagöz et al. 2015). These plant-derived medicines are rich in antioxidant activity, and plant-derived medicines are enriched with flavonoids, tannins and phenolic compounds (Khanzadi 2012; Maha et al. 2015). The plant redesignated a massive natural source for new drug establishment (Sunitha et al. 2018).

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Plant tissue culture is an applied biotechnology that is used for *in vitro* cloning of plant secondary metabolites, production, mass propagation, and virus elimination to overcome the insufficiency of these types of plants. In vast areas of horticulture, forestry, agriculture, and plant breeding, tissue culture has been extended. Plant tissue culture has been specifically applied for the conservation of endangered plant species by using quick and medium-term conservation to explore the untapped assortment of medicinal plants, and there has been growing interest worldwide. Previously, we reported that *Abroma augusta* seed extract has significant antibacterial, cytotoxic and pesticidal activities (Sujaye et al. 2023; Goswami et al. 2023).

There are no reports on the *in vitro* regeneration of *A. augusta* plants in the literature. Siddique (2005) reported *Abroma augusta* as an *e*ndangered medicinal plant in the Barind tract. Currently, many of these medicinal plants are endangered, and sufficient *in vitro* regeneration-related research is lacking (Sunitha et al. 2018). Hence, this study aimed to compare and evaluate the *in vitro* development and growth of *A. augustum* from seeds on MS media containing different plant growth regulators.

2. Materials and methods

2.1. Materials

2.1.1. Study area and design

The research was conducted during late summer (July 2022) at the Professor Joarder DNA & Chromosome Research Lab., Dept. of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi 6205, Bangladesh.

2.1.2. Plant material collection

Abroma augustum plant seeds were collected from Rajshahi University Campus, Rajshahi, Bangladesh. The plant was identified by Dr. Md Mahbubur Rahman, Department of Botany, University of Rajshahi, Bangladesh (Botany Herbarium, Voucher No. AC205). The mature seeds of the plant were used as explants for the present investigation.

2.1.3. Chemicals and reagents used

The chemicals ethanol, Murashige and Skoog (MS) medium, benzyladenine (BAP), indole-3-butyric acid (IBA) and α-naphthalene (NAA) were purchased from Sigma–Aldrich, USA. NaOCl was purchased from Himedia, India. All the reagents and chemicals used were of analytical grade.

2.2. Methods

2.2.1. Culture media preparation

The growth media were prepared according to the procedure of Hailu et al. (2020). Murashige and Skoog (1962) media were used as base nutrients for plant growth and development at different concentrations. MS media were prepared by placing a 500 ml beaker containing 250 ml of autoclaved distilled water. A total of 30 g/L MS powder and different plant growth hormones were added individually or in combination. Agar (9 g/L) was added as a solidifying agent. The total volume of the mixture was autoclaved, and the mixture was kept in a cool place for explant culture.

2.2.2. Surface sterilization of explants

To ensure surface sterilization, the seeds were distilled with one drop of savlon for 5 minutes. To ensure surface sterilization, the seeds were rinsed with 0.1% NaOCl for 1 min and then washed with distilled water 5 times.

2.2.3. Culture incubation

In the present study, mature seeds were placed in MS media supplemented with different concentrations and combinations of auxin and cytokinins for direct and indirect regeneration. Finally, the regenerated shoots were placed on MS media supplemented with auxin for root induction. The culture was maintained at 25+2°C under warm fluorescent light intensities ranging from 2000--3000 lux. The photoperiod was generally maintained at 16 hours light and 8 hours dark.

2.2.4. Subculture of shoots

In vitro germinated plantlets were rescued aseptically from the culture vessels on a sterile petri dish, cut into several small pieces with auxiliary buds and again cultured in freshly prepared media containing different combinations of hormonal supplements for shoot multiplication.

2.2.5. Subculture for rooting

When these shoots were approximately 5–8 cm in length, they were separated aseptically from the culture vessels, and the separated individuals were transferred to freshly prepare rooting media containing different combinations of hormonal supplements. For root induction, the newly transferred cultures were kept in dim light for three days and then kept in full light as in shooting.

2.2.6. Statistical analysis

The mean values were calculated from three different experiments, and the data are presented as the standard error of the mean (mean ± SE) via one-way ANOVA with SPSS software version 17 (SPSS, Chicago, IL, USA). Duncan's multiple range test (DMRT) was used to determine the significance of differences between the treatments at $p < 0.05$. Three repetitions were performed for each culture and treatment. The rates of culture response were calculated as follows:

Response (%) = (number of inoculations/number of total responses) × 100

3. Results and Discussion

3.1. Standardization and germination of seeds

For standardization of the seed surface, treatment with 0.1% NaOCl for 2 min was the most effective. The percentages of contamination-free seeds and germination-susceptible seeds were 90.0±1.0 and 95.0±0.0, respectively. Details are presented in Table 1 and Figure 1A. Eliwa et al. (2024) reported the highest responsiveness (82.81%) and survival (96.61%) to 20% sodium hypochlorite (NaOCl) for 15 min. Yildiz and Er (2002) also reported the effects of NaOCl solutions on the germination and viability of hypocotyl explants. In contrast, Kabir et al. (2015) reported 90% contamination-free cultures using 0.1% mercuric chloride solution treated for 7 minutes. De Stefano et al. (2022) reported the *in vitro* micropropagation of an endangered orchid via seed culture and germination. Alatar et al. (2023) reported similar results for *in vitro* plantlet regeneration of *Maerua crassifolia* from seed explants.

Table 1 Effects of different durations of 0.1% NaOCl treatment

Treatment time (Min.)	No. of seeds treated	% of contamination free (M±SE)	% of seed germination (M±SE)
0.5	8	73.3±1.2	100.0±0.0
1.0	10	80.0±1.0	96.6±0.4
1.5	11	86.6±0.8	80.0±1.0
2.0	10	90.0±1.0	95.0±0.0
2.5	12	93.3±0.9	73.3±1.2
3.0	11	96.6±0.4	70.0±0.0
3.5	10	100.0±0.0	66.6±1.3

3.2. Shoot proliferation

3.2.1. Effects of BAP

The highest percentage of shoot response was 60.0% for MS supplemented with 2.0 mg/L BAP, followed by 36.6% supplemented with 1.5 mg/L BAP. On the other hand, the lowest percentage of shoot induction was 10.0%, which was observed for MS containing 0.5 /L BAP. The greatest mean length of shoots was 6.6 cm, followed by 6.0 cm, which consisted of 2.5 mg/L BAP. On the left hand, the shortest mean shoot length was 5.0, which was observed on MS media supplemented with 3.0 mg/L BAP (Table 2 and Figure 1C). Similar results were reported by Iacuzzi et al. (2023).

3.2.2. Effects of BAP with NAA

For shoot proliferation, seeds were cultured in MS media supplemented with different concentrations of BAP in combination with NAA. The different hormonal treatments used to regenerate shoots included 2.0 mg/L BAP and 0.1–1.5 mg/L NAA. In this combination, almost 70.0% of the inoculated explants regenerated within 14 days of inoculation. The second highest percentage of regenerated shoot culture was 60.0% with 2.0 mg/L BAP+0.5 mg/L NAA. On the other hand, the lowest percentage of shoots was 23.3%, which was observed on MS media supplemented with 2.0 mg/L BAP+1.5 mg/L NAA (Table 2 and Figure 1B, D). MS media supplemented with 1.0 mg/L BAP+ 0.50 mg/L NAA presented the greatest number and length of shoots in *Aloe elegans* (Welehaweria and Sbhatu 2023). Similar results were reported by Komakech et al. (2020) in the endangered medicinal tree *Prunus africana*. Rimy et al. (2024) reported the highest number of shoots per explant and longest shoot length in the micropropagation of strawberry in combination with 1.5 mg/L BAP + 1.0 mg/L KIN. Sarkar et al. (2015) reported *in vitro* plantlet regeneration of A. Augusta from root tips on MS media supplemented with 4 mg/L 2,4-D+2 mg/L NAA+2 mg/L KN+1 g/L YE. This result contrasts with the present findings.

Table 2 Effects of different concentrations and combinations of BAP and NAA in MS media on shoot proliferation

Growth regulators (mgl^{-1})	% of culture response (M±SE)	Shoot length (cm) (M±SE)
BAP		
1.0	23.3±1.6	5.3±0.3
1.5	36.6±0.9	5.3±0.3
2.0	60.0±0.0	6.6±0.6
2.5	26.6±1.2	6.0±1.0
3.0	23.3±0.8	5.0±1.0
BAP +NAA		
2.0+0.1	26.6±1.2	5.6±0.6
2.0+0.2	46.6±0.6	7.0±1.0
2.0+0.3	70.0±0.0	7.6±0.6
2.0+0.5	60.0±0.0	7.0±1.0

Note: The experiments were repeated three times, each consisting of 10 replicates.

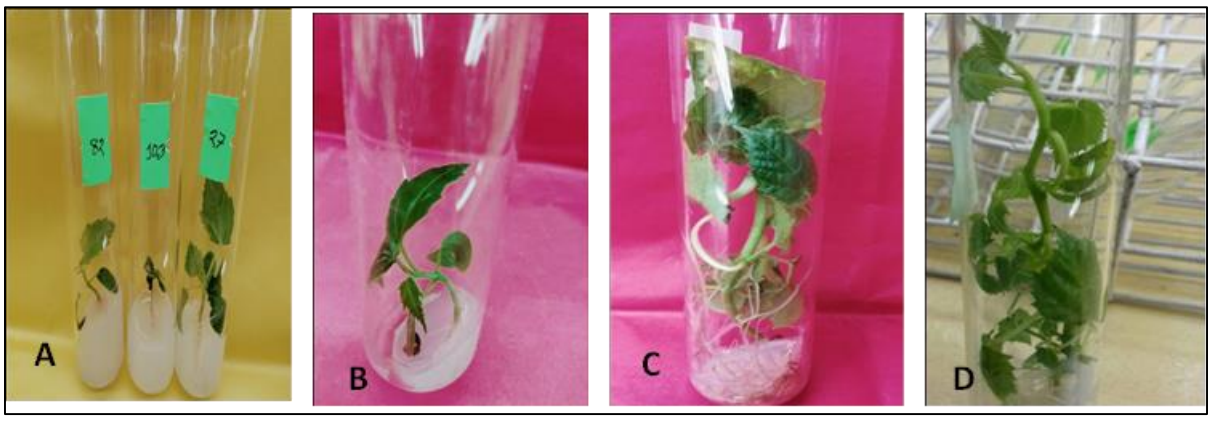

Figure 1 Shoot proliferation, (A) seed germination, (B) shoot initiation on MS media supplemented with BAP, (C) shoot elongation on MS supplemented with BAP and (D) shoot elongation on MS supplemented with BAP+NAA

3.3. Root proliferation

3.3.1. Effects of NAA on root initiation and elongation

The highest percentage, 80.0%, of root regeneration was recorded in MS media supplemented with 2.0 mg/L NAA, followed by 66.6% in MS media supplemented with 1.5 mg/L NAA. The lowest percentage of root induction was 23.3% in MS supplemented with 3.0 mg/L NAA. A total of 6.6 shoots/roots were found via MS, with 2.0 mg/L NAA. The lowest number of roots was 2.0 in MS with 3.0 mg/L NAA. The greatest mean length of roots was 5.0 cm in MS media supplemented with 2.0 mg/L NAA, followed by 5.2 cm in media supplemented with 2.5 mg/L NAA. The shortest mean length of the shoot was 3.0 cm on MS supplemented with 0.5 mg/L NAA. At these concentrations, the induced roots were long and thick in nature (Table 3 and Figure 2A). Similar mean root lengths were reported at NAA concentrations ranging from 0.75 to 1.50 mg/L (Welehaweria and Sbhatu 2023). Hailu et al. (2020) reported that the number of roots increases with increasing NAA concentration from 0.50 to 1.0 mg/L in *Aloe trichosantha.*

3.3.2. Effects of IBA on root initiation and elongation

The highest degree of root induction was 90.0% in MS with 1.0 mg/L IBA, and the lowest degree of root induction was 43.3% in MS supplemented with 2.0 mg/L NAA. The highest number of roots per shoot was 12.0 on 1.0 mg/L IBA, and the lowest number of roots was 4.0 in MS media supplemented with 0.1 mg/L IBA. The longest root length was 6.5 cm in MS, with 1.0 mg/L IBA, while the shortest root length was 2.0 cm, and the induced roots were long and thick in nature. The data are given in Table 3 and Figure 2B. Biswas et al. (2008) reported similar responses to root induction in *Abrus precatorius*. Gangaprasad et al. (2005) reported the same result on ½-strength MS media supplemented with 0.2 mg/L IAA for rooting, which supports our present findings. For root induction and elongation, IBA was best for both root induction and shoot elongation. Similar results were reported by Kabir et al. (2015) for cassava root induction and elongation via MS containing IBA. Here, 1.0 mg/L IBA was the best treatment for root induction as well as shoot elongation in MS. Shoot induction and elongation, as well as root induction and elongation, were investigated via *in vitro* micropropagation approaches. The results of our investigation are primarily proven, but more investigations are needed to confirm that the protocol of direct micro propagation may help with further investigations of this valuable endangered medicinal plant.

Table 3 Effects of NAA and IBA on root initiation and elongation in induced shoots

Growth regulators (mgl^{-1})	% of culture response (M±SE)	No. of roots/shoot (M±SE)	Root length (cm) (M±SE)	Nature of roots
NAA				
0.5	30.0±0.0	2.5±0.9	3.0±1.0	Long, thin
1.0	43.3±1.9	4.0±1.0	4.7±0.8	Long, thin
1.5	66.6±2.0	4.2±0.7	5.0±1.0	Long, thin
2.0	80.0±0.0	6.6±0.8	5.5±1.3	Long, thin
2.5	46.6±1.4	5.9±1.4	5.2±1.3	Long, thin
IBA				
0.1	56.6±2.2	4.0±1.0	2.0±1.0	Long, thin
0.5	73.3±1.6	6.4±1.2	3.0±1.0	Long, thin
1.0	90.0±0.0	12.0±1.0	6.8±1.6	Long, thin
1.5	66.6±1.0	6.9±1.5	4.0±1.0	Long, thin
2.0	43.3±1.9	5.0±1.0	3.3±0.6	Long, thin

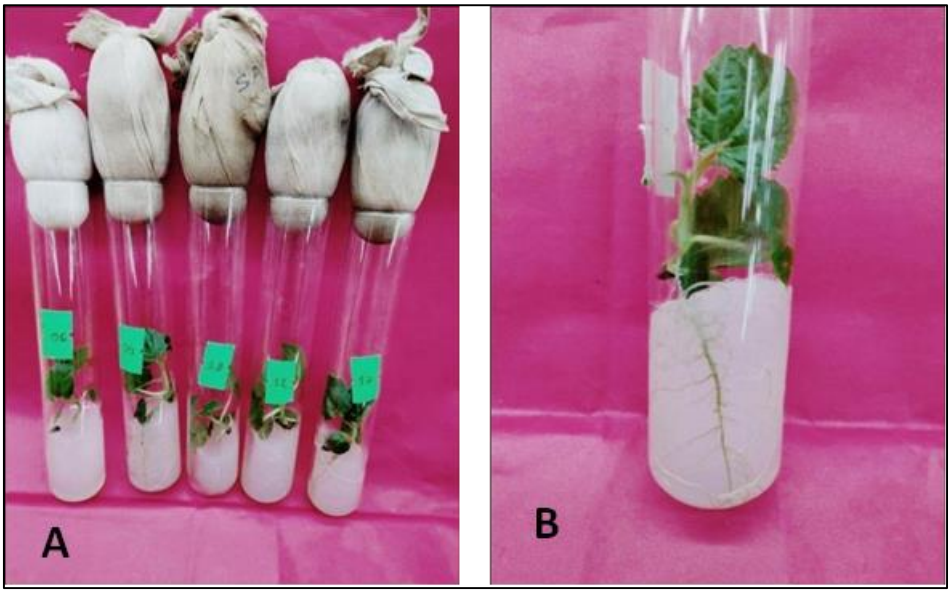

Figure 2 Root initiation and elongation from induced shoots. (A) NAA and (B) IBA

4. Conclusion

To overcome the large demand of this plant, various *in vitro* plantlet regeneration methods have been used to produce sufficient amounts of the plant. These micropropagation methods, whether reported or further utilized, can help further. The *in vitro* regeneration of plants holds tremendous potential for the production of high-quality plant-based medicines. Advances in plant cell culture could provide new means for the commercial production of even rare or exotic plants, their cells, cost-effectiveness and the chemicals that they produce. In general, there has been good progress in protocol optimization for medicinal plants in developed countries. However, some bindings exist in developing countries, and there is a need for research in these areas to conserve and maximize the use of these medicinal plants. The present research may help further investigations of *in vitro* regeneration.

Compliance with ethical standards

Acknowledgments

The authors are thankful to the Director, Professor Joarder DNA & Chromosome Research Lab., Dept. of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh, for technical support during the research.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author contributions

MEH and HBJ performed the material collection, methodology, and investigation and wrote the main manuscript. BS, UKA and MK performed the formal analysis, data curation and figure preparation. MFH ensured conceptualization, manuscript revision, editing and supervision. All the authors reviewed the manuscript.

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