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# Micropropagation of mint (*Mentha spicata*)

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## Abstract

*Mentha spicata* is a valuable, medicinally important, economic, essential oil-yielding perennial herb that is grown worldwide both in cultivated and wild forms. The present experiment was conducted at Biotechnology Laboratory in the Department of Biotechnology, Sher-e-Bangla Agricultural University to evaluate the effect of different concentrations of Benzyl adenine (BA) (1.0, 1.5, 2.0 and 2.5 mg/l) and Indole-3-butyric acid (IBA) (0.5, 1.0, 1.5 and 2.0 mg/l) either alone or in combination on micropropagation of mint. The treatment of 2.0 mg/L BA performed best in respect of percent response of explants (80.00%), number of shoots per explant (19.75) and shoot length (12.12 cm). In contrast, the maximum shoot number per explant (20.33) and shoot length (13.0 cm) was found in 2.0 mg/L BAP+1.0 mg/L IBA treatment. The maximum number of roots (3.4 and 5.2) and root length (7.50 and 7.67 cm) was observed in 1.0 mg/L BA and 2.0 mg/L BA in combination with 1.5 mg/L of IBA. Survival rate of regenerated plantlets 80 % in open atmosphere. Finally, feasible micropropagation protocol of mint has been developed that can be used for further improvement programme of breeding.

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## Introduction

Mint (*Mentha spicata*, Lamiaceae) is a valuable medicinal, aromatic, herbaceous perennial plant, having significant therapeutic effects. Garden mint or spearmint has proven therapeutic effects including: antioxidant, antiviral, antibacterial, spasmolytic, carminative, antiseptic, antiparasitic, analgesic, and antitumor (Trevisan *et al*., 2017). Currently, three major commercial producers of spearmint are: The United States, India and China. The United States of America is the main producer of peppermint and spearmint followed by India.

Due to its stimulant, stomachic properties, the plant is used for alleviating nausea, headache and vomiting. However, mentha contains a variety of ingredients that are classified as peppermint essential oil (PEO) and non-essential components including steroids, flavonoids, triterpenoids, phenolic acids, etc. The PEO, consisting mainly of menthol, menthone, neomenthol and iso-menthone, is a mixture of biologically active secondary metabolites which may pharmacologically protect gastrointestinal, liver, kidney, skin, respiratory, brain and nervous systems and exert hypoglycemic and hypolipidemic effects (Zhao *et al.,* 2022). Because of its therapeutic potential and diversified uses, mint oil demand is constantly increasing throughout the world. Further, the ruthless exploitation has threatened the availability of this natural resource as a matter of extinction or genetic erosion of the plants. A severe reduction in natural resources has been observed due to ruthless exploitation.

Generally, mint is propagated vegetatively rather than by seeds which are a slow process and the plant is susceptible to various fungal, bacterial and viral diseases (Safaeikhorram, 2008). The conventional breeding of mint propagation is unsuccessful due to commercially available cultivars are mostly pollen sterile, high in ploidy number, an insufficient number of seedling production as a result of poor overwintering in tropical and sub-tropical countries like Bangladesh. Micropropagation has proven to be an excellent method for overcoming the conventional breeding problems (Pati *et al*., 2006). The main advantage of *in vitro* propagation is the production of high quality and uniform planting materials in the large scale. These planting materials can be multiplied on a year-round basis under disease free conditions anywhere irrespective of the season and weather (Zayova *et al*., 2021).

For *in vitro* regeneration of peppermint different plant growth regulators (PGRs) such as BA (6-benzyl adenine), BAP(6-benzylaminopurine), TDZ (thidiazuron), KIN (kinetin), ZEA (zeatin) and 2,4-D (2,4-dichlorophenoxy acetic acid), NAA (α-naphthylacetic acid), IAA (indole-3-acetic acid), or IBA (indolyl-3-butyric acid) can be used, all of which have different effects and functions on morphology and organogenesis.

The present research was carried out to find out the performance of two different hormones (BA and IBA) for micropropagation of mint. The ultimate aim of this study was to develop a micropropagation protocol that could ensure a high frequency of regeneration within a short time.

## Materials and methods

*Plant material*

The disease-free healthy mother plant of *Mentha spicata* L. (Mint) were used as experimental materials in the current research where a nodal segment (1-2 cm) of mint was used as explants. For sterilization, the explants were treated with 70% ethanol and rinsed with autoclaved sterilized distilled water for 3 to 4 times. After that explants were soaked in 0.1% mercuric chloride solution containing 2 to 3 drops of surfactant tween-20 for 5 min followed by 3 to 4 times washing with sterilized distilled water. Explants of suitable size (0.5-1 cm) were transferred to culture vial containing 20 mL MS medium with plant growth regulators. MS medium supplemented with 4 level of BA (1, 1.5, 2 and 2.5 mg/L BA) and 4 level of IBA (0.5, 1 and 1.5 mg/L IBA) either separately or in combinations which were used as treatments. Initial subculturing was done after 30 days followed by plantlets were transferred to room

temperature for acclimatization.

**Results and discussion**

*Effect of BA on in vitro shoot regeneration of Mint*

Significant variations were observed among different concentrations of BA on percent response of explants, shoot number and length of shoot. It is revealed from Fig. 1 that percentage of shoot initiation increased as the concentration of BA increased up to certain level. 2.0 mg/l BA that had produced the highest frequency of shoot (80.00%), however, further increase in BA concentration had led to decrease percentage of shoot regeneration (Fig. 1). The highest number of shoots (19.75) was noticed from the 2.0 mg/l BA at 42 Days after initiation (DAI) (Fig. 2) whereas maximum length of shoot (12.12 cm) was observed at 2 mg/l BA concentration (Fig. 3). Najafianashrafi, (2021) observed the maximum stimulation of shoots at 3 mg/L BA. According to Liu *et al.* (2018), 1.0 mg/L BA supplemented with MS medium was optimal for inducing adventitious shoots. Akter and Hoque (2018) conducted a similar experiment for the selected mint genotype (*Mentha* sp.), in which the same 1 mg dm−3 BA concentration results showed much better shoot proliferation rates for both apical and lateral meristems.

**Table 1.** Effect of IBA on *in vitro* root regeneration in Mint.

| IBA (mg/l) | Number of roots per plantlet | Length of root |
|---|---|---|
| 0.0 | 2.25 d | 1.50 c |
| 0.5 | 5.75 d | 4.37 b |
| 1.0 | 16.25 a | 7.50 a |
| 1.5 | 11.75 b | 4.62 b |
| 2.0 | 8.25 c | 3.62 b |
| CV (%) | 8.69 | 10.77 |
| LSD (0.05) | 2.016 | 1.196 |

Figures in a column followed by different letter(s) differ significantly whereas figures having common letter (s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

**Table 2.** Combined effect of BA and IBA on *in vitro* regeneration potentiality in mint.

| Treatment | Number of shoot/explants | Length of shoot (cm) | Number of roots | Length of root (cm) |
|---|---|---|---|---|
| 1.0mg/l BA+ 0.5 mg/l IBA | 8.00 f | 6.83 e | 9.67 e | 3.50 fg |
| 1.0mg/l BA+ 1.0 mg/l IBA | 19.66 a | 12.63 a | 19.33 a | 7.46 a |
| 1.0mg/l BA+ 1.5 mg/l IBA | 13.33 b-d | 9.8 b | 14.00 b | 6.16 c |
| 1.5mg/l BA+ 0.5 mg/l IBA | 9.00 f | 6.50 ef | 9.67 e | 4.00 e-g |
| 1.5mg/l BA+ 1.0 mg/l IBA | 19.67 a | 12.83 a | 20.00 a | 7.00 a |
| 1.5mg/l BA+ 1.5 mg/l IBA | 14.66 b | 9.83 b | 13.67 b | 6.33 bc |
| 2.0mg/l BA+ 0.5 mg/l IBA | 9.00 f | 8.00 d | 10.33 de | 3.00 g |
| 2.0mg/l BA+ 1.0 mg/l IBA | 20.33 a | 13.00 a | 20.67 a | 7.67 a |
| 2.0mg/l BA+ 1.5 mg/l IBA | 14.33 bc | 9.16 bc | 13.67 b | 6.17 c |
| 2.5mg/l BA+ 0.5 mg/l IBA | 8.00 f | 6.00 f | 9.67 e | 4.00 e-g |
| 2.5mg/l BA+ 1.0 mg/l IBA | 19.66 a | 12.50 a | 19.67 a | 7.50 ab |
| 2.5mg/l BA+ 1.5 mg/l IBA | 13.33 b-d | 9.33 bc | 13.33 bc | 5.67 cd |
| CV (%) | 4.49 | 6.43 | 4.56 | 7.88 |
| LSD (0.05) | 1.866 | 0.696 | 1.902 | 1.332 |

Figures in a column followed by different letter(s) differ significantly whereas figures having common letter (s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference. LSD (0.05) = Least significant difference.

*Effect of IBA on in vitro root regeneration in Mint*

For rooting different concentrations of IBA were applied and the highest number of root (16.25) was observed at 42 DAI with 1.0 mg/l IBA (Table 1). The highest rooting was recorded on MS medium with 2.0 mg/l IBA by Mehta *et al.* (2012) whereas Bariya and

Pandya (2014) observed a similar result with us that 1.0 mg/l IBA was required for rooting of *in vitro* regenerated plantlets after sub-culturing at 4 weeks. However, the disparity in different experiment may be due to differences in species or explant or culture condition.

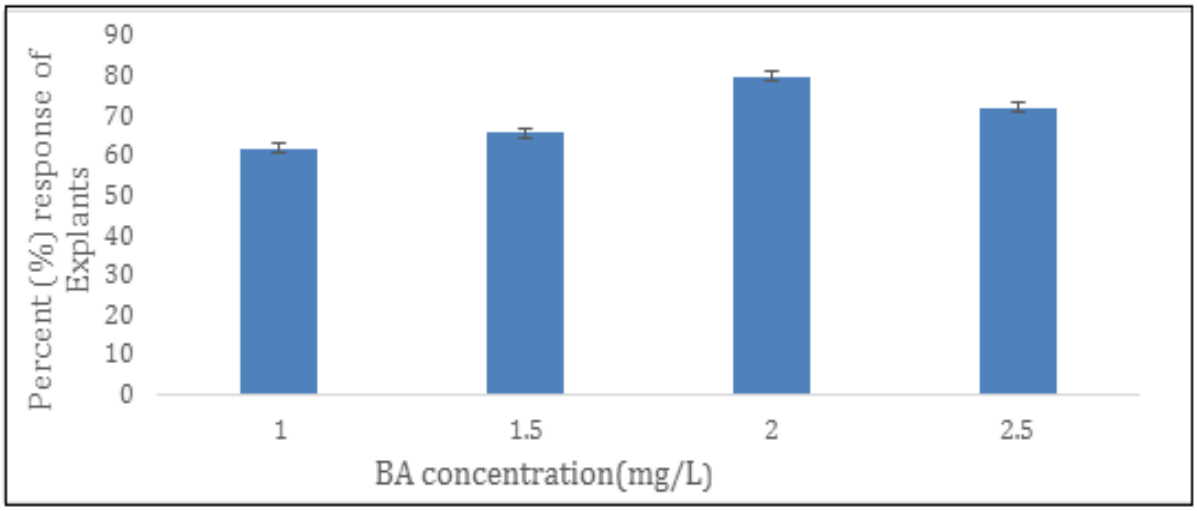


**Fig.1.** Percent response of explants at different BA concentration (mg/L).

On the other hand, the maximum length of root (7.50 cm) was noticed from the same 1.0 mg/l IBA treatment and the lowest length of root (1.5 cm) at 42 DAI was noticed in control without hormone (Table 1). However, Lyczko *et al.* indicated the addition of 0.5 mg dm−3 indolyl-3-butyric acid (IBA) with MS medium showed the best overall effects on rooting.

The dissimilarity in different experiment may lead to open up the door of further experimentation.

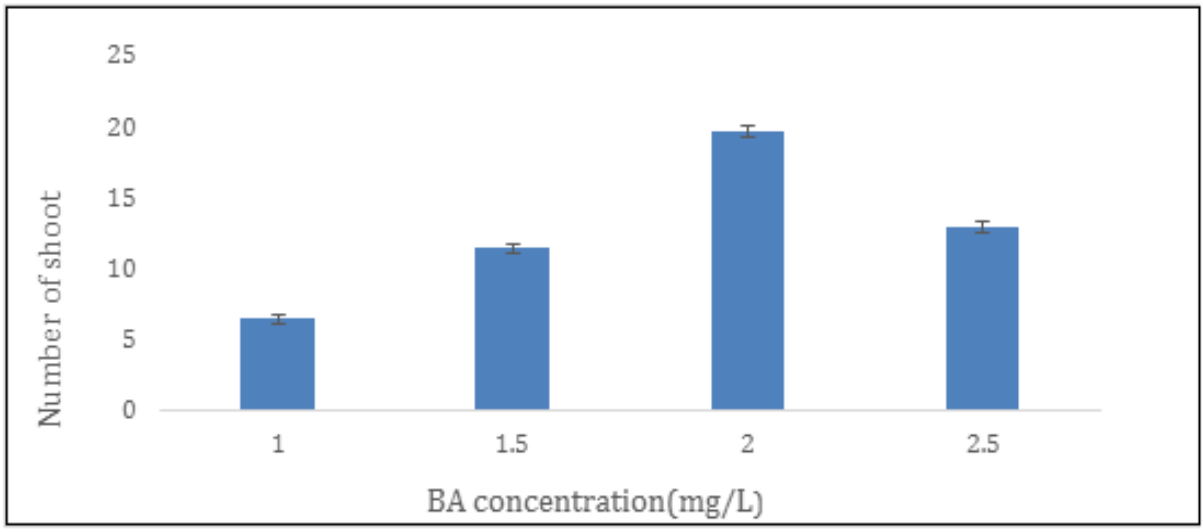


**Fig. 2.** Number of shoots at different BA concentration (mg/L).

*Combined effect of BA and IBA on in vitro plantlet regeneration*

Maximum number of shoots per explant (20.33) was observed on MS medium containing 2.0 mg/l BA + 1.0 mg/l IBA whereas minimum number of shoots (4.50) was noticed in control treatment. At the same time, the highest length of shoot (13.00 cm) was recorded from the 2.0 mg/l BA+ 1.0 mg/l IBA (Table 2). These findings are partially similar to the results of Sharma *et al.* (2012) that each inoculated explant produced 18.10 ± 0.66 shoots inoculated on MS medium supplemented with 4.44 µM BA in

combination with 2.85 µM IBA in Mint. This variation may be due to growth regulators in the culture media, genetic, physiological and morphological change in vitro (Chaturvedi *et. al.*, 2007). The treatment combination 2.0 mg/l BA+ 1.0 mg/l IBA provided the highest rooting (20.67) and root length (7.67 cm) (Table 2). Zayova (2021) reported that the maximum number of roots/explant (4.1) was obtained on half strength MS medium with 1.0 mg/l BA and 0.1 mg/l IBA treatments.

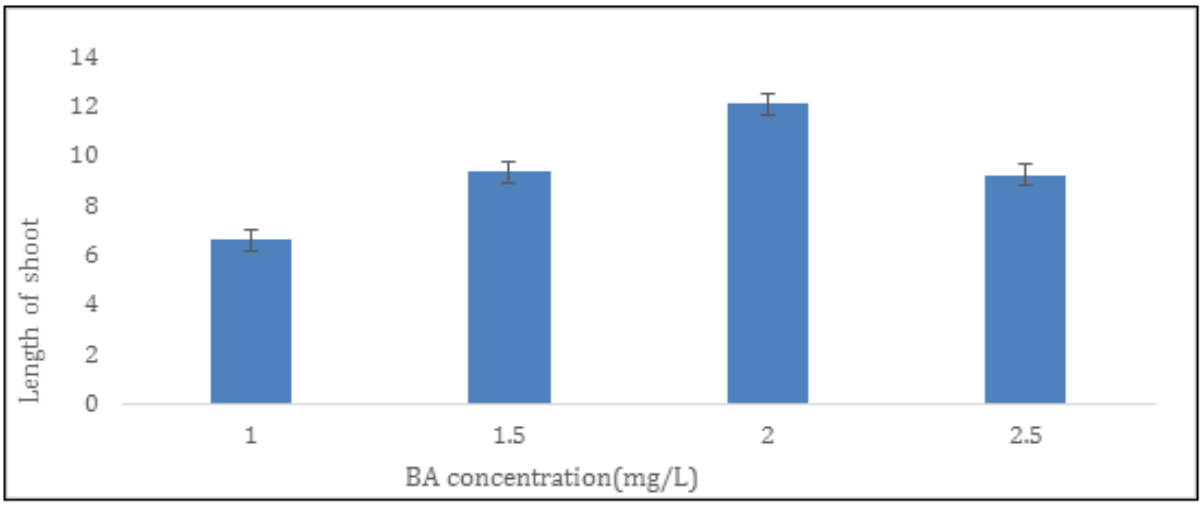


**Fig. 3.** Length of shoot at different BA concentration (mg/L).

*Ex vitro establishment of regenerated plantlets*

The regenerated plantlets were removed from cultural vial carefully and placed in growth chamber for acclimatization after 6-8 weeks of culture. The plantlets transferred to vermiculite pots filled with sterilized soil: cow dung (1:1). After that, they were moved to a shade house where they would grow at room temperature for 14 days while receiving biweekly irrigations. Finally, in the shade house, the survival rate was 83.33% which was ultimately 80% at normal atmospheric conditions. A similar finding was also observed by Sharma *et al.* (2012) that plantlets of mint were successfully transferred to the soil where survivability was 80%.

**Fig. 4.** *In vitro* regenerated Plantlets and regenerated plantlets in natural condition.

**Conclusion**

From the present research work it was concluded that the moderate dose, 2.0 mg/l BA performed best for in vitro shoot regeneration while 1.0 mg/l IBA gave the best results in case of root regeneration. MS medium supplemented with 2.0 mg/l BA with 1.0 mg/l IBA was recommended for satisfactory growth of mint *in vitro*. Overall, a reliable protocol has been established

that can be used for crop improvement programs of mint. Further research work can be done with different concentrations and combinations of auxins and cytokinin for *in vitro* regeneration of Mint.

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