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Clonal Propagation of *Mentha arvensis* L. Through Nodal Explant

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Abstract: Nodal explants of *Mentha arvensis* L. from naturally grown plants were cultured on MS basal medium fortified with various combinations of auxins and cytokinins. The best response for multiple shoot regeneration (4±1.58) and callogenesis was observed in MS+4.4 μM BAP. Whereas, explants cultured on MS+8.9 μM BAP+4.4 μM IAA exhibited the best direct multiple shoot regeneration (14±2.74) and rhizogenesis (1±0.70). The well developed micro shoots were successfully acclimated.

Key words: *In vitro*, nodal segments, explant, *Mentha arvensis*, clone

INTRODUCTION

Mint is valued for its multipurpose uses in the field of pharmaceuticals, cosmetics as well as for flavoring foods, beverages and tobacco (Ohloff, 1994). The plant is aromatic, stimulant and carminative. The infusion of leaves affords a remedy for rheumatism and indigestion (Anonymous, 1962). Because of mints sterility, improvement of their tolerance or resistance to pests is not possible using conventional breeding techniques. However, variation generated by use of tissue culture has resulted in improvement of diverse commercial crops (Reisch, 1983). An alternative method for improving mint may involve the use of somaclonal variants (Larkin and Scowcroft, 1981). To date, orange mint and peppermint embryos (Van Eck and Kitto, 1990), Japanese mint (*M. arvensis var.* piperascens) and stem segments (Ono, 1982) have been regenerative. In addition, shoots from 5 mint genotypes (*M. arvensis* L., *M. piperita* L., *M. pulegium* L., *M. spicata*., *M. viridis* L.) have been proliferated *in vitro*, rooted and acclimatized *in vivo*. (Rech and Pires, 1986). Ruthless exploitation and lack of organized cultivation has resulted in drastic decrease of this natural resource. Hence, it became imperative to establish a suitable regeneration protocol for rapid *in vitro* propagation of this medicinally important plant species.

MATERIALS AND METHODS

Nodal explants were taken from healthy *in vivo* grown plants grown in gene bank repository of RRL, Srinagar (J and K). The study was conducted in Biotechnology division of the institute during the summer season of the year 2004. The nodal explants were dipped in 5% teepol solution (commercially available detergent) for about 15 min followed by continuous shaking and then rinsed 4-5 min with sterilized distilled water (DDW). The nodal explants were then surface sterilized by using 0.1% mercuric chloride ($HgCl_2$) solution for about 5 min and then finally rinsed 5-6 times with DDW. These explants were inoculated in (Murashige and Skoog, 1962) MS basal medium augmented with various addenda i.e., (4.4-8.9 μM) BAP, (4.6-9.3 μM) Kn, (4.4 μM) IAA and (5.4 μM) NAA singly. To evaluate the effect of auxin - cytokinin, the nodal explants were also cultured on MS basal medium fortified with (8.9 μM) BAP+(4.4 μM) IAA/ (5.4 μM) NAA, (9.3 μM) Kn+(4.4 μM) IAA/ (5.4 μM) NAA (Table 1).

The pH of the medium was adjusted to 5.8 prior to autoclaving at 1.06 kg cm^{-2} pressure for 20 min. Ten replicates were taken for each treatment. The cultures

Table 1: Effect of growth regulators on organogenesis of *Mentha arvensis* L. through nodal explant

Treatments	Regeneration (%)	No. of shoots/ explant±SD	No. of roots/ shoots±SD
MS Full	30	-	3±0.70
Half MS	40	-	5±1.58
MS+4.4 μM IAA	70	-	14±2.92
MS+5.4 μM NAA	40	-	5±1.58
MS+4.4 μM BAP	50	4±1.58	white compact callus
MS+8.9 μM BAP	80	8±1.58	-
MS+4.6 μM Kn	40	4±1.58	-
MS+9.3 μM Kn	50	2±0.70	-
MS+8.9 μM BAP+4.4 μM IAA	80	14±2.74	1±0.70
MS+8.9 μM BAP+5.4 μMIAA	70	10±1.58	2±1.41
MS+9.3 μM Kn+4.4 μM IAA	60	3±1.58	1±0.0
MS+9.3 μM Kn+5.4 μM NAA	50	2±0.70	-

Evaluation was made after four weeks of inoculation. Values represent means±standard deviation of 10 replicates per treatment in the three repeated experiments

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were incubated under cool white light provided through fluorescent tubes at a photon flux rate of 400 μmol $m^{-2}S^{-1}$. All the cultures were transferred to fresh medium after every four weeks. Number of microshoots per explant was recorded for statistical analysis.

In vitro differentiated shoots measuring 3-4 cm in length were excised from shoot clump and cultured on auxin free full and half strength MS basal medium. The shootlets were also cultured on MS basal medium supplemented with (4.4 μM) IAA and (5.4 μM) NAA. Data were recorded on percentage of rooting, mean number of roots four weeks after transferring onto rooting media. Rooted plantlets were transferred to polycups containing sterile soil, vermiculite (1:1) and covered with plastic bags to maintain 85-90% humidity. Subsequently, the plantlets were transferred to greenhouse (Vista Biocell Limited) for a period of one month and finally transferred to the field conditions.

RESULTS AND DISCUSSION

Nodal explants were cultured on MS basal medium supplemented with BAP and Kn Shoots were proliferated from cut end of the explants on lower concentration of (4.4 μM) BAP 50% cultures formed shoots (4±1.58) along with white compact callus formation after 16 days of incubation (Fig. 1). These results are in consonance with the earlier observations on 44.4 μM BA+250 mL^{-1} Coconut Water (CW) in *Mentha spicata* (Van Eck and Kitto, 1990), Mints (Berry *et al*., 1997) and in *Mentha piperita*, *Mentha spicata* (Faure *et al*., 1998). When BAP concentration was doubled (8.9 μM) the nodal explants produce highly proliferating shoots (8±1.58) after 18 days of incubation in almost in 80% of the cultures (Fig. 2). Among various concentrations of kinetin tested the highest regeneration frequency, 40% and highest number of shoots (4±1.58) were recorded at kinetin (4.6 μM) after 20 days of inoculation. Increasing the concentration of Kn from (4.6-9.3 μM) resulted decrease in the rate of shoot regeneration ability (2±0.70), Table 1.

Cytokinins, especially BAP were reported to overcome apical dominance, release lateral buds from dormancy and promote shoot formation (George, 1993). Hence different concentrations of BAP and Kn were evaluated on shoot formation. The results are presented in Table 1. Further increase in the concentration of both reduced the number of shoots indicating an upper limit of BAP for *Mentha arvensis*. On the other hand, kinetin showed poor response to multiple shoot induction as compared to BAP in the present study.

Regeneration may also be affected by age (Welander, 1988), explants origin and culture maintenance

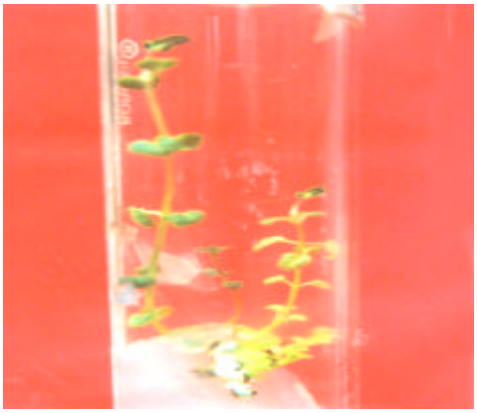

Fig. 1: Sixteen days old culture showing regeneration and white compact callus from the nodal explants cultured on MS+4.4 μM BAP

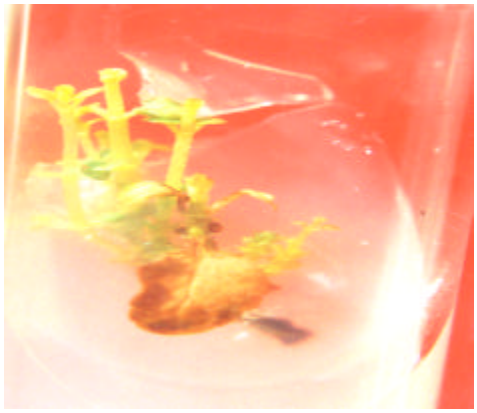

Fig. 2: Eighteen days old culture showing direct shoot proliferation from nodal explants cultured on MS+8.9 μM BAP

Fig. 3: Fifteen days after inoculation complete plant regeneration was observed on MS+4.4 μM IAA

Fig. 4: *In vitro* raised plants transferred to earthen pots having garden soil

conditions (Pierik, 1987). This genotype regeneration may be due to difference in genetic control of organogenesisby the different mint genotypes (Baronecelli *et al*., 1974). *Mentha arvensis* proliferated *in vitro* on a modified mint proliferation media (Rech and Pires, 1986) rooted and acclimated readily under green house conditions.

The addition of IAA or NAA with optimal concentration of kinetin significantly reduced the frequency of shoot formation (Table 1) in the present investigation similar results were obtained on same combinations in *Mentha piperita* by Ghanti *et al.* (2004). Multiple shoot formation in *Mentha* was also reported from calli derived from mature and immature embryos and leaves on MS basal medium supplemented with either BAP+NAA or BAP alone in two separate studies (Van and Kitto, 1990, 1992).

Individual shoot when planted in half or full strength MS basal medium free from growth regulators few number of roots were elicited (3±0.70) and (5±1.58) with low frequency. This may be due to the presence of endogenous auxins which were sufficient to initiate rooting. The best rhizogenesis was induced on MS basal fortified with IAA (4.4 μM) having 70% regeneration with 14±2.92 roots after 15 days of incubation (Fig. 3). These *in vitro* raised plantlets were then transferred in green house (Vista Biocell Limited) for hardening for a period of one month. Finally the acclimated plants were then shifted to the field conditions having 60% survivality (Fig. 4). Similar results were obtained from stem explants in Mint on NAA (0.5 mg^{-1}), by Mariska *et al.* (1987), (Ravishankar and Venkataraman (1988), Van and Kitto (1990, 1992) Faure *et al.* (1998) and Ghanti *et al.* (2004) which is in consonance with the present findings on *Mentha arvensis* L.

The present study emonstrates the procedure for the clonal propagation of *Mentha arvensis* from nodal explant, which can ensure a stable supply of this medicinally important oil yielding plant irrespective of any seasonal variation and may serve as the better source for biologically active compounds.

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