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Micropropagation of *Mentha viridis* L.: An aromatic medicinal plant

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Abstract

An efficient *in vitro* propagation technique was developed for *Mentha viridis* L. a commercially important medicinal herb, using nodal segments as explants. Explants were cultured on MS medium enriched with various combinations and concentrations of PGRs (BAP, NAA and IAA). The highest average number (5.70±0.36) and length (4.90±0.34) of microshoot was achieved on MS medium with 2.0 mg/l BAP and 0.5 mg/l IAA. The rooting experiments were carried out on ½MS using different concentrations of IBA, IAA and NAA (0.5 – 1.5 mg/l). The maximum root formation (5.0±0.60) with highest elongation of roots (5.39±0.23) was achieved in the medium containing 1.5 mg/l IAA. The rooted plantlets were hardened and transferred to the pots. The survival rate was 90%.

Key-Words: *Mentha viridis*, Aromatic medicinal plant, Micropropagation

Introduction

Mentha viridis L., commonly known as spearmint, belongs to the family Lamiaceae. In Bengali it is known as '*Pudina*'. It is a glabrous perennial, 30 to 90 cm high, with creeping rhizomes, indigenous to the north of England, but grown all over the world. In Bangladesh it is cultivated throughout the country. The leaves and essential oils have high commercial and medicinal values. Different species of mint are used for their medicinal and culinary properties and is usually taken after a meal for its ability to reduce indigestion and colonic spasms by reducing the gastrocholic reflux [1]. Leaves can be used as herbal tea which has traditionally been used in the treatment of several minor ailments such as fever, headache, and digestive disorder; decoction of leaves is used as a lotion in aphthae[2]

The herb is considered to be stimulant, carminative and antispasmodic[3]. It is given in the treatment of hiccup, bilious vomiting, flatulence, colicky pain and cholera. Juice of leaves is given in treating fever and bronchitis. Oil is a local anesthetic it relieves toothache, pain of neuralgia and herpes[4]. Spearmint oils are used for the manufacture of food confectioneries and pharmaceuticals. The plant is known to be endowed with variety of biological properties due to the high content of secondary metabolites[2].

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The development of rapid large scale propagation systems for medicinally important plants is a necessity in order to meet pharmaceutical needs and also to prevent the plants from becoming endangered and extinct[5]. Plant tissue culture techniques have been extensively used for commercial production, conservation and multiplication of medicinally valued plants[6-8]. In this perspective it was considered important to develop protocol for rapid propagation of *M. viridis*. Direct shoot regeneration from explants is of great importance in order to get true type progenies[9]. In the present research a repeatable protocol for *in vitro* mass propagation of *Mentha viridis* was established, using nodal segments as explants, which can be exploited for further advanced biotechnological researches such as development of transgenic plants.

Material and Methods

Nodal explants were collected from juvenile shoot apices of two to three months old pot grown plants those were then cut into short pieces and thoroughly washed under running tap water for 30 minutes followed by 10% savlon solution with few drops of Tween 80 for 5-10 minutes with constant shaking. The materials were then washed 3- 4 times with distilled water and then rinsed with 70% ethanol for 60 seconds followed by surface sterilization using 0.1% (w/v) mercuric chloride for 6 minutes and washed thoroughly 3-4 times with sterile distilled water to remove every trace of the sterilent. The sterilized tissues were cut into appropriate sizes (0.5-1.0 cm) using sterile forceps

and knife. For the direct shoot induction, the explants were cultured on MS medium supplemented with different levels of plant growth regulators, 3% sucrose as carbon source and solidified with 8% agar and the pH was adjusted to 5.8 before autoclaving. The medium was autoclaved at 1.1 kg/cm^2 pressure at a temperature of 121°C for 30 minutes. After inoculation cultures were kept in growth room exposed to artificial fluorescent light with a cycle of 14/10 hours light/dark photoperiod at 25±2°C.

In order to produce complete plantlets, elongated multiple shoots at a length of 3-5 cm were individually transferred to rooting media composed of half strength MS with various concentrations (0.5 – 2.0 mg/l) of IBA, NAA, IAA. Rooting experiments were also conducted in the culture room under the same environmental condition as used for multiple shoot induction.

All cultures were observed at regular intervals of one week and subcultures were made and data were recorded at 21-28 days intervals.

Each treatment consisted of 15 explants, and the experiment was repeated tree times. The results presented are the means of the replications with the standard error (±SE).

Results and Discussion

Nodal segments were cultured on MS medium supplemented with different concentrations (1.0-3.0 mg/l) of BAP individually and in combination with BAP (1.5-2.0 mg/l) + NAA (0.2-1.0 mg/l) and BAP (1.5-2.0 mg/l) + IAA (0.2-1.0 mg/l). Explants gave differential response in different PGR supplemented media and results are presented in the Table-1. Shoot buds were induced within 15 - 21 days of culture and produced green and dense multiple shoot buds (MSBs). When BAP was applied singly, 50% explants responded with highest average number (3.40±0.24) of shoot buds per explant in 2.0 mg/l BAP but the average length of shoot buds were maximum in 3.0 mg/l BAP. Shoot bud induction boosted up considerably while explants were cultured on MS fortified with low concentrations of NAA or IAA. In earlier researches on *in vitro* propagation of medicinal plants, proliferation efficiency of nodal explants was found significantly higher than that of other explants and BAP was most effective cytokinin for inducing multiple shoots in *in vitro* techniques[10-11]. Combinations of low concentrations of auxins with cytokinins were used in many earlier researches[12-13]. In the present experiment, multiplication of shoots along with shoot elongation was noted in various media combinations. The induction of MSBs was highest (100%) in MS + 2.0 mg/l BAP+0.5 mg/l IAA with highest number (5.70±0.30) of shoot buds per explant (Plate-1a, b). *In vitro* propagation of different medicinal plants using BAP and IAA was also reported by other researchers.[14-17]. A previous experiment reported maximum (95%) proliferation of nodal explants of *Mentha viridis* in MS + 3.0 mg/l BAP[12]. In another experiment 91% shoot bud proliferation was obtained in MS + 3.5 mg/l BAP[3]. These results did not corroborate with the present investigation and the variation might be due to the differences in genotype used and age of the explants. Prompt elongation of microshoots was taken place in some media combinations along with subculture (Table-1). Subcultures were done at an interval of 28-30 days. The highest elongation (4.90 ± 0.34 cm) was recorded in 2mg/l BAP + 0.5mg/l IAA (Plate 1b).

For root induction well developed microshoots were individually transferred to half strength MS without or with auxins (IBA, IAA and NAA) at a range of 0.5 – 1.5 mg/l. Rooting response were varied considerably depending on the concentrations of the auxins. Microshoots rooted in all the media combinations but ½ strength MS with 1.5 mg/l IAA was found most effective in respect of per cent (100%) of microshoot rooted, average number of root per shoot (5.0±0.60) and average length (5.39 ± 0.23) of roots (Table-2, Plate 1c). Microshoots cultured on rooting medium produced profuse aerial roots. Half strength MS has been widely used in *in vitro* rooting in various plants including many medicinal plant species and low concentration of IAA was proven efficient in rooting experiment in many previous researches[18-19]. Divergent result, however, has been reported during *in vitro* root proliferation of *Mentha viridis*[5], which did not correspond to the present investigation.

With the aim of hardening of *in vitro* raised plantlets in outside environment, the culture vessels were kept outside the culture room with gradual increase the time duration up to five days. The plantlets were then taken out from the vessels, washed with running tap water for removal of agar and then transferred to small earthen pots containing garden soil, compost and sand in the ratio of 1:1:1 and kept in room temperature for 4-5 days. Finally the plants were transferred to outside natural environment and the grownup plants were healthy and fully vigorous (Plate 1d) and the survival rate was 90%.

This research article described an *in vitro* mass propagation protocol of *Mentha viridis* that can be used reliably for multiplication and in the advanced biotechnological experiments on this important medicinal plant species.

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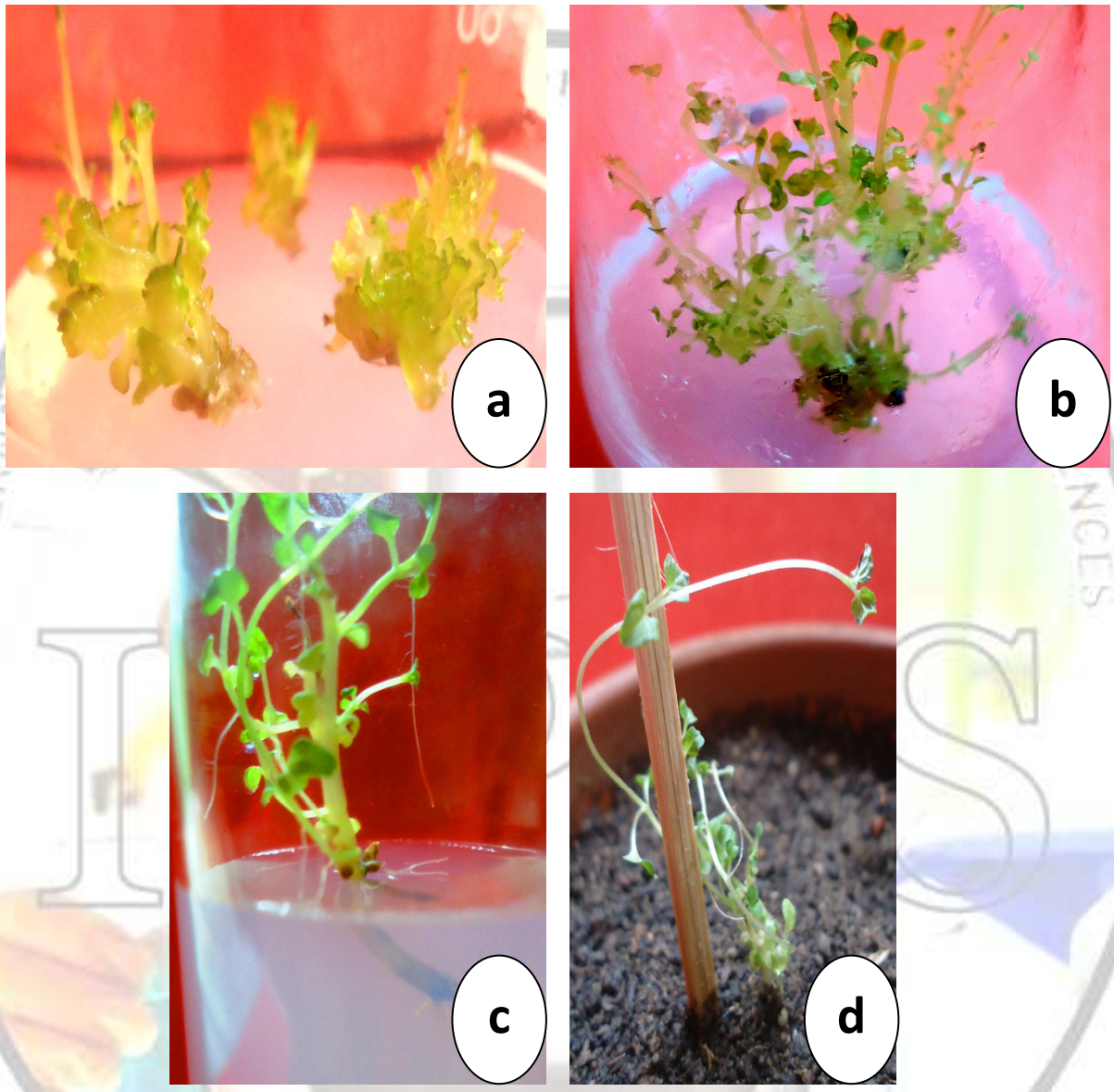

Fig. 1: *In vitro* propagation of *Mentha viridis* from nodal explants

a. Induction of shoots in three weeks of culture
b. Multiplication and elongation of shoots
c. Rooting of *in vitro* generated shoots
d. Regenerated plant after acclimatization

Table 1: Effect of cytokinins and auxins on multiple shoot buds proliferation in nodal explants of *Mentha viridis*

Plant growth regulators concentration (mg/l)				Frequency (%) of response	Average* No. of MSBs/ explant (Mean ± SE)	Average* length of individual shoot bud (cm) (Mean ± SE)
BAP	Kn	NAA	IAA			
1.0				35	2.20 ± 0.23	1.50 ± 0.11
2.0				50	3.40 ± 0.24	1.60 ± 0.14
3.0				40	2.06 ± 0.16	1.70 ± 0.12
	1.0			24	2.00 ± 0.16	1.50 ± 0.14
	2.0			28	2.10 ± 0.17	1.50 ± 0.11
	3.0			35	2.80 ± 0.24	1.50 ± 0.17
1.5		0.2		70	3.10 ± 0.19	2.86 ± 0.31
1.5		0.5		80	3.00 ± 0.17	4.50 ± 0.21
1.5		1.0		50	3.10 ± 0.20	4.60 ± 0.24
2.0		0.2		85	4.40 ± 0.37	3.50 ± 0.35
2.0		0.5		90	4.10 ± 0.26	4.70 ± 0.21
2.0		1.0		90	4.20 ± 0.37	4.50 ± 0.24
1.5			0.2	83	4.10 ± 0.20	3.17 ± 0.33
1.5			0.5	56	5.50 ± 0.40	4.70 ± 0.22
1.5			1.0	56	4.80 ± 0.20	4.60 ± 0.33
2.0			0.2	83	4.10 ± 0.21	3.24 ± 0.32
2.0			**0.5**	**100**	**5.70 ± 0.36**	**4.90 ± 0.34**
2.0			1.0	100	4.50 ± 0.19	4.40 ± 0.26

* Values are the mean of three replicates with 15 explants.

Table 2: Effect of different concentrations of auxins on root induction of *Mentha viridis*

Growth regulators (mg/l)			Days to induction of root	Frequency (%) of rooting	Average No. of root/ shoot bud (Mean ± SE)	Average* length (cm) of root (Mean ± SE)
½ MS without PGRs			20-28	50	2.4 ± 0.49	3.4 ± 0.49
IBA	IAA	NAA				
0.5			15-20	60	3.5 ± 0.50	4.16 ± 0.19
1.0			15-20	75	4.0 ± 0.40	4.59 ± 0.56
1.5			16-20	80	4.0 ± 0.58	4.66 ± 0.65
	0.5		15-20	80	4.5 ± 0.50	4.93 ± 0.19
	1.0		14-20	90	4.8 ± 0.56	5.13 ± 0.46
	1.5		**14-20**	**100**	**5.0 ± 0.60**	**5.39 ± 0.23**
		0.5	16-20	60	4.0 ± 0.30	4.50 ± 0.41
		1.0	15-20	75	4.3 ± 0.30	4.59 ± 0.56
		1.5	17-20	70	4.4 ± 0.51	4.66 ± 0.65

* Values are the mean of three replicates with 15 explants.