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Direct organogenesis of *Mentha piperata* L. from shoot tip, nodal and sucker explants

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The efficient protocol for rapid multiplication of *Mentha piperata* L. through shoot tip, nodal and sucker explants was established using Murashige and Skoog's (MS) medium. Multiple shoots were obtained without the intervention of callus. Highest number of shoots i.e.4.25±0.85was observed from nodal explants at Kin 1.5 mg/l and highest shoot length i.e. 4.72±0.87 cm was observed from shoot tip explants at BAP 3.0+NAA 0.50 mg/l. Highest number of roots (6.5±2.67) and highest root length (5.07±1.22 cm) was encountered at IAA 2 mg/l and IBA 2 mg/l respectively. The rooted plantlets were transferred to plastic cups containing sterile garden soil, farmyard soil and sand (2:1:1). The survival rate was 90% after 10 weeks of acclimatization.

Key words: callus, Lamiaceae, organogenesis, sucker explant

Introduction

Mentha piperata L. (Peppermint) is an aromatic, herbaceous, perennial and strongly scented herb, belonging to family Lamiaceae. The plant is 50-60 cm (3-4 feet) high, stems are usually reddish-purple, smooth and leaves are short, oblong-ovate and serrate. The flowers are purple-pinkish and appear in the summer months. It grows well in humid, temperate climate and is very sensitive to drought. It is widely cultivated in temperate region of Europe, Asia, United States, India and Mediterranean. It is thought to be a natural hybrid between spearmint *(Mentha spicata)* and water mint *(Mentha aquatic)* (Foster, 1996; Peirce, 1999). Ancient Greek, Roman, and Egyptian cultures used the herb in cooking and medicine. Peppermint leaf and oil are used for folk medicine, as flavoring agents and in cosmetic and pharmaceutical products throughout the world (Foster, 1996). Peppermint oil is the most extensively used of all the volatile oils (Murray, 1995). Peppermint is taken internally as a tea, tincture, oil or extract, and applied externally as a rub or liniment.

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Herbalists consider peppermint an astringent, antiseptic, antipyretic, antispasmodic, carminative, diaphoretic, mild bitter, analgesic, ant catarrhal, antimicrobial, rubefacient, stimulant and emmenagogue (Hoffman, 1996; Bove, 1996). Peppermint oil vapor is used as an inhalant for respiratory congestion. Peppermint tea is used to treat coughs, bronchitis, and inflammation of the oral mucosa and throat. It has traditionally been used to treat a variety of digestive complaints such as colic in infants, flatulence, diarrhea, indigestion, nausea and vomiting, morning sickness and anorexia, and as a spasmolytic to reduce gas and cramping. Peppermint is currently used to treat irritable bowel syndrome, Crohn's disease, ulcerative colitis, gallbladder and biliary tract disorders, and liver complaints (Blumenthal, 1998; Fleming, 1998). Peppermint oil is used to relieve menstrual cramps (Tyler, 1992). Peppermint oil is used externally for neuralgia, myalgia, headaches, migraines and chicken pox (Bove, 1996; Blumenthal, 1998). Peppermint contains about 1.2-1.5% essential oil. The volatile oil, also known as Mentha piperita aetheroleum, contains 30-70% free menthol and menthol esters (Dombek, 1990) and more than 40 other compounds. The principal components of the oil are menthol (29%), menthone (20-30%), and menthol acetate (3-10%). Ruthless exploitation has resulted in severe reduction of this natural sensational scented herb. Hence, it became extremely important to develop an *In vitro* protocol for rapid regeneration of *Mentha piperata* L. The present study regeneration through sucker explants is reported for first time.

Materials and methods

Mentha piperata L. plants were collected from Botanical Garden, Sant Gadge Baba Amravati University, Amravati. The plants were multiplied in different earthen pots. Different types of explants i.e., shoot tips, nodal and sucker explants were used as explant source. The various explants were washed sterilized with soap solution for 2 minutes, rinsed with 70 % alcohol for 1-2 minutes. The explants were then surface sterilized using 0.1 % $Hgcl_2$ (30 seconds for shoot tip, 1 minute for nodal portions and 1.30 minutes for sucker explants). After repeated washing with sterile double distilled water, the explants were cultured on sterile nutrient medium. The basal medium used during the entire studies was of Murashige and Skoog's (MS) salt solution (1962). Different growth regulators viz. BAP, IAA, NAA, IBA and Kinetin were used at different concentrations singly or in combination. All the culture media was fortified with 3% sucrose solidified with 0.8% agar. The PH of the media was adjusted to 5.8 before inoculation. The media was dispensed in test tubes as well as in culture bottles containing 20 and 50 ml respectively of media respectively. Cultures were kept inside growth chambers, appropriate photo

period (16 hours light and 8 hours dark) and temperature (20-25^0 C) were maintained. Shoot tips, nodal and sucker explants were inoculated in sterile culture tubes and in sterile culture bottles containing MS media supplemented with different concentrations of BAP, NAA and Kinetin singly or in combination with each other (Table 1) for shoot induction. Multiple shoots formed were transferred to different concentrations/combinations of auxins (Table 2) to induce multiple rooting in them. Regenerated plantlets were isolated and transferred to plastic cups containing sterile garden soil, farmyard soil and sand (2:1:1). The potted plantlets were initially maintained in programmable environmental chamber for 4 weeks. The plantlets were then transferred to normal laboratory conditions for 2 weeks. Finally they were transplanted to Departmental Botanical Garden and placed under shade for further growth and development. The morphological growth characteristics and survival efficiency was observed.

Results and Discussion

Multiple shooting

Multiple shoot initiation started after 13 days from shoot tips, nodal and sucker explants without the intervention of callus phase (Table 1). Among the various practiced explants, highest number of shoots i.e.4.25±0.85was observed from nodal explants at Kin 1.5 mg/l (Fig. 1 a) and highest shoot length i.e. 4.72±0.87 cm was observed from shoot tip explants at BAP 3.0+NAA 0.50mg/l (Fig. 1 b). BAP has been commonly used for the induction of organogenesis in many plants (Zilis *et al*., 1979). Cytokinins, particularly BAP is reported to overcome apical dominance, release lateral buds from dormancy and promote shoot formation (George, 1993). Venkatramalingam (2011) showed regeneration of *Mentha piperata* shoots on MS medium after 10 days of culture initiation. In conclusion *Mentha piperata* is enriched with essential oils which are widely used in food flavor, cosmetic and pharmaceutical industry. In addition to nodal and shoot tip explants, sucker plants are used for rapid regeneration protocol. Further this will be helpful for genetic transformation and extraction of active principle from the plant.

Table 1. Multiple shoot induction from shoot tip, nodal and sucker explants at different concentrations and combinations of growth regulators

PGR	Conc. (mg/l)	Average number of shoots and shoot lenght of various explants					
		Shoot tip explant		Nodal explant		Sucker explant	
		Average no. of shoots[x]	Average shoot length[x] (cm)	Average no. of shoots[x]	Average shoot length[x]	Average no. of shoots[x]	Average shoot length[x](cm)
BAP	1.0	1.75±0.25	2.5±0.64	1.5±0.28	2.65±0.69	1.5±0.90	2.37±0.55
	2.0	1.75±0.25	2.75±0.95	3±0.40	2.92±0.87	2.75±0.5	2.82±0.52
	3.0	1.5±0.28	2.7±0.68	2.5±0.28	2.82±0.69	1.5±0.28	2.52±0.69
	4.0	1.75±0.48	1.75±0.25	1.5±0.28	1.45±0.48	0.75±0.25	2±0.58
BAP+NAA	1.0 + 0.5	1±0	1.62±0.51	1±0	1.77±0.50	1±0	1.12±0.30
	2.0 + 0.5	3.25±0.47	4.07±1.01	3.75±0.28	4.07±0.93	3±0.40	3.57±0.90
	3.0 + 0.5	3±0.40	4.72±0.87	3.5±0.28	3.62±0.87	2.75±0.47	3.025±0.67
	4.0 + 0.5	1±0.40	1.075±0.36	1.75±0.25	0.92±0.24	1±0	1.95±0.48
Kin	1.0	3.25±1.31	3.3±1.0	3.25±0.85	3.02±0.60	2.5±0.87	2.6±0.39
	1.5	2.5±0.64	3.87±0.85	4.25±0.85	2.25±0.72	1.75±0.48	3.05±0.78
	2.0	2.75±1.03	3.85±0.90	3±0.91	3.5±0.84	3.5±1.19	2.73±0.55

[x]Mean±standard error.

Table 2. Response percentage of shoot tip, nodal and sucker explants at different concentrations and combinations of growth regulators

Plant growth regulator	Concentration (mg/l)	Response percentage (%)		
		shoot tip explant	Nodal explant	Sucker explant
BAP	0.5	48.4	60	68
	1.0	36.2	68	72
	2.0	60	76	80
	3.0	80	50	46
BAP+NAA	0.25 + 0.5	80	78	52
	1.0 + 0.5	90	86	82
	2.0 + 0.5	96	90	90
	3.0 + 0.5	88	92	96
Kin	0.75	78	48	88
	1.0	90	80	82
	1.5	92	86	84
	2.0	88	78	92

Multiple Rooting

In vitro shoots were excised from the micropropagated shoots after 30 days and dried on sterile tissue paper to remove adhering media and then transferred to rooting medium augmented with various auxin concentrations (Table 3). Roots were visible within 10-14 days following the transfer of elongated shoots to the rooting media. Highest number of roots i.e. 6.5±2.67

(Fig. 1 c) and highest root length i.e. 5.07±1.22 cm was encountered at IAA 2 mg/l and IBA 2 mg/l respectively. Kiran *et al.,* 2004 induced rooting from in vitro excised shoots on MS medium supplemented with different concentrations of NAA and IBA.

Table 3. Multiple root induction from *In vitro* grown shoots at different concentrations of auxins

Plant Growth Regulator	Concentration (mg/l)	Response Percentage (%)	Average no. of roots[x]	Average root length[x] (cm)
IAA	1.0	88	3.75±1.75	0.45±0.17
	2.0	78	6.5±2.67	0.62±0.19
	3.0	92	5±2.38	1.13±0.44
	4.0	82	3±1.22	1.92±0.52
NAA	0.5	76	0.5±0.29	0.97±0.70
	0.75	72	2.75±0.48	2.8±0.48
	1.0	68	2.5±0.65	2.7±0.85
	1.5	90	1.92±0.52	1.5±0.28
IBA	0.5	92	2.75±0.77	0.57±0.20
	1.0	96	3.5±1.04	1.02±0.37
	1.5	90	3.25±1.31	3.25±1.31
	2.0	92	3±1.08	5.07±1.22

[x]Mean ± standard error.

Acclimatization

Rooted plantlets were isolated from the culture media and washed with sterile double distilled water to remove adhering medium. These plantlets were transferred to plastic cups that contained sterile garden soil, farmyard soil and sand (2:1:1). The survival rate after hardening for first 5 weeks was 100 %.

However it decreased to 90% after 10 weeks of acclimatization. All of the micropropagated plants were free from external defects (Fig. 1 d) and are maintained in Departmental garden.

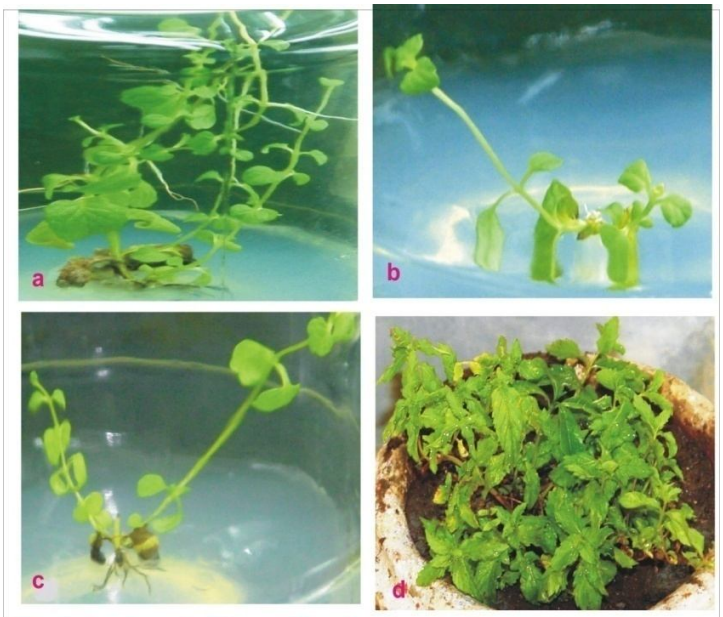

Fig. 1. *In vitro* studies on *Mentha piperata* L.

a) Shoot formation from nodal explant at Kinetin 1.5 mg/l.
b) Shoot formation from shoot tip explant at BAP 3 + NAA 0.50 mg/l.
c) Rooting of in vitro grown shoots at IAA 2 mg/l.
d) Acclimatized plant.

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