



Journal of
Plant Sciences

ISSN 1816-4951



Academic
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An Efficient *in vitro* Culture Method of Shoot Regeneration for a Medicinally Important Plant *Mentha piperita*

¹K. Venkatramalingam and ²M.G. Ebbie

¹Department of Zoology, Thiru Kolanjiyappar Government Arts College, Vriddhachalam

²Department of Biotechnology, Sengunthar Arts and Science College, Tiruchengode

Corresponding Author: M.G. Ebbie, Department of Biotechnology, Sengunthar Arts and Science College, Tiruchengode, Namakkal Dt, Tamil Nadu, India Tel: +9843429166

ABSTRACT

Mentha piperita is a very important medicinal plant used in preparations of various food products. At present a high frequency regeneration protocol has been developed from nodal explants of *Mentha piperita* using Murashige and Skoog's (MS) medium supplemented with varying combination of 0.20, 0.30, 0.40 and 0.50 mg L⁻¹ of Naphtalene Acetic Acid (NNA) and Kinetin (KN). The highest number of shoots (3) and shoot length (9.1 cm) was obtained in medium containing 0.40 mg L⁻¹ of NAA and 0.40 mg L⁻¹ of KN combination. The shoot length was increased up to 5.5, 7.0, 9.1 and 8.7 cm on 30 days of culture. After 30 days, the whole regenerated plants were transferred to rooting medium. This study is found to be an efficient and effective method for the conservation of this plant.

Key words: *Mentha piperita*, shoot induction, regeneration, plant growth regulator, kinetin, NAA

INTRODUCTION

Mentha Piperita is a perennial herb of the Lamiaceae and contains essential oil that has been used as an ingredient in medicine. It thrives well in humid and temperate climate and mostly cultivated in temperate region of Asia, Europe, United States and India. The plant is aromatic, stimulant, stomachic, carminative and used for allaying nausea, headache and vomiting. *Mentha piperita* oil is widely used in food products, cosmetics, pharmaceuticals, mouth washes, soaps, chewing gums, candies, confectionery and liquors (Kiran *et al.*, 2004; Scavroni *et al.*, 2005). It is also a chemopreventive and antimutagenic agent (Samarth *et al.*, 2006).

The leaves and flower tops of the plant are the major part utilized. The essential oil synthesized and stored in leaf glandular trichomes are valued commercially as additives for food products, cosmetics and pharmaceuticals. This herbal spice is cultivated mainly for the essential oil, traded as peppermint oil which contains menthol, viridiflorol, menthofuran, menthone, isomenthone, isomenthol, neomenthol, neoisomenthol, pulegone, piperitone, α -pinene, limonene, terpinene, phellandrene, cineole, carvone, menthoside and several sesquiterpenes (Anonymous, 1962; Rastogi and Mehrotra, 1993). The export of peppermint (*M. piperita*) oil from April 2000-March 2001 was about 1000 tonnes with an estimated value of Rs. 35 crores. The Indian price of peppermint oil is Rs. 500 per kg. Propagation of medicinal plants through axillary bud proliferation has been shown to be the simple and reliable method for rapid mass production of the desired clones (Elangomathavan *et al.*, 2003).

Mentha piperita proliferates generally through vegetative propagation. Technical advancements in the field of biology have resulted in the potential use of biotechnology in improving the peppermint crop. Plant tissue culture plays an important role in the production of agricultural and ornamental plants and in the manipulation of plant for improved agronomic performance. Plant cell cultures were introduced as an important tool for studying and producing plant secondary metabolites in the mid 1960s. Improved cell and tissue culture technologies would help in producing the active compounds *in vitro* with better productivities without cutting down the natural resources (Siwach *et al.*, 2011). *In vitro* culture of plant cells and tissue has attracted considerable interest over the recent years because it provides the means to study the physiological and genetic processes of plants in addition of offering the potential to assist in breeding improved cultivars by increasing the genetic variability (Benderradji *et al.*, 2007). The lack of organized cultivation has resulted in drastic decrease of this natural resource. Hence, it becomes imperative in establishing a suitable regeneration protocol for rapid *in vitro* propagation of this medicinally important plant species. In this present study an attempt has been made to establish experimental conditions for rapid multiplication of *Mentha piperita* using combination of kinetin (KN) and alpha naphthalene acetic acid (NAA) in the nutrient medium (MS basal medium).

MATERIALS AND METHODS

Mentha piperita plants were collected from Niligiris hills of Western Ghats, Tamil Nadu, India. Plant specimens were identified, authenticated and voucher specimens have been deposited at the department of biotechnology, SASC (F. No. 6). The plants were grown in the department herbal garden and were used as a source of explant. The study was carried in the year March 2010.

Explant preparation and surface disinfection: About 4 cm long axillary buds from plants each containing at least one node were collected for the preparation of nodal explants. The explants were dipped in 5% teepol solution for about 10 min followed by continuous shaking and then rinsed for 4-5 min in sterilized distilled water. The explants were then surface sterilized by using 0.1% mercuric chloride (HgCl_2) solution for about 5 min then finally rinsed 5-6 times using sterilized distilled water.

Media preparation and explant inoculation: The shoot tip and nodal explants were inoculated by inserting their cut ends in the MS medium supplemented with the combination of NAA and KN (0.20, 0.30, 0.40 and 0.50 mg L⁻¹ each) to induce multiple shoots. The medium contained 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH was adjusted to 5.6 and autoclaved at 121°C (i.e., 15 lb pressure) for 15 min. The cultures were maintained at 25±1°C under a light intensity of 3000 lux provided by cool white fluorescent lamps. Three cultures were raised for each concentration of Plant Growth Regulator (PGR) and all experiments were done twice. *In vitro* initiated shoots, length and number of shoots were analysed after 10, 20 and 30 days. The cultured plants were then transferred to rooting medium followed by hardening. Statistical analysis was carried out by following the method of Zar (1974).

RESULTS

Different concentrations and combinations of NAA and KN were utilized to find out the shoot regeneration capacity of *Mentha piperita*. The highest frequency (100%) with higher number of shoots (3) and shoot length (9.1 cm) was recorded in 0.40 mg L⁻¹ of NAA and 0.40 mg L⁻¹ of KN

combination media. This was followed by a frequency of 92% with 2 shoots and 8.7 cm shoot length in a combination of 0.50 mg L⁻¹ NAA and 0.50 mg L⁻¹ of KN supplemented media after 30 days of culture initiation. The number of shoots raised was 2, 3, 3 and 2 in 0.20, 0.30, 0.40 and 0.50 mg L⁻¹ combination of NAA and KN, respectively. The highest shoot number was recorded in 0.30 and 0.40 mg L⁻¹ concentration of NAA and KN combination. Increase in the concentration of both (NAA and KN) reduced the frequency of shoot induction, shoot number and shoot length. This clearly indicates an upper limit in combination of NAA and KN at 0.40 mg L⁻¹ for *Mentha piperita* (Table 3).

After 20 days of incubation two fold increase in shoot length (4.0, 4.5, 5.9, 5.1 cm) was observed in all combinations (Table 2). Where as, after 10 days of incubation highest shoot length (2.8 cm) was observed in 40 mg L⁻¹ concentration (NAA and KN each) supplemented media (Table 1). The minimum shoot length (2.1, 4.0, 5.5 cm) was noticed in 0.20 mg L⁻¹ NAA and 0.20 mg L⁻¹ KN containing media after 10, 20 and 30 days of culture, respectively.

The minimum concentration of plant growth regulators (0.20 mg L⁻¹ of NAA and 0.20 mg L⁻¹ of KN) has promoted a shoot induction of about 60% which seems to be the lowest when compared with other concentrations (Fig. 1a). During the study period, the number of shoots propagated remained the same except 0.40 mg L⁻¹ of NAA and 0.40 mg L⁻¹ of KN media combination. In this media one more shoot was regenerated after 10 days of incubation and it reached to four numbers (Fig.1 b). The maximum shoot length of 0.20, 0.30 and 0.40, 0.50 mg L⁻¹ concentration combination (NAA and KN) was 5.5, 7.0, 9.1 and 8.5 cm, respectively (Fig. 1c, d).

Table 1: Regeneration of *Mentha piperita* shoots on MS medium after 10 days of culture initiation

Concentration and combination of NAA and KN (mg L ⁻¹)	Regeneration frequency (%)	No. of shoots/explant	Shoot length/explant (cm)
0.20±0.20	60	2±0.10	2.1±0.20
0.30±0.30	88	2±0.60	2.7±0.10
0.40±0.40	100	3±0.20	2.8±0.19
0.50±0.40	90	3±0.33	2.4±0.41

Values are Mean±SD

Table 2: Regeneration of *Mentha piperita* shoots on MS medium after 20 days of culture initiation

Concentration and combination of NAA and KN (mg L ⁻¹)	Regeneration frequency (%)	No. of shoots/explant	Shoot length/explant (cm)
0.20±0.20	60	2±0.33	4.0±0.50
0.30±0.30	90	2±0.33	4.5±0.61
0.40±0.40	100	3±0.00	5.9±0.50
0.50±0.40	92	2±0.33	5.1±0.26

Values are Mean±SD

Table 3: Regeneration of *Mentha piperita* shoots on MS medium after 30 days of culture initiation

Concentration and combination of NAA and KN (mg L ⁻¹)	Regeneration frequency (%)	No. of shoots/explant	Shoot length/explant (cm)
0.20±0.20	60	2±0.33	5.5±0.19
0.30±0.30	90	3±0.33	7.0±0.33
0.40±0.40	100	3±0.33	9.1±0.10
0.50±0.40	92	2±0.10	8.7±0.33

Values are Mean±SD

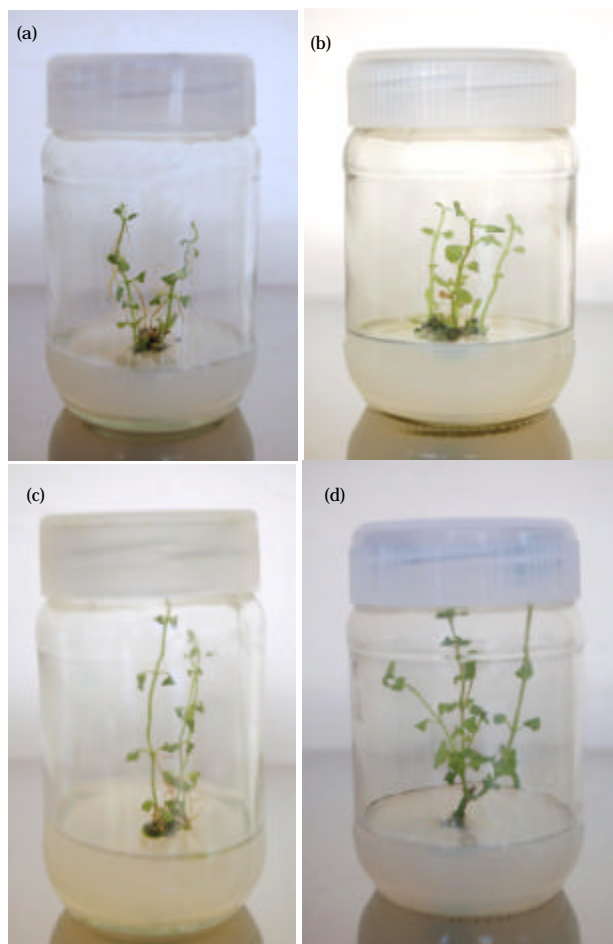


Fig. 1: (a) 30 days of incubation on 0.20 mg L^{-1} NAA and 0.20 mg KN , (b) 30 days of incubation on 0.30 mg L^{-1} NAA and 0.30 mg KN , (c) 30 days of incubation on 0.40 mg L^{-1} NAA and 0.40 mg KN and (d) 30 days of incubation on 0.50 mg L^{-1} NAA and 0.50 mg KN

DISCUSSION

In the present study an attempt has been made to culture *Mentha piperita* from nodal explants with the help of different plant growth regulators (NAA and KN) with varying combination and concentration. Poovaiah *et al.* (2006 a, b) reported that nodal explants exhibited better regeneration capacity than leaf explants in *M. piperita*. Nodal explants are the best source of explants used for the multiplication of shoots. This has been suggested earlier in the case of other medicinal plants such as *Rauwolfia serpentina* (Roy *et al.*, 1995), *Emblica officinails* (Rahman *et al.*, 1999). Plant growth regulators NAA and kinetin have proved to be fruitful in shoot regeneration of *Mentha piperita*. NAA and kinetin (0.40 mg L^{-1} each) medium triggers the shoot regeneration stimulus upto 100%, that continued for more than two subcultures. Several studies have demonstrated that an excess of cytokines can promote the regeneration of shoots in tissue cultured plants (Kevers *et al.*, 1984). Healthy shoots (3) having more than 9 cm length with their

basal end was observed in 0.40 mg L⁻¹ of NAA and kinetin supplemented media. This result was similar to that observed in spearmint culture on medium containing combination of TDZ and ZT. The addition of NAA with optimal concentration of Kinetin (0.40 m L⁻¹) significantly reduced the frequency of shoot formation (Chishti *et al.*, 2006). In the present investigation, similar results were obtained on 0.50 mg L⁻¹ combination and concentrations. Most of the reports are supportive but the concentration of PGR utilized is higher. Moreover, in this study a lesser concentration of PGR has been successively utilized. This media composition has been found to be an efficient and effective method in conserving this plant.

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