African Journal of Biotechnology Vol. 11(7), pp. 1644-1650, 24 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1336
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Evaluation of different densities of auxin and endophytic fungi (*Piriformospora indica* and *Sebacina vermifera*) on *Mentha piperita* and *Thymus vulgaris* growth

Hossein Kari Dolatabadi[1]*, Ebrahim Mohammadi Goltapeh[1], Ahmad Moieni[2] and Ajit. Varma[3]

[1]Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modarres University, Tehran, Iran.
[2]Department of Plant Breeding, Faculty of Agriculture, Tarbiat Modarres University, Tehran, Iran
[3]Amity Institute of Herbal and Microbial Studies, Sector125 New Super Highway, Noida, India.

Accepted 15 November, 2010

***Mentha piperita* and *Thymus vulgaris* are two important species of the family Lamiaceae. Two distinct experiments were conducted and examined; the first evaluate the effect of different auxin levels on *M. piperita* and *T. vulgaris* growth, while the second examined the effect of two fungi *Piriformospora indica* and *Sebacina vermifera* on plant height, root length, shoots and root weight in *in vitro* in a completely randomized design (3 and 20 replication in first and second experiments, respectively). The first experiment showed that the most effective hormone dose for the *M. piperita* growth was 1 mgl^{-1} of indole-3-butyric acid (IBA) and in the *T. vulgaris*, it was 1 mgl^{-1} of indole acetic acid (IAA). Increase of the hormone doses resulted in reduced growth. The second experiment showed that growth of the plants inoculated with fungi increased significantly. The data indicated that the plants inoculated with *S. vermifera* were of maximum height and the plants inoculated with *P. indica* had maximum weight also. The nodes of *M. piperita* and numbers of shoots in *T. vulgaris* increased significantly.**

Key words: *In vitro* culture, auxin, peppermint, thyme, *Piriformospora indica*, *Sebacina vermifera*.

INTRODUCTION

Peppermint (*Mentha piperita*) belonging to the Labiatae family is an important aromatic plant. This plant is native to the Mediterranean regions but can be commercially cultured in temperate regions of the world especially in America, Canada and China. Its extracts and essential oil are used for making gum and confectionery industries to produce different flavors and drugs (Foster, 1990). This plant is used to treat nausea, bronchitis, flatulence and anorexia (Fonseka-Kruel and Fernandes, 2003). Essential oil is also recognized for its carminative, stimulant, antiseptic, antispasmodic, antifungal and antibacterial properties (Guedón Pasquier, 1994; Gershenzon et al., 2000; Inoue et al., 2002; Samarth and Kumar, 2003; Ruiz del Castillo et al., 2004; Duarte et al., 2005). Another important plant of Labiatae family that is used by food and drug industries is thyme (*Thymus vulgaris*). The essential oil was reported to have antimicrobial (bacteria and fungi), carminative and expectorant activities (Leung

*Corresponding author. Email: emgoltapeh@modares.ac.ir or emgoltapeh@yahoo.com.

Abbreviations: PGR, Plant growth regulator; **AMF,** arbuscular mycorrhizal fungi; **IAA,** indole acetic acid; **NAA,** 1-naphthaleneacetic acid; **IBA,** indole-3-butyric acid.

and Foster, 1996; Chao et al., 2000; Horne et al., 2001). Soil microorganisms could increase the food production and plant growth regulator (PGR), and have antagonistic properties against plant pathogens (Bolton et al., 1993). One of these beneficial microorganisms is arbuscular mycorrhizal fungi (AMF). They form symbiotic relationships with roots of about 90% land plants in natural and agricultural ecosystems (Brundrett, 2002). AMF symbiosis improved nutrient (especially phosphate) and water uptake, increased resistance to water stress, increased levels of available soil, increased root durability, increased plant growth, prevention of the toxicity of heavy metals, and improved soil structure (Marschner and Dell, 1994; Cooper, 1984; Huang et al., 1985; Ellis et al., 1985; Dela Cruz, 1991; Abbott and Robson, 1982; Jeffries et al., 2003; Harley and Smith 1983). Gupta et al. (2002) reported that *Glomus fasciculatum* increased plant height, fresh and dry weight of three cultivars of *Mentha arvensis* (Kalka, Shivalik and Gomti). In contrast to most mycorrhizal fungi, *Piriformospora indica* (Verma et al., 1998) *and Sebacina vermifera* (Warcup, 1988) are cultivable fungi and can grow on synthetic or complex media without hosts (Varma et al., 2001; Peskan-Berghofer et al., 2004). Many researchers have reported that *P. indica* and *S. vermifera* can improve the growth rate of various host plants (Varma et al., 1999; Sahay and Varma, 1999; Rai et al., 2001; Waller et al., 2005; Peskan-Berghofer et al., 2004; Kumari et al., 2003). In this study, various concentrations of auxin, indole acetic acid (IAA), 1-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) and endophytic fungi *P. indica* and *S. vermifera* have been tested for their effect on the growth and development of peppermint (*M. piperita*) and thyme (*T. vulgaris*) in *in vitro* conditions.

MATERIALS AND METHODS

Plant material

Pieces of mint and thyme were prepared from cultivated farms of the Institute of Medicinal Plants and Natural Products Research, Karaj, Iran and transferred to an environmentally controlled chamber (25/20°C day/night with 16-h photoperiod). Then plants grown in pot culture were used as source material for *in vitro* cultures.

In vitro stock plant

Stock cultures were obtained from stem node explants. These explants, excised from green house thyme and mint plant, were washed in running water. Surface sterilization was obtained by immersion for 5 min in 70% ethanol, followed by soaking for 10 min in sodium hypochlorite (1%) plus 0.01% Tween 20 as surfactant and rinsing four times in sterile distilled water. Nodes were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 30 g/l sucrose, 1 mg/l thiamine and 8 g/l agar in glass bottles. The pH was adjusted to 5.8 before autoclaving at 120°C for 20 min. The explants were placed in a growth chamber at 25°C under cool white fluorescent lamps in 16 h light/ 8 h dark.

Root endophyte cultures

P. indica and *S. vermifera* were grown on aspergillus broth and agar medium at 30°C for 7 days and grown on PDA medium too (Pham et al., 2003).

In vitro experiments

Thirty days old micro propagated plants were employed as the source of apex to be used in experiments for *in vitro* plant inoculation. Two distinct experiments were carried out separately; first, the effect of auxin (NAA, IBA and IAA) and its various combinations were assessed on plant height. Three replications were maintained for each treatment. Experiment was evaluated in completely randomized design (CRD). Micro propagated plants were analysed after 30 days.

Treatments: T1: 1 mgl^{-1} IBA; T2: 2 mgl^{-1} IBA; T3: 4 mgl^{-1} IBA; T4: 1 mgl^{-1} NAA; T5: 2 mgl^{-1} NAA; T6: 4 mgl^{-1} NAA; T7: 1 mgl^{-1} IAA; T8: 2 mgl^{-1} IAA; T9: 4 mgl^{-1} IAA; T10: 2 mgl^{-1} IAA + 2 mgl^{-1} IBA; T11: 4 mgl^{-1} IBA + 4 mgl^{-1} NAA; T12: 4 mgl^{-1} IAA + 4 mgl^{-1} NAA; T13: 2 mgl^{-1} IAA + 2 mgl^{-1} IBA +2 mgl^{-1}NAA and T14: Control.

In the next test, the effect of two fungi *P. indica* and *S. vermifera* were evaluated on plant growth. The transparent culture glass bottles destined for plant-fungus co-culture were inoculated with a 5 mm diameter mycelial disk of 7 days old culture of *P. indica* and *S. vermifera* at the center into culture glass bottle each containing 50 ml of MS medium, immediately afterward, mint and thyme shoots, were excised behind the second pair of leaves from the top and individually inserted inside the fungal disk. For this experiment, 20 inoculated shoots with *P. indica*, 20 inoculated shoots with *S. vermifera* and 20 non-inoculated shoots (20 replication) were used in a completely randomized design. Micro propagated plants were analysed for mint and thyme after 45 and 60 days, respectively.

Statistical analysis

The collected data were statistically computed using software Statistical Package for the Social Sciences (SPSS). Data were subjected to analyses of variance and treatment means were compared by an approximate Duncan's multiple test (DMR).

RESULTS

In the first experiment, plant height was analysed after 30 days (Table 1). The most effective hormone was seen at 1 mgl^{-1} of IBA for *M. piperita*. Increasing concentration of IBA reduced mint growth. Plant height in glass bottle containing 4 mgl^{-1} IBA, 2 mgl^{-1} IAA and 4 mgl^{-1} IAA were compared with control and showed statistically similar performance (Table 1). The most effective hormone in *T. vulgaris* was seen at 1 mgl^{-1} of IAA. Increasing

Table 1. Effect of different densities of auxin on plant height (cm) in *T. vulgaris* and *M. piperita* after 30 days.

Treatment	*T. vulgaris*	*M. piperita*
1 mgl^{-1} IBA	4.0 ± 0.26^{a}	7.4 ± 0.70^{a}
2 mgl^{-1} IBA	3.2 ± 0.56^{bc}	6.3 ± 1.37^{abc}
4 mgl^{-1} IBA	3.1 ± 0.50^{c}	5.6 ± 0.71^{cd}
1 mgl^{-1} NAA	2.9 ± 0.70^{cd}	4.2 ± 1.35^{de}
2 mgl^{-1} NAA	2.1 ± 0.70^{e}	3.1 ± 0.53^{ef}
4 mgl^{-1} NAA	1.9 ± 0.36^{e}	3.2 ± 0.40^{ef}
1 mgl^{-1} IAA	4.2 ± 0.17^{a}	7.2 ± 0.35^{ab}
2 mgl^{-1} IAA	3.9 ± 0.20^{ab}	5.2 ± 0.82^{cd}
4 mgl^{-1} IAA	3.1 ± 0.36^{c}	5.4 ± 0.87^{cd}
2 mgl^{-1} IAA + 2 mgl^{-1} IBA	2.3 ± 0.53^{de}	5.9 ± 0.78^{bc}
4 mgl^{-1} IBA + 4 mgl^{-1} NAA	1.6 ± 0.36^{e}	3.2 ± 0.60^{ef}
4 mgl^{-1} IAA + 4 mgl^{-1} NAA	2.1 ± 0.30^{e}	2.2 ± 0.32^{f}
2 mgl^{-1} IAA + 2 mgl^{-1} IBA +2 mgl^{-1}NAA	2.0 ± 0.17^{e}	2.1 ± 0.36^{f}
Control	2.2 ± 0.26^{de}	5.4 ± 0.57^{cd}

Values followed by a different letter within rows are significantly different using Duncan's multiple range test ($P < 0.05$). Values are reported in mean ± standard deviation.

Figure 1. Effects of endophytic fungi on plant growth in micropropagated peppermint. P: *Piriformospora indica*; S: *Sebacina vermifera*; C: control.

concentration of IAA reduced thyme growth. Plants height in glass bottle containing 2 mgl^{-1} IAA + 2 mgl^{-1} IBA were compared with control statistically which showed similar performance (Table 1).

In the second experiment, mint growth was significantly affected by *P. indica* and *S. vermifera*. To assess the influence of *P. indica* and *S .vermifera* on plant morphology, plant height and root length, numbers of nodes, green and root weights were analysed. The increase resulted from the higher mean number of stem nodes and leaf pairs per plants, as well as increase in total green fresh and dry weight (Figure 1). Forty-five days after co-culture, treatments differed for plant height and root length. The tallest plants and roots were recorded from plants inoculated with *S. vermifera* (18.15 and 9.65 cm, respectively) and the shortest from the control (9.60 and 6.05 cm, respectively). Plant height and root lengths of plants inoculated with *S. vermifera* were 89 and 60%,

Table 2. Effect of two endophytic fungi on plant height, root length, green fresh weight, root fresh weight and number of nodes in *M. piperita* after 45 days.

Parameter	*P. indica*	*S. vermifera*	Control	Mean differences respectively
Plant height (cm)	12.45 ± 2.14[b]	18.15 ± 3.73[a]	9.60 ± 2.30[c]	+2.85- 8.55
Root length (cm)	9.10 ± 1.68[a]	9.65 ± 2.01[a]	6.05 ± 1.47[b]	+3.05- 3.60
Green fresh weight (mg)	581.90 ± 88.89[a]	546.80 ± 89.09[a]	163.20 ± 20.30[b]	+418.70- 383.60
Root fresh weight (mg)	304.25 ± 61.77[a]	294.25 ± 59.63[a]	23.00 ± 5.59[b]	+281.25-271.25
Root : Green	0.53 ± 0.14[a]	0.55 ± 0.11[a]	0.14 ± 0.04[b]	+0.39- 0.41
Number of nodes	20.30 ± 3.10[a]	19.70 ± 3.85[a]	12.30 ± 2.25[b]	+ 8.00- 7.40

Values followed by a different letter within rows are significantly different using Duncan's multiple range test ($P < 0.01$). Values are reported in mean ± standard deviation.

Figure 2. Effects of endophytic fungi on plant growth in micropropagated thyme. P: *Piriformospora indica*; S: *Sebacina vermifera*; C: control.

higher than that of non-inoculated control plants, respectively (Table 2). The highest green fresh weight biomasses were produced by *P. indica*, followed by *S. vermifera* and the least by the controls. *P. indica* and *S. vermifera* increased green fresh weight (257 and 352%) in comparison with control plants, respectively (Table 2). In micro propagated plants inoculation with *P. indica* and *S. vermifera*, the intense lateral branching of roots resulted in a well-developed herringbone root system. The mean fresh root ranged from 23.00 to 304.25 mg. The highest root fresh produced by the plants inoculated with *P. indica* and the shortest plants were from the control plants (Table 2). In addition, inoculation with *P. indica* and *S. vermifera* significantly increased the number of nodes. The average number of node with *P. indica* and *S. vermifera* (20.3 and 19.7, respectively) were 65 and 60% higher in comparison with control plants (Table 2).

Thyme growth was significantly affected by *P. indica* and *S. vermifera* (Figure 2). To assess the influence of *P. indica* and *S. vermifera* on plant morphology, several parameters including plant height and root length, number of shoots, fresh weight of plant and root were analysed 60 days after co-culture, the mean plant height ranged from 6 to 7.15 cm. The tallest plants were produced with the plants inoculated with *S. vermifera* and the shortest plants were the control plants (Table 3). Similarly, the root length ranged from 1.8 to 3.10 cm, where the longest roots were those of the plants inoculated with *S. vermifera* and the shortest roots were those of the control plants (Table 3). The highest green and root fresh

Table 3. Effect of two endophytic fungi on plant height, root length, green fresh weight, root fresh weight and number of shoots in *T. vulgaris* after 60 days.

Parameter	*P. indica*	*S. vermifera*	Control	Mean differences respectively
Plant height (cm)	6.10 ± 1.33^{ab}	7.15 ± 1.23^{a}	6.00 ± 1.21^{b}	+0.10 - 1.15
Root length (cm)	2.90 ± 0.85^{a}	3.10 ± 0.79^{a}	1.80 ± 0.52^{b}	+1.10 - 1.30
Green fresh weight (mg)	420.65 ± 98.46^{a}	411.85 ± 92.38^{a}	130.95 ± 17.36^{b}	+289.70 - 280.90
Root fresh weight (mg)	179.00 ± 20.35^{a}	157.35 ± 17.13^{b}	51.55 ± 19.09^{c}	+127.45- 105.80
Root : Green	0.45 ± 0.12^{a}	0.40 ± 0.1^{a}	0.40 ± 0.16^{a}	+0.05 - 0.00
Number of shoots	4. 15 ± 0.88^{a}	4.00 ± 0.79^{a}	2.05 ± 0.76^{b}	+2.10 - 1.95

Values followed by a different letter within rows are significantly different using Duncan's multiple range test ($P < 0.01$). Values are reported in mean ± standard deviation.

weight were produced with the plants inoculated with *P. indica* and the shortest were those of the control plants. *P. indica* and *S. vermifera* increased green fresh weight (221 and 215%), and increased root fresh weight (247 and 205%) in comparison with control plants, respectively (Table 3). In addition, the average number of shoots with *P. indica* and *S. vermifera* (4.15 and 4, respectively) was 102 and 95% higher in comparison with control plants (Table 3).

DISCUSSION

Micro propagation of many plant species, including medicinal plants, has been done in the last few decades Sarwar et al., 2009; Pandeya et al., 2010). Micro propagation of medicinal plants has been achieved through rapid proliferation of shoot-tips and axillary buds in culture Hashemabadi and Kaviani, 2008; Meena et al., 2010; Al-Sulaiman and Barakat, 2010). The role of plant hormones has been proven in tissue culture. Auxins and cytokinins influence micro propagation of medicinal and aromatic plants. Cytokinins were shown to be the most critical for micro propagation of many medicinal plants (Chen et al., 1995; Saxena et al., 1998). In the first experiment, the best hormones on peppermint and thyme growth were observed as 1 mgl^{-1} IBA and IAA, respectively.

Various fungi colonizing the root surface have positive effects on host performance by the production of growth-promoting substances (Hause et al., 2002; Clay, 1984). Mucciarelli et al. (2003) reported that plant height, number of nodes, biomass, leaf area and leaf perimeter in *M. piperita* were enhanced in the presence of endophyte. Many researchers have proved that mycorrhizal fungi colonize the roots of a wide variety of plant species which promotes their growth. Cabello et al. (2005) showed that mycorrhizal fungus *Glomus mossae* increased growth peppermint (*M. piperita*). Freitas et al. (2004) showed that mycorrhizal fungi increased plant growth and development of *M. arvensis*. Silveira et al. (2006) reported that development of vegetative parts, fresh and dry weight in inoculated plants with *Glomus clarum*, *Glumus etunicatom* and *Acaulospora scrobiculata* were increased in *M. piperita*.

In the second experiment, *P. indica* and *S. vermifera* increased thyme and mint growth. Sirrenberg et al. (2007) reported that auxin production affecting root growth is responsible for, or at least contributes to, the beneficial effect of *P. indica* on its host plants. *P. indica* was shown to produce IAA in liquid culture (Sirrenberg et al., 2007). Also *P. indica* produces low amounts of auxins and produce relatively high levels of cytokinins, and the cytokinin levels are higher in colonized roots when compared with the uncolonized controls (Vadassery et al., 2008). In this study, we showed that plants treated with *P. indica* and *S. vermifera* were superior in development to control plants. Rai et al. (2001) reported that root length and stem weight, leaf size and seed production of medicinal plants *Spilanthes calva* and *Withania somnifera* were enhanced in the presence of *P. indica*. This study confirmed the finding of Prasad et al. (2008) who reported that *P. indica* increased growth of *Bacopa monniera* in *in vitro* conditions.

ACKNOWLEDGMENT

This study was supported by the Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modarres University, Tehran, Iran.

REFERENCES

Abbott IK, Robson AD (1982). The role of Vesicular-Arbuscular Mycorrhizal fungi in agriculture and the selection of fungi for inoculation. Aust. J. Res. 33: 389-408.

Al-Sulaiman MA, Barakat MN (2010). *In vitro* shoot multiplication of Ziziphus spina-christi by shoot tip culture. Afr. J. Biotechnol. 8: 3782-3788.

Bolton H, Fredrickson JK, Elliot LF (1993). Microbial ecology of the rhizosphere. Microbial production of plant growth regulators. In Soil microbial ecology. Applications in agricultural and environmental management. F.B. Metting, Jr. (edit) Marcel Dekker, Inc., New York, pp. 27-63.

Brundrett MC (2002). Coevolution of roots and mycorrhizas of land plants. New Phytol. 154: 275-304.

Cabello M, Irrazabal G, Bucsinszky AM, Saparrat M, Schalamuk S (2005). Effect of an arbuscular mycorrhizal fungus, *Glomus mossae*, and a rock-phosphate-solubilizing fungus, *Penicillium thomii*, on *Mentha piperita* growth in a soillessn medium. J. Basic Microbiol. 45: 182-189.

Chao SC, Young DG, Oberg CJ (2000). Screening for inhibitory activity of essential oils oil selected bacteria, fungi and viruses. J. Essent. Oil Res. 12: 639-649.

Chen LJ, Hu TW, Huang LC (1995). A protocol toward multiplication of the medicinal tree, Eucommia ulmoides Oliver. *In vitro* Cell Dev. Biol. Plant, 31: 193-198.

Clay K (1984). The effect of the fungus *Atkinsonella hypoxylon* (Clavicipitaceae) on the reproductive system and demography of the grass *Danthonia spicata*. New Phytol. 98: 165-175.

Cooper KM (1984). Physiology of VA mycorrhizal associations. In: *VA* Mycorrhiza (Ed.): C.L. Powell and D.J. Bagyaraj, CRC Press, Inc., Boca Raton, Florida. pp. 155-186.

Dela Cruz RE (1991). Status of Bio-reforestation in the Philippines. Paper presented during the pre-Workshop on Bio-Reforestation sponsored by IUFRO Japan, Bogor, Indonesia March 25-29.

Duarte MC, Figueira GM, Sartoratto A, Rehder VL, Delarmelina C (2005). Anti-*Candida* activity of Brazilian medicinal plants. J. Ethnopharmacol. 97: 305-311.

Ellis JR, Larsen HJ, Boosalis MG (1985). Drought resistance of wheat plants inoculated with vesicular mycorrhizae. Plant and Soil, 86: 369-378.

Fonseka-Kruel VS, Fernandes PV (2003). Coleção de Plantas Medicinais, Wrst ed. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro.

Foster S (1990). Peppermint, *Mentha piperita*. In *Botanical Series*; American Botanical Council: Austin, TX, p. 306.

Freitas MSM, Martins MA, Vieira IJC (2004). Produçãone qualidade de óleos essenciais de *Mentha arvensis* em resposta à inoculação de fungos micorrízicos arbusculares. Pesquisa Agropecuária Brasileira, Brasilia, 39: 887-894.

Guedón DJ, Pasquier BP (1994). Analysis and distribution of flavonoid glycosides and rosmarinic acid in 40 *Mentha piperita* clones. J. Agric. Food Chem. 42: 679-684.

Gupta ML, Prasad A, Ram M, Kumar S (2002). Effect of the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition in the crop of different cultivars of menthol mint (*Mentha arvensis*) under field conditions. Biol. Technol. 81: 77-79.

Harley JL, Smith SE (1983). Mycorrhizal symbiosis. Academic Press, New York, p. 483.

Hashemabadi D, Kaviani B (2008). Rapid micro-propagation of Aloe vera L. via multiplication. Afr. J. Biotechnol. 7: 1899-1902.

Hause B, Maier W, Miersch O, Kramell R, Strack D (2002). Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots. Plant Physiol. 130: 1213-1220.

Horne D, Holm M, Oberg C, Chao S, Young PG (2001). Antimicrobial effects of essential oils on *Streptococcus pneumonia*. J. Essent. Oil Res. 13: 387-392.

Huang RS, Smith WK, Yost RE (1985). Influence of vesicular arbuscular mycorrhizae on growth, water relation and leaf orientation in *Lcucaena lcucocephala* (Lam.) de wit. New Phytol. 99: 229-243.

Inoue T, Sugimoto Y, Masuda H, Kamei C (2002). Antiallergic effect of flavonoid glycosides obtained from *Mentha piperita* L. Biol. and Pharm. Bull. 25: 256-259.

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol. Fert. Soils, 37: 1-16.

Kumari R, Kishan H, Bhoon YK, Varma A (2003). Colonization of cruciferous plants by *Piriformospora indica*. Curr. Sci. 85: 1672-1674.

Leung AY, Foster S (1996). Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics, John Wiley and Sons, New York, p. 492.

Marschner H, Dell B (1994). Nutrient uptake in mycorrhizal symbiosis. Plant and Soil, 159: 89-102.

Meena MC, Meena R, Patni V (2010). High frequency plant regeneration from shoot tip explants of Citrullus colocynthis (Linn.) Schrad. – An important medicinal herb. Afr. J. Biotechnol 9: 5037-5041.

Mucciarelli M, Scannerini S, Berrtea C, Maffei M (2003). *In vitro* and *in vivo* peppermint (*Mentha piperita*) growth promotion by nonmycorrhizal fungal colonization. New Phytol. 158: 579-591.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Pandeya K, Tiwari KN, Singh J, Verma JP, Dubey SD (2010). *In vitro* propagation of *Clitoria ternatea* L.: A rare medicinal plant. J. Med. Plant. Res. 4: 664-668.

Peskan-Berghoefer T, Shahollaria B, Giong PH, Hehl S, Markerta C, Blanke V, Kost G, Varma A, Oelmeuller R (2004). Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmatic reticulum and at the plasma membrane. Physiol. Plant, 122: 465-477.

Pham GH, Singh An, Malla R, Kumari R, Saxena AK, Rexer K-H, Kost G, Luis P, Kaldorf M, Buscot F, Herrmann S, Peskan T, Oelmuller R, Mittag M, Declerck S, Hehl S, Varma A (2003). Interaction of *Piriformospora indica* with diverse microorganisms and plants. *In* Varma, A., Abbott, L., Werner, D. and Hampp, R. (eds.), Plant Surface Microbiology. Springer- Verlag, Germany (in press).

Prasad R, Bagde US, Puspangadan P, Varma A (2008). *Bacopa monniera* L.: Pharmacological Aspects and Study Involving *Piriformospora indica*. Int. J. Integ. Biol. 3: 100-110.

Rai M, Acharya D, Singh A, Varma A (2001). Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. Mycorrhiza, 11: 123-128.

Ruiz Del Castillo ML, Blanch GP, Herraiz M (2004). Natural variability of the enantiomeric composition of bioactive chiral terpenes in *Mentha piperita*. J. Chromatogr. 29: 87-93.

Sahay NS, Varma A (1999). *Piriformospora indica*: a new biological hardening tool for micropropagated plants. FEMS Microbiol. Lett. 181: 297-302.

Samarth RM, Kumar A (2003). Radioprotection of Swiss albino mice by plant extract *Mentha piperita* (Linn.). J. Radiat. Res. 44: 101-109.

Sarwar S, Zia M, Rehman RU, Fatima Z, Sial RA, Chaudhary MF (2009). *In vitro* direct regeneration in mint from different explants on half strength MS medium. Afr. J. Biotechnol. 8: 4667-4671.

Saxena C, Rout GR, Das P (1998). Micropropagation of Psoralea corylifolia Linn. J. Med. Aromatic Plant Sci. 20: 15-18.

Silveira SV, Lorscheiter R, Barros IBI, Schwarz SF, Souza PVD (2006). *Mentha piperita* as a multiplying of arbuscular mycorrhizal fungi. Botucatu, 8: 91-97.

Sirrenberg A, Gobel C, Grond S, Czempinski N, Ratzinger A, Karlovsky P, Santos P, Feussner I, Pawlowski K (2007). *Piriformospora indica* affects plant growth by auxin production. Physiol. Planta. 131: 581-589.

Vadassery J, Ritter C, Venus Y, Camehl I, Varma A, Shahollari B, Novák O, Strnad M, Ludwig-Müller J, Oelmüller R (2008). The Role of Auxins and Cytokinins in the Mutualistic Interaction Between *Arabidopsis* and *Piriformospora indica*. Mol. Plant-Microbe Interact. 21: 1371-1383.

Varma A, Singh A, Sudha N, Sahay S, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharti K, Franken P, Hurek T, Blechert O, Rexer K-H, Kost G, Hahn A, Hock B, Maier W, Walter M, Strack D, Kranner I (2001). *Piriformospora indica*: A cultivable mycorrhiza-like endosymbiotic fungus. In: Mycota IX, Springer Series, Germany, pp. 123-150.

Varma A, Verma S, Sudha N, Sahay S, Bütehorn B, Franken P (1999). *Piriformospora indica*, a cultivable plant growth-promoting root endophyte. Appl. Environ. Microbiol. 65: 2741-2744.

Verma S, Varma A, Rexer KH, Kost G, Sarbhoy A, Bisen P, Butehorn B, Franken P (1998). *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. Mycologia, 95: 896-903.

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Heuckelhoven R, Neumann C, von Wettstein D, Franken P, Kogel KH (2005). The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc. Natl. Acad. Sci. USA, 38: 13386-13391.

Warcup JH (1988). Mycorrhizal associations of isolates of *Sebacina vermifera*. New Phytol. 110: 227-231.