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CLONAL PROPAGATION AND EVALUATION OF PEROXIDASE ACTIVITY DURING *IN VITRO* RHIZOGENESIS IN *MENTHA ARVENSIS* L.

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Abstract: A rapid and efficient plant propagation system through nodal explants was developed for *in vitro* propagation of *Mentha arvensis*. Nodal explants exhibited high frequency shoot proliferation on Murashige and Skoog's (MS) basal medium supplemented with 0.5 mg l^{-1} BAP (6-benzylaminopurine). Microshoots were best rooted on ½ strength MS medium with 0.5 mg l^{-1} IBA (indole-3-butyric acid) and 2% (w/v) sucrose. Plantlets with high (≥ 0.8) Fv/Fm (φ_{P0} – maximum quantum yield of primary photochemistry) were successfully shifted to natural conditions. The overall survival rate from *in vitro* growth to field transfer was 74%. The developed micropropagation protocol can be successfully used for large-scale multiplication and genetic modification of this high value medicinal plant species. In addition, the results of this study also indicate the key role of Gpx (guaiacol-peroxidase) as a marker of *in vitro* rhizogenesis in *M. arvensis*.

Keywords: Fv/Fm, guaiacol-peroxidase, *in vitro* rhizogenesis, *Mentha arvensis*, Micropropagation.

Introduction

Mentha L. is a genus of aromatic perennial herbs belonging to the family Lamiaceae, distributed widely in temperate and sub-temperate areas of the world. Several *Mentha* species are considered industrial herbs as they are a source of essential oils enriched in certain monoterpenes, commonly used in food, flavor, cosmetic and pharmaceutical industries. *Mentha arvensis* L., known as common mint, is often used as a folk medicine to treat indigestion, nausea, sore throat, and toothache since prehistoric time. The fresh leaves contain essential oils consisting of monoterpenes like menthol, menthone, carvone and pulegone as major constituents, which are widely used by industries in food, pharmaceutical, flavor and/or fragrance formulations. The plant also contains anti-viral [ALI & al. 1996], anti-inflammatory [VERMA & al. 2003], anti-mycotic [PANDEY & al. 2003], anti-fungal [DUARTE & al. 2005], anti-oxidative [ZAKARIA & al. 2008] and anti-bacterial [WANNISSORN & al. 2008; COUTINHO & al. 2008; NASCIMENTO & al. 2010; JOHNSON & al. 2011] activities.

Owing to the diverse biological activities and increasing consumer interest, few efforts were carried out to propagate this valuable plant species through tissue culture techniques [CHISHTI & al. 2006; ISLAM & BARI, 2012]. In spite of earlier studies, more efficient and rapid *in vitro* propagation techniques are required for the genetic transformation and conservation of this medicinal plant. In the present investigation, we report a new plant

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regeneration method for *M. arvensis* through nodal explants. This efficient, rapid and simple regeneration system facilitates the application of plant tissue culture and genetic engineering approach in *M. arvensis*. In addition, efforts were also carried out to understand the involvement of guaiacol-peroxidase (Gpx) in rhizogenesis *in vitro* in this plant species.

Materials and methods

Culture media and growth conditions: The young shoots of *M. arvensis* were collected from plants grown at the botanical garden of Jaipur National University, Jaipur, India. The stem segments dissected and washed thoroughly under running tap water for 30 min to eliminate dust particles and then with 5% teepol for 8-10 min and rinsed several times in sterile distilled water. Thereafter, the explants were surface sterilized under a laminar flow chamber with aqueous solution of 0.1% (w/v) solution of $HgCl_2$ for 3 min and finally washed with sterile distilled water for 2–3 times.

After trimming both the cut ends, the sterilized stem explants were inoculated vertically on MS Medium [MURASHIGE & SKOOG, 1962] supplemented with 3% (w/v) sucrose and various concentrations (0.5 – 5.0 mg l^{-1}) of BAP and KIN (kinetin), singly or in combinations for multiple shoot induction. MS medium lacking plant growth regulators served as control. These cultures were transferred to a culture room and maintained at 25 ± 2 °C and 65 – 70% relative humidity with photoperiod of 16 h using a photosynthetic photon flux density (PPFD) of 40 µmol/m^2 s^1 provided by cool white fluorescent tubes (Philips, India). To induce rhizogenesis, elongated shoots with 2–3 pairs of healthy leaves were cultured on MS supplemented with various auxins.

Hardening and acclimatization: Well rooted plantlets were gently washed in sterile water and transferred to plastic cups (10 cm × 8 cm) containing sterilized mixture of sterile soil, sand and coco peat (1:2:1). The plantlets covered in transparent polyethylene bags were kept for 3 weeks in growth chamber at 25 ± 2 °C with 16 h photoperiod and 40 µmol/m^2 s^1 of irradiation. The plantlets were irrigated with tap water. The hardened plantlets were subsequently transferred to earthen pots containing normal garden soil and were maintained in greenhouse conditions.

Measurement of Fv/Fm (φ_{P0}): Photosynthetic screening during hardening and acclimatization process can help to improve the performance and survival of micropropagated plants. Thus, the maximum quantum yield of primary photochemistry (φ_{P0}= Fv/Fm) of plantlets growing under growth chamber and green house conditions was regularly measured using a Plant Efficiency Analyser, PEA (Hansatech Instruments, Kings Lynn, Norfolk, U.K.) according to HEBER & al. (2011). The leaf samples were dark adapted for 2 h before the fluorescence measurements. The plants with high φ_{P0} ($\geq$ 0.8) were subsequently transferred in natural conditions. The survival rate of plantlets was recorded after 1 month of transfer to natural conditions.

Guaiacol peroxidase (GPx) activity (1.11.1.7): To determine the Gpx activity during the rhizogenesis *in vitro*, the nodal explants were cultured on root-induction medium [½ strength MS medium + 2% (w/v) sucrose + 0.5 mg l^{-1} IBA] and withdrawn daily, until the emergence of root primordia, to homogenize in pre-chilled mortar and pestle using phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000 rpm for 20 min and used to essay GPx activity. GPx activity was measured spectrophotometrically at 25 °C by following the method of RACUSEN & FOOTE (1965). The reaction mixture consisted of 1.5 ml of 50 mmol l^{-1} potassium phosphate buffer (pH 7.0), 0.2 ml of enzyme extract, and 1

ml of 1% guaiacol, and the absorbance was set zero at 470 nm. The reaction was started by the addition of 0.3% hydrogen peroxide (0.2 ml) and changes in absorbance were recorded for every 15 s up to 5 min. Enzyme activity was calculated in terms of units g^{-1} fresh wt. One unit of enzyme was defined as the amount of enzyme necessary to decompose 1 mmol of substrate per minute at 25 °C.

Experimental design and statistical analysis: MS medium without hormone was treated as control in all experiments. All experiments were conducted with a minimum of 30 replicates per treatment and each experiment was repeated thrice. The data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Tukey's test at P = 5 % using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA) and data represented as mean ± standard error (SE).

Results and discussion

***In vitro* establishment and multiplication**: Among the two different cytokinins tested, BAP proved better than KIN for accelerating the multiple shoot induction in nodal explants of *M. arvensis*. KIN at low concentration (0.5 mg l^{-1}) could provoke the development of single shoot per node after 12 days of culture (Fig. 1 A). Regenerated shoots elongated further on same medium (Fig. 1 B). Higher concentrations of KIN could not alter the frequency of shoot bud proliferation *in vitro*. All concentrations of BAP (0.5-5.0 mg l^{-1}) induced multiple shoot induction in nodal explants of *M. arvensis* (Tab. 1). The best response in terms of explants response (%), mean number of shoots and mean shoot length was achieved on MS medium supplemented with 0.5 mg l^{-1} BAP (Fig. 1 C). Frequency of shoot induction was drastically decreased with increasing concentration of BAP. Low frequency of shoot bud induction and callus formation at basal part of nodal explants was noted on medium enriched with high concentration of BAP (Fig. 1 D). Similar, inhibitory effect of higher concentrations of BAP on *in vitro* shoot proliferation has also been reported earlier in *Pterocarpus marsupium* [ANIS & al. 2005], *Arachis hypogaea* [BANERJEE & al. 2007], *Doritis pulcherrima* [MONDAL & al. 2013] and *Salvia splendens* [SHARMA & al. 2014]. Combinations of BAP and KIN could not initiate any significant morphogenetic responses in nodal explants of *M. arvensis*.

Tab. 1. Influence of BAP on direct shoot organogenesis in *M. arvensis* L.
Values represent the mean of 30 replicates ± SE, n= 3.

BAP concentration (mg l^{-1})	Explant response (%)	Mean number of shoots per node	Mean shoot length (cm)
0.5	92.05 ± 4.1	8.20 ± 1.04	3.45 ± 0.33
1.0	81.84 ± 2.9	6.44 ± 0.81	3.31 ± 0.53
2.0	41.88 ± 4.3	2.83 ± 0.93	3.26 ± 0.44
3.0	33.68 ± 4.2	2.38 ± 0.49	0.92 ± 0.12
4.0	20.51 ± 2.7	2.24 ± 0.05	0.66 ± 0.08
5.0	11.30 ± 2.1	2.24 ± 0.09	0.66 ± 0.76

***In vitro* rhizogenesis:** The rooting characters like percentage of cultured shoots producing roots, mean number of root/shoot and mean root length (cm) have been significantly affected by the MS medium strength and IBA concentration. Overall, MS

medium of ½ strength proved better for induction of *in vitro* rhizogenesis as compared to full strength MS medium whether with or without IBA of any concentration (Tab. 2). ½ strength MS medium supplemented high concentrations of IBA (3.0-5.0 mg l^{-1}) could lead the development of aerial roots (Fig. 1 E). High frequency (96 ± 6.2) induction of rooting was obtained on ½ strength MS medium with 0.5 mg l^{-1} IBA and 2% (w/v) sucrose. Similarly, IBA-induced rhizogenesis *in vitro* has been reported in many plant species, *i.e. Tectona grandis* [MENDOZA DE GYVES & al. 2007], *Phaseolus vulgaris* [KWAPATA & al. 2010], *Gentiana kurroo* [SHARMA & al. 2014], *Cattleya* [DEWIR & al. 2015]; *Hemidesmus indicus* [SHEKHAWAT & MANOKARI, 2016].

Tab. 2. Influence of IBA and MS strength on *in vitro* rhizogenesis in *M. arvensis* L. after 4 weeks of culture. Values represent the mean of 30 replicates ± standard error (SE).

Media Combinations [(MS strength + IBA (mgl^{-1})]	**% Rooting**	**Mean no. of root/shoot**	**Mean root length (cm)**
Full + 0.0	14.0 ± 2.1	1.43 ± 0.7	0.77 ± 0.2
Full + 0.5	28 ± 3.3	1.84 ± 0.3	0.97 ± 0.2
Full + 1.0	26 ± 4.1	1.89 ± 0.5	2.26 ± 0.7
Full + 2.0	21 ± 2.9	1.96 ± 0.4	2.29 ± 0.4
Half + 0.0	36 ± 3.7	3.58 ± 0.3	2.61 ± 0.8
Half + 0.5	96 ± 6.2	8.50 ± 1.1	6.28 ± 0.3[e]
Half + 1.0	77 ± 4.9	6.50 ± 1.4	3.83 ± 0.5
Half + 2.0	48 ± 3.2	2.66 ± 0.5	2.91 ± 0.4

Hardening and acclimatization: The increased use and efficiency of plant tissue culture techniques is still limited by the high percentage of plants which are lost or damaged when transferred from *in vitro* to *in vivo* conditions [POSPÍŠILOVÁ & al. 1999]. It is due to a poor photosynthetic capacity of *in vitro*-cultured plantlets, caused by insufficient inflow of carbon dioxide and the sucrose added to growth medium causing negative feedback for photosynthesis. Therefore, screening of photosynthetic potential of *in vitro* raised plants is essential to optimize hardening and acclimatization duration to ensure their high survival rate in field conditions. Plantlets regenerated *in vitro* were successfully acclimatized in the growth chamber (84% survival) and then in the greenhouse (88% survival). The φ_{P0} value of *in vitro* developed plantlets was found 0.42, which was slightly increased during hardening process in culture chamber (Fig. 2 A). Micropropagated plants achieved φ_{P0} value 0.73 in first week of their transfer at green house conditions. All the plants gained φ_{P0} value $\geq$ 0.8 within three weeks of acclimatization process. Fully hardened plants having high PSII photochemical efficiency ($\varphi_{P0} \geq 0.8$) were shifted from green house to natural conditions with 100% survival rate (Fig. 2 B). The regeneration method standardized in the present investigation relies on the fact that it is efficient, quick and highly reproducible method for micropropagation and genetic transformation studies of *M. arvensis*.

GPx activity during *in vitro* rhizogenesis: The biochemical changes drive cellular differentiation and organization as tissues and finally growth as organs. As per GASPAR & al. (1992) rhizogenesis includes three biochemical phases: *1*) an induction phase, characterized by a sharp reduction in peroxidase activity, *2*) a phase of root initiation with corresponding increase in peroxidase activity, and *3*) an expression phase, characterized by a gradual decline in peroxidase activity, which is followed by the emergence of root

primordia. In this study, we determined the possible involvement of Gpx in rhizogenesis *in vitro* in *M. arvensis*. In the present study, nodal explants of *M. arvensis* showed a slight decline in the Gpx activity on the 2th day, a gradual increase till the emergence of root primordia (8th day) and a drastic reduction on the 10th day on the root-induction medium (½ strength MS medium with 0.5 mg l^{-1} IBA and 2% (w/v) sucrose). An increase followed by a decrease in peroxidase activity preceding root appearance has also been observed in *Psoralea corylifolia* [ROAT & al. 2000], *Plumbago zeylanica* [SAXENA & al. 2000] and *Petunia* × *hybrida* microshoots [KOTIS & al. 2009]. According to the GASPAR & HOFINGER (1988), the increase in Gpx activity during the induction phase is related with a reduction in the content of endogenous auxin level; the reduction of peroxidase activity during the initiation phase is associated to an increase in the endogenous auxin level, leading to the differentiation of root primordia. Increase in peroxidase activity could also be related to the lignification of root primordia cells [MARJAMAA & al. 2009; NOVO-UZAL & al. 2013]. The results of this study suggest a key role of Gpx in *in vitro* rooting of microshoots of *M. arvensis*.

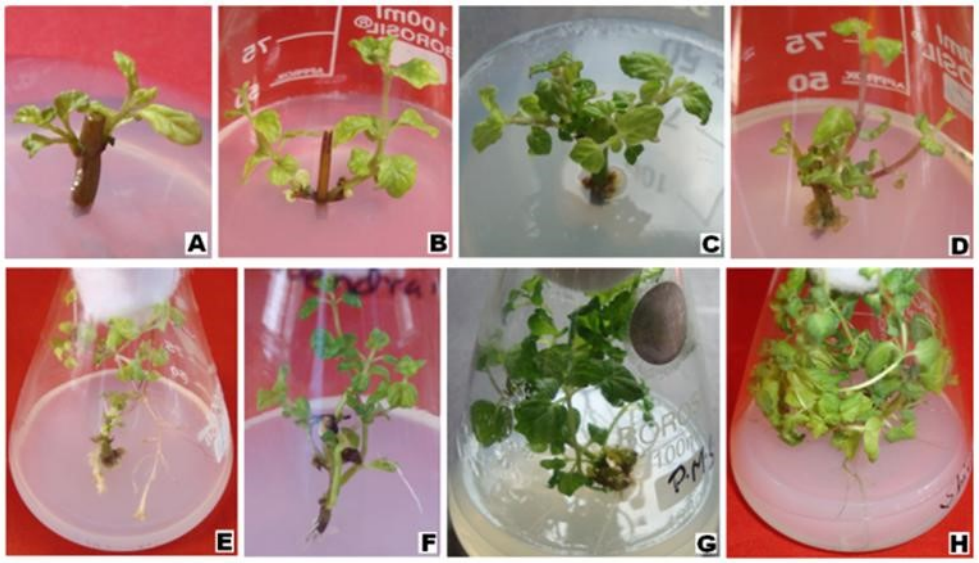

Fig. 1. *In vitro* propagation of *M. arvensis*. **A- B.** Shoot induction from nodal explant on MS medium fortified with 0.5 mg l^{-1} KIN. **C.** Multiple shoot proliferation on MS medium containing 0.5 mg l^{-1} BAP, 4-week-old culture. **D.** Shoot bud induction and callus formation at basal part of nodal explants on MS medium augmented with 5.0 mg l^{-1} BAP. **E.** *In vitro* development of aerial roots on MS medium supplemented with IBA (5.0 mg l^{-1}). **F.-H.** *In vitro* rhizogenesis on ½ strength MS medium with 0.5 mg l^{-1} IBA and 2% (w/v) sucrose.

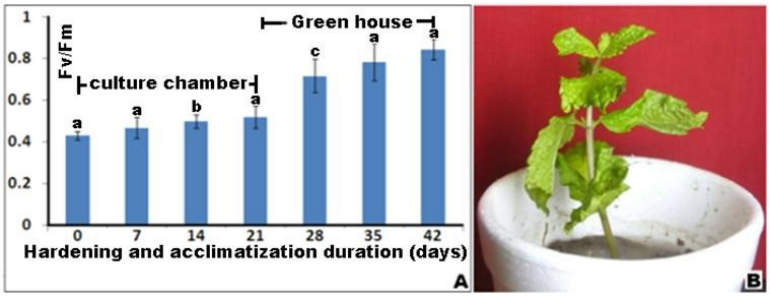

Fig. 2. A. Fv/Fm (φ_{P0}) values of *in vitro* raised plantlets during hardening and acclimatization [Different letters mean that there are significant differences among treatments. Tukey Test HSD ($p > 0.05$) n=30]. **B.** Acclimatized plantlet.

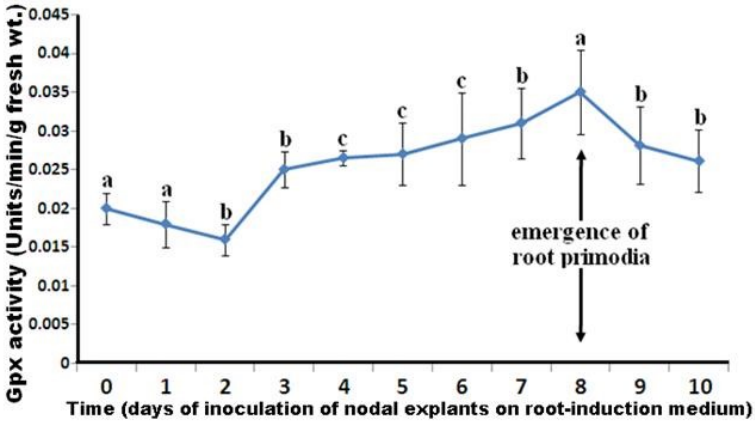

Fig. 3. Gpx activity during *in vitro* rhizogenesis in nodal explants of *M. arvensis.* [Different letters mean that there are significant differences among treatments. Tukey Test HSD ($p > 0.05$) n=30].

Conclusions

The present study describes an efficient and reproducible protocol for *in vitro* propagation of *M. arvensis* using nodal explants. The protocol can be used for clonal propagation and genetic modification of this high value medicinal plant species. In addition, biochemical study also indicates the key role of Gpx as a marker of rhizogenesis in *M. arvensis*.

References

ALI A. M., MACKEEN M. M., EL-SHARKAWY S. H., ABDUL HAMID J., ISMAIL N. H., AHMAD F. B. H. & LAJIS N. H. 1996. Antiviral and cytotoxic activities of some plants used in Malaysian indigenous medicine. *Pertanika J. Trop. Agric. Sci.* **19**(2/3): 129-136.

ANIS M., HUSAIN M. K. & SHAHZAD A. 2005. *In vitro* plantlet regeneration of *Pterocarpus marsupium* Roxb., an endangered leguminous tree. *Curr. Sci.* **88**(6): 861-863.

BANERJEE P., MAITY S., MAITY S. S. & BANERJEE N. 2007. Influence of genotype on *in vitro* multiplication potential of *Arachis hypogaea* L. *Acta Bot. Croat*. **66**(1): 15-23.

CHISHTI N., SHAWL A. S., KALOO Z. A., BHAT M. A. & SULTAN P. 2006. Clonal propagation of *Mentha arvensis* L. through nodal explants. *Pak J Biol Sci.* **9**(8): 1416-1419.

COUTINHO H. D. M., COSTA J. G. M., FALCÃO-SILVA V. S., LIMA E. O. & SIQUEIRA-JÚNIOR J. P. 2008. Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. and chlorpromazine. *Chemotherapy*. **54**(4): 328-330.

DEWIR Y. H., EL-MAHROUK M. E., MURTHY H. N. & PAEK. K. Y. 2015. Micropropagation of *Cattleya*: Improved *in vitro* rooting and acclimatization. *Horticulture, Environment, and Biotechnology*. **56**(1): 89-93.

DUARTE M. C., FIGUEIRA G. M., SARTORATTO A., REHDER V. L. & DELARMELINA C. 2005. Anti-*candida* activity of Brazilian medicinal plants. *J. Ethnopharmacology*. **97**(2): 305-311.

GASPAR T. & HOFINGER M. 1988. Auxin metabolism during adventitious rooting. Pp. 117-131. In T. D. DAVIS & al. (eds). *Adventitious root formation in cuttings.* Portland: Dioscorides Press. ISBN: 0-931146-10-0 (HB).

GASPAR T., KEVERS C., HAUSMAN J. F., BERTHON J. Y. & RIPETTI V. 1992. Practical uses of peroxidase activity as a predictive marker of rooting performance of micropropagated shoots. *Agronomie.* **12**: 757-765.

HEBER U., SONI V. & STRASSER R. J. 2011. Photoprotection of reaction centers: thermal dissipation of absorbed light energy vs charge separation in lichens. *Physiologia Plantarum*. **142**(1): 65-78.

ISLAM M. S. & BARI M. A. 2012. *In vitro* regeneration protocol for artificial seed production in an important medicinal plant *Mentha arvensis* L. *J. Bio-Sci*. **20**: 99-108.

JOHNSON M., WESELY E. G., KAVITHA M. S. & UMA V. 2011. Antibacterial activity of leaves and inter-nodal callus extracts of *Mentha arvensis* L. *Asian Pacific J. Tropical Medicine*. **4**(3): 196-200.

KOTIS M., YUPSANIS T. A., SYROS T. D. & ECONOMOU A. S. 2009. Peroxidase, acid phosphatase, RNase and DNase activity and isoform patterns during *in vitro* rooting of *Petunia* × *hybrida* microshoots. *Biologia Plantarum*. **53**: 530-538.

KWAPATA K., SABZIKAR R., STICKLEN M. B. & KELLY J. D. 2010. *In vitro* regeneration and morphogenesis studies in common bean. *Plant Cell, Tissue and Organ Culture.* **100**(1): 97-105.

MARJAMAA K., KUKKOLA E. M. & FAGERSTEDT K. V. 2009. The role of xylem class III peroxidases in lignifications. *J. Exp. Bot.* **60**(2): 367-376.

MENDOZA DE GYVES E., ROYANI J. I. & RUGINI E. 2007. Efficient method of micropropagation and *in vitro* rooting of teak (*Tectona grandis* L.) focusing on large-scale industrial plantations. *Ann. For. Sci.* **64**(1): 73-78.

MONDAL T., ADITYA S. & BANERJEE N. 2013. *In vitro* axillary shoot regeneration and direct protocorm-like body induction from axenic shoot tips of *Doritis pulcherrima* Lindl. *Plant Tissue Culture and Biotechnology*. **23**(2): 251-261.

MURASHIGE T. & SKOOG. F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*. **15**: 473-497.

NASCIMENTO M. M., RODRIGUES F. G., CAMPOS A. R. & COSTA G. M. 2009. Phytochemical prospection, toxicity and antimicrobial activity of *Mentha arvensis* (Labiatae) from northeast of Brazil. *J. Young Pharm*. **1**(3): 210-212.

NOVO-UZAL E., FERNÁNDEZ-PÉREZ F., HERRERO J., GUTIÉRREZ J., GÓMEZ-ROS L. V., BERNAL M. A., DÍAZ J., CUELLO J., POMAR F. & PEDREÑO M. A. 2013. From *Zinnia* to *Arabidopsis*: Approaching the involvement of peroxidases in lignification. *J. Exp. Bot*. **64**(12): 3499-3518.

PANDEY A. K., RAI M. K. & ACHARYA D. 2003. Chemical composition and antimycotic activity of the essential oils of corn mint (*Mentha arvensis*) and lemon grass (*Cymbopogon flexuosus*) against human pathogenic fungi. *Pharmaceutical Biology.***41**(6): 421-425.

POSPÍŠILOVÁ J., TICHÁ I., KADLEČEK P., HAISEL D. & PLZÁKOVÁ Š. 1999. Acclimatization of micropropagated plants to *ex vitro* conditions. *Biologia Plantarum.* **42**(4): 481-497.

RACUSEN D. & FOOTE M. 1965. Protein synthesis in dark grown bean leaves. *Can. J. Bot.* **43**(7): 817-824.

ROUT G. R., SAMANTARAY S. & DAS P. 2000. *In vitro* rooting of *Psoralea corylifolia* Linn: Peroxidase activity as a marker. *Plant Growth Regulation*. **30**(3): 215-219.

SAXENA C., SAMANTARAY S., ROUT G. R. & DAS P. 2000. Effect of auxins on *in vitro* rooting of *Plumbago zeylanica*: Peroxidase activity as a marker for root induction. *Biologia Plantarum*. **43**(1): 121-124.

SHARMA A., KAUR R. & SHARMA N. 2014. *In vitro* morphogenic response of different explants of *Gentiana kurroo* Royle from Western Himalayas – an endangered medicinal plant. *Physiol. Mol. Biol. Plants*. **20**(2): 249-256.

SHARMA S., SHAHZAD A., KUMAR J. & ANIS M. 2014. *In vitro* propagation and synseed production of scarlet salvia (*Salvia splendens*). *Rend. Fis. Acc. Lincei.* **25**(3): 359-368.

SHEKHAWAT M. S. & MANOKARI M. 2016. *In vitro* regeneration frequency, micro-morphological studies and *ex vitro* rooting of *Hemidesmus indicus* (L.) R. Br.: a multi-potent endangered climber. *Ind. J. Plant Physiol.* **21**(2): 151-160.

VERMA S. M., ARORA H. & DUBEY R. 2003. Anti–inflammatory and sedative-hypnotic activity of the methanolic extract of the leaves of *Mentha arvensis*. *Anc. Sci Life.* **23**(2): 95-99.

WANNISSORN B., JARIKASEM S., SIRIWANGCHAI T. & THUBTHIMTHED S. 2005. Antibacterial properties of essential oils from Thai medicinal plants. *Fitoterapia*. **76**(2): 233-236.

ZAKARIA Z., AZIZ R., LACHIMANAN Y. L., SREENIVASAN S. & RATHINAM X. 2008. Antioxidant activity of *Coleus blumei*, *Orthosiphon stamineus*, *Ocimum basilicum* and *Mentha arvensis* from Lamiaceae family. *Int. J. Nat. Eng. Sci.* **2**(1): 93-95.

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