IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
e ISSN-0976-593X Volume **12** | Issue 2 | July, 2017 | 149-155

DOI: 10.15740/HAS/IJPS/12.2/149-155
Visit us - *www.researchjournal.co.in*

RESEARCH ARTICLE

Evaluation study of micropropagation stages of patchouli plant

■ **SAILAJA INAMPUDI, LAXMAN BHOSALE, ANURADHA ROHINIKAR, IVVALA ANAND SHAKER, C. GONJARI KOMAL AND AJIT GANGAVANE**

SUMMARY

An effective means for rapid multiplication of plant species of clonal origin is micro propagation. Various *in vitro* studies have been reported on different species of patchouli, one such method of propagation that can be usefully employed to produce relatively uniform plantlets in a short time is via *in vitro* culture but there is limited effort to study direct organogenesis, which supports cultivation by providing true type plants in large numbers.Therefore this study determines the effect of different concentrations of growth hormones on patchouli, micropropagation and rapid multiplication stages of patchouli plant within a short time with good results of micropropagation stages for regeneration of patchouli were successfully initiated.

Key Words : Patchouli, Alpha naphthalene acetic acid (NAA), Indol acetic acid (IAA), Benzyl amino purine (BAP), Kinetin (kin)

How to cite this article : Inampudi, Sailaja, Bhosale, Laxman, Rohinikar, Anuradha, Shaker, Ivvala Anand, Komal , C. Gonjari and Gangavane, Ajit (2017). Evaluation study of micropropagation stages of patchouli plant. *Internat. J. Plant Sci.*, **12** (2): 149-155, **DOI: 10.15740/HAS/IJPS/12.2/149-155**.

Article chronicle : Received : 04.03.2017; **Revised :** 01.05.2017; **Accepted :** 20.05.2017

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
SAILAJA INAMPUDI, Department of Biotechnology, Parul Institute of Applied Sciences, Parul University, Limda, Waghodia, VADODARA (GUJARAT) INDIA
Email : inampudisailaja@rediffmail.com

Address of the Co-authors:
LAXMAN BHOSALE AND ANURADHA ROHINIKAR, Department of Biotechnology, Parul Institute of Applied Sciences, Parul University, Limda, Waghodia, VADODARA (GUJARAT) INDIA

IVVALA ANAND SHAKER, Department of Biochemistry, Parul Institute of Medical and Research, Parul University, Limda, Waghodia, VADODARA (GUJARAT) INDIA

C. GONJARI KOMAL, College of Agricultural Biotechnology, Loni, PRAVARANAGAR (M.S.) INDIA

AJIT GANGAVANE, Parul Institute of Applied Sciences, Parul University, Limda, Waghodia, VADODARA (GUJARAT) INDIA

Plant tissue culture is the process whereby small pieces of living tissue (explants) are isolated from a desired cultivar and grown aseptically on a nutrient medium. For successful plant tissue culture, it is best to start with an explants rich in undetermined cells because such cells are capable of rapid proliferation. Roots and shoots contain meristematic cells, which is the source of all dividing cells. Root and shoot tips can be excised and cultured directly on medium, and this can initiate new organs (via somatic organogenesis) that can be clonally propagated.Patchouli is a tropical aromatic plant that belongs to the mint family, Lamiaceae. It is a hardy perennial herb adapted to hot and humid climatic condition .The plant has erect stems, reaching two or

three feet (about 0.75 meters) and is mainly cultivated in Southeast Asia, India and Brazil. *Pogostemon cablin*, known as patchouli or nilam in Indonesia and Malaysia, has rounded leaves and is heart shaped. The leaves and stems of patchouli plants are soft and very hairy. The leaves of this plant have the highest content of patchouli oil (2.5-5%) and the fragrance is strong and heavy. For this reason, the oil is used for the perfumery industry.

Production of true to type plant of *Pogostemon heyneanus* through differentiated axillary buds. However, there are limited effort to study direct organogenesis, which supports cultivation by providing true type plants in large number patchouli oil has fixative properties which make the smell last longer on the skin (Sugimura *et al*., 1995).This suggests that although the addition of auxin is beneficial for rooting,their use is not essential in patchouli. The similar result is reported in *Ulmus* species (Paula *et al.*, 2008).

Propagation through vegetative cutting is slow and insufficient for large scale cultivation. The rapidness of tissue culture techniques can be advantageous for the continuous provision of plantlets stock for field cultivation and may further complement breeding programmes (Reddy *et al*., 2001). Higher effect of the combination of BAP and Kin may be due to the synergy of cytokinins as reported in *Rollinia mucosa* and *Solanum surattenes*. Activated charcoal is an antioxidant and knows to induce rhizogenesis in *Decalepis hamiltonii* (Obul *et al*., 2001) and *Annona cherimoya*.

The oil is used as a base material in perfumery industries as it has strong fixative properties. It is also used as flavoring agent in many food products. It also possesses many antifungal and bacteriostatic properties (Sharma *et al.*, 1992). In aromatherapy, it is used to calm nerves, relive depression and stress. Fimrinolytic and antithrombotic activity of this essential oil is also been reported (Sumi, 2003 and Park *et al*., 2002). Plant regeneration has been accomplished from protoplast derived callus, leaf-and node-derived callus of *in vitro* derived plants of *Pogostemon cablin* (Meena, 1996).

Several studies using tissue culture techniques have been reported such as clonal propagation (Sharma *et al.*, 1992). Patchouli oil is a natural fixative used in aromatic industries and important ingredient in many fine fragrance products like perfumes, as well as in soaps and cosmetics product (Bauer *et al*., 1997).The patchouli oil, obtained by steam distillation of shade dried leaves is commercially used in perfumes and cosmetics (Hasegawa *et al*., 1995 and Maheshwari *et al.*, 1993). Anti-fungal and bacteriostatic properties (Kukreja *et al*., 1990). Anti-insecticidal activities, antifungal and bacteriostatic properties (Yang *et al*., 1996).

Shoot bud like structure, leaves clump, micro shoots, these three phases were also reported when new individuals of plantlets from the leaves callus of *Pogostemon cablin* regenerated using NAA and IBA plant growth regulators (Meena, 1996). The formation of *in vitro* roots prior to acclimatization is not needed. *In vitro* rooting could be eliminated to reduce tie and cost. Mass production of patchouli through conventional method has been limited due to recurrence of mosaic viruses.The highest shoot length and fresh weight of shoot was evidenced in the treatment of BAP over kin when used singly, and in combination has demonstated for the axillary bud proliferation in many medicinal plants of Lamiaceae like *Mentha spicata* and *Lavendula viridis* (Hirata, 1990). Using *Bixa orellana* demonstrated that the combination of NAA and BAP produced white, friable callus with glossy surface, which later developed into white compact callus and finally regenerated green globular structure (Sha-valli-Khan *et al.*, 2002). Shoot meristem culture and *Agrobacterium* – mediated transformation (Yukio *et al*., 2005).Tissue culture has been applied to a large variety of ornamental and medicinal plants. Plant regeneration has been accomplished from protoplast derived callus (Kageyama *et al.*, 1995). Explants from differentage plants may have different levels of endogenous hormones and, therefore, the age of explants would have a critical impacton the regeneration success, similar results have been reported in other plants, including *Platanus occidentalis* (Sun *et al*., 2009).

In Dianthus, it is reported that the youngest leaves just below the apical meristem, when used as explants, gave the highest regeneration response, and the regeneration response declined towards the third pair of leaves. Similar observations have also been reported in *Prunus serotina* (Liu and Pijut, 2008). *P. cablin* is a fast growing perennial herb, oil production with plant regeneration may be more efficient than with cell culture or callus culture (Jones and Krishnadethan, 1973).The presence of NAA along with BAP in the medium has been reported to further improve the shoot induction from leaf disc explants in *Curculigo orchioides* (Thomas, 2007). The fact that NAA treatment could possibly eliminate the secretion of phenolic substances by

competing for the active sites of the auxinoxidase enzyme involved in oxidization of phenols (Perez-Tornero *et al.*, 2000). Proposed that the higher regeneration rates of apical leaves in apple might be because the youngest leaves, still developing, have less differentiated and more metabolically active cells, with a more suitable hormonal and nutritional situation that could improve organogenesis (Famiani *et al.*, 1994). Benzyl adenine (BA) in the medium was shown to have a positive effect on the capacity of *Lavandula dentata* plantlets to produce and/or accumulate essential oils; the amount was 150 per cent more than that produced in the absence of BA (Sudria *et al.*, 1999). Leaves have been most widely studied for adventitious shoot formation in diverse plants. The observations regarding the importance of position of leaves on the plant agree with what has been observed by Sankhla *et al*. (1993).

Economic importance :

While 92 per cent of the oil is non-odoriferous, the rest is made up of mixture of sesquiterpenes of which norpatchoulenol and β - patchoulene and γ-guaislene are major aroma compounds making it a complex contributing to its characteristic odour. These are difficult to synthesize or substitute and hence natural oil from cultivation remains the only source of oil.

***In vitro* propagation of patchouli :**

In vitro micropropagation is an effective means for rapid multiplication of plant species of clonal origin. Many *in vitro* studies have been reported on different patchouli species, using nodal segment explants.One such method of propagation that can be usefully employed to produce relatively uniform plantlets in a short time is via *in vitro* culture. This method ensures a steady supply of planting material for the perfumery and pharmaceutical industries. Production of true to type plant of *Pogostemon heyneanus* through differentiated axillary buds. However, there are limited effort to study direct organogenesis, which supports cultivation by providing true type plants in large numbers patchouli oil has fixative properties which make the smell last longer on the skin (Sugimora *et al*., 1995). This suggests that although the addition of auxin is beneficial for rooting,their use is not essential in patchouli. The similar result is reported in *Ulmus* species (Paula *et al*., 2008).

Several studies using tissue culture techniques have been reported such as clonal propagation (Sharma *et al*., 1992). Patchouli oil is a natural fixative used in aromatic industries and important ingredient in many fine fragrance products like perfumes, as well as in soaps and cosmetics product (Bauer *et al*., 1997). Explants from different age plants may have different levels of endogenous hormone sand, therefore, the age of explants would have a critical impact on the regeneration success, similar results have been reported in other plants, including *Platanus occidentalis* (Sun *et al.*, 2009). Shoot bud like structure, leaves clump, micro shoots these three phases were also reported when new individuals of plantlets from the leaves callus of *Pogostemon cablin* regenerated using NAA and IBA plant growth regulators (Meena, 1996). The formation of *in vitro* roots prior to acclimatization is not needed. *In vitro* rooting could be eliminated to reduce tie and cost. Mass production of patchouli through conventional method has been limited due to recurrence of mosaic viruses.The highest shoot length and fresh weight of shoot was evidenced in the treatment of BAP over Kin when used singly,and in combination has demonstated for the axillary bud proliferation in many medicinal plants of Lamiaceae like *Mentha spicata* and *Lavendula viridis* (Hirata *et al.*, 1990 and Kukreja, 1910). Using *Bixa arellana* demonstrated that the combination of NAA and BAP produced white, friable callus with glossy surface, which later developed into white compact callus,and finally regenerated green globular structure (Sha-valli-Khan *et al.*, 2002).

Therefore, the present study was undertaken to determine the effect of different concentrations of growth hormones on *Pogostemon cablin* Benth and to study the micropropagation and Rapid multiplication stages of patchouli plant within a short time.

MATERIAL AND METHODS

Plant material:

Pogostemon cablin* Benth.*:

The stock plant selected from Sunrise Agro Nursery, Wakad, Pune

The chemicals, glassware and the equipment's used for experiments are presented below:

Media chemicals :

MS media, sucrose, agar and plant growth hormones (PGRs).

Sterilants :

Sodium hypo chloride, gentamycin/streptomycin, savlon, bavistin.

Growth regulators:

6-Benzyl amino purine (BAP), indole acetic acid (IAA), alpha-napthalene acatic acid (NAA), kinetin (KIN)

Source of explants :

Mother plants of Patchouli (*Pogostemon cablin* Benth). Nodal segment 1-2 cm explants taken from mother plants as that long was suitable for sterilization procedures.

Sterilization of glassware :

The glassware such as conical flask, culture tubes, Petridishes, measuring cylinders and the other equipment like forceps, scalpel and blade holder were washed in tap water using detergent. Wrapped in Plastic bag subsequently autoclaved at 120°C at 15 lbs pressure for 20 minutes.

Media preparation :

Nutritional requirement for optimal growth of a tissue *in vitro* may vary with the species. As such, no single medium can be suggested as changes being entirely satisfactory for all types of plant tissues and organs. When starting with a new system it is essential to work out a medium that would fulfill the requirement of that tissue. In order to formulate a suitable medium for a new system it would be better to start with a well-known medium such as MS. By making minor changes, through a series of experiments, a new medium may be involved to accommodate specific requirements of the plant material in question. The concentrated stocks of the major salts, minor salts and growth regulator were prepared and stored under refrigeration. Auxins were normally prepared by dissolving in 1N KOH and cytokinins in 1N HCl before making the final volume with distilled water. Auxins were generally used in plant cell culture at a concentration range of 0.01-10.0 mg/ml. When added in appropriate concentrations they may regulate cell elongation, tissue swelling, cell division, formation of adventitious roots, inhibit adventitious and axillary shoot formation, callus initiation and growth, and induction of embryogenesis. Cytokinins are generally used in plant cell culture range of 0.1-10.0 mg/l. when added in appropriate concentrations they may regulate cell division,stimulate auxiliary and adventitious shoot proliferation, regulate differentiation, inhibit root formation, activate RNA synthesis, and stimulate protein and enzyme activity. The medium was prepared by adding required amounts of stock solution and final volume was made up with distilled water. The pH was adjusted to 5.8 and agar was used for solidifying the medium. The media was poured into washed test tubes (approx. 20ml). They were then autoclaved at 121°C for 20 minutes at 15 psi pressure and transferred to the media storage room where they were kept under aseptic conditions till their further use.

Media composition:

Murashige and Skoog (1962) medium

Macronutrients (g/l):

NH_4NO_3, KNO_3, $CaCl_2$,$2H_2O$, $MgSO_2$,$7H_2O$, KH_2PO_4

Micronutrients(mg/l) :

$MnSO_4 4H_2O$, $ZnSO_4 H_2O$, H_3BO_3, $Na_2MoO_4 2H_2O$, $CoCl_2 6H_2O$, $CuSo_4 5H_2O$.

Vitamins(mg/l):

Nicotine acid, pyridoxine HCl, thiamine HCl

Iron (mg/l) :

$FeSO_4$, $7H_2O$, NaEDTA, $2H_2O$, KI

Amino acid:

Glycine

Stock solution of inositol :

Myo-inositol, sugar, agar.

Growth regulators:

6-benzyl amino purine (BAP), indole acetic acid

(IAA), alpha-napthalene acatic acid (NAA), kinetin (kin).

Explants preparation and sterilization:

The disease free, young and healthy nodal explants were selected.The young explants were excised, cut into 5-7 cm long pieces having 3 or 4 nodes; internodes; leaves and shoot tips were washed thoroughly in the running tap water.

Explant sterilization :

The leaves were removed from the explants and washed under running tap water for 2-3 times in order to wash off the external dust/contaminants.In the next step explants were soaked in an aqueous solution containing 0.2 per cent bavistin for 10 min.This was followed by gentle wash in sterile double distilled water for 2-3 times. Then the bottle was covered with muslin cloth, tight with rubber and washed for 20 min with continuous running water, the explants were immersed in aqueous solution of savlon for 3 min. Then the explants were washed thoroughly with sterile double distilled water for 5 minutes (two cycles). After this treatment, the explants were sterilized with 0.01 per cent $HgCl_2$ aqueous solution for 3 minutes under laminar hood. Then the explants were removed from the sterilizing solution and rinsed thoroughly for 2 times with sterile double distilled water. Then treatment of explants with gentamycin for 3 min was given.

Inoculation of explants:

After sterilization of explants, explants were inoculated in culture bottles aseptically. All these operation were carried out in laminar air flow cabinet. Trimming and leaves were removed with sterile scalpel blade. After cutting explants into suitable size (2-3cm), explants were transferred to culture bottles containing (1) MS medium, (2)MS medium with 0.5mg/lit. BAP, (3) MS medium with 1.0mg/lit. BAP, (4) MS medium with 1.5mg/lit. BAP, (5) MS medium with 2.0mg/lit. BAP. The surface sterilized explants were placed on media. The inoculation was performed by placing explants on the surface of medium with the help of heat sterilized long forceps and replacing the cap of the bottle. During inoculation explants were properly positioned on medium and were gently pressed with forceps to secure their firm contact with the medium. After proper labeling clearly mentioning media, date of inoculation etc., the bottles were transferred to growth room.The inoculated culture bottles were incubated at 25°± 2°C under 80 per cent relative humidity and 14hr photoperiod/day in a culture room. All the cultures were kept in 2500 Lux light intensity under cool fluorescent white light.

Shoot multiplication:

After 6 weeks of initiation the initiated culture shifted to a medium that was for shoot multiplication. The shoot multiplication medium was 3 types (1) MS medium with 0.5 mg/lit. BAP, (2) MS medium with 1.0 mg/lit. BAP, (3) MS medium with 1.5 mg/lit. BAP, initiated cultures were transferred to autoclaved medium within bottles in laminar air flow hood only. Every possible care has been taken to prevent any further contamination. Culture bottles were transferred to growth room for 5 weeks.

Rooting:

The 4 to 5 cm long shoot were cut under aseptic condition and transferred in to rooting medium.The root medium was 2 types: 1. Half MS Media + activated charcoal 100 mg/l, 2. Half MS Media + NAA 0.5 mg/l

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Influence of PGR(s):

Shoot development of Pogostemon cablin in different concentration of BAP :

The nodal explants underwent direct regeneration when cultured on MS using various concentrations of BAP and kin (0.5, 1.0, 1.5, 2.0mg/l) separately or in combinations. The use of MS supplemented with 0.5mg/l BAP was best suitable for bud break and resulted

A B C D

(A) MS+2.0(mg/l) BAP+0.5(mg/l) NAA
(B) MS+1.5(mg/l) BAP + 0.5(mg/l) NAA.
(C) MS+1.0(mg/l) BAP+0.5(mg/l) NAA.
(D) MS+0.5(mg/l) BAP+0.5(mg/l) Kinetin

maximum number of shoots/explants.

Initiation :

Average number of shoots and length of shoots developed after 6 weeks per explants

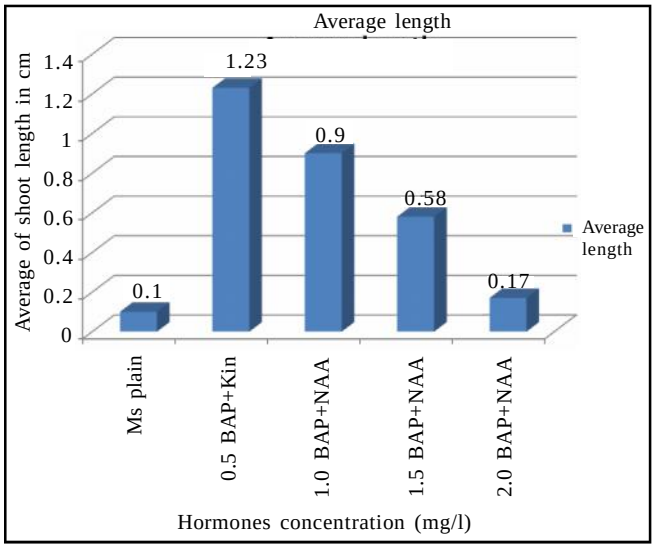

Fig. 1 : Effect of different conc. of BAP in micropropogation of patchouli

Multiplication :

Average numbers of multiplication of shoots and length of shoots in cm after 5 weeks.

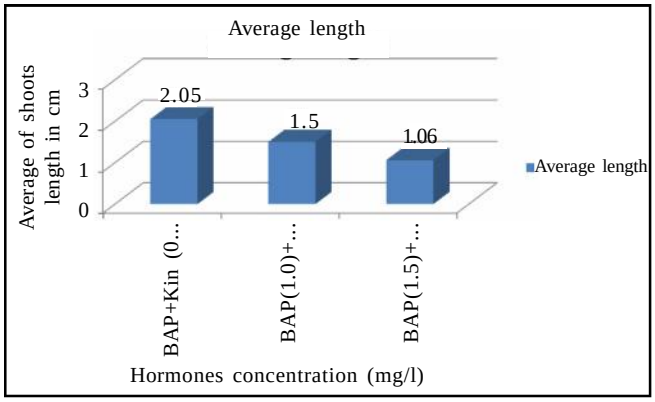

Fig. 2 : Effect of different concentration of BAP in shoot multiplication

Rooting :

Observations of roots and length of roots after 2 Weeks

In the present study, young shoots upto 4-5 nodes were taken for the experiment. But it was observed that young nodes (upto 2-3) responded better than the later nodes. It was observed that in older nodes the contamination rate and death of the tissue was very high

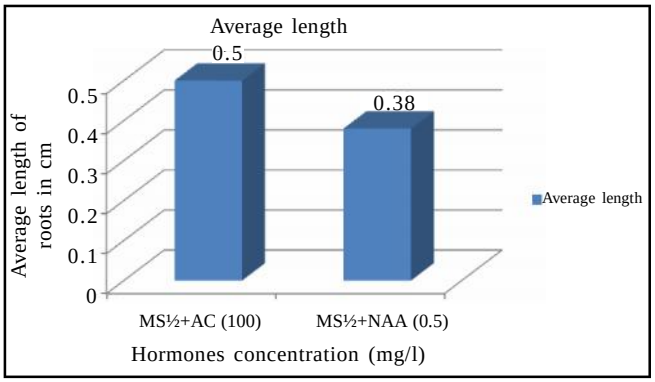

Fig. 3 : Effect of MS ½ +Activated charcoal on rooting of patchouli

while in case of young node it was very less because older tissues were very hard. It was observed that when the concentration of BAP was lower then shoot increased. In higher concentration in BAP there was reduction in the number of shoots per explants. In the present study micro shoots were observed in some concentration of BAP, kin, NAA concentration. Micro shoots were formed maximum in BAP with kin, NAA concentration. The above result indicates that combination of BAP and kin was a better choice for patchouli.Nodal explants were inoculated on MS half media fortified with 100 mg/l activated charcoal and 0.5 mg NAA was better choice for rooting stage. The highest rate of response of shoot proliferation was obtained at 0.5 mg/lit. BAP; 0.5 mg/lit. kin combination, maximum 85 per cent explants show shoot proliferation. MS half with combination of activated charcoal and NAA were used for efficient rooting. The micro shoots were rooted efficiently in half strength MS medium supplemented with 100mg/lit. concentration of charcoal. This is useful for to reduction of the phenolic browning problem, and gives healthy growth of plantlet at rooting stage.

Conclusion :

Micropropagation stages for regeneration of patchouli were successfully initiated by using tissue culture technique which can further be used in large scale as leaves of this plant have the highest content of patchouli oil and the fragrance is strong and heavy. For this reason, the oil is used for the perfumery industryas as well as in soaps and cosmetics.

REFERENCES

Bauer, K., Garve, D. and Surburg, H. (1997). Common fragrance and flavor materials:Prepration, properties and used,

Wiley-VCH, Weinheim, **3** : 205.

Famiani, F., Ferradini, N., Staffolani, P. and Standardi, A. (1994). Effect of leaf excision time and age, BA concentration and dark treatments on *in vitro* shoot regeneration of M26 apple rootstock. *J. Hort. Sci.*, **69**, 679–685.

Hasegawa, Y., Tajama, K., Toi, N. and Sugimura, Y. (1995). Plant regeneration from patchouli protopasts encapsulated in alginate beads. *Plant Cell Tiss. Org. Cult.*, **41** (1) : 65-70.

Hirata, T., Murakami, S., Ogihara, K. and Suga, T. (1990). Volatile monoterpenoid constituents of the plantlets of *Mentha spicata* produced by shoot tip culture. *Phytochemistry,* **29** : 493–495.

Jones, L.H. and Krishnadethan, P.P.S. (1973). Factors influencing production of patchouli sesquiterpenes in cultured cells and regenerated plantlets. *Phytochemistry,* **12** : 1513-1514.

Kageyama, Y., Honda, Y. and Sugimura, Y. (1995). Plant regeneration from patchouli protoplasts encapsulated in alginate beads. *Plant Cell Tiss.Organ Cult.,* **41** : 65–70.

Kukreja, A.K., Mathur, A.K. and Zaim, M. (1990). Mass production of virus –free patchouli plants [*Pogostemon cablin* (Blanco) Benth.] by *in vitro* culture. *Trop. Agric.,* **67** : 101-104.

Liu, X. and Pijut, P.M. (2008). Plant regeneration from *in vitro* leaves of mature blackcherry (*Prunus serotina*). *Plant Cell Tiss.Organ Cult.,* **94** : 113–123.

Maheshwari, M.L., Vasantha, K.T., Neelam, S. and Chadel, K.P.S. (1993). Patchouli-An Indian perspective. *Indian Perfumer,* **37** : 9-11.

Meena, M. (1996). Regeneration of patchouli (*Pogostemon cablin* Benth.) plants from leaf and node callus and evolution after growth in the field. *Plant Cell Rep*., **15** : 991-994.

Obul, R.B., Giridhar, P. and Ravishankar, G.A. (2001). *In vitro* rooting of *Decalepis hamiltonii*. Wight and Arn, an endangered shrub, by auxins and root promoting agents. *Curr. Sci.*, **81**(11):26-29.

Park, E., Yoon, H.K. and Kim, D.H. (2002). Antithrombotic activity of sunghyangjunggisan. *Natural Products Sci.*, **8**(2):71-75.

Paula, C., Alexandra, C. and Conceicao, S. (2008). A protocol for *Ulmus minor* Mill. Micropropafation and acclimatization. *Plant Cell Tiss.Org.Cult.*, **92** (1):113-119.

Perez-Tornero, O., Egea, J., Vanoostende, A. and Burgos, L. (2000). Assessment of factors affecting adventitious shoot regeneration from *in vitro* cultured leaves of apricot. *Plant Sci.,* **158** : 61–70.

Reddy, P.S., Rodrigues, R. and Rajasekharan, R. (2001). Shoot organogenesis and mass propagation of *Coleus forskohlii* from leaf derived callus. *Plant Cell Tiss. Org. Cult.,* **66** : 183-188.

Sankhla, D., Davis, D.T., Sankhla, N. and Upadhyaya, A. (1993). Clonal multiplication of Germanred carnation. *Proc. Plant Growth Reg. Soc. Am.,* **20** : 167–174.

Sha-Valli-Khan, P.S., Prakash, E. and Rao, K.R. (2002). Callus induction and plantlet regeneration in *Bixa oreliana* L., an annatto-yielding tree. *In vitro Cell. Dev. Biol.-Plant,* **38** (2) : 186-190.

Sharma, R.N., Gupta, A.S., Patwardhan, S.A., Hebbalkar, D.S. and Tare, V. (1992). Bioactivity of Lamiaceae plants against insect's. *Indian J.Exp.Biol.,* **30** : 244-246.

Sudria, C., Pinol, M.T., Palazon, J., Cusido, R.M., Vila, R., Morales, C., Bonfill, M. and Canigueral, S. (1999). Influence of plant growth regulators on the growth andessential oil content of cultured *Lavandula dentata* plantlets. *Plant Cell Tiss. Organ Cult.,* **58** : 177–184.

Sugimora,Y.M., Padayhag, S., Ceniza, K.N., Eguchi, S., Natsuaki, T. and Okuda, S. (1995). Essential oil production increased by using virus free patchouli plants derived from meristem-tip culture. *Plant Pathol*., **44** : 510-515.

Sumi, H. (2003). Fibrinolysis-accelerating activity of the essential oils and Shocho aroma. *Aroma Res.,* **4**(3) : 264-267.

Sun, Y., Zhao, Y., Wang, X., Qiao, G., Chen, G., Yang, Y., Zhou, J., Jin, L. and Zhuo, R. (2009). Adventitious bud regeneration from leaf explants of *Platanus occidentalis* L. and genetic stability assessment. *Acta Physiol. Plant.,* **31** : 33–41.

Thomas, T.D. (2007). High-frequency, direct bulblet induction from rhizome explants of *Curculigo orchioides* Gaertn. An endangered medicinal herb. *In Vitro Cell. Dev. Biol.,* **43** : 442–448.

Yang, D., Michel, Madin, Chaumont, Andriamboavonjypoitry and Mellet, C. (1996). Antifungal and antibacterial properties *in vitro* of three patchouli oils from different origins. *Acta Botanica Gallica,* **14** (2):29-35.

Yukio, S., Naoto, K., Yoshiko, Kaori, S., Sakihito, K., Toshiharu, F. and Masato, I. (2005). Tansgenic patchouli plants produced by *Agrobacterium*-mediated transformation. *Plant Cell, Tissue & Organ Culture,* **82** : 251-257.