IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 7, Issue 3 (Jul. – Aug. 2013), PP 96-98
www.iosrjournals.org

Protocol for Rapid Clonal Multiplication Using In Vitro Apical Bud of Lavandula Angustifolia

Dr. D. Leelavathi[1], Narendra Kuppan[2]
[1](Department of botany, MES Degree college, Karnataka, India)
[2](Dos in botany, Mysore University, Mysore, Karnataka, India)

Abstract : *The relevance of the present work is highlighted to produce large number of disease free planting material available to the farmers at an affordable price and also the establishment of repeatable protocol for clonal multiplication using in vitro apical bud of Lavandula angustifolia.*
Keywords: *BAP: 6-Benzyl Amino Purine, MSBM: Murashige and Skoog Basal Medium (1962), NAA: α-Napthalene Acetic Acid, µM: micro molar*

I. Introduction

Aromatic plants ***Lavandula angustifolia*** **L**. is commercially important perennial plant and are conventionally propagated by seed and stem cuttings**.** ***Lavandula angustifoli*****a L.** is a perennial shrub belonging to the family Lamiaceae. It is in great demand for the lavender oil it yields which is used in perfumery, cosmetics, flavouring and pharmaceutical industries.Such aromatic plants are gift of nature it should be protected and propagated. Although these aromatic plants can be propagated vegetatively, the poor rooting ability of the stem cuttings, as well as the lack of selected clones, restrain industrial exploitations. Further, limited tissue culture work has been done on aromatic plants to date as suggested by Segura and Calvo (1991) [10] and Quazi (1980) [9]. Therefore, it is imperative to develop efficient protocols using explants

In order to meet the growing demand of lavender oil, in vitro techniques are being used as alternative method for large scale multiplication and ex-situ conservation. In the present investigation, in vitro axillary bud explants of ***Lavandula angustifolia*** **L**. was cultured

Lavandula angustifolia **L**. commonly known as Lavender belongs to the class Dicotyledon order Tubiflorae family Lamiaceae The genus **Lavandula** of the family Labiatae (Lamiaceae) comprises of 32 species. They are distributed from the Canary Islands, Maderia, Mediterranean Basin, North Africa, South West Asia, Arabian Peninsula and tropical North East Africa and India.

Lavandula is a small genus of perennial aromatic herbs**, semi-shrubs or shrubs of the Labiatae or Lamiaceae family.** Three species of **Lavandula** are extensively utilized for extraction of essential oils.

In the present investigation, aromatic plant ***Lavandula angustifolia*** **L** was chosen for direct regeneration of apical bud These plants were collected from the Department of Horticulture G.K.V.K., Bangalore and the plants were maintained in the green house, Plant Biotechnology Unit, Department of Botany, Bangalore University, Bangalore-560056, Karnataka.

II. Material and Methods

The success of tissue culture protocols ultimately depends on the plant chosen, size of the explant, age and the manner in which it is cultured (George and Sherrington, 1984)[11].so explants was carefully chosen The apical bud measuring 0.5 cm was excised from 20 days old **in vitro** plants of ***Lavandula angustifolia*** (Fig.1) and cultured on MSBM supplemented with BAP in different concentrations ranging from 4.44 µM, 6.66µM, 8.88 µM, 11.11 µM, and 13.32 µM and Kn in different concentrations ranging from 4.64 µM, 6.96 µM, 9.28 µM, 11.60 µM and 13.92 µM and NAA 2.68 µM separately to study their effect on apical bud multiplication (Table 1,Graph 1) After 8 days of culture, tiny leaf primodium (Fig.2) was observed and by 15 days of culture 4-6 leaves were formed (Fig. 3). Further growth of the shoot was noticed after 23 days of culture (Fig.4) on all the concentrations of growth regulators studied, which ranges from 55-90 % of response (Table 1,Graph 1) . The highest percentage of response (90%) was noticed on MSBM+ BAP (8.88 µM) + NAA (2.68 µM) and the lowest percentage of response (55%) was observed on MSBM + Kn (13.92 µM) + NAA (2.68 µM) (Table 1,Graph 1)

After 38 days of culture, 5-6 multiple shoots (Fig.5) was noticed. Further, the number of shoots increased to 10-12 multiple shoots after 14 days of subculture on the same media (Fig.6). Upon subculture the number of shoots increased from 10-20 multiple shoots after 35 days of culture (Fig.7), 20-30 multiple shoots were observed after 49 days of subculture (Fig.8).

The 49 days old culture when subcultured on the same medium exhibited large number of multiple shoots this was conducted to produce large number of healthy multiple shoots of 4-5cms in height (Fig.8). These

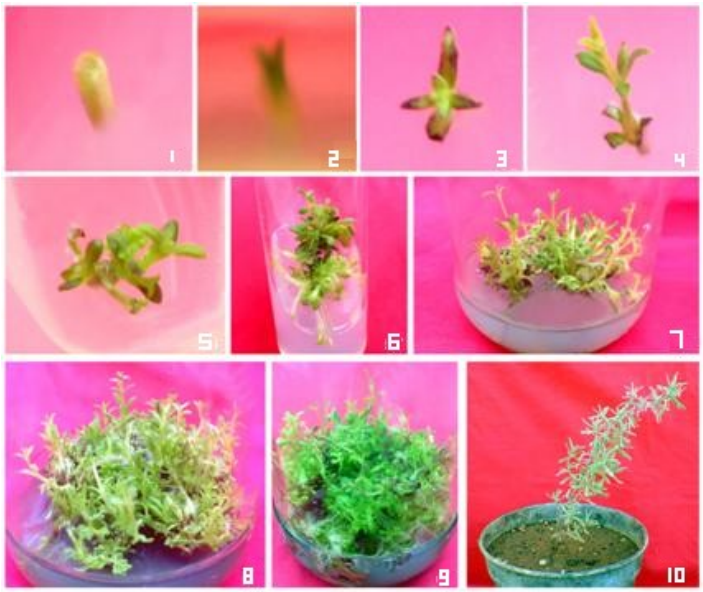
multiple shoots developed roots on the root initiating media on MSBM fortified with BAP (8.88µM) + NAA (2.68µM) + IBA (4.92 µM) (Fig.9). 30-40 multiple shoots with roots were observed after 63 days of culture (Fig.9). Later the cultured plants were subjected to hardening (Fig.10).

In the present study, the statistical data obtained from different experiments were compiled and analyzed. The results revealed that the mean number of shoots per explant ranged from 16.00-30.20 (Table 1,Graph 1)

III. Result & Discussion

It was noticed in the present study that the cultures responded differently in different treatments. However, the highest mean number of shoots 30.20 was observed on MSBM + BAP (8.88 µM) + NAA (2.68 µM) and the lowest mean number 16.00 on MSBM + Kn (13.92 µM) + NAA (2.68 µM) (Table 1,Graph 1)

IV. Figure Showing Various Stages Of Apical Bud Culture Invitro



V. Table 1: Effect Of Different Concentrations Of Growth Regulators For Initiation And Multiplication Of Shoots From In Vitro Apical Bud Explant Of Lavandula Angustifolia

Basal media	BAP (µM)	BAP (mg/l)	NAA (µM)	Response (%)	No. of shoots / explant X ± SD
MS	4.44	1.0	2.68	70	18.0 ± 1.41
MS	6.66	1.5	2.68	85	22.5 ± 2.33
MS	**8.88**	**2.0**	**2.68**	**90**	**30.2 ± 2.63**
MS	11.11	2.5	2.68	80	24.8 ± 2.85
MS	13.32	3.0	2.68	70	20.5 ± 1.50
	Kinetin (µM)	**Kinetin (mg/l)**			
MS	4.64	1.0	2.68	60	16.30 ± 1.84
MS	6.96	1.5	2.68	72	18.30 ± 1.41
MS	**9.28**	**2.0**	**2.68**	**78**	**22.30 ± 2.23**
MS	11.60	2.5	2.68	62	18.00 ± 1.62
MS	13.92	3.0	2.68	55	16.00 ± 1.94

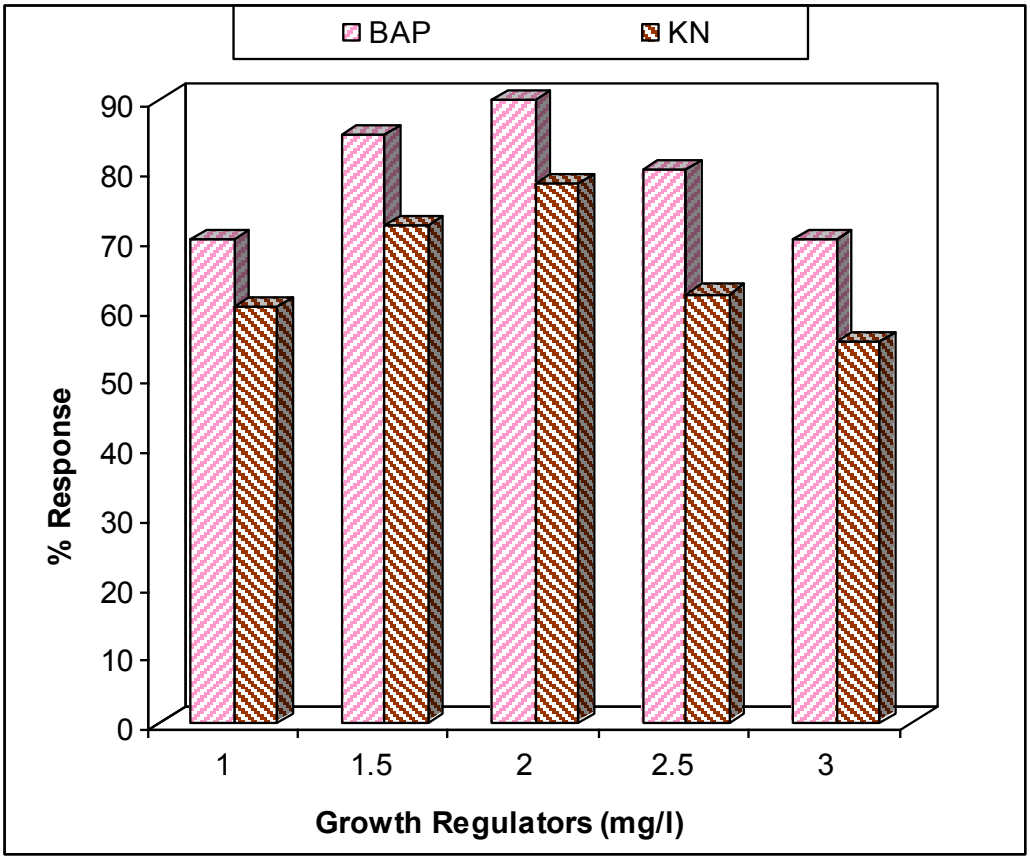
ANOVA TABLE (number of shoots/explant)

SV	DF	SS	MSS	F_{cal} ratio	F_{tab}value**	CD
Treatment	9	1746.89	194.09	**41.73**	**2.00**	**4.28**
Errors	90	418.50	4.65			
Total	99	2165.39				

Note: * : Mean of 10 replication

**** : Significance F Value @ 5%level**

VI Graph 1 : Effect of different concentrations of growth regulators on initiation and multiplication of shoots from *in vitro* apical bud explant of *Lavandula angustifolia* L



VII.Conclusion

In the present study, it was found that **MSBM + BAP (8.88 µM) + NAA (2.68 µM)** was the most suitable medium for initiation and multiplication of shoots from apical bud. Further, it was significantly superior when compared to other treatments with respect to multiple shoot formation.

References

[1] Anamaria Jordan, M. C., Calvo M. C., And Segura, J. 1998. Micropropagation Of Adult Lavandula Dentata Plants. Journal Of Horticultural Sciences And Biotechnology, 73(1): 93-96.
[2] Andrade, L.B., Echeverrigaray, S., Fracaro, F., Pauletti G. F. And Rota, L. 1999. The Effect Of Growth Regulators On Shoot Propagation And Rooting Of Common Lavender (Lavandula Vera Dc). Plant Cell, Tissue and Organ Culture, 56: 79-83.
[3] Calvo, M.C. And Segura, J. 1988. In Vitro Morphogenesis From Explants Of Lavandula Latifolia And Lavandula Stoechas Seedlings. Scientia Horticulturae, 36: 131-137.
[4] Derek, V. Banthorpe, Hilary J. Bilyard. And David G. Watson. 1985. Pigment Formation By Callus Of Lavandula Angustifolia. Phytochemistry, 24(11) : 2677-2680.
[5] Dias, M.C., Almeida,. R. And Romano A. 2002. Rapid Clonal Multiplication Of Lavandula Viridis L'he'r Through In Vitro Axillary Shoot Proliferation. Plant Cell, Tissue And Organ Culture, 68: 99-102.
[6] Dronne, S., Colson, M.., Moja, S. And Faure, O. 1999. Plant Regeneration And Transient Gus Expression In A Range Of Lavandin (Lavandula X Intermedia Emeric Ex Loiseleur) Cultivars. Plant Cell, Tissue And Organ Culture, 55 : 193-198.
[7] Leelavathi.D., ex-situ conservation of *lavandula angustifolia* using in vitro techinques, lake 2010: wetlands, biodiversity and change 22 nd-24 th december 2010 IISC Bangalore.
[8] Miguel Carlos Sanchez- Gras And Maria Del Carmen Calvo. 1996. Micropropagation Of Lavandula Latifolia Through Nodal Bud Culture Of Matured Plants. Plant Cell,Tissue And Organ Culture, 45: 259-261.
[9] Quazi M. H. 1980. In Vitro Multiplication Of Lavandula Spp. Annuals Of Botany, 45: 361-362.
[10] Segura And Calvo (1991). Micropropagation Of Lavandula Latifolia Through Nodal Bud Culture Of Mature Plants

Book:

[11] E. F. George and P. D. Sherrington, Plant Propagation By Tissue Culture — Handbook And Directory Of Commercial Laboratories.