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IN VITRO ASEPTIC, REGENERATION OF *LAVANDULA ANGUSTIFOLIA* L.- AN IMPORTANT MEDICINAL PLANT USING AXILLARY BUD EXPLANTS

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ABSTRACT

The prime objective of the present investigation was to develop a repeatable protocol for rapid clonal multiplication using *in vitro* axillary bud of *Lavandula angustifolia* explants on Murashige and Skoog's MS basal medium supplemented with BAP (8.88 µM) + NAA (2.68 µM) with 80% response. Further, by repeated subculture resulted in rapid shoot multiplication on the same medium. These shoots developed roots when inoculated on rooting medium on MSBM fortified with BAP (8.88µM) + NAA (2.68µM) + IBA (4.92µM). The axenic plants were subjected to hardening, the hardened acclimatized plants were transferred to the soil with 80% survival frequency.

Keywords:- *Lavandula angustifolia* Axillary bud Multiple shoots Murashige and Skoog's medium etc.

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INTRODUCTION

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 (Fig. 1) of the Labiatae or Lamiaceae family.Lavender (2n = 42 or 48) is a perennial, herbaceous bushy plant with straight, woody branches (Fig.2), lower branches are leafless giving out numerous herbaceous shoots about 1m in length which are quadrangular, pubescent, greyish in colour and glands in the stem emits an aromatic fragrance. Aromatic plants are gift of nature with greater uses to mainkind this plants should be propagated, propagation of this plant should be promoted.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) and

Therefore, it is imperative to develop efficient protocols using Explants, Such aromatic plants are gift of nature it should be protected and Propagated Leelavathi.D and Narendra Kuppan.(2013)

MATERIAL AND METHODS

The axillary bud measuring 0.5 cm with the stem was excised from 20 days old ***in vitro*** plants derived from direct regeneration apical bud explants of ***Lavandula angustifolia*** (Fig.1) and cultured on MSBM fortified with BAP different concentrations of ranging from 4.44 µM, 6.66µM, 8.88 µM, 11.11 µM, 13.32 µM and Kn ranging from 4.64 µM, 6.96 µM, 9.28 µM, 11.60 µM, 13.92 µM and NAA 2.68 µM separately to study their effect on axillary bud multiplication (Table.1).(Leelavathi.D and Narendr Kuppan)(2013)

After 10 days of culture, shoot initiation with 2-3 leaves (Fig.2) were observed on all the concentrations of growth regulators studied with varying percentage (46-91 %) of response (Table.1, Graph.1). The highest (91%) and lowest (46%) percentage of response was observed on MSBM + BAP (8.88 µM) + NAA (2.68 µM) and MSBM + KN (13.92 µM) + NAA (2.68 µM) respectively.

After 20 days of culture 2-3 multiple shoots were noticed (Fig.3). After 27 days of culture 3-5 elongated multiple shoots (Fig.4) were observed. After 36 days of culture, the shoots were subcultured on the same medium to obtain more number of multiple shoots. After 14 days of subculture, 6-8 multiple shoots were observed (Fig.5) on MSBM + BAP (8.88 µM) + NAA (2.68 µM) 10-12 multiple shoots (Fig.6) were formed after 28 days of subculture. 32-36 elongated multiple shoots which attained the height of 3-5 cm (Fig.7) were noticed after 42 days of subculture. These shoots developed roots when inoculated on rooting medium on MSBM fortified with BAP (8.88µM) + NAA (2.68µM) + IBA (4.92µM) after 28 days of culture (Fig.8). The axenic plants were subjected to hardening (Fig.9).

In the present investigation, the statistical analysis of the data revealed highly significant differences existing between and within the treatments. The mean number of shoots per explant ranged from 18.40 to 36.50 (Table.1,Graph.1). The highest mean number 36.50 was observed on MSBM + BAP (8.88 µM) + NAA (2.68 µM) and the lowest mean number 18.40 on MSBM + Kn (13.92 µM) + NAA(2.68 µM) respectively.

RESULT AND DISCUSSION:

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). Most of the studies carried out on several plants reveals that seeds and juvenile tissues were utilized for ***in vitro*** multiplication than the tissues from matured plants as suggested by Bonga,(1987).The selection of appropriate nutrient medium is also important for the success of all experimental system in plant tissue culture. Bhojwani and Razdan (1983) In the present study ***in vitro*** axillary bud ***L. angustifolia,*** **was** cultured on MS basal basal medium supplemented with various combinations and concentrations of growth regulators BAP, Kn, IAA, NAA, IBA and 2,4-D separately regeneration and subsequent retrieval of axenic plants.

It was found in the present study, that MS basal medium supplemented with BAP (8.88 µM) and NAA (2.68 µM); and MSBM with BAP (8.88 µM) was the best medium for ***in vitro*** axillary bud initiation and shoot multiplication of ***L. angustifolia***. This findings does not coincides with the findings of Panizza and Tognoni (1992) and Miugel and Maria (1996) who have reported that the micropropagation of ***L. latifolia*** is through

axillary bud culture on MSBM supplemented with BAP and NAA. The present findings are in accordance with the findings of Anamaria ***et al.,***(1998) and Andrade ***et al***., (1999) and Dias ***et al***., (2002) and Leelavathi ***et al***.,(2013)

TABLE.1 Effect of different concentrations of growth regulators for initiation and multiplication of shoots from *in vitro* axillary bud explant of *Lavandula angustifolia*

Basal media	BAP (µM)	BAP (mg/l)	NAA (µM)	Response (%)	No.ofshoots /explant X *± SD
MS	4.44	1.0	2.68	50	20.10 ± 2.02
MS	6.66	1.5	2.68	73	29.50 ± 1.50
MS	8.88	2.0	2.68	91	36.50 ± 2.29
MS	11.11	2.5	2.68	71	28. 30 ± 2.45
MS	13.32	3.0	2.68	66	26.50 ± 2.15
	Kinetin (µM)	Kinetin (mg/l)	2,4-D (µM)		
MS	4.64	1.0	2.26	46	18.50 ± 1.74
MS	6.96	1.5	2.26	51	20.40 ± 2.87
MS	9.28	2.0	2.26	71	28.50 ± 1.80
MS	11.60	2.5	2.26	51	20.60 ± 1.56
MS	13.92	3.0	2.26	46	18.40 ± 2.10

ANOVA TABLE (number of shoots/explant)

SV	DF	SS	MSS	F_{cal} ratio	F_{tab}value**	CD
Treatment	9	3250.05	361.11	74. 45	2.00	4.46
Errors	90	436.70	4.85			
Total	99	3686.75				

Note: * Mean of 10 replication ** Significance F Value @ 5%level

Graph.1 Effect of different concentrations of growth regulators on initiation and multiplication of shoots from ***in vitro*** axillary bud explant of ***Lavandula angustifolia***

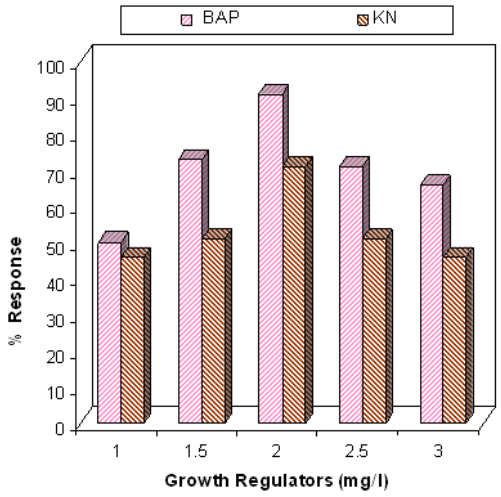
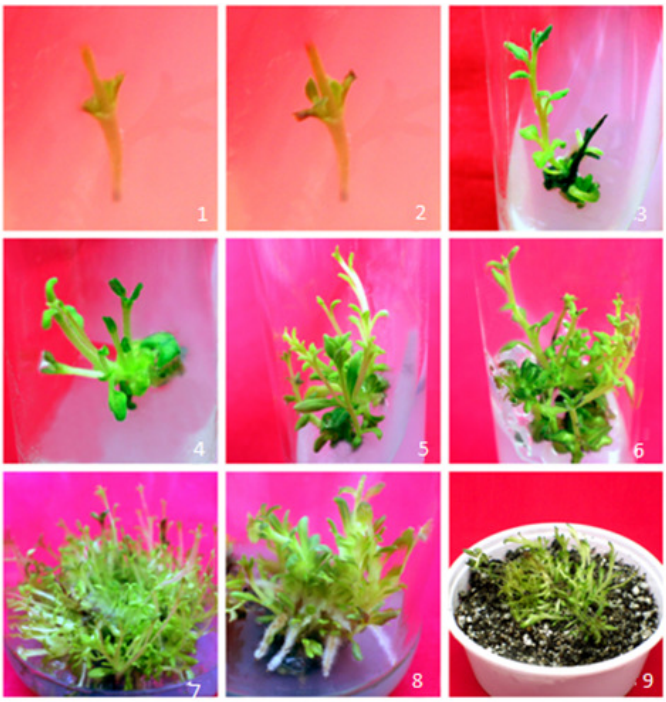


Figure showing various stages of growth of axillary bud of ***Lavandula angustifolia*** in vitro nutrient medium



CONCLUSION

In the present study, it was observed that **MSBM + BAP (8.88 µM) + NAA (2.68 µM)** was the best medium for axillary bud intiation and shoot multiplication. Further, it was significantly superior than the other concentrations of growth regulators tested with respect to multiple shoots formation.

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