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Research Article

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An efficient protocol for *in vitro* aseptic shoot multiplication and plant regeneration of *Rosmarinus officinalis*- An Important Medicinal Plant Using axillary bud

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ABSTRACT

The prime objective of the present investigation was to build up a repeatable protocol for rapid clonal multiplication using in vitro axillary bud of Rosmarinus officinalis. In vitro axillary buds were cultured on MS basal medium supplemented with BAP (8.88 µM) + IAA (2.85 µM) to induce multiple shoots. Further, these shoots were subcultured on the same medium to produce more number of multiple shoots. Well developed multiple 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.6) and the axenic plants are subjected to hardening (Fig.7). The acclimatized plants were transferred to soil with 75% survival frequency.

Keywords: *Rosmarinus officinalis L, axillary bud Murashige and Skoog Basal Medium.*

INTRODUCTION

Rosmarinus officinalis L. (Rosemary) belongs to the class Dicotyledon order Tubiflorae family Lamiaceae. Rosemary is a native of Mediterranean regions of Europe, Asia Minor and North Africa. Rosemary is grown in Spain, Italy, France, Algeria, Morocco and Portugal for its essential oil. In India, Rosemary is cultivated to a limited extent in the Nilgiris in South India.

Rosmarinus officinalis L is an aromatic plant. Due to excessive collection and exploitation has depicted the wild species of aromatic plants. Therefore, it is imperative to attempt exploration, collection, maintenance, evaluation, multiplication and conservation of aromatic plants for the present and future use[1].

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[2].

MATERIAL AND METHODS

The axillary buds measuring 0.5-1cm with stem was excised from *in vitro* plants retrieved from direct regeneration of apical bud explants of *Rosmarinus officinalis* (Fig.1) and cultured on MSBM fortified with different concentrations of BAP ranging from 4.44 µM, 6.66µM, 8.88 µM, 11.11 µM, 13.32 µM ; KN ranging from 4.64 µM, 6.96 µM, 9.28 µM, 11.60 µM, 13.92 µM and IAA 2.85 µM separately to study their effect on axillary bud multiplication (TABLE 1).

After 23 days of culture, shoot initiation with 1-2 leaves (Fig.2) were observed on all the concentrations of growth regulators studied with varying percentage (49-93%) of response (TABLE 1, Graph.1).The highest (93%) and lowest (49 %) percentage of response was observed on MSBM + BAP (8.88 µM) + IAA (2.85 µM) and MSBM + Kn (13.92 µM) + IAA (2.85 µM) respectively.

After 30 days of culture 2-5 multiple shoots were noticed (Fig.3). Further, the 45 days old shoots were subcultured on the same medium to obtain more number of shoots. 10-25 multiple shoots were observed (Fig.4) by 40 days of subculture. 30-32 multiple shoots with a height of 2-4cms. were noticed (Fig.5) after 70 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.6) and the axenic plants are subjected to hardening (Fig.7).

In the present investigation, the statistical analysis of the data revealed highly significant differences exists between and within the treatments. The mean number of shoots per explant ranged from 17.30 to 32.60 (TABLE 1, Graph.1). The highest mean number 32.60 was observed on MSBM + BAP (8.88 µM) + IAA (2.85 µM) and the lowest mean number 17.30 on MSBM + Kn (13.92 µM) + IAA (2.85 µM) respectively.

RESULT AND DISSCUSION

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[3]. 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[4].

Totipotency is potential of the cell or living protoplasm to form an complete organism when provided with ideal micro and macro environment which is specific to that particular cell or protoplasm and this forms the basis of tissue culture. And greater developments in biotechnology as taken place because of tissue culture[17].

The selection of appropriate nutrient medium is also important for the success of all experimental system in plant tissue culture[5].

In general, juvenile explants such as apical bud, axillary bud, embryos, cotyledon and hypocotyl explants from seedlings are more responsive than the other tissues. Best results were achieved when they are from juvenile parts of the plants as suggested by Bonga[4].

In the present study, *in vitro* vegetative propagule, axillary bud of was cultured supplemented with various combinations and concentrations of growth regulators BAP, Kn, IAA, NAA, IBA and 2,4-D separately.

In the present study, it was observed that MS basal medium supplemented with BAP(8.88 µM) and IAA (2.85µM) was the best medium for initiation and multiple shoot formation from *in vitro* axillary bud in *R.officinalis.* This does not agrees with the findings of Dias[6].

In the present observation, it was found that high concentration of BAP will not enhance the multiplication and elongation of shoots from apical bud and axillary buds of *R.officinalis.* This coincides with Sugimora *et al*.,(1998) have suggested that to achieve high frequency of shoot initiation it is essential to supply cytokinin BAP with auxins NAA and IAA to the culture medium And the findings coincides with findings of Leelavathi (2010) and Narendra Kuppan (2013)

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

Basal media	BAP (µM)	BAP (mg/l)	IAA (µM)	Response (%)	No. of shoots /explant X *± SD
MS	4.44	1.0	2.85	55	19.30±1.95
MS	6.66	1.5	2.85	66	23.30±0.90
MS	**8.88**	**2.0**	**2.85**	**93**	**32.60±1.80**
MS	11.11	2.5	2.85	69	24.40±1.62
MS	13.32	3.0	2.85	64	22.40±1.62
	Kinetin (µM)	**Kinetin (mg/l)**			
MS	4.64	1.0	2.85	52	18.50±2.87
MS	6.96	1.5	2.85	58	20.50±1.56
MS	**9.28**	**2.0**	**2.85**	**69**	**24.40±1.68**
MS	11.60	2.5	2.85	58	20.60±2.28
MS	13.92	3.0	2.85	49	17.30±3.43

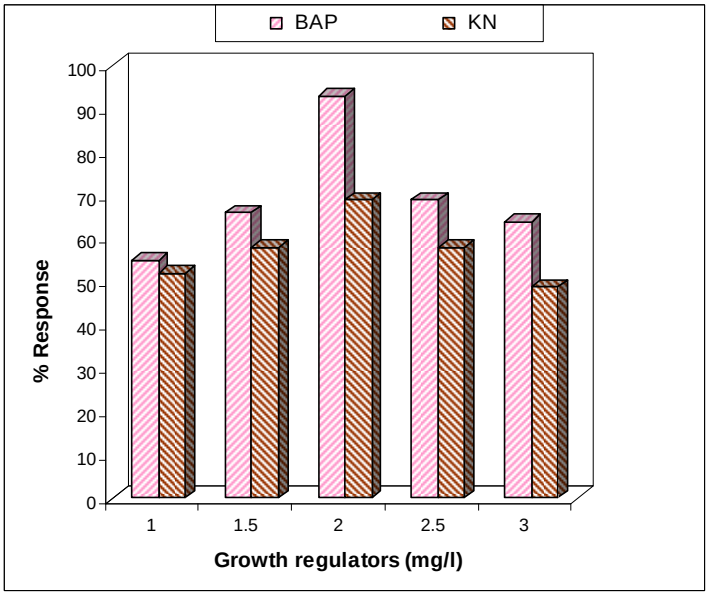
ANOVA TABLE (number of shoots/explant)

SV	DF	SS	MSS	F_{cal} ratio	F_{tab}value**	CD
Treatment	9	1704.81	189.42	**26.75**	**2.00**	**6.52**
Errors	90	637.30	7.08			
Total	99	2342.11				

Note: * : Mean of 10 replication

** : Significant 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 *Rosmarinus officinalis*



Figures showing various stages of growth of the callus from in vitro axillary bud of *Rosmarinus Officinalis*.L

CONCLUSIONS

In the present study, it was observed that MSBM + BAP (8.88 µM) + IAA (2.85 µM) was the best medium for axillary bud initiation 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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