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Research Article

CALLUS INDUCTION AND REGENERATION OF MULTIPLE SHOOTS FROM *IN VITRO* APICAL BUD EXPLANT OF *ORIGANUM VULGARE.* AN IMPORTANT MEDICINAL PLANT

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ABSTRACT

Apical bud explant measuring 0.5-1.0 cm was excised from ***in vitro*** plant of ***Origanum vulgare*** where cultured on MSBM fortified with cytokinin BAP (8.88µM) and auxins in different concentrations of NAA (2.68 µM, 5.36 µM, 8.05 µM, 10.74 µM, 13.42 µM) and 2,4-D (2.26 µM, 4.52 µM, 6.78 µM, 9.04 µM, 11.30 µM) respectively in order to induce callus, whitish green compact callus were observed on MSBM + BAP (8.88µM) + 2,4-D(2.26 µM).Which developed shoots after 42 days of subculture, after several subcultures, 20-30 well developed healthy, elongated multiple shoots were observed. These shoots developed roots on the same medium thus avoiding an additional step of ***in vitro*** rooting after 70 days of subculture these ***in vitro*** plants were subjected to hardening, Hardened plants were transferred to the soil with 90% survival frequency.

Keywords: ORIGANUM VULGARE, MULTIPLE SHOOTS, IMPORTANT MEDICINAL PLANT, APICAL BUD

MSBM: Murashige and Skoog Basal Medium.

INTRODUCTION

The use of aromatic plants in health and beauty care traced back 1500 years before Christ. Aromatic plants are economically important because of the essential oil they produce. In the event of repeated failure of essential crops due to diseases and vagaries of weather aromatic plants can provide alternate and regular source of income to farmers. There is a strong need of promoting and popularizing this wealth among the farmers entrepreneur and pharmaceutical industry. Further, the number of aromatic crops in commercial cultivation is limited and information available on their multiplication and cultivation technique is scanty. Under such circumstances tissue culture technique becomes more relevant in handling large scale multiplication and crop specific problems. India has enjoyed unique position in the manufacture of superior perfumes and aromatics since ancient times (Atal and Kapur, 1997).

Origanum vulgare L oregano, wild or common majorum belongs to the class Dicotyledon order **Tubiflorae** family **Lamiaceae** is a herbaceous ,ornamental perennial herb, which grows wild in Western and Southern Turkey and other East Mediterranean countries (Davis 1982) it is also native to Europe, but often used in Italian dishes. In India, it is grown in temperate Himalayas from Kashmir to Sikkim, common in Simla hills and Kashmir valley. The leaves are used as vegetable in Lahaul, Punjap and Kashmir. It is cultivated on a small scale in several states. Origanum species have been used in medicine and as spices since antiquity mainly because of their essential oils, which consists of a considerable amount of caravacrrol and thymol ***O. vulgare*** is well known as a plant with a medicinal value and as such is accepted officially in a number of countries.

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

Apical bud explant measuring 0.5-1.0 cm was excised from ***in vitro*** plant of ***Origanum vulgare*** and cultured (Fig. 1) on MSBM fortified with cytokinin BAP (8.88µM) (Fig. 1) and auxins in different concentrations of **NAA (2.68 µM, 5.36 µM, 8.05 µM, 10.74 µM, 13.42 µM) and 2,4-D (2.26 µM, 4.52 µM, 6.78 µM, 9.04 µM, 11.30 µM**) respectively in order to induce callus (Table1).
At the cut end, callusing of the explant was observed (Fig. 2) after 23 days of culture on all the concentrations studied with varying frequency of response (34-90 %) (Table1, Graph.1). The highest frequency of response (90%) was observed on **MSBM + BAP (4.44 µM) + 2,4-D (9.04µM)** and the lowest (34%) on **MSBM + BAP (4.44µM) + NAA (13.42 µM)** (Table1, Graph.1).
After 46 days of culture, moderate greenish friable and large amount of whitish green compact callus was observed on MSBM supplemented with BAP (4.44 µM) + NAA 10.74 µM (Fig. 3) and BAP (4.44 µM) + 2,4-D 9.04µM (Fig. 4) respectively.
The amount of callus obtained was recorded and analyzed statistically (Table 2). Analysis of the results revealed that there exist highly significant differences between within the treatments. The highest amount of callusing was observed on **MSBM + BAP (4.44 µM) + 2,4-D (9.04µM)** with fresh weight 13.64 gm and dry weight 2.50 gm when compared to NAA in which moderate amount of callusing was observed on **MSBM + BAP (4.44 µM) + NAA (13.42µM)** with fresh weight 5.14gm and dry weight 0.41 gm (Table 2, Graph.2).
It was observed in the present study that the increase in concentrations of **2,4-D to 11.30µM** (Table 2, Graph.2) resulted in decrease in the amount of callus formation and it was also found that increase in the concentration of NAA decreased the amount of callus formed.
46 days old moderate creamish green friable and whitish green compact callus were subculture on MSBM fortified with different concentrations of **BAP (2.22µM,4.44 µM,6.66µM, 8.88µM and 11.11µM)** and **NAA(2.68µM) and 2,4-D (2.26µM)** respectively (Table 3) to study their morphogenetic ability.
After 28 days of subculture, shoot initiation was observed on all the concentrations of **BAP, NAA and 2,4-D** tried with varying frequency of response (17-85 %) (Table 3).The highest frequency of response (85%) was observed on **MSBM + BAP (8.88µM) + 2,4-D (2.26 µM)** and the lowest frequency of response (17 %) on **MSBM +BAP (2.22 µM) + NAA (2.68 µM)**
After 35 days of subculture (Fig. 5) the maximum number of shoot bud (5-10) formation was observed only from whitish green compact callus on **MSBM + BAP (8.88µM) + 2,4-D(2.26 µM).7-9** multiple shoots were noticed by 42 days of subculture, (Fig. 6). Further growth of the multiple shoots were observed after 49 days of subculture (Fig. 7). 20-30 well developed healthy, elongated multiple shoots were observed after 60 days of subculture (Fig. 8).These shoots developed roots on the same medium thus avoiding an additional step of ***in vitro*** rooting after 70 days of subculture (Fig. 8) these ***in vitro*** plants were subjected to hardening (Fig. 9).

RESULT AND DISSCUSION

Totipotency is potential of the cell or living protoplasm to form a 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 cell or protoplasm tissue culture. And greater developments in biotechnology as taken place because of tissue culture.(*Narendra Kuppan et al.)*.
The establishment of micropropagation protocols on a sound commercial proposition, especially in aromatic and economically important crop using shoot tip to produce a novel stock, which are free from pathogens, viruses and the approach of variety of processes including anther, pollen, gynoecium, ovule, protoplast culture and somatic hybridization to speed the process of producing better varieties have all contributed to the acceptance of plant tissue culture techniques as viable and valuable tool.
The cell and tissue culture techniques have tremendous advantages in agriculture, horticulture, forestry and other commercially important aromatic crop plants, especially those, which are propagated vegetatively.
In the present study, ***in vitro*** apical bud of ***O .vulgare*** was cultured Further, analysis of the results indicates that MS basal medium supplemented **BAP (8.88 µM)** was the most suitable medium for initiation and multiplication of shoots from ***in vitro*** apical bud of ***O. vulgare*** and it is significantly superior when compared to other treatments with respect to multiple shoot formation. This does not coincides with the findings of Goleniowski ***et***

al., (2003). They have reported micropropagation of Oregano (***Origanum vulgare x applii***) using MS basal medium supplemented with NAA and BAP in low concentrations.

It was found that higher concentration of BAP will not enhance the multiplication and elongation of in ***vitro*** shoots raised from apical buds of field grown plants and also aseptic plants of ***Oreganum vulgare***.

In the present observation, it was found that high concentration of BAP will not enhance the multiplication and elongation of shoots from apical bud ***O. vulgare .*** The present findings showed 2-3 and 1-3 multiple shoots per culure from ***in vitro*** apical bud explants on MS basal medium fortified with BAP alone and also in combination with auxins NAA, IAA and 2,4-D. However, there was three-fold, increase in the formation of multiple shoots from apical bud (20-30) explants when cultured on MS basal medium fortified with BAP with incorporation of auxin to the medium.

CONCLUSION

It was found in the present study that **MSBM+ BAP (8.88µM) + 2,4- D (2.26 µM)** was the best medium for multiple shoot formation from the callus and elongation of shoot bud when compared to other concentrations of BAP tested.

Table 1: Effect of different concentrations of cytokinin and auxins on *in vitro* apical bud explant of *Origanum vulgare* for callus induction

Basal media	BAP (µM)	2,4-D (mg/l)	2,4-D (µM)	Culture duration	Response (%)
MS	4.44	0.5	2.26	23 days	47
MS	4.44	1.0	4.52	23 days	56
MS	4.44	1.5	6.78	23 days	64
MS	4.44	**2.0**	**9.04**	**23 days**	**90**
MS	4.44	2.5	11.30	23 days	61
		NAA (mg/l)	**NAA (µM)**		
MS	4.44	0.5	2. 68	23 days	41
MS	4.44	1.0	5.36	23 days	47
MS	4.44	1.5	8.05	23 days	50
MS	4.44	**2.0**	**10.74**	**23 days**	**67**
MS	4.44	2.5	13.42	23 days	34

Table 2: Effect of different concentrations of cytokinin and auxins for the growth of the callus from *in vitro* apical bud explant of *Origanum vulgare*

Basal media	BAP (µM)	2,4-D (mg/l)	2,4-D (µM)	Culture duration	Quantity of callus	Fresh Weight X *± SD	Dry Weight X *± SD
MS	4.44	0.5	2.26	46	+	07.11±0.26	0.43 ±0.10
MS	4.44	1.0	4.52	46	+++	08.40±0.40	0.63 ±0.10
MS	4.44	1.5	6.78	46	++	09.56 ±0.43	0.83 ±0.14
MS	4.44	**2.0**	**9.04**	**46**	**++**	**13.64±0.53**	**2.50 ± 0.31**
MS	4.44	2.5	11.30	46	++	09.15 ±0.24	0.61±0.10
		NAA (mg/l)	**NAA (µM)**				
MS	4.44	0.5	02.68	46	+	06.19±0.43	0.47 ±0.17
MS	4.44	1.0	05.36	46	++	07.19±0.40	0.59±0.31
MS	4.44	1.5	08.05	46	++	07.54±0.47	0.69 ±0.30
MS	4.44	**2.0**	**10.74**	**46**	**++**	**10.15±0.68**	**1.02 ±0.31**
MS	4.44	2.5	13.42	46	+	05.14**±0.37**	0.41 ±0.10

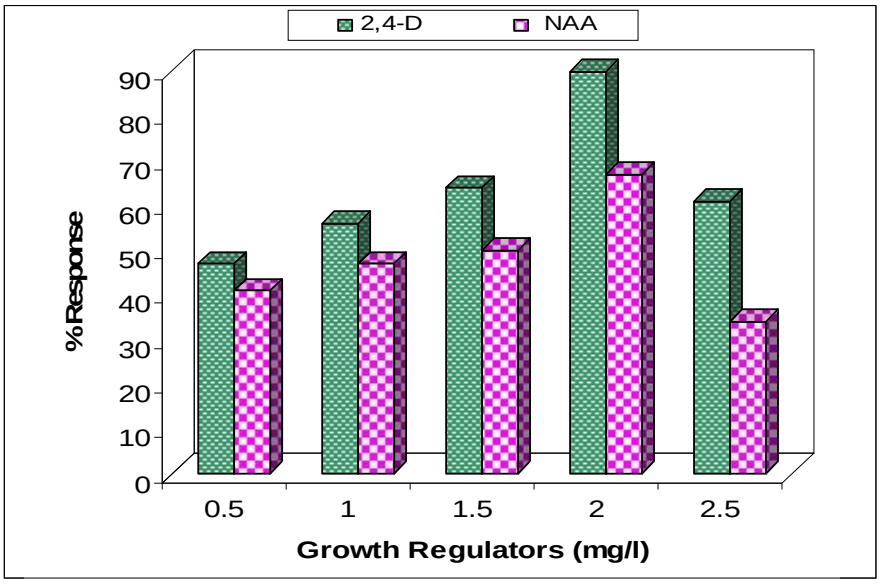
ANOVA TABLE (Fresh Weight)

SV	DF	SS	MSS	F_{cal} ratio	F_{tab} value**	CD
Treatment	9	518.18	57.57	**45.69**	**2.00**	**1.16**
Errors	90	114.00	1.26			
Total	99	632.18				

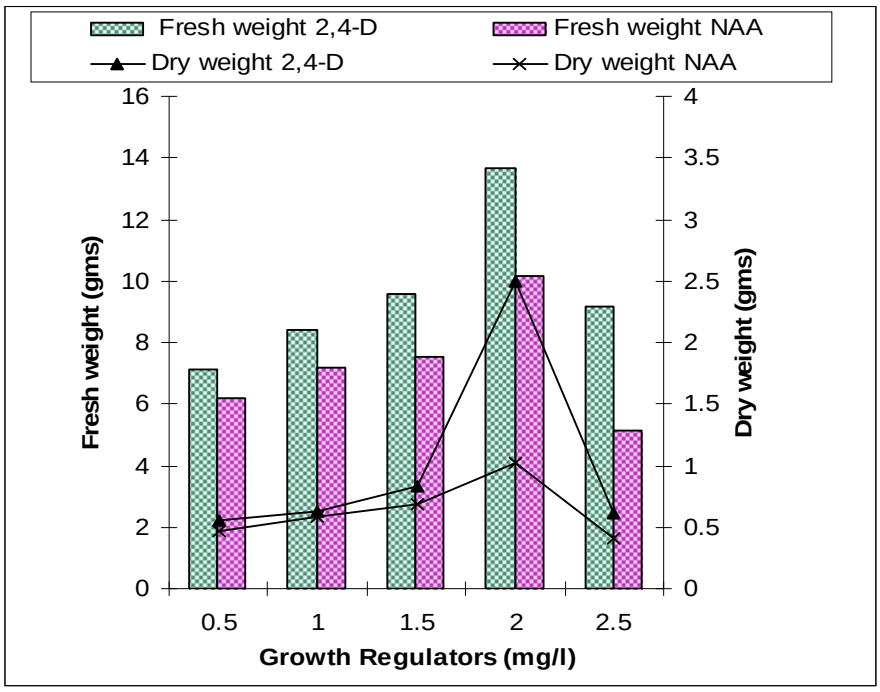
ANOVA TABLE (Dry Weight)

SV	DF	SS	MSS	F_{cal} ratio	F_{tab} value**	CD
Treatment	9	61.27	6.80	**61.81**	**2.00**	**0.10**
Errors	90	10.53	0.11			
Total	99	71.80				

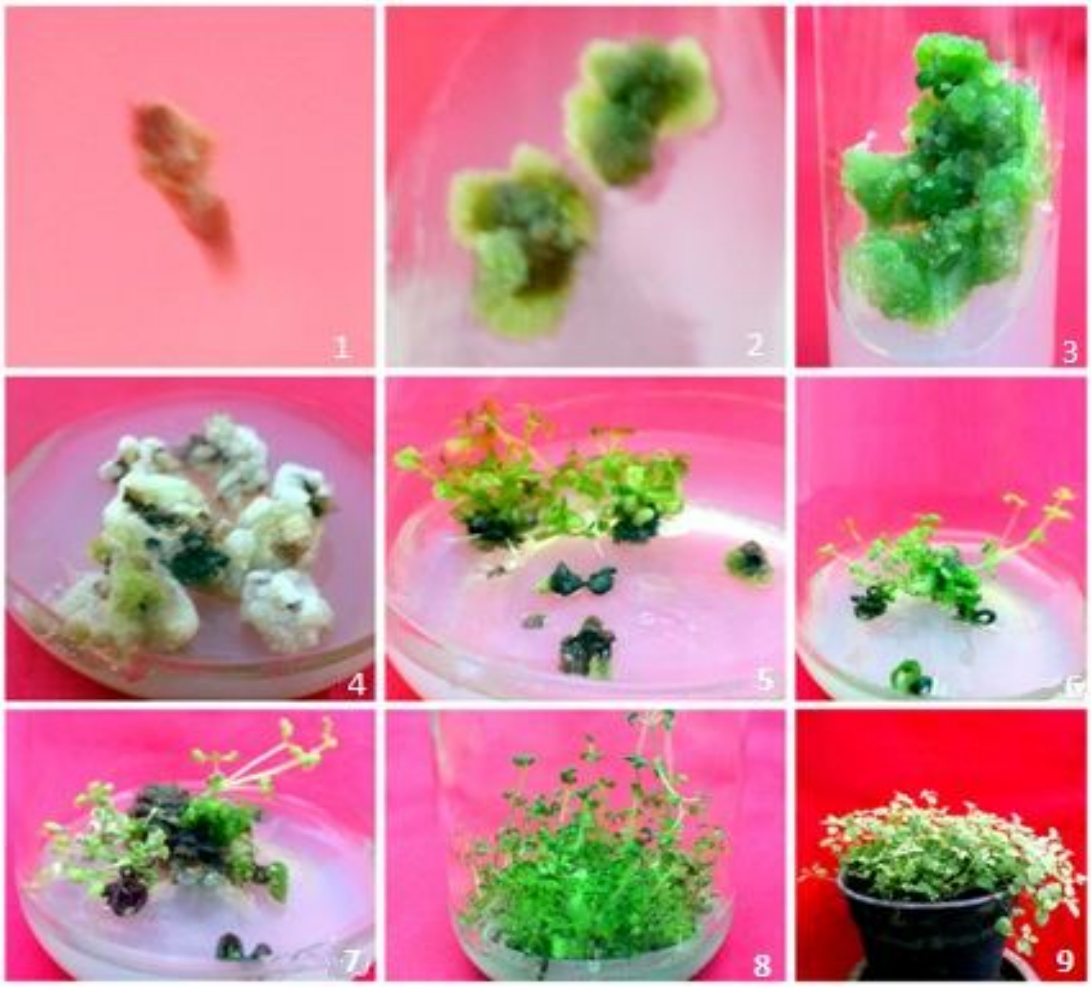
Note: * : Mean of 10 replication
**** : Significance F Value @ 5%level**
\+ : Scanty++ : Moderate +++ : Abundant



Graph. 1: Effect of different concentrations of auxins on *in vitro* apical bud explant of *Origanum vulgare* for callus induction



Graph. 2: Effects of different concentrations of auxins on the growth of the callus from *in vitro* apical bud explant *Origanum Vulgare*



Figures showing various stages of growth of the callus from *in vitro* apical bud of *Origanum Vulgare.*L

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