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Organogenesis in fennel (*Foeniculum vulgare* Mill.)

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ABSTRACT

Organogenesis was achieved in fennel (*Foeniculum vulgare* Mill.) using hypocotyls explant from *in vitro* germinated seedlings. Banzylaminopurin, Naphthaleneacetic acid and Indoleacetic acid are able to induced shoot regeneration but the effect of combination of Banzylaminopurin with Naphthaleneacetic acid was more pronounced. High frequency shoot regeneration (8 shoots explant^{-1}) was obtained on combination of Banzylaminopurin 0.1 mg l^{-1}, Naphthaleneacetic acid 0.1 mg l^{-1} and Indoleacetic acid 0.5 mg l^{-1}. Interaction of Banzylaminopurin with Naphthaleneacetic acid and Indoleacetic acid resulted in increase in number of regenerated shoots. Different kind of callus morphology was observed but it had no relationship with regeneration potential. The regenerated plants are normal and healthy.

Key words : fennel, callus, organogenesis, *Foeniculum vulgare*, shoot regeneration

Abbreviations: BAP=Banzylamino purine; IAA=Indoleacetic acid; NAA=Naphthaleneacetic acid

INTRODUCTION

Application of tissue culture techniques in the improvement of fennel (*Foeniculum vulgare* Mill.) crop is limited. A reproducible and highly regenerating *in vitro* protocol is a pre- requisite before tissue culture system actually used in crop improvement programme for gene insertion and expression. Limited information is available on response of somatic tissues to differentiate and develop plantlets *in vitro* from callus culture of fennel. Tissue culture studies in fennel is however, limited and reported protocols are using different PGR combination and explants (Maheshwari and Gupta 6, Hunault 5, Manoir *et al*. 7, Anzidei *et al*. 1, Anzidei *et al*. 2, Fiore *et al*. 4). The purpose of this study was to develop regeneration protocol and exploit somaclonal variation in regenerated plants for crop improvement.

MATERIALS AND METHODS

Clean and healthy seeds of fennel variety AF 1 were obtained from seed store of National Research Centre on Seed Spices, Ajmer and surface sterilized with 0.1% mercuric chloride for 4 minutes and thoroughly washed with sterilized distilled water 4-5 times. These surface sterilized seeds were placed in a sterilized test tube on a filter paper for germination in a BOD at 25^{o}C. The lower half of filter paper was immerged in distilled water. Hypocotyl segment from germinated seedlings (10-15 days old) were used as explants and inoculated on MS medium (Murashige and Skoog, 8) supplemented with various concentration of BAP, NAA and IAA (0.1 -1.0 mg l^{-1}) alone and in combination. Cultures were incubated at 27±1^{0}C under fluorescent light in a 14/10 hrs. photo period. Light intensity was maintained at 35 µmol M^{-2}S^{-1} at bench level. Observations were recorded periodically on number of explants inoculated and responded, morphology of callus and number of shoots explant^{-1}.

RESULTS AND DISCUSSION

Response was increased from 50 to 75 per cent as the concentration of BAP increased in the medium from 0.1 to 1.0 mg l^{-l}. Number of days to callus induction was slightly less on higher concentration of BAP. In all the treatment produced callus was of good quality with yellow and friable morphology. None of the treatment having BAP alone showed shoot regeneration from callus (Table 1).

Almost 100 per cent explants responded on medium supplemented with high concentration of NAA i.e. 1.0 mg l^{-1}. At all the concentration produced callus was of good quality with yellow and compact callus morphology. The shoot initiation was observed on either low or higher concentration of NAA in the medium. Three shoots explant^{-1} were recovered on these treatments. Response was further improved when NAA was replaced with IAA as alternative auxin source. As compare to NAA, 100 per cent explants responded on the medium supplemented with low concentration (0.1 mg l^{-1}) of IAA (Table 1). Higher concentration of IAA produced less response; however, both low and high concentration of IAA resulted in good number of regenerated shoots (Table 1).

Combination of similar concentration of BAP and NAA (0.1, 0.5 and 1.0 mg l^{-1}) in the medium produced

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good quality callus with 5 shoot explants^{-1} on medium supplemented with each BAP and NAA 0.1 mg l^{-1}. The quality of produced callus was also very good with yellow and friable callus morphology. However, no shoots were produced on higher concentration of BAP and NAA while 6 shoots explant^{-1} were produced on 0.5 mg l^{-1} each of BAP and NAA. Combination of BAP and IAA produced poor response. Callus produced was of good quality on all the concentration with yellow and compact morphology but number of shoots explant^{-1} was significantly less as compare to BAP + NAA combination. This may be due to the fact that IAA is a natural auxin which is less stable in the medium as compare to NAA.

To see the combinational effect of BAP, NAA and IAA on regeneration, MS medium was supplemented with all three PGR's with different concentration. All treatments

Table 1. Effect of BAP, NAA and IAA on regeneration from hypocotyl explant of fennel

Treatments Details (MS medium + BAP/IAA/NAA mg l^{-1})	% response	No. of days to callus induction	Callus morphology	No. of shoot explant^{-1}
0.1 BAP	50.00	9	YF	0
0.5 BAP	62.50	9	YF	0
1.0 BAP	75.00	8	YF	0
0.1 NAA	54.54	9	YC	3
0.5 NAA	77.77	9	YC	Callus
1.0 NAA	100	9	YC	3
0.1 IAA	100	9	YC	6
0.5 IAA	100	9	YC	5
1.0 IAA	78.57	9	GF	6
0.1+0.1 BAP/NAA	55.55	9	YF	5
0.5+0.5 BAP/NAA	100	9	YF	6
1.0+1.0 BAP/NAA	100	9	YF	0
0.1+0.1 BAP/IAA	69.23	9	YC	3
0.5+0.5 BAP/IAA	75.00	8	YC	3
1.0+1.0 BAP/IAA	50.00	9	YC	1
0.5+1+0.5(BAP/NAA/IAA)	92.30	9	YF	6
1+0.1+1.0 (BAP/NAA/IAA)	90.90	9	YF	callus
0.1+0.5+0.1(BAP/NAA/IAA)	100	9	BC	2
0.1+0.5+1.0 (BAP/NAA/IAA)	100	9	YF	callus
0.1+1.0+0.1 (BAP/NAA/IAA)	100	9	YF	6
0.5+1.0+0.5 (BAP/NAA/IAA)	93.75	9	GF	callus
0.5+0.5+0.1 (BAP/NAA/IAA)	100	9	GF	2
1.0+1.0+0.1 (BAP/NAA/IAA)	100	9	GF	Rooting
0.1+0.1+0.5 (BAP/NAA/IAA)	100	9	GF	8
1.0+1.0+0.5 (BAP/NAA/IAA)	100	9	GF	Callus
0.1+0.1+1.0 (BAP/NAA/IAA)	100	9	GF	2
0.5+0.5+1.0 (BAP/NAA/IAA)	84.21	9	GF	Callus
Mean	84.97	8.92	-	2.38
SD (±)	18.07	0.27	-	2.61

YF = Yellow Friable, YC = Yellow Compact, GF = Green Friable, BC = Brown Compact

combination produced 100 per cent response with good to very good amount of callus. Produced callus was green and friable in most of the treatments. However, not all the treatments proved organogenic as many treatments produced only callus. Treatment having low concentration of BAP and NAA (0.1mg l^{-1}) and slightly high concentration of IAA (0.5mg l^{-1}) produced significantly high number of shoots explants^{-1} (8 shoots explants^{-1}). Regenerated shoots were successfully rooted on MS medium supplemented with IBA 0.5 mg l^{-1}

Plant regeneration from cultured cells and tissues is achieved largely by application of exogenous plant growth regulators. There are a number of reports regarding *in vitro* regeneration and callus induction of fennel by using various explants such as hypocotyl or stem explants (Bennici *et al*. 3). Anzidei *et al*. (1) reported that callus induction and organogenic response of several fennel populations was clearly genotype and growth regulator dependent. In present investigation we tried commonly used plant growth regulators in fairly good number of combination by taking hypocotyl explants. Days to callus induction and time taken in shoot regeneration were far less as compare to previous reports.

In present study we used BAP as the source of cytokinin. BAP is the most commonly used synthetic cytokinin in tissue culture. Being a synthetic hormone it remain stable in the system for longer time. The BAP was also tested in combination with IAA/NAA to see their synergistic effect on regeneration frequency. It is clearly evident the synergistic effect of BAP and auxin on regeneration frequency. Alone BAP was not resulted in shoot regeneration. Between NAA and IAA, NAA proved better, Combination of all three PGR i.e. BAP, IAA and NAA was however more efficient but concentration dependent.

It was also observed that callus morphology is an important trait for assessment of morphogenic potential of callus. Friable callus generally proved non organogenic while green compact callus is indicative of organogenic callus. In present investigation we observed almost all types of callus morphology and it had no relation with regeneration potential in fennel. The regenerated plants were normal and such high frequency regeneration protocol can effectively be used for gene transformation.

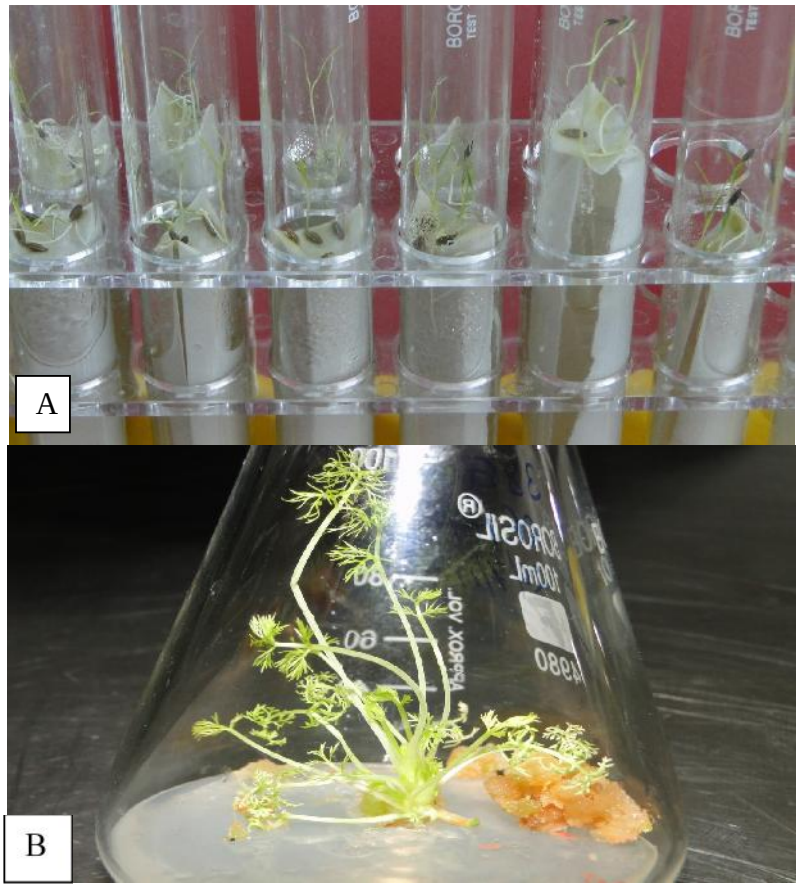

Fig 1 A: *In vitro* germinated seeds of fennel B: Well developed shoots from hypocotyls callus of fennel

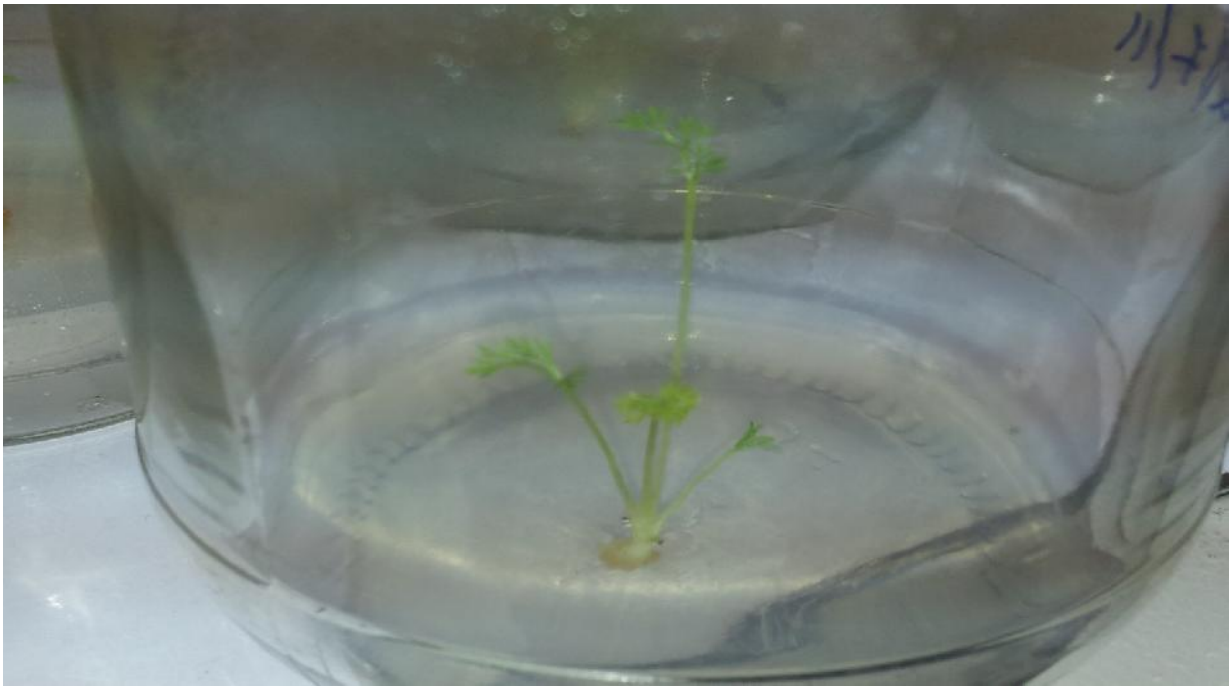

Fig 2: Regenerated shoot with rooting

REFERENCES:

1. Anzidei, M., Vivona, L., Schifff, S., Bennici, A. 1996. *In vitro* culture of *Foenicumlum vulgare:*Callus characteristics in relation to morphogenesis, *Plant Cell Tiss. Org. Cult.* **45**: 263-68.
2. Anizidei, M., Bennici, A., Schiff, S., Tani, C., Mori, B. 2004. Embryogenesis in *Foeniculum vulgare* histological observation of developing Embryogenic callus. *Plant cell, Tiss. and Organ cult*. **61**: 69-79.
3. Bennici, A., Anzidei, M. and Vendramin, G.G. 2004. Genitic stability and uniformity of *Foeniculum Vulgare* Mill. regenerated plants through organogenesis and somatic embryogenesis. *Plant Sci.* **166**: 221-27.
4. Fiore, M. C., Sunseri, F., Carra, A. and Carimi, F. 2010. Somatic embryogenesis and plants regeneration from immature inflorescence of Sicilian fennel ecotypes. Proceeding of the 54th Italian Society of Agricultural Genetics Annual Congress Matera, Italy. 27-30.
5. Hunault, G. 1984. *In vitro* culture of fennel tissues (*Foeniculum Vulgare*) from cell suspension to mature plant. *Scientia Horti.* **22**: 55-65
6. Maheshwari, S.C., Gupta, G.R.P. 1965. Production of adventitious embryoids *in vitro* from stem callus of *Foeniculum vulgare*. *Planta* **67**: 384-86.
7. Manoir, J. D., Desmarest, P. and Saussay, R. 1985. *In vitro* propagation of fennel (*Foeniculum vulgare*). *Scientia Hort.* **27**: 15-19.
8. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco culture. *Physiol. Plant*. **15**: 473-97.

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