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ORIGINAL ARTICLE

In Vitro Regeneration in Fenugreek (*Trigonella foenum-graecum* L.)

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ABSTRACT
The present investigation was carried out with the objectives of optimization of the condition for callus induction in shoot apex explants and explore the possibility of regeneration in callus culture. Shoot apex explants were inoculated on MS medium containing varying concentrations of cytokinins and auxins either singly or in combinations. The cultures were incubated at 25 ± 2^{0}C with a light intensity of 3000 – 3500 lux. Maximum callus induction was observed in shoot apex explant among all the explants, when MS medium supplemented with 0.5 mg/l BAP + 0.5 mg/l 2,4-D with 100 per cent frequency. Among genotypes maximum callus induction was observed in RMt-305 in shoot apex explant on MS medium at responsive level (0.5 mg/l BAP + 0.5 mg/l 2,4-D). De novo shoot development was observed in callus derived from shoot apex explant on 0.5 mg/l BAP + 0.5 mg/l 2,4-D upon subsequent subculturing on 0.5 mg/l BAP. Root induction in de novo developed shoots were also observed at 0.2 mg/l IAA with 70 per cent frequency.
Keywords:- *Micropropogation, in vitro, shoot apex, subculturing.*

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INTRODUCTION

Fenugreek (*Trigonella foenum- graecum* L.) is an important annual herb belonging to the sub-family papilionaceae of the family fabaceae. It is a self-pollinated crop. The plant grows up to 50 to 60 cm height. It is diploid species with chromosome number of 2n = 16 [1]. The place of origin of fenugreek supposed to between Iran and North India [2]. It is mainly a condiment crop, popularly known by its vernacular name "methi". It can be grown under wide range of climatic conditions, requires a cool climate and it can tolerate frost and high humidity.

Fenugreek is an important vegetable, spice and medicinal legume plant used as fresh and dried leaves and seeds in many parts of the world [3]. It is assumed to possess nutritive and restorative properties. The young leaves and sprouts are good source of protein, mineral and vitamin C [4, 5]. Rao and Sharma [6] reported that fenugreek seeds contain 25.5% protein, 7.9% fat, 20% mucilaginous matter and 4.8% saponins. Seeds from fenugreek have been extracted for polysaccharide, galactomannan, different saponins such as diosgenin, yamogenin, mucilage, volatile oil and alkaloids such as choline and trigonelline [7]. Trigonelline, coumarin and nicotinic acid have been isolated from fenugreek seeds and shown to be useful in diabetes [8]. Productivity of fenugreek is low which is due to several causes such as its cultivation on marginal lands with poor fertility, susceptibility to diseases like powdery mildew, wilt, root rot and poor adoption of improved agronomic packages and practices. The present study have been undertaken to explore *in vitro* potentials of plant cell culture techniques and development of regeneration protocol in fenugreek.

MATERIAL AND METHODS

Seed were taken from five genotypes of fenugreek i.e. RMt-1, RMt-303, RMt-305, RMt-365 and Hissar sonali, mainly the genotype RMt-1 was used for various studies. All other genotypes were tested on responsive levels of plant growth regulators.

To obtain aseptic seedlings, seeds of different genotypes were surface sterilized with 0.1% mercuric chloride for 2.5 minutes then washed thoroughly with sterilized double distilled water for 3-4 times. The seeds were then transferred aseptically to sterilized culture tubes containing paper bridge partly dipped

in ½ MS medium. The scalpel, inoculation needle, forceps were kept in rectified spirit and flamed before use. Shoot apex explants, excised from 17-19 days old seedling of different genotypes and were placed in sterilized distilled water. While inoculating, care was taken to obtain explants only from healthy seedlings, avoiding pre-existing meristems on nodal region and to keep uniformity in size. These explants were inoculated in 100 ml, wide neck Erlenmeyer conical flasks and test tubes, each dispensed with 40 ml and 20 ml of the culture medium, respectively. All the aseptic manipulation was done in a laminar flow chamber. The chamber was sterilized by ultraviolet irradiation for about 30 minutes.

Different concentrations of auxin (BAP, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) and cytokinin (2,4-D, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0mg/l) were incorporated singly and in combinations BAP (0.5 mg/l) + 2,4-D (0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) in the MS medium to induce the callus in shoot apex, cotyledon and hypocotyl explant of fenugreek.

Different fenugreek genotypes were assessed at responsive level of plant growth regulator for callus induction and *de novo* organogenesis. To initiate organogenesis, the level of auxin (NAA- 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) and cytokinin (BAP- 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) incorporated singly was worked out empirically. The *de novo* developed shoot was subjected to different levels of (IAA- 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 mg/l) for induction of roots.

RESULTS AND DISCUSSION

When shoot apex explants of fenugreek incubated on MS medium without any plant growth regulator did not show any response. When BAP (0.1-5.0 mg/l) added singly in medium callus induction was initiated within 13-15 days of incubation. Maximum callus induction were observed at 4.0 mg/l BAP followed by 3.0 mg/l BAP with 80 per cent frequency (Table 1). Multiple shoots and callus have been induced under the influence of cytokinin from shoot tip of *Foeniculum vulgare* [9-15].

Table 1 : Morphogenetic effects of various concentrations of cytokinin (BAP) added singly in the MS medium on shoot apex explants of fenugreek variety RMt-1

Concentr-ation BAP (mg/l)	Response (%)	Days taken in callus initiation	Colour of induced callus	Texture of callus	Morphogenetic response	Fresh weight of callus (mg)
Explant - shoot apex						
0.1	60	13.66	Pale yellow	Compact	C^{+}	147.66
0.5	60	14.00	Brown	Loose	C^{++}	370.00
1.0	70	14.14	Green	Loose	C^{+++}	620.85
2.0	70	12.87	Brown	Compact	C^{+++}	792.50
3.0	80	14.37	Light green	Compact	C^{+++}	801.50
4.0	80	13.57	Light green	Loose	C^{+++}	1281.14
5.0	100	14.30	Green	Compact	C^{++}	550.60

C+++ = Profuse callus, C++=medium callus, C+=slight callus

Likewise BAP, auxin (2,4-D) also induced callus in shoot apex explants when added singly in basal medium. However, the callus induction was comparatively low in all the explants of fenugreek (Table 2). 2,4-D also induced maximum callus on fresh weight basis at 2.0 mg/l level in shoot apex explants of fenugreek with 80 per cent frequency. Similar observation of callus induction on 2,4-D was also observe in fennel by Anzidei *et al.,* [16], Grzebelus *et al.,* [17] in carrot leaf and hypocotyls explants and Jamshidi *et al.,* [18] in fenugreek leaf explant.

When the combination of BAP (0.5 mg/l) with 2,4-D (0.1-5.0 mg/l) added to the basal medium callus induction was observed in shoot apex explants (Table 3). Maximum fresh callus weight was observed in shoot apex explants at 0.5 mg/l BAP + 0.5 mg/l 2,4-D followed by cotyledon at 0.5 mg/l + 1.0 mg/l 2,4-D (Fig. 1). Thus shoot apex derived callus at 0.5 mg/l BAP+ 0.5 mg/l 2,4-D was considered as most responsive combination of plant growth regulator for callus induction in fenugreek. Combination of cytokinin and auxin has been reported to induce callus from shoot apex explant in many plant species [15]. This callus remained potent during subsequent subculturing without any morphogenesis.

Shoot apex explants obtained from aseptically grown seedlings of genotypes ((RMt-1, RMt-303, RMt-305, RMt-365 and Hissar Sonali were incubated on MS medium supplemented with best responsive level (0.5 mg/l BAP + 0.5 mg/l 2,4-D) of plant growth regulators to induce callus. Profuse callus induction was observed in all genotypes except Hissar Sonali (Fig. 2). The callus induction delayed in Hissar Sonali along with decrease in the frequency of callus induction. Maximum callus induction was observed in RMt-305 followed by RMt-1. These results are also similar with the earlier observation of Sanatombi and Sharma, [21] in Capsicum, Michel *et al.,* [19] and Payghamzadeh and Kazemitadar, [20] in cotton. Frequency of

callus induction was 100 per cent in all genotypes except Hissar Sonali (80 per cent). Perusal of Table 4 indicated significant difference in callus induction among different genotypes.

Table 2 : Morphogenetic effects of various concentrations of auxin (2, 4-D) added singly in the MS medium on shoot apex explants of fenugreek variety RMt-1

Concentration 2,4-D (mg/l)	Response (%)	Days taken in callus initiation	Colour of induced callus	Texture of callus	Morphogenetic response	Fresh weight of callus (mg)
Explant - shoot apex						
0.1	80	14.38	Yellow	Friable	C^{+}	112.50
0.5	80	14.37	Yellow	Friable	C^{+}	217.38
1.0	80	14.25	Light green	Semi-compact	C^{+}	258.62
2.0	80	11.50	Light green	Friable	C^{++}	360.50
3.0	80	11.87	Pale yellow	Friable	C^{+++}	608.87
4.0	80	10.75	Pale yellow	Friable	C^{+}	297.62
5.0	90	10.88	Light green	Friable	C^{++}	363.22

C^{+++} = Profuse callus, C^{++}=medium callus, C^{+}=slight callus

Table 3 : Morphogenetic effects of various concentrations of cytokinin (BAP) and auxin (2,4-D) added in combination in the MS medium on shoot apex explants of fenugreek variety RMt-1

Concentration BAP + 2,4-D (mg/l)	Response-se (%)	Days taken in callus initiation	Colour of induced callus	Texture of callus	Morpogenetic response	Fresh weight of callus (mg)
Explant - shoot apex						
0.5+0.1	100	12.60	Green	Compact	C^{+++}	1258.20
0.5+0.5	100	9.33	Green	Semi-compact	C^{+++}	2297.44
0.5+1.0	100	9.77	Green	Semi-compact	C^{+++}	1387.30
0.5+2.0	80	9.12	Pale green	Compact	C^{+++}	1180.37
0.5+3.0	90	9.11	Green	Compact	C^{+++}	793.11
0.5+4.0	100	9.40	Pale green	Loose	C^{+++}	844.90
0.5+5.0	70	10.14	Pale green	Semi- compact	C^{++}	517.71

C^{+++} = Profuse callus, C^{++}=medium callus, C^{+}=slight callus

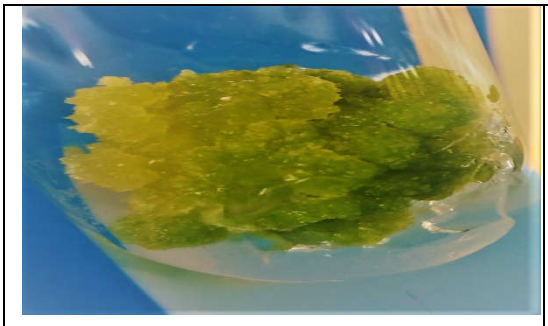

Fig. 1 Profuse callus induction in shoot apex explants on MS Medium supplemented with 0.5 mg/l BAP+0.5 mg/l 2,4- D.

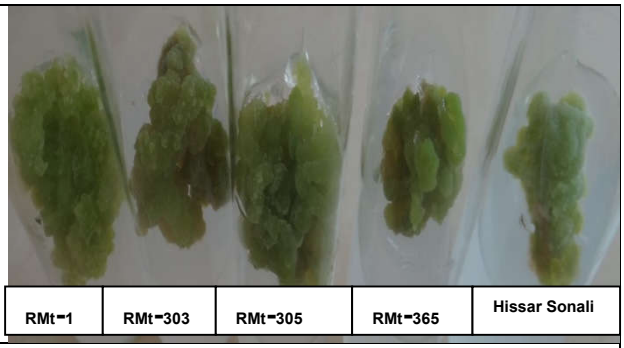

Fig. 2 Callus induction in shoot apex explants of different fenugreek genotypes on MS medium supplemented with 0.5 mg/l BAP + 0.5 mg/l 2,4-D.

Upon subculture of stock callus cultures, shoot morphogenesis was started after 15-18 days of incubation of shoot apex derived callus on MS medium supplemented with 0.5 mg/l BAP. The reproducibility of this morphogenesis was 20-70 per cent in different genotypes of fenugreek. Genotype RMt-305 showed 70 per cent *de novo* shoot morphogenesis followed by RMt-1and RMt-303. Genotype RMt-305 induced maximum *de novo* shoots from callus followed by RMt-1(Fig.3). There was significant difference among the genotypes to induced *de novo* shoots from callus (Table 5). The shoot apex derived callus upon subculture did not differentiate shoot bud in genotype Hissar Sonali on the media conducive for shoot

organogenesis in all other genotypes. Only callus growth was observed. Anzidei *et al.* [22] established regenerating callus from hypocotyl explants of the fennel. Limited shoot organogensis also reported in cotyledon derived callus of coriander [23], shoot apex derived callus of cumin [24], embryo derived callus of cumin [25] and regeneration in somatic embryos derived from protoplast in coriander [26].

Table 4 : Effect of 0.5 mg/l BAP + 0.5 mg/l 2,4-D on callus induction in shoot apex explants of different fenugreek genotypes

Genotype	Response (%)	Days taken in callus initiation	Colour of callus	Texture of callus	Morphogenetic response	Fresh weight (mg)
RMt-1	100	13.20	Pale green	Compact	C^{+++}	46.66 # (2178.50)
RMt-303	100	14.20	Pale green	Compact	C^{+++}	43.46 (1898.10)
RMt-305	100	14.70	Green	Compact	C^{+++}	49.66 (2460.00)
RMt-365	100	14.00	Green	Compact	C^{+++}	40.85 (1644.80)
Hissar sonali	80	14.62	Green	Compact	C^{++}	19.73 (588.37)
CD at 5 %						5.64

C^{+++} = Profuse callus, C^{++}=medium callus, # = transformed values, ( ) = values in parenthesis represents mean callus weight

Table 5 : Regeneration in shoot apex explant derived callus (0.5 mg/l BAP + 0.5 mg/l 2,4-D) in different genotypes of fenugreek in MS medium supplemented with 0.5 mg/l BAP

S. No.	Genotypes	Number of *de novo* developed shoot	Frequency (%)
1	RMt-1	1.41# (1.48)	50
2	RMt-303	1.22 (0.98)	60
3	RMt-305	1.49 (1.72)	70
4	RMt-365	0.81 (0.15)	20
5	Hissar sonali	0.7 (-)	-
CD at 5 %		0.54	

= transformed values, (-) = No response, ( ) = values in parenthesis represents mean *de novo* developed shoots

Fig. 3 *De novo* shoot regeneration from shoot apex derived callus on 0.5 mg/l BAP + 0.5 mg/l 2,4-D after subculture on 0.5 mg/l BAP.

Fig. 4 Induction of root at 0.2 mg/l IAA.

Induction of thick/thin and short root morphology was observed in *de novo* regenerated plantlets on MS medium supplemented with 0.1- 0.4 mg/l level of IAA. Higher level of IAA (0.5-0.7 mg/l) completely inhibited root induction in regenerated shoots (Table 6). Maximum root induction was observed at 0.2 mg/l IAA with 70 per cent frequency (Fig. 4). Auxins when incorporated singly in the culture medium have been reported to induce roots in carrot [27], *Ananas Comosus* [28], *Gmelina arborea* [29], *Solanum melongena* [30] and fenugreek [31, 32, 33].

Table 6 : Effect of IAA on root induction

Concentration (mg/l)	Morphogenetic response (%)	No. of root/ plant	Root morphology
0.1	50	1.2	Thick & medium
0.2	70	1.4	Thick & short
0.3	20	0.9	Thick & short
0.4	10	0.6	Thin & short
0.5	-	-	-
0.6	-	-	-
0.7	-	-	-

(-) = no response

REFERENCES

1. Frayer, J. R. (1930). Chromosome Atlas of flowering plants. George Allen and Urwin, London, pp: 519.
2. Smith, A. (1982). Selected materials for turmeric, coriander seed, cumin seed, fenugreek seed and curry powder. Tropical Product Institute, London Co. 165: 7-45.
3. Lewis, G., Schrire, B., MacKinder, B. and Lock, M. (eds). (2005). Legumes of the world. Royal Botanical Gardens, Kew, UK.
4. Khan, M. B., Khan, M. A. and Sheikh, M. (2005). Effect of phosphorus levels on growth and yield of fenugreek. International Journal of Agriculture Biology, 7: 504-507.
5. Chhibba, I. M., Nayyar, V. K. and Kanwar, J. S. (2007). Influence of mode and source of applied Iron on fenugreek (*Trigonella corniculata* L.) in a typic ustochrept in Punjab, International Journal of Agricultural Biology, 9: 254-256.
6. Rao, P.U. and Sharma, R.D. (1987). An evaluation of protein quality in fenugreek seed and their supplementary effect. Food Chemistry, 24(1): 1-9.
7. Aasim, M., Hussain, N., Umer, E. M., Zubair, M., Hussain, S. B., Saeed, S., Rafique, T. S. and Sancak, C., (2010). *In vitro* shoot regeneration of fenugreek (*Trigonella foenum-graecum* L.). American Journal of Biotechnology, 9(42): 7142-7179.
8. Moorthy, R., Prabhu, K. M. and Murthy, P. S. (2010). Antihyperglycemic compound (G) from fenugreek seeds, its purification and effect in diabetes mellitus. Indian Journal of Experimental Botany, 48: 1111- 1118.
9. Miura, Y., Fukui, H. and Tabata, M. (1987). Clonal propagation of chemically uniform fennel plants through somatic embryos. Plant Medica, 53: 92-94.
10. Hunault, C., Desmarest, P. and Du Manior, (1989). *Foeniculum vulgare* : Cell culture, regeneration and the production of anethole. In : Biotechnology in Agriculture and Forestry, Medicinal and Aromatic Plants II (Ed. Y.P.S. Bajaj) Springer-Verlag, Berlin, 7: 185-209.
11. Hunault, C.. and Du Manior, (1992). Micropropagation of fennel (*Foeniculum vulgare* Mill). In: Biotechnology in Agriculture and Forestry, High-Tech Micropropagation III (Ed. Y.P.S. Bajaj) Springer-Verlag, Berlin,19: 199-217.
12. Lourdes, B. C. and Alfermann, A. W. (1994). Somatic embryogenesis in some apiaceae species. In: Abstracts 8th Asian Symposium on Medicinal Plants and Spices National production, pp 12-16.
13. Didel, R. P., Jakhar, M. L., Rathore, V. S., Jain, U. K. and Rajput, S. S. (2004). Effect of plant growth regulators on *in vitro* cultures of fennel (*Foeniculum vulgare* Mill.) genotypes. In National Seminar on New Perspectives in Commercial Cultivation, Processing and Marketing of Seed Spices and Medicinal Plant Organized by Rajasthan Agricultural University at Jobner from 25-26 March, 2004., pp. 4.
14. Jakhar, M. L. and Choudhary, M. R. (2012). Regeneration of *in vitro* plantlets through organogenesis in fennel (*Foeniculum vulgare* Mill.). The Journal of Plant Science Research, 28(2): 65-71.
15. Torabi S., Far, M. S., Omidi, M., Gharakhanlou, H. and Saffar, Z. N., (2014). A survey of the best growing conditions of *in vitro* culture in fennel different genotypes for mass production. International Journal of Biosciences, 5(4): 7-17.
16. Anzidei, M., Vivona, L., Schiff, S. and Bennici, A. (1996). *In vitro* culture of *Foeniculum vulgare* : callus characteristics in relation to morphogenesis. Plant cell Tissue and Organ Culture, 45(3): 263-268.
17. Grzebelus, E., Szklarezyk, M. and Baranski, R. (2012). An improved protocol for regeneration from leaf and hypocotyls derived protoplasts of carrot. Plant Cell Tissue Organ Culture, 109(1): 101-109.
18. Jamshidi, S., Lahouti, M. and Ganjeali, A. (2014). Assessment of callus growth and bio-production of diosgenin in callus culture of *Trigonella foenum-graecum* L. bulletin of environment, Pharmacology and Life Sciences, 3: 191-198.
19. Michel, Z., Hilaire, K. T., Mongomaké, K., Georges, A. N. and Justin, K. Y. (2008). Effect of genotype, explants, growth regulators and sugars on callus induction in cotton (*Gossypium hirsutum* L.). Australian Journal of Crop Science, 2(1): 1-9.
20. Payghamzadeh, K. and Kazemitadar, S. K. (2011). Effects of genotype and explants on callus induction in cotton. African Journal of Biotechnology, 10(3): 290-311.
21. Sanatombi, K. and Sharma, G. J. 2008. *In vitro* plant regeneration in six cultivars of Capsicum spp. Using different explants. Biologia Plantarum, 52(1): 141-145.
22. Anzidei, M., Bennici, A.., Schiff, S., Tani, C. and Mori, B. (2000). Organogenesis and somatic organogenesis in *Foeniculum vulgare*, histological observation of developing embryogenic callus. Plant Cell Tissue and Organ Culture. 61(3): 69-79.

23. Kim, S. W., Park, M. K. and Liu, J. R. (1996). High frequency plant regeneration via somatic somatic embryogenesis in cell suspension cultures of coriander (*Coriandrum sativum* L.). Plant Cell Reports. 15(10): 751-753.
24. Jakhar, M. L., Bhargava, S. C.,Rathore, V. S.; Raje, R. S. and Rajput, S. S. (2004). *In vitro* regeneration in cumin (*Cuminum cyminum* L.). In National Seminar on New Perspectives in Commercial Cultivation, Processing and Marketing of Seed Spices and Medicinal Plants Organized by Rajasthan Agricultural University at Jobner from 25-26 March, 2004. pp. 22-23.
25. Valizadeh, M., Kazemi Tabar, S. K. and Nematzadeh, G. A. (2007). Effect of plant growth regulators on callus induction and regeneration of cumin (*Cuminum cyminum*). Indian Journal of Crop Science, 2(2): 287-290.
26. Mujib, A., Tonk, D. and Ali, M. (2014). Plant regeneration from protoplasts in Indian local *Coriandrum sativum* L. scanning electron microscopy and histological evidences for somatic embryogenesis. Plant Cell Tissue Organ Culture, 117: 323-334.
27. Pant, B. and Manandhar, S. (2007). *In vitro* propagation of carrot (*Daucus carota* L.). Scientific World, 5(5): 51-53.
28. Soneji, J. R., Rao, P. S. and Khatre, M. 2002. *In vitro* regeneration from leaf explants of pine apple (*Ananas comosus* L. Merr.). Journal of Plant Biochemistry, 11: 117-119.
29. Thirunavoukkarasu, M. and Debata, B. K. (1998). Micropropagation of *Gmelina arborea* (Roxb) through axillary bud culture. Indian Journal of Plant Physiology, 3(2): 82-85.
30. Narayanaswamy, S. (1994). Tissue (callus) cultures. In: Plant Cell and Tissue Culture. Madras Science foundation, Madras, pp 51-93.
31. Elnour, M. E. M., Mohammed, L. S. and Saeed, B. E. A. T. (2013). *In vitro* callus induction of fenugreek (*Trigonella foenum-graecum* L.) using different media with different auxins concentration. Agriculture and Biology Journal of North America, 4(3): 243-251.
32. Elaleem, K. G. A., Ahmed, M. M and Saeed, B. E. A. (2014). Study of the *in vitro* callus induction in *Trigonella foenum-graecum* L. from cotyledons and hypocotyls explants supplemented with various plant harmons. International Journal of Current Microbiology and Applied Sciences, 3(12): 486-493.
33. Vaezi, Z., Daneshvar, M. H., Heidari, M. and Chehrazi, C. (2015). Indirect regeneration plant fenugreek (*Trigonella foenum-graecum* L.), with the use of plant growth regulators *in vitro*. Bulletin of Environment, Pharmacology and Life Sciences, 4(5):103-108.

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