Factors affecting *In Vitro* Callus Induction in Fenugreek (*Trigonellafoenum-graecum* L.)

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Abstract
The study investigates the effect of genotypes and culture media on callus induction of fenugreek (Trigonellafoenum-graecumL.). Shoot apex explants of different genotypes 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.

Among all the 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). MS medium induced maximum callus in shoot apex explants in comparison to other media supplemented with 0.5 mg/l BAP + 0.5 mg/l 2,4-D.

Keywords: Fenugreek, MS media, *in vitro*, shoot apex**.**

Introduction

Fenugreek (*Trigonellafoenum-graecum* L.) is an important legume, self-pollinated crop. It is diploid species with chromosome number of 2n = 16.[5] It belongs to the sub-family papilionaceae of the family Fabaceae. The place of origin of fenugreek is supposed to be between Iran and North India.[16] It can be grown under wide range of climatic conditions, requires a cool climate and it can tolerate frost and high humidity. Plants grow erect, semi-erect or branched based on its varieties. It has compound pinnate, trifoliate leaves, axillary white to yellow flowers. It bears light green coloured slender shaped pods having 10-20 seeds. Fenugreek is an important vegetable, spice and medicinal legume plant and its fresh and dried leaves and seeds are used in many parts of the world.[10] Fenugreek seeds contain 25.5% protein, 7.9% fat, 20% mucilaginous matter and 4.8% saponins.[14]

General tendency of humans to traditional medicine is the concern of the harmful health effects of chemical agents that are associated with primary health care and cosmetic ingredients in the plants. One of the many medicinal properties of plants that has been mentioned here is fenugreek. It is assumed to possess nutritive and restorative properties. The young leaves and sprouts are good source of protein, mineral and vitamin C.[2,8] 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.[1] Trigonelline, coumarin and nicotinic acid have been isolated from fenugreek seeds and shown to be useful in diabetes.[12] The productivity of fenugreek is low which is due to several abiotic and biotic factors. The present study has been undertaken to explore *in vitro* potentials of plant cell culture techniques in fenugreek.

Material and Methods

Present study was conducted at Department of Plant Breeding and Genetics of Sri Karan Narendra Agriculture University, Jobner. Seeds were taken from five genotypes of fenugreek i.e. RMt-1, RMt-303, RMt-305, RMt-365 and Hissarsonali, 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 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 explant of fenugreek. Different fenugreek genotypes and culture media were assessed at responsive level of plant growth regulator for callus induction.

Results and Discussion

Effect of Genotypes: To study the effect of genotypic control on morphogenesis, five genotypes of fenugreek (RMt-1, RMt-303, RMt-305, RMt-365 and HissarSonali) were assessed. Shoot apex explants obtained from aseptically grown seedlings of these genotypes 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.

Greenish, compact callus proliferation was observed from the base of shoot apex explant after 13-15 days of incubation in all the genotypes (Fig. 1). Perusal of table 1 indicated significant difference in callus induction among different genotypes. Profuse callus induction was observed in all genotypes except Hissar Sonali. 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. Frequency of callus induction was 100 per cent in all genotypes except Hissar Sonali (80 per cent).

Although once considered, primarily a function of exo-endogenous hormone interaction, callus induction is now known to be highly influenced by the genotype of the plant. Genotypic specificity to callus induction has been reported in a number of plants and variations occur between varieties and even within varieties in an out breeding species.

Results of present investigation indicated that different genotypes exhibited medium to profuse callus at the base of shoot apex explant at most responsive level (Table 1). Genotype RMt-305 produced maximum callus at 0.5 mg/l BAP + 0.5 mg/l 2,4-D on shoot apex explants. Dieterat et al[3] while studying morphogenesis in *Brassica spp.* concluded that variability for *in vitro* expression depends both on the genotype and medium. They suggested that the relative uniformity of behaviour of callus tissues within each genotype strongly indicates that the differences observed between genotypes have a genetic rather than a physiological origin.

Kumar et al[9] reported that effectiveness of callus induction as well as regeneration in cotton reflected the degree of genotypic diversity when cultures were grown on same media and plant growth regulators and same observations were also noticed in the present investigation.

Kale[7] also reported significant genotypic difference for callus induction in ten field bean genotypes under influence of IAA + BA. In the present investigation, strong genotypic influence on the callus induction was observed. Fenugreek variety RMt-305 induced maximum callus at 0.5 mg/l BAP + 0.5 mg/l 2,4-D followed by RMt-1.

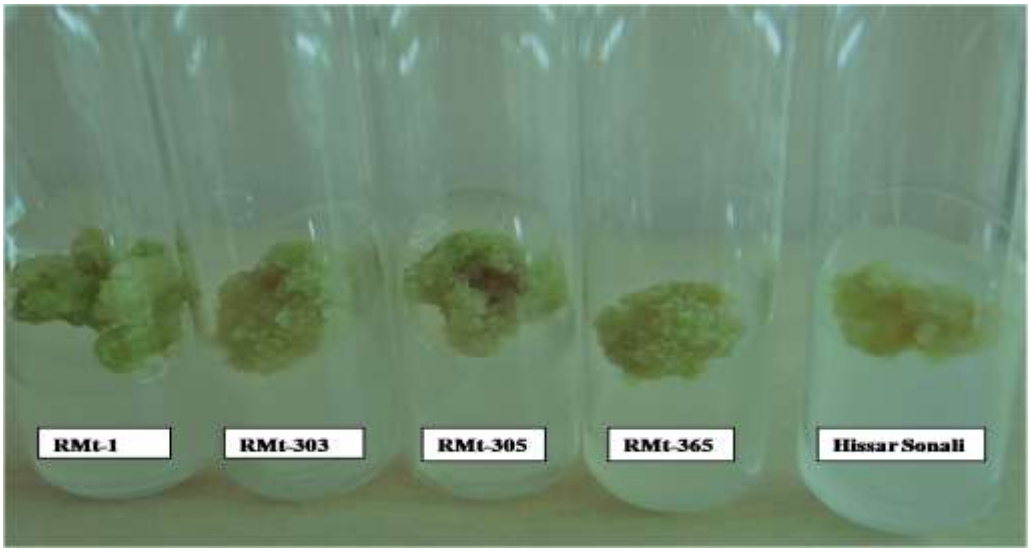

Fig. 1: Callus induction in shoot apex explants of different fenugreek genotypes on MS medium supplemented with 0.5 mg/l BAP + 0.5 mg/l 2,4-D

Table 1
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	Morphoge-netic 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)
Hissarsonali	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

These results are also similar with the earlier observation of Sanatombi and Sharma[15] in *Capsicum*, Michel et al[11] and Payghamzadeh and Kazemitadar[13] in cotton.

Effect of Different Media: To see the effect of different media on callus induction, mainly three types of media (MS medium, White's medium and Nitsch's medium) were used. Different culture media were supplemented with most responding level of 0.5 mg/l BAP in combination with 0.5 mg/l 2,4-D for callus induction. Maximum callus induction was observed in MS medium in shoot apex explant with 100 per cent frequency followed by White's medium and Nitsch's medium. There was significant difference among the media to induced callus. Green and compact callus induction was observed in MS medium after 12-13 days of incubation (Table 2).

Yellow, semi-compact and compact callusing was observed in White's medium and Nitsch's medium respectively after 13-14 days of incubation with 70-80 per cent frequency. Callus induction was comparatively very low in White's medium and Nitsch's in comparision to MS medium (Fig. 2).

Composition of culture medium largely affects the callus induction in different explants. Plant materials do vary in their nutritional requirements and therefore it is necessary to modify the medium to suit a particular tissue. Elnour et al[4] observed that callusing day initiation in fenugreek (*Trigonellafoenum-graecum* L.) from hypocotyl and cotyledon explants was faster in B_5 medium than that culture on MS medium. However, there is no significant difference of callus rate and mean of callusing index. Growth regulator type and concentration have significant effects on the callus induction, the increment of callus index and callus physical appearance. Jamshidi et al[6] observed maximum callus weight on MS medium. These findings were close in agreement to the results obtained in the present study.

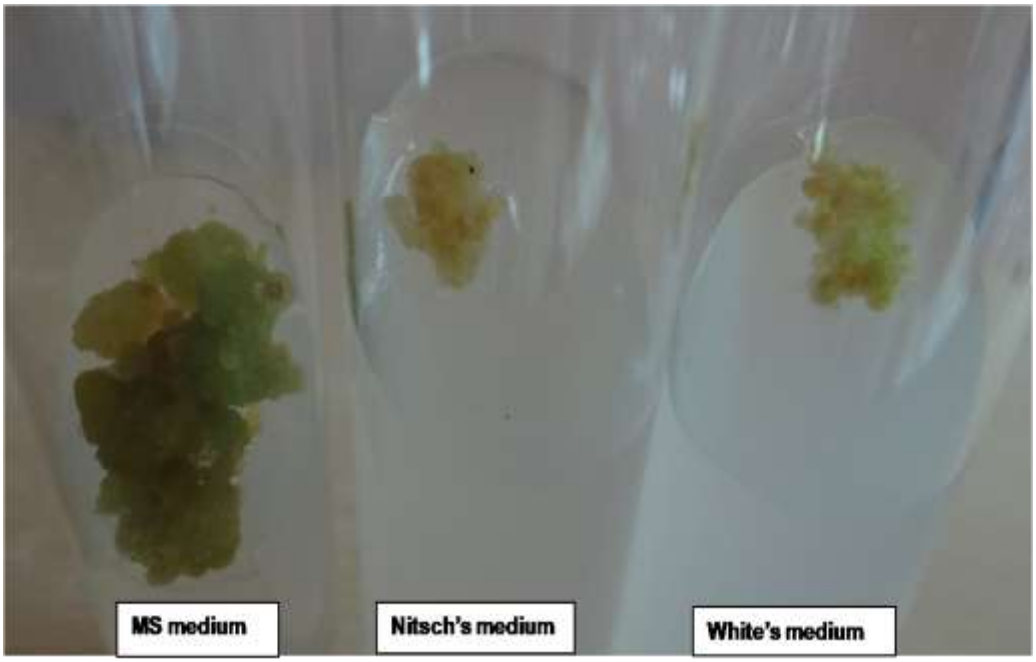

Fig. 2: Callus induction in shoot apex explants of genotype RMt-1on different culture media supplemented with 0.5 mg/l BAP + 0.5mg/l 2,4-D

Table 2
Effect of 0.5 mg/l BAP + 0.5 mg/l 2,4-D on callus induction supplemented with different culture media in shoot apex explants of fenugreek genotype RMt-1

Culture media	Response (%)	Days taken in callus initiation	Colour of callus	Texture of callus	Morphoge-netic response	Fresh weight (mg)
MS medium	100	12.70	Green	Compact	C^{+++}	47.13 # (2168.90)
White' medium	80	14.00	Yellow	Semi-compact	C^{+++}	24.44 (659.25)
Nitsch's medium	70	13.87	Yellow	Compact	C^{+}	10.70 (226.00)
CDat 5 %						**9.38**

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

Conclusion

Based on the present study, it is recommended that callus induction is not only influenced by level of plant growth regulators but also affected by other factors like genotypes and media. Among all the genotypes, maximum callus induction was observed in RMt-305 followed by RMt-1, RMt-303, RMt-365 and HissarSonaliin shoot apex explant supplemented with 0.5 mg/l BAP + 0.5mg/l 2,4-D. Maximum callus induction in shoot apex explants was observed in MS medium followed by White's and Nitsch's media supplemented with 0.5 mg/l BAP + 0.5 mg/l 2,4-D.

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