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Effect of different media and genotypes on *In Vitro* Callus Induction in Fenugreek (*Trigonella foenum-graecum* L.)

Anita Burdak[1], Anurag Tripathi[2] and Jeet Ram Choudhary[3]

[1]Jobner, Distt- Jaipur (Raj.) 303329
[2]GBPUAT, Pantnagar, Uttrakand.
[3]Indian Agricultural Research Institute, New Delhi-110012

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Abstract

The study examines the effect of genotypes and culture media on the induction of fenugreek callus (Trigonella foenum-graecum L.). Shoot apex explants of different genotypes were inoculated into MS medium containing concentrations of cytokinins and auxins individually or in combination. The cultures were incubated at a temperature of 25 ± 2° C and an intensity of 3000-3500 lux. Among all genotypes, the maximum callus induction was found in RMt-303 in a shoot apex explant on MS medium at the responsive level (0.75 mg / L BAP + 0.75 mg / L 2, 4-D). MS medium induced maximal callus in shoot apex explants compared to other media supplemented with 0.75 mg / L BAP + 0.75 mg / L 2,4-D.

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

Introduction

Fenugreek (trigonellafoenum-graecum l.) crop is an important legume, self-pollinated crop. It is a diploid species with 2n = 16 chromosomes (frayer, 1930). It belongs to the papilionaceae subfamily of the fabaceae family. The origin of fenugreek has been suggested to be between Iran and northern India (smith, 1982). It can be grown in a wide range of climates, requires a cool climate, and tolerates frost and high humidity. Plants grow erect, semi-erect, or branched, depending on the variety. It has compound feathery, trifoliate leaves, axillary flowers from white to yellow. It bears light green thin pods with 10-20 seeds. Fenugreek is an important vegetable, spice, and medicinal legume used as fresh and dried leaves and seeds in many parts of the world (Lewis et al. 2008). Fenugreek seeds contain 25.5% protein, 7.9% fat, 20% mucous substances and 4.8% saponins (Rao, Sharma, 1987). One of the many medicinal properties of plants mentioned here is fenugreek, which is believed to have nutritional and regenerating properties. Young leaves and sprouts are a good source of protein, minerals and vitamin C (Khan et al. 2005, Chhibbaetal. 2007). Polysaccharide, galactomannan, various saponins such as diosgenin, yamogenin, mucus, volatile oils, and alkaloids such as choline and trigonelline have been isolated from fenugreek seeds (Aasimetal. 2010). Trigonellin, coumarin and niacin have been isolated from fenugreek seeds and have

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been shown to be beneficial in diabetes (Moorthy et al. 2010). Fenugreek yields are low due to several abiotic and biotic factors. The present study was undertaken to explore the possibilities of in vitro methods for culturing plant cells on fenugreek.

Material and Methods

This study was conducted at the Department of Breeding and Genetics at Sri Karan Narendra Agricultural University, Jobner, in 2016-17. The seeds were taken from five fenugreek genotypes: RMt-303, RMt-1, RMt-365, RMt-305 and Hissar sonali, mainly the RMt-1 genotype was used for various studies. At the time of inoculation, an attempt was made to obtain explants only from healthy seedlings, avoiding the pre-existing meristems in the nodal area and maintaining uniformity in size. These explants were inoculated into 100 ml wide-necked Erlenmeyer flasks and tubes, to each of which were added 40 ml and 20 ml of culture medium, respectively. All aseptic manipulations were performed in a laminar flow chamber. The chamber was sterilized with ultraviolet irradiation for about 30 minutes.

Various concentrations of auxin (BAP, 0.5, 0.75, 1.0, 1.25 1.50, 1.75 and 2.0 mg / L) and cytokinin (2.4-D, 0.5, 0.75, 1.0, 1.25 1.50, 1.75 and 2.0 mg / L) were incorporated individually and in combinations BAP (0.75 mg / L) + 2.4-D (0.5, 0.75, 1.0, 1.25, 1.50, 1.75 and 2.0 mg / k) in MS medium to induce callus in the explant of the shoot apex of the fenugreek. Various fenugreek genotypes and nutrient media were assessed at the level of response of the plant growth regulator to callus induction.

Result and Discussion

Effect of Genotypes

To study the effect of genotypic control on morphogenesis, five fenugreek genotypes were evaluated (RMt-303, RMt-1, RMt-365, RMt-305 and Hissar Sonali). Shoot apex explants obtained from seedlings of these genotypes grown under aseptic conditions were incubated in MS medium with the addition of the most responsive level (0.75 mg / L BAP + 0.75 mg / L 2, 4-D) of plant growth regulators for callus induction.

Greenish compact proliferation of callus was observed from the base of the shoot apex explant after 13-15 days of incubation in all genotypes (Fig. 1). Examination of Table 1 showed a significant difference in callus induction between different genotypes. Abundant callus induction was observed in all genotypes except for Hissar Sonali. Callus induction in Hissar Sonali was delayed along with a decrease in callus induction. Maximum callus induction was observed in RMt-303 and then in RMt-1. The callus induction rate was 100 percent for all genotypes except for Hissar Sonali (80 percent). While it was once thought that callus induction was primarily a function of exoendogenous hormonal interactions, it is now known that callus induction is strongly influenced by the plant genotype. The genotypic specificity of callus induction has been reported in a number of plants, and there are differences between cultivars and even within cultivars in alien species.

The results of the present study showed that different genotypes showed moderate to abundant callus at the base of the shoot apex explant at the most response level (Table 1). The

RMt-303 genotype produced the maximum amount of callus at 0.75 mg / L BAP + 0.75 mg / L 2,4-D on shoot apex explants. Dieterat et al. (1982), studying the morphogenesis of Brassica spp. came to the conclusion that the variability of expression in vitro depends on both the genotype and the medium. They suggested that the relative homogeneity of callus tissue behavior within each genotype strongly suggests that the differences observed between genotypes are of genetic rather than physiological origin. Kumar et al. (2003) reported that the efficiency of callus induction as well as cotton regeneration reflected the degree of genotypic diversity when crops were grown in the same media and plant growth regulators, and the same observations were noted in the present study. Kale (2004) also reported a significant genotypic difference in callus induction in ten genotypes of field beans under the influence of IAA + AD. In the present study, a strong genotypic effect was observed on callus induction. Fenugreek cultivar RMt-305 caused the maximum amount of callus at 0.75 mg / L BAP + 0.75 mg / L 2,4-D followed by RMt-1. These results are also similar to earlier observations by Sanatombi and Sharma, 2008 in Capsicum, Michel et al. et al. 2008 and Paygamzadeh and Kazemitadar 2011 on cotton.

Effect of Different Media

To see the effect of different media on callus induction, mainly three types of media were used (MS medium, White's medium and Nitsch's medium). 0.75 mg / L BAP was added to various culture media in combination with 0.75 mg / L 2,4-D to induce callus. Maximum callus induction was observed in MS medium in the shoot apex explant at 100% frequency, followed by White and Nitsch medium. There was a significant difference between media for induced callus. Induction of green and compact callus was observed in MS medium after 12-13 days of incubation (Table 2). Yellow, semi-compact and compact callus were observed in White's and Nitsch's media, respectively, after 13-14 days of incubation with a frequency of 70-80%. Callus induction was comparatively low for White's and Nitsch's media compared to MS medium (Fig. 2).

The composition of the nutrient medium significantly affects the induction of callus in different explants. Plant materials do differ in nutrient requirements, so the environment needs to be modified to suit the specific tissue. Elnour et al. (2013) observed that the beginning of the callus day in fenugreek (Trigonella foenum-graecum L.) from hypocotyl and cotyledon explants proceeded faster in B5 medium than in culture on MS medium. However, there is no significant difference in callus rate and average callus index, as in the case of growth regulator type and concentration, which have a significant effect on callus induction, increase in callus index and callus appearance. Jamshidi et al. In 2014, the maximum callus weight was observed on MS medium. These results were close to those obtained in the present study.

Conclusion

Based on the present study, it is recommended that callus induction should be influenced not only by the level of plant growth regulators, but also by other factors such as genotypes and media. Among all genotypes, maximum callus induction

was observed in RMt-303, followed by RMt-1, RMt-305, RMt-365 and Hissar Sonali in Shoot apex explant supplemented with 0.75 mg / L BAP + 0.75 mg / L 2.4 -D. Maximum callus induction in Shoot apex explants was observed in MS medium and then in White and Nitsch medium supplemented with 0.75 mg / L BAP + 0.75 mg / L 2,4-D.

Fig. 1 Callus induction in shoot apex explants of different fenugreek genotypes on MS medium supplemented with 0.75 mg/l BAP + 0.75 mg/l 2,4-D.

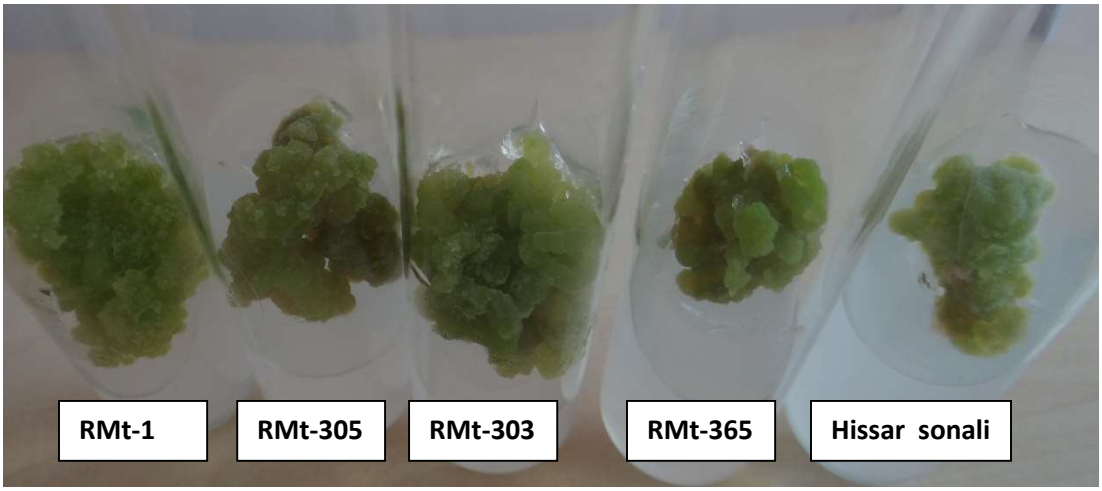

Fig.2. Callus induction in shoot apex explants of genotype RMt-1 on different culture media supplemented with 0.75 mg/l BAP + 0.75 mg/l 2,4-D.

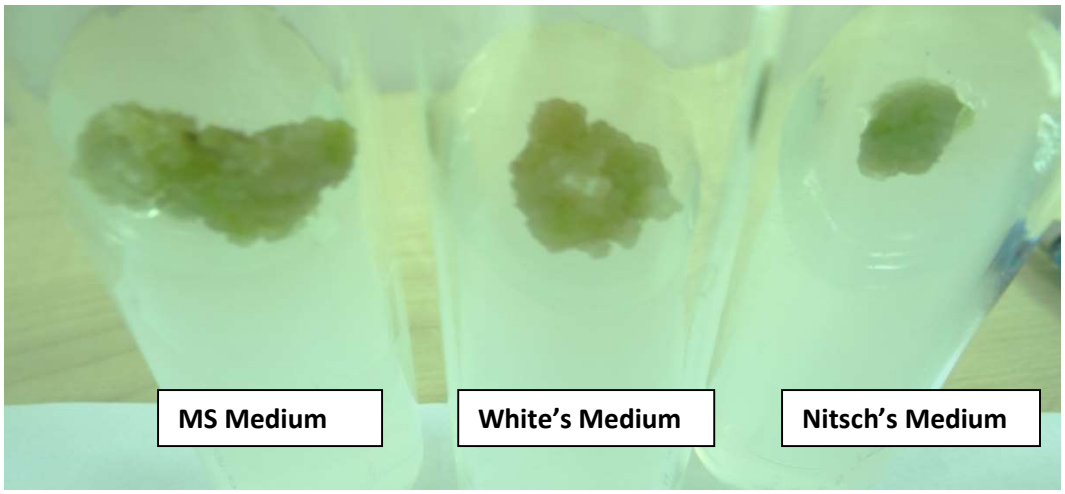

Table 1: Effect of 0.75 mg/l BAP + 0.75 mg/l 2, 4-D on callus induction in shoot apex explants of different fenugreek genotypes

Genotype	Response (%)	Days taken in callus initiation	Colour of callus	Texture of callus	Morphogenetic response	Fresh weight (mg)
RMt-1	100	13.30	Pale green	Compact	C^{+++}	2179.60
RMt-305	100	14.30	Pale green	Compact	C^{+++}	2545.10
RMt-303	100	14.90	Green	Compact	C^{+++}	1919.40
RMt-365	100	14.20	Green	Compact	C^{+++}	1675.50
Hissarsonali	80	14.82	Green	Compact	C^{++}	589.77

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

Table 2: Effect of 0.75 mg/l BAP + 0.75 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	Morphogenetic response	Fresh weight (mg)
MS medium	100	12.90	Green	Compact	C^{+++}	2189.95
White's medium	80	14.20	Yellow	Semi-compact	C^{+++}	665.95
Nitsch's medium	70	14.00	Yellow	Compact	C^{+}	228.20

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

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