International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 02-01-2022, Accepted: 17-01-2022, Published: 02-02-2022
Volume 7, Issue 2, 2022, Page No. 68-71

Rapid growth of *Trigonella foenum–graecum* by *In-vitro* propagation methods

Thirtha M Shetty, Triveni*, Tejashwini B N, Jayarama Reddy
Department of Botany, St. Joseph`s College, 36, Langford Road, Bengaluru, Karnataka, India

Abstract
Fenugreek (*Trigonellafoenum – graecum* L.) is an annual herb and are widely cultivated. It is used as important vegetable, spice and mainly as medicinal plant that are used in some disease therapy (Moradi kor*et al*. 2013).Fenugreek seeds contain a good amount of fiber and minerals, including iron and magnesium. The study Presents an efficient shoot generation protocolfrom within 8-10 days by using the fenugreek seeds for inoculation which is then cultured on Murashige and Skoog (MS) media with varied concentrations of IAA, BAP. The seeds are surface sterilized and inoculated into cultured medium with different concentrations of growth regulators. Presence of auxins in to the culture medium shows the positive increase in shoot length (Aasim*et al*.2010). The MS medium supplemented with hormone 6- Benzyl adenine at the concentration of required pH. In this study, the fenugreek seed shows the shoot initiation with the callus formation. There are few research papers shows that even the callus induction with different cytokinin and other different concentrations in hormones. This paper also shows the shoot generation and then callus formation after 20days with optimal concentrations of the hormones (IAA and BAP).

Keywords: *Trigonella foenum-graecum* l. micropropagation, *In vitro*, hormones, fenugreek seeds, fenugreek

Introduction
Fenugreek (*Trigonella foenum – graecum* L.) is an important annual herb that belongs to the sub-family Papilionaceae of the family fabaceae. India is the one of the largestproducers of fenugreek and occupies the prime position in the world, grown extensively in Rajasthan. It is traditional spice crop that has been grown for centuries and it is self-pollinated crop (Moradi kor*et al*. 2013). Mainly it is a condiment crop, commonly known by its name "methi". The plant grows upto a height of 50-60 cm. It is mainly rich in several phytochemicals, aminoacids and minerals in fenugreek, it can also be used for nutraceutical (Yadav *etal*. 2011) nutritional and therapeutic purposes. Fenugreek can reduce the risk of developing heart conditions and improve the health of the heart and it help`s to improve the blood pressure and regulates the cholesterol levels. The seeds of fenugreek have a good amount of fiber and minerals, including the magnesium and iron content. The leaves are rich in source of vitamin K, Strongly aromatic, flovorful, also used as a carminative, tonicas ayurvedic of Indian medicins. Due to the presence of important phytochemical, they have arich medicinal property. Research on medicinal aspects of fenugreek biproducts has been taken a greater importance in the past few years. Hence, fenugreek productivity is low because of poor fertility in lands and susceptibility to few diseases like root rot and powdery mildew. So, the present study has been undertaken to explore the invitro propagation and development of protocols in fenugreek.

Materials and Methods

Fig 1: A showing the entire plant of fenugreek, Fig-B flower of fenugreek and Fig- c showing fenugreek seeds.

Surface sterilization of explants
Part 1: Outside the LAF or initial part of washing
1. Selected explant must be washed under tapwater thoroughly until all the dirt and debris are washed off.
2. Cut the explant into suitable sizes.
3. Prepare 10% teepol or Tween-20, 0.4 peepol

4. Teepol wash for selected explant for 15 minutes wash it with vigorous shake.
5. Wash off Teepol under running tap water until no foam wash is seen. (foam formation not seen).
6. Transfer the explants into a container or culture bottle having doubled distilled water.

Part 2: Surface sterilization of explant under LAF
1. Shift the explants from part 1 to a bottle containing 0.1% mercuric chloride.
2. Wash the explant in 0.1% mercuric chloride for 15minutes.
3. After 15 minutes of washing, transfer the explants to autoclave double distilled water with a wash for less than one minute.
4. Transfer the explants to hydrogen peroxide solution with a wash of less than one minute.
5. Shift the explants into autoclave double distilled water.

Culture equipment's, such as culture vessels, metallic instruments, aluminum foils, etc. are sometimes sterilized in hot air oven by exposing them to hot air at a temperature of 160^0-180^0C for a period of 2-4 hours. This is a method of 'dry heat sterilization'. Autoclaving is the sterilization of both empty and medium containing culture vessels and some instruments such as micro pipettes, by heating in an autoclave to 121˚C at 15p.s.i (pound per square inch) for 15 to 40 min.

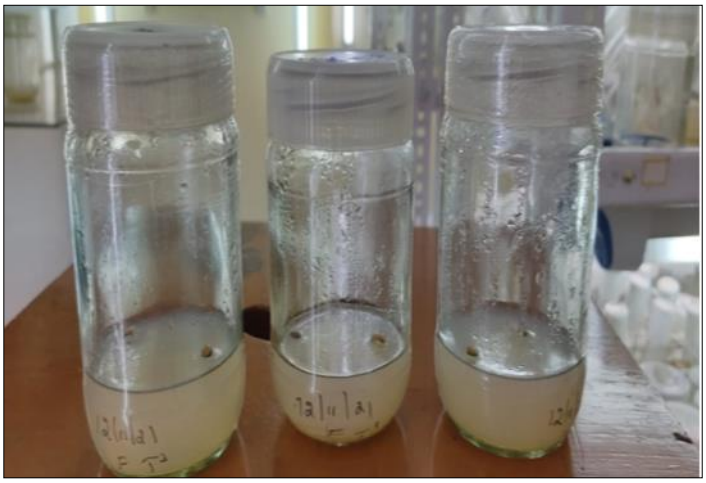

Fig 2: Showing the inoculation of fenugreek seeds in Culture bottles.

Result and Discussion

Table 1

Media	Hormone added	Callus growth	Shoot regeneration	After 10 days	After 20-25 days	After 30-35 days
MS- 1	BAP, IAA	-	Seen	germination	Little callus with germination	Active callus with germination

From the above table, the obtained results were, when MS media is added but, there was no callus growth seen, but actually direct growth of small plantlet was observed from the seed. The plantlet was seen after 10days of inoculation procedure under the maintained conditions. The callus induction and shoot regeneration were observed when kept under MS basal media, this was studied and reported by the previous researchers (Mahmood valizadeh, 2018) [2] and in previous other research papers, there was a callus induction with regeneration with the usage of different cytokinins cited by (Mahammad Aasim *et al* 2010) [13] But this study presents at first only the shoot regeneration was there and no growth of callus was seen so we came to some thought that because this paper doesn`t have different cytokinin components. But after few days I.e, 13-15days we have seen shoot regeneration and also callus induction is obtained. The shoot apex explants of fenugreek incubated on MS medium without any plant growth regulator did not show any response. But when BAP added singly in medium callus induction was indicated within 13-15 days of incubation. This was related to previous research study by Anita Burdak *et al* (2017) [1].
The obtained callus is light green in colour and fragile. But this study shows only shoot regeneration, as the explant was fenugreek seed, but not any other part as explant used in previous studies. The observed plantlet which was obtained can be subcultured for further.

Fig 3: Showing the germination of fenugreek seeds after 10 days of inoculation.

Fig 4: Showing the regeneration of shoot along with the callus induction after 20 days.

Fig 5: Showing after 30 days of inoculation

Acknowledgement
The authors are thankful to St. Joseph's College (Autonomous), Bengaluru for providing us with lab facilities. The guidance received from Dr. Jayarama Reddy and Dr. Martin Paul is also acknowledged and the authors are grateful.

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