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Original Research Article

Study of the in vitro callus induction *Trigonella foenum-graecum* L. from cotyledons and hypocotyls explants supplemented with various plant hormones

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Keywords

Trigonella foenum-graecum, explants, callus, B5 and MS media, hormones

A B S T R A C T

The present study was undertaken to examine the in vitro callus induction using cotyledons and hypocotyls explants of Trigonella foenum-graecum. The explants from in vitro culture were taken and cultured in B5 and MS media supplemented with different concentrations of 2,4-D and NAA, to determine the effect of media and hormones on callus induction. The maximum callus formation was observed in the B5 media containing 2.0 mg/l 2, 4-D is (4.0±0.19) in cotyledon segments, within 5 days followed by 3.4±0.16 induced by concentration 2.0 mg/l NAA within 5 days. MS and B5 media supplemented with 2, 4 D and NAA induced 100% callus with all level of auxins, it was compact and friable and the colors was varv between concentrations.

Introduction

Trigonellafoenum-graecum is medicinal plant extensively distributed in most regions of the world. The genus *trigonella* belongs to the family leguminoceae. Its seeds and leaves are believed to have not only antidiabetic effect [17], but have also been studied for nutritional [15] and therabutical properties widely in the world. The leaves are consumed as rich source of calcium, iron, B carotene and other vitamins [18]. *Trigonella sp* extract is also reported to have Immunomodulatory effects in mice [5]. Seeds are reported to have nutritive properties such as stimulating the digestive processes and treating a number of gastrointestinal disorders in the Indian system of medicine [14]. Plant tissue culture is a practice used to propagate plants under sterile conditions, often to produce clones of plants. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation [22].

Plant callus is a mass of undifferentiated cells derived from plant tissues (explants) for use in biological research and biotechnology. In plant biology, callus cells are those cells that cover a plant wound. Plant hormones, such as auxins, cytokinins,

and gibberellins, are supplemented into the medium to initiate callus formation or somatic embryogenesis [10].

Fenugreek seeds have been extracted for Polysaccharide, galactomannan, different saponins such as Diosgenin, yamogenin, mucilage, volatile oil and alkaloids such as choline and trigonelline [16]

Fenugreek seeds mixed with yogurt are used as a conditioner for hair. Seeds are used for making oily pickles in South Asia. Galactagogue in fenugreek seeds are used to increase milk supply in lactating women [3]. The T. *foenum –graecum*) has many traditional uses in Sudan country, like it used for digestive system attractions, and many other extra uses [7].

Materials and Methods

Seed Material

The seeds of *T.graecum* used in this study were purchased from the local market healthy and uniform, Khartoum in Sudan.

Seed surface sterilization medium and growth regulator's

Seeds free of injuries were washed under running tap water, then treated with 70% alcohol for 1min, then it washed quickly with sterile distilled water, then sterilized for 15 min in 15% Clorox solution (containing 5.25% of sodium hypochlorite) with few drops of liquid soap (tween twenty). Finally the seeds were culture on [18] MS media composed of Macro, micro elements, 30g/l sucrose and 7g/l agar. The pH adjusted to 5.8±0.02. And B5 medium composed of Macro, micro elements, 20 g/l sucrose and 7.0g /l agar. The pH adjusted to5.5±0.02 in MS media prepared by adding basal medium salt+ 30 g/l sucrose and7.0g/l agar as given in section 3.2.1.1; the pH of media was set at 5.8±2. For B5 media the components as given in section 3.2.1.2 were used and the pH of the medium was set at 5.5 ±0.02. Agar was added, and then melted and dispensed in the tissue culture jars. These jars were then autoclaved at 121ºC for 15 minutes at 15 psi, and stored at incubation room. Contained two type of auxins 2, 4, - dichlorophenoxy acetic acid (2, 4-D), naphthalene acetic acid (NAA). 50 mg of the powder of 2, 4-D dissolved in 0.5 ml of 1N NaOH, then heated and the volume was made up to 50 ml with sterilized distilled water stored in a refrigerator as stock for use. The final Concentration is: 1.0 mg/ml.50 mg of the powder of NAA was weighed and dissolved in 0.5 ml of 1N NaOH, then heated and the volume was made up to 50 ml with sterilized distilled water stored in a refrigerator as stock for use. The final Concentration is: 1.0 mg/ml.

Source of Explants

Cotyledons and hypocotyls were obtained from one week old in vitro micro plants above for callus induction. B5 medium were used for all callus induction experiments and supplemented with 20 g/l sucrose and 7 g/l agar and the pH was adjusted to 5.5±2 with 0.1 N NaOH or 0.1 N HCL before adding agar, the agar was melted by heating and the medium was dispensed into culture jars, and then autoclaved. All cultures were incubated at 25±2°C under cool white fluorescent lamp at 16h light and 8h dark.

Effect of Explants and Auxin on MS Media and B5 Media

Cotyledons and hypocotyls segments which used for callus induction were inoculated in a culture bottle (5x9 cm) containing 25 ml of MS media and B5 media, four explants /jar fortified with different concentrations of 2,4-

D and NAA (0.0, 0.5, 1.0, 1.5, and 2.0 mg/l). Cultures were maintained in a growth room at 25±2°C. Callus induction observed regularly. The final data was documented which include day of callus initiation, callus percentage, callus color, callus texture and callus degree.

Effect of Explants and Auxin on Callus Induction on MS Media B5 Media:

Different auxins namely (2, 4-D, NAA) with different concentrations for each one (0.0, 0.5, 1.0, 1.5, and 2.0 mg/l) were tested to evaluate their effects on callus induction from cotyledons and hypocotyls of the *T.graecum* on Cotyledon segments were cut off about 0.5 cm; while hypocotyls segments were cut off about 1.0 - 2.0 cm, then cultured in Murashige and Skoog (MS) medium, (B5) medium supplemented by different hormones media. For each treatment four explants /jar were used, all in B5 media. All cultures were incubated at 25± 2°C. The final data was documented which include day of callus initiation, callus percentage, callus color, callus texture and callus degree.

Statistical Analysis:

Two-way Analysis of Variance (ANOVA) was used to analyze the effects of genotype and the concentrations of the PGRs as well as their interaction on the number of hormones. The means were compared by Duncan's Multiple Range Test using SPSS v. 16.0 package software.

Results and Discussion:

Effect of two medium on *T.graecum* seed germination:

The present study of *Trigonellafoenum-graecum* with two types medium (MS) medium [11], (B5)[8] additional to Different auxins namely (2, 4-D, NAA) and different concentrations for each one (0.0, 0.5, 1.0, 1.5, and 2.0 mg/l) tested and showed in plate(1);to evaluate their effects on callus induction from cotyledons and then cultured in Seed germination rate is an important factor for establishing plant tissue culture and is particularly useful when there is a need to submit a uniform set of seedlings to a treatment or to a series of experiments [21].

Preparation and effect of explants type:

Both hypocotyls and cotyledon explants, from ten-day-old seedlings, were tested using the two of hormones and the two types of explants showed comparable results in their capacity to initiate shoots in a given concentration/combination of growth regulator (Table 1).

Results in Table (1) also showed that the effect of different concentrations of the two types of the hormones 2. 4-D and NAA auxin on the percentage of callus induction of *T. graecum L.* explants after three weeks, using hypocotyls and leaves explants. The hypocotyls explants induced by NAA auxin, were initiated callus after six days only and the percentage of callusing was 100% by two auxin concentrations level. However, the same thing happened in cotyledons stem segments although sometimes non-significant, in the explants, cotyledon and hypocotyls explants showed the highest percentages of callusing this consistent with [20]. Inconsistent with this, [2].

Effect of growth regulators on texture and color of explants

Figure (1); showed the color obtained from *T.-graecum* in MS medium supplemented by 2, 4-D and NAA was initiated after 12 days

from culturing, they gives the various callus induction color was recorded in cotyledon, hypocotyls segments (90%, 70) as yellow-green, yellowish and white respectively, this agree with[9]

I agree with [4] in using cotyledon explants and MS medium supplemented with NAA for callus induction color. Also I agree with [5] in using MS medium supplemented with 2, 4 –D for callus induction color. The color of callus was varying among different concentrations of hormones, and the texture was compact in the cotyledon segments, and friable in the hypocotyls segments.

The results in figure (2) showed that callus generation increased significantly (<0.05) through the observation data explain: Callus induced from *T.graecum* in B5 medium supplemented by 2, 4- D were initiated after 5 days from culturing. The maximum callus degree was recorded in cotyledon segments, and it was 4.0 by the concentration 2.0, and the minimum callus degree was recorded in hypocotyls segments and it was 1.9 by the concentration 0.5.

Figure (3); Callus induction obtained from *T.graecum in* B5 medium supplemented by NAA was initiated after 5 days from culturing. The maximum callus degree was recorded in cotyledon segments, and it was 3.4 by the concentration 2.0, and the minimum callus degree was recorded in hypocotyls segments, and it was 1.8 by the concentration 0.5 (see figure .3). These result are in line with the results of [12].

Table.1 Effects of different concentrations of 2, 4-D, and NAA hormones on MS and (B5) medium on the induction of callus of *T. graecum* L. explants

2,4 D					
Growth regulators mg/l	**Explants**	**%of callusing**	**callus(Means±SE)(B5)**	**callus(Means±SE)(MS)**	**Mean**
CONTROL	**Cotyledon**	**000**	**0.0±0.0**	**0.0±0.0**	**0.0±0.0**
0.5		**100**	**1.6±0.22**	**3.0±0.24**	**2.3 a±46 a**
1.0		**100**	**1.3±0.15**	**3.8±0.29**	**5.1 a ±0.44b**
1.5		**100**	**1.4±0.16**	**2.2±0.14**	**3.6 a ±0.30 a**
2.0		**100**	**1.4±0.16**	**4.0±0.19**	**5.4 a ±0.35 b**
CONTROL	**Hypocotyls**	**000**	**0.0±0.0**	**0.0±0.0**	**0.0±0.0**
0.5		**100**	**1.0±0.0**	**1.9±0.23**	**2.9 a ±0.23 b**
1.0		**100**	**1.0±0.0**	**2.3±O.30**	**3.3 b ±0.3 b**
1.5		**100**	**1.0±0.0**	**2.6±.16**	**3.6 a ±0.16 a**
2.0		**100**	**1.4±0.16**	**2.5±0.17**	**3.9 a ±0.33 b**
NAA					
CONTROL	**Cotyledon**	**000**	**0.0±0.0**	**0.0±0.0**	**0.0±0.0**
0.5		**100**	**1.2±0.13**	**1.6±0.16**	**2.8 b ±0.29 a**
1.0		**100**	**1.4±0.16**	**3.2±0.25**	**4.6 b ±0.4 b**
1.5		**100**	**1.4±0.16**	**2.6±0.16**	**4.00 b ±0.32a**
2.0		**100**	**1.2±0.13**	**3.4±0.16**	**4.6 a ±0.29 a**
CONTROL	**Hypocotyls**	**000**	**0.0±0.0**	**0.0±0.0**	**0.0±0.0**
0.5		**100**	**0.5±0.16**	**1.8±0.25**	**2.3 b ±0.41 a**
1.0		**100**	**0.5±0.16**	**2.8±0.20**	**3.3 a ±0.36 a**
1.5		**100**	**0.5±0.16**	**2.4±0.16**	**2.9 b ±0.32 b**
2.0		**100**	**0.5±0.16**	**2.7±0.15**	**3.2±0.31**

* Means with different letters are significantly different at $p = 0.05$.

Fig.1 Callus induced from *T. graecum* L. explants with different concentrations of 2,4-D, and NAA hormones on MS

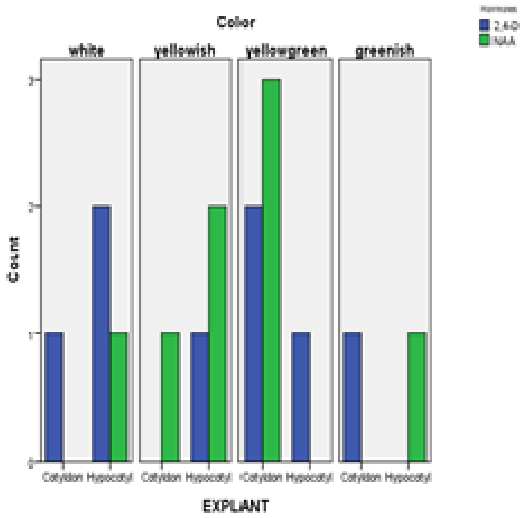

Fig.2 Callus induced from *T. graecum* L. explants with different concentrations of 2,4-D, and NAA hormones on B5

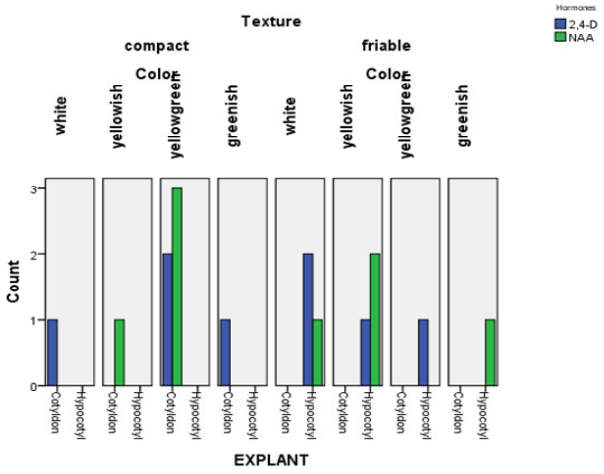

Figure.3 Callus induction from cotyledon segment by 2,4D at 0.5 mg/l concentration on B5 media

Figure.4 Effect of Explants and Auxins on Callusing Day on B5 Media

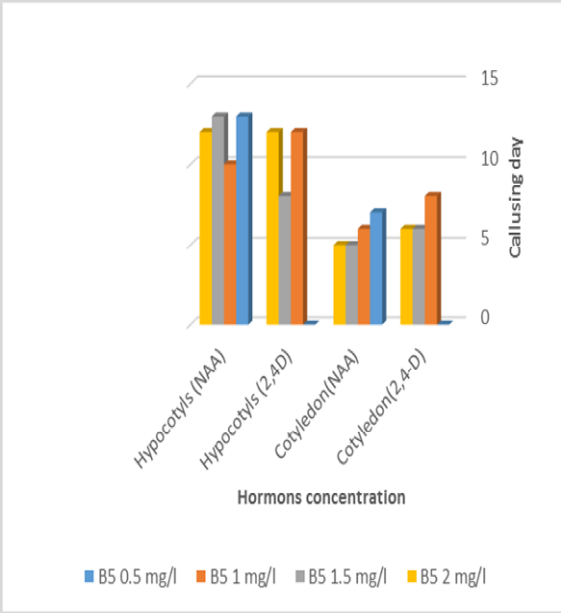

Figure.5 Effect of Explants and Auxins on Callusing Day on MS Media

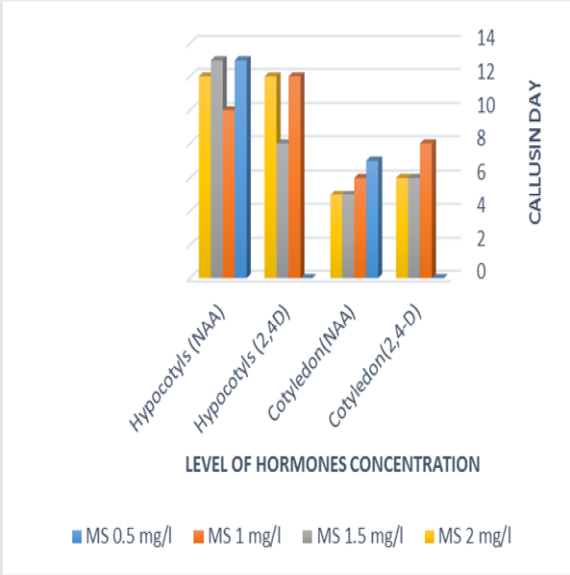

Figure (4); Callus induction obtained from *T.graecum in* MS medium supplemented by NAA was initiated after 5 days from culturing. The maximum callus degree was recorded in cotyledon segments, and it was 3.4 by the concentration 2.0, and the minimum callus degree was recorded in hypocotyls segments, and it was 1.8 by the concentration 0.5 (see figure .4). These result are in line with the results of [13].

Figure (5); Callus induction obtained from *T.graecum in* MS medium supplemented by NAA was initiated after 5 days from culturing. The maximum callus degree was recorded in cotyledon segments, and it

was 3.4 by the concentration 2.0, and the minimum callus degree was recorded in hypocotyls segments, and it was 1.8 by the concentration 0.5 (see figure .5). I agree with [21] in using hypocotyls explants and MS medium supplemented with NAA for callus induction color. Also I agree with [7] in using MS medium supplemented with NAA for callus induction color.

The best sterilization way for *Trigonellafoenum-graecum* seeds was recorded by Clorox 15% for 15 minutes.
The maximum callus degree (4.0±0.19) was recorded by cotyledon segment cultured in B5 media, supplemented with 2, 4-D at concentration 2.0 mg/l, within 5 days.

The minimum callus degree (0.5±0.16) was recorded by hypocotyls segment cultured in MS media, supplemented with NAA by all the concentrations.

MS and B5 media both produce 100% callus percentage with all concentration of auxin, texture of callus is compact with all cotyledon segments and friable with hypocotyls, and the colour was vary due to type and concentration of auxin .

References

[1]Aasim M, Nazim H, Ejaz M, Zubair U M, Hussain N, Hussain S B, Saeed S, Rafique T S and Sancak C (2010). In vitro shoot regeneration of fenugreek (Trigonellafoenum-graecum L.) using different cytokinins. African Journal of Biotechnology Vol. 9(42), pp. 7174-7179.

[2]Acharya S N, Thomas J E, Basu S K)2006(.Fenugreek: an "old world" crop for the "new world". Biodiversity (Tropical, Conservancy).7 (3 & 4): 27– 30.

[3]Arfan M, Gul S, Usman R, Khan A, Rauf A, Muhammad N, Ali Shah S U, Khan A, Ali M (2013). The Comparative Free Radical Scavenging Effect of Trigonella foenum- graecum, Solanum nigram and Spinacia oleracea. Academic. J. of Plant Sci. 6 (3): 113-116.

[4]ASHA P. K., SHAILA M. S. and VAIDYANATHAN C. S. (1979). Effect of Phytohormones on Nuclear RNA Synthesis in Germinating Seeds of Trigonella Foenumgraeceum and its Callus. J. Biosci., Vol. 1, Number 3: 327–334.

[5]Bin-Hafeez B, Haque R, Parvez S, Pandey S, Sayeed I, Raisuddin S(2003). Immunomodulatory Effects of Fenugreek (Trigonellafoenumgraecum L.) extract in mice. Int. Immunopharmacol. 3: 257-265.

[6]Dangi, R.S., Lagu, M.D., Choudhary, L.B., Ranjekar, P.K. and Gupta, V.S. (2004). Assessment of Genetic Diversity in Trigonella foenum-graecumand Trigonellacaeruleausing ISSR and RAPD Markers BMC Plant Biology.4:13.

[7]ELNour , Mawahib,E.M , Mohammed, Lamia S. and.Saeed. Bader Eldin A. (2013) In vitro Callus induction of Fenugreek (Trigonellafoenum-graecumL.)Using Different Media with Different Auxins Concentrations. AGRICULTURE AN D BIOLOGY JOURNAL OF NORTH AMERICA .4 :(3) 243.251

[8]Gamborg OL, Miller RA, Ojima K (1968). Nutrient requirements of suspension cultures of soyabean root cells. Exp. Cell Res. 50: 151-158.

[9]Jongebloed, M. (2004). Coriander and Fenugreek. p. 229-235. In S. Salvin

et al. (ed.). The New Crop Industries Handbook, Rural Industries Research and Development Corporation (RIRDC), Australian Government, Australia.

[10]Kalidass C, Mohan V R, Daniel A (2010). Effect of auxin and cytokinin on vincristine production by callus cultures of Catharanthus roseus L. (apocynaceae). Trop. Subtrop. Agroecosystems, 12: 283-288.

[11]Murashige T, Skoog F (1962). A revised Medium for Rapid Growth and Biossays with Tobaco Tissue Cultures. Physiol. Plant. 15: 473-479.

[12]Phitos, D., and Damboldt, J. (1985). Die Flora Der InselKeffllinia (Griechenland). BotanikaChronika. 5(1-2): 1-204.

[13]Pribac1, C., A. Ardelean. (2008). In vitro Culture of Trigonellafoenum-graecum Plantules and Their Anatomic Characterization. EMC 2008 14th European Microscopy Congres s 1-5 Septembe r 2008, Springer Berlin Heidelberg, Aachen, Germany, 3: 181-182.

[14]Puri, D., (1998). Therapeutic Ptentials of Fenugreek. Indian J. Physiol. Pharmacol., 42: 423-424. 332-334.

[15]Rajagopalan MS. (1998). Fenugreek, What Can This Herb Offer? Naturally, 1: 1-4

[16]Seasotiya L, Siwach P, Bai S, [13]Malik A, Bharti P (2014).Free radical scavenging activity and phytochemical analysis of seed s of Trigonella foenum-graecum. Asian Pac. J. Health Sci. 1(3):219-226.

[17]Shani J., Gold Schmied A., Joseph B., Abronson Z. and Sneman F.G. (1974). Hypoglycemic Effects of TrigonellafaenumgraecumandLupin ustermis(Leguminosae) Seed and Their Major Alkaloids in Alloxan-Diabetic and Normal Rats. Arch. Int. PharmacodynTher. 210, 27-37.

[18]Sharma RD (1986). Effect of Fenugreek Seeds and Levels on Blood Glucose and Serum Insulin Response in Human Subjects. Nutr. Res. 6:1353-1364.

[19]Sinskaya, E. (1961). Flora of the Cultivated Plants of the U.S.S.R. XIII. InPerennial Leguminous Plants. Part I: Medicago, Sweet clover, Fenugreek. Israel Programme for Scientific Translations, Jerusalem.

[20]Smith, A. (1982). Selected Markets for Turmeric, Coriander, Cumin and Fenugreek Seed and Curry Powder. Pub. No. G165 .Tropical Product Institute, London.

[21]Snedecor,G.W.,Cochran :statistical methods univ .press , Lowa state .(1967). pp189-199

[22]Wang, X.-D., Nolan, K. E., Irwanto, R. R., Sheahan, M. B., Rose, R. J. (10 January 2011). "Ontogeny of embryogenic callus in Medicagotruncatula: the fate of the pluripotent and totipotent stem cells". Annals of potany 107 (4): 599–609.