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ORIGINAL ARTICLE

Indirect Regeneration plant Fenugreek (*Trigonella foenum-graecum* L), with the use of Plant growth Regulators *in vitro*

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ABSTRACT
Fenugreek is a plant of leguminous family. This plant is a vegetable, spices; aromatic and medicinal plants are important and can be used in many countries. Different parts of the plant can treat diabetes, bronchitis, digestive disorders and wound healing helpful. In order to investigate the effect of explant and hormone treatments on Callus Induction and Plant regeneration (Trigonella foenum-graecum L). In vitro, two factorial experiment in a completely randomized design with three replications. The experiment callus speciation, cotyledon and hypocotyl explants of two and 2,4-D (1,0 and 1.5 mg per liter) and Kin (0, 0.25, and 0.5 mg per ml) was used. Environmental regeneration; 16 combined hormonal includes BAP (0, 0.5, 1.5 and 2 milligrams per liter) and NAA (0, 0.25, 0.5, 0.75 milligrams per liter), respectively. The highest callus of the cotyledon explants on MS medium containing 5.1 mg per liter of 2,4-D and 5.0 mg l Kin was obtained. Combined hormonal 5.1 mg l BAP and 5.0 mg per liter NAA best combination hormonal shoot regeneration from callus was obtained from hypocotyl explants. The cotyledon callus derived from small samples, there is no rebirth.
Keywords*: Fenugreek (Trigonella foenum-graecum L), regeneration, callus, hypocotyl.*

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INTRODUCTION

General Tendency of humans to traditional medicine, the concern of the harmful health effects of chemical agents that are associated with primary health care and cosmetic ingredients in the plants, resulting in more of them is not. One of the many medicinal properties of plants that have been mentioned here is fenugreek [1].

More than 100 species of wild and cultivated plants in the world have been identified, of which 33 species in many parts of Iran, including Isfahan, Damghan, Central, Azerbaijan, Fars and Khorasan distributed [2]. Much research has shown that different parts of the plant can be used in the treatment of diabetes, bronchitis, digestive disorders and wound healing useful [3]. Fenugreek seed, which is a rich source of diosezhin drug frequently used in the synthesis of steroids. Other substances found in fenugreek seeds, can mucilage, volatile oils and alkaloids named. Trigonelline, an alkaloid found in fenugreek seeds in addition to anti-cancer properties and antiseptic in the treatment of diseases such as migraine, hyperlipidemia and diabetes. By altering the genes causing genetic engineering to improve the quality of agricultural products, the introduction of plant cell and tissue culture and genetic engineering is to transfer genes [4].

Now, using the *in vitro* culture of the possibility of producing secondary metabolites, that is important for the pharmaceutical industry, such as alkaloids, amino acids and steroids etc [5]. In the field of in vitro culture of TF, studies have been conducted. Provorov et al [6] using hormone 2,4-D, Kin and BAP successfully motivated callus were at TF. Khawarai et al [7] for in vitro culture of fenugreek, a fine example of hypocotyl, cotyledon and root were used. Oncina [8] metabolite production in callus explants stems, leaves and roots were examined TF. Rezaian [9] that Changes metabolite accumulation in callus from leaf explants, the shoot apex and root, fenugreek review. The accumulation of these metabolites in callus from leaf explants reported.

Luo and Jia [10] for shoot regeneration in a legume, a combination of BAP and NAA hormones used. Zhang et al [11] for regeneration beans, the MS medium supplemented with BAP used. Barna and Bakhlo [12] for

stimulation of plant callus and leaf explants in medium containing 2,4-D applied them. Lu et al [13], in a study conducted on *Trigonella corniculata*, the highest of somatic embryogenesis from leaf explants on MS medium containing 2 mg of mesospheric l NAA and 5.0 mg l BAP observed. Hoda et al [14] conducted a study on regeneration indirect peas and the highest number of shoots formed on MS medium containing 2 mg l BAP and 5.0 mg per liter NAA reported. Study; to indirect regeneration plant using different combinations of hormones in vitro was performed.

MATERIALS AND METHODS

The study of 2012 in tissue culture laboratory at the University of Agriculture and Natural Resources Ramin Khuzestan. In this study, the two cotyledon and hypocotyl explants were used. The seeds were purchased from the company of pure seed. To obtain sterile seedlings, seeds with running water for 30 minutes and then were washed once with sterile distilled water. The rest of the interior of the room was Air fluency transfer under laminar hood. To disinfect seeds under laminar hood, a solution of 70% ethanol for 1 minute and 5% sodium hypochlorite for 5 min was used. Then washed three times with distilled water, seeds MS medium (MS) without hormones, with 7 grams per liter and 30 grams per liter sucrose agar, were transferred. Germination in the growth chamber, with constant temperature 2 ± 26 ° C and equipped with a fluorescent lamp with a light intensity of 1500 lux (16 hours light and 8 hours dark) was performed. Germination and seedling establishment after the 15-day-old seedlings in sterile conditions after removal of agar and cotyledon and hypocotyl explants were prepared with a scalpel blade. In this study, the formation of callus from 9 hormonal composition containing 2,4-D (1,0 and 1.5 mg per liter) and Kin (0, 0.25, and 5.0 mg per ml) was used.

In order to motivate more callus explants, using a scalpel blade to cut on the underside of the samples was made. After cultivation, the explants for callus induction in conditions of darkness and constant temperature 2 ± 25 ° C, for 1 month. After induction, callus within 1 month, callus weight per explant using a digital scale (resolution 0.01 g) was measured. Callus production, to produce shoots were transferred to regeneration medium. The regeneration of callus produced by the combination of hormones BAP (0, 0.5, 1.5 and 2 milligrams per liter) and NAA (0, 0.25, 0.5 and 0.75 mg per liter) was used. Samples were transferred to regeneration medium in home growth, with constant temperature 2 ± 26 ° C and equipped with a fluorescent lamp with 1500 lux illumination (16 hours light and 8 hours dark) were maintained. After 8 weeks, the number of shoots formed were recorded. Subculture in all phases of operation was performed once every 4 weeks. In this study, a factorial experiment based on randomized complete block design with 3 replications of 3 glass planted. Analysis and comparison of data using SAS software using MSTAT-C software using multiple range test of Duncan 0.01 Done. Graphs were plotted using Excel software.

RESULTS

Callusing

MS medium in each of plant growth regulators, explant and their interaction showed significant differences at 0.01. Table 1 compares the effects of 2,4-D and Kin simple hormones on callus weight shows.

Table 1. Comparison of the effects of simple 2.4-D and Kin hormones on callus weight

callus weight (g)	hormones
0.64	A_1
0.74 b	A_2
1.07 a	A_3
0.74 c	B_1
0.80 b	B_2
0.91 b	B_3

A1, A2, A3, respectively 0, 0.5, and 1.5 mg per liter of 2,4-D

B1, B2 and B3, respectively 0, 0.25 and 0.5 milligrams per liter of Kin

* Means at least one letter in common are significantly different at the 1% level by Duncan test

The Effect of 2,4-D and Kin hormone interactions were observed with respect to Figure 1, the maximum weight of callus on MS medium containing 5.1 mg per liter, 2.4-D and 5.0 mg l Kin was obtained. And then MS medium containing 1.5 mg per liter of 2,4-D and l Kin was 0.25. Color callus from hypocotyl explants on MS medium with a concentration of 5.1 mg per liter of culture medium containing 2,4-D, yellow and concentration of less than 5.1 mg per liter of 2,4-D is milky. Color pale green cotyledon callus explant (Figure 1 and 2).

Effect of 2,4-D in the interaction of three factors: the Kin in explant was observed that most of the cotyledon explant callus on MS medium containing 1.5 mg per liter of 2,4-D and 5.0 mg per liter of Kin that the weight of callus produced significant differences in the other treatments.

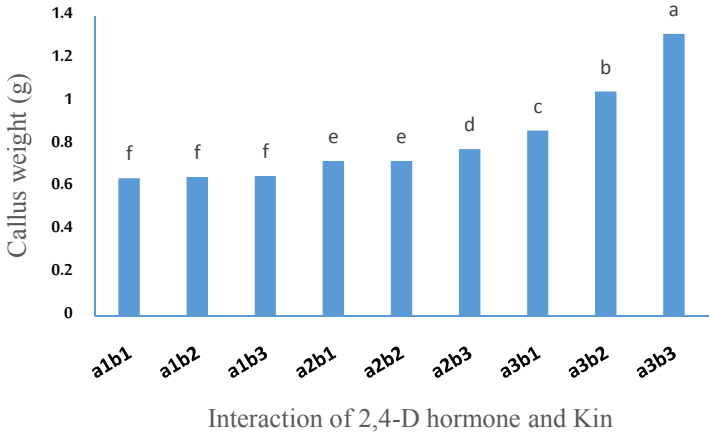

Figure 1. Interaction of 2,4-D and Kin hormones on callus weight
a1, a2 and a3, respectively, 0, 1 and 1.5 mg per liter of 2,4-D
b1, b2 and b3, respectively, 0, 0.25 and 1 mg l-Kin
* Means at least one letter in common are significantly different at the 1% level by Duncan test.

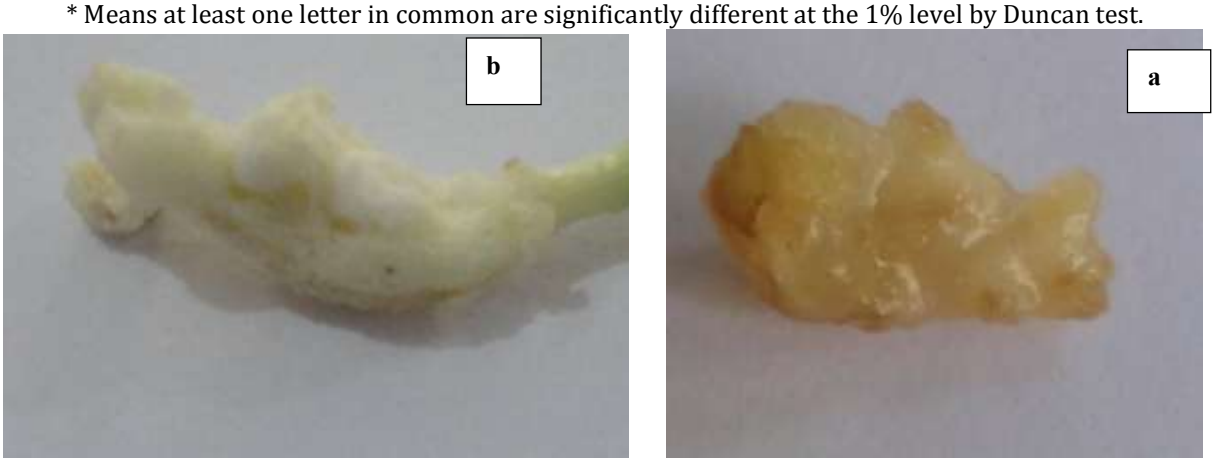

Figure 1. A and B hypocotyl explants formed callus on MS medium containing 1.5 mg per liter, 2.4-D and 5.0 mg l-Kin, in the first and second crop

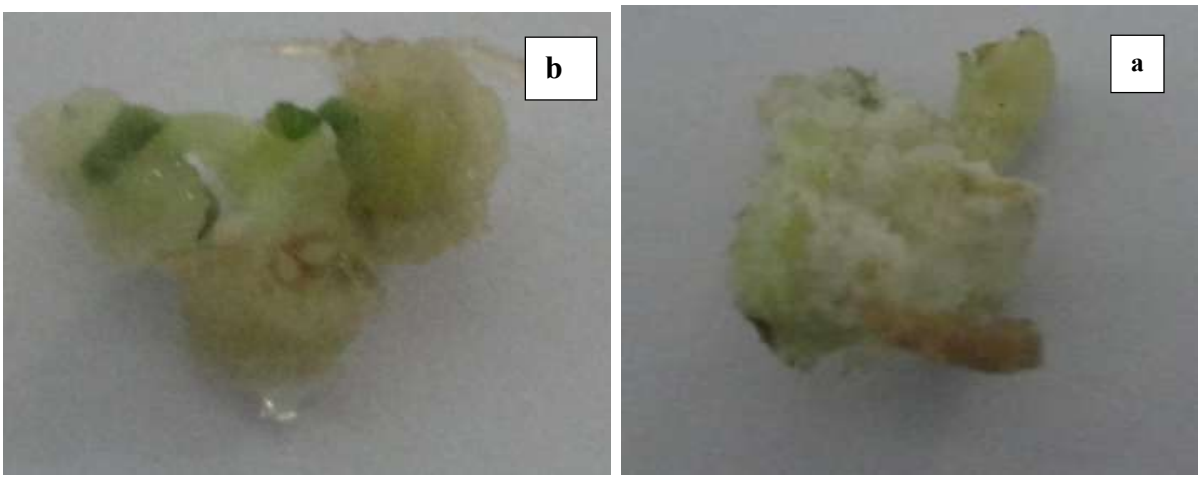

Figure 2 (a) and (b) cotyledon explants formed callus on MS medium containing 1.5 mg per liter, 2.4-D and 5.0 mg l-Kin, in the first and second crop

Regeneration

Number of shoots

Table 2 showed Comparison of the independent effects of BAP and NAA on shoot number. According to Table 2, it was observed that with increasing levels of BAP from 0 to 1.5 milligrams per liter increase in the number of regenerated shoots. 1.5 mg l BAP hormone concentrations were reduced number of shoots.

Table 2. Comparison of the simple effects of hormones BAP and NAA on shoot number

shoot number	hormones
0 d	A_1
0.33 c	A_2
2.88 a	A_3
2.34 b	A_4
0.99 c	B_1
1.85 b	B_2
2.32 b	B_3
0.37 d	B_4

a1, a2, a3 and a4, respectively, 0, 0.5 and 1.5 and 2 mg per liter of 2,4-D

b1, b2, b3 and b4, respectively, 0, 0.25, 0.5 and 0.75 mg l-Kin

* Means at least one letter in common are significantly different at the 1% level by Duncan test.

Effect of NAA, BAP interaction in small sample showed the highest number of shoots per callus from hypocotyl explants on MS medium containing 1.5 mg l BAP and 5.0 mg per liter NAA arose with other treatments no significant difference was found (Figure 2 and Figure 3). Callus derived from cotyledon explants were no regeneration. Lack of regeneration in callus derived from cotyledon explants could be due to poor lighting conditions and the composition or properties of the explant is used hormones.

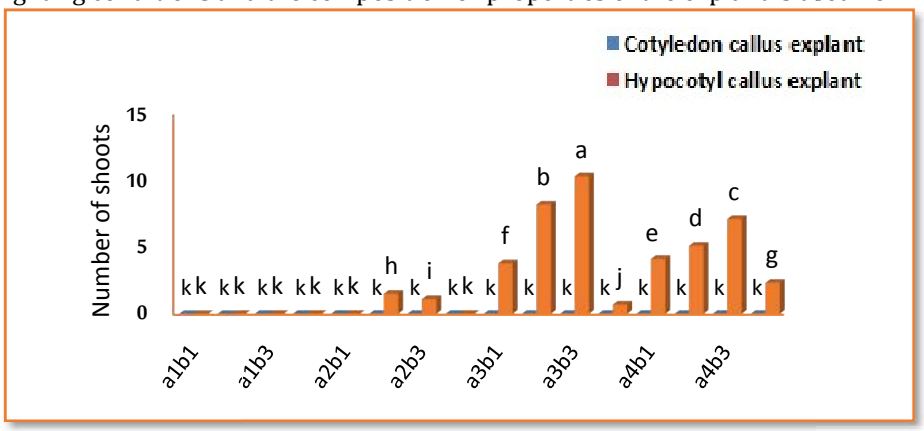

Figure 2. Interaction between BAP and NAA hormones and callus explants on number of shoots

a1, a2, a3 and a4, respectively, 0, 0.5 and 1.5 and 2 mg per liter of 2,4-D

b1, b2, b3 and b4, respectively, 0, 0.25, 0.5 and 0.75 mg l-Kin

* Means at least one letter in common are significantly different at the 1% level by Duncan test.

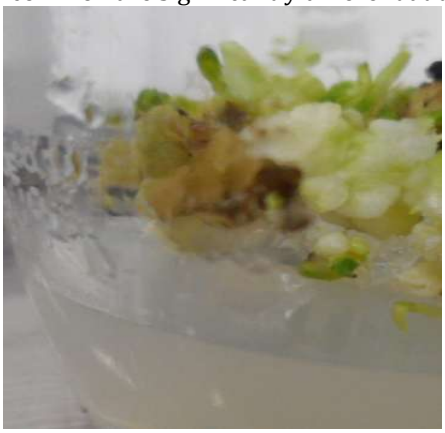

Figure 3. Growth of callus formation from hypocotyl explants on MS medium containing 1.5 mg l BAP and 5.0 mg per liter NAA

DISCUSSION

Callusing

According Hoori et al [15], the best combination hormonal stimulation of callus on MS medium containing 2,4-D Medics and Kin. Provorov et al [6], the interaction between hormones and 2,4-D as the best combination hormonal Kin, fenugreek reported to form callus. The results of the present study corresponded with the results. In all the hormonal composition of the cotyledon explants, callus than the hypocotyl explant has produced. In studies conducted in tissue culture plants, explants of different answers to the same hormonal compounds at different levels within the physiological properties explant been reported [16]. In this study, different responses to stimulation of callus explants could be the reason. In this study, the concentration of 2,4-D hormone cotyledon and hypocotyl explants for callus formation at 5.1 milligrams per liter have been reported. These results lead to the study of Elnor, et al [17] on Fenugreek is consistent. Simultaneous presence of 2,4-D and Kin hormones in the culture medium MS, increased callus weight was compared with other treatments. Right combination hormone cytokinin and auxin are leading to increased motivation callus.

Regeneration

Luo and Jia [10] for shoot regeneration in a legume combination of BAP and NAA hormones used the results of the study and Luo and Jia [10] confirm these results. According to Table 2, it was observed that the increase in NAA concentration of 5.0 milligrams per liter 0.25 to further increase the number of shoots produced. While the concentration of 75/0 l NAA result was to reduce the number of shoots. It was observed at concentrations of 0 mg l BAP did not regenerate. BAP hormone increases to 5.1 milligrams per liter increase in the number of shoots formed. With increasing concentration of 5.1 to 2 mg BAP decreased number of shoots formed. The shoots of the two hormones BAP and NAA treatments were simultaneously more effective than other treatments and that the simultaneous presence of auxin and cytokinin in shoot formation states. The number of shoots was reduced. In this Faisal et al [18] on the herb *Mucuna pruriens* high concentrations of BAP on shoot number was reduced in the absence of auxin, which corresponded with the results. Effect of BAP and NAA on shoot formation BAP in combination with low concentrations of NAA increased the number of shoots. The presence of the hormone in vitro BAP for shoot formation is essential for the simultaneous presence of BAP and NAA in the culture medium increased the number of shoots. In this study, the highest callus was induced in cotyledon explants. Hormonal composition contains 5.1 milligrams per liter of 2,4-D and 5.0 mg per liter Kin best combination of cotyledon and hypocotyl explants for callus induction was both. In callus derived from cotyledon explant regeneration after transfer to the environment, there is no rebirth. The results showed that the most suitable for the formation of shoots in MS medium containing 1.5 mg l BAP and 5.0 mg l NAA.

REFERENCES

1. Salehi Surmaghi, M. H. (2008). Medicinal Plants and Herbal Therapy, Iran, (1): pp: 253 -254.
2. Mozafarian, V. (1995). Plants Names Dictionary of Iran. Farhang Moaser Publication, 558p.
3. Al-Habori, A. and Raman, A. 1998. Antidiabetic and hypocholerstrolaemic effects of fenugreek. phytotherapy. Res., 12: 233-242.
4. Evans, D.E., Coleman, J. O. D. and Kearns, A. (2003). Plant Cell Culture. Bios scientific publishers, London and New York. 277p.
5. Vanisree, M. and Tsay, H. S. (2004). Plant Cell Cultures - An Alternative and Efficient Source for the Production of Biologically Important Secondary Metabolites. International J. Appl. Sci. Engin., 2: 29-48.
6. Provorov, N. A., Soskov, Y. D., Lutova, L. A., Sokolova, O. A. and Bairamov, S. S. (1996). Investigation of the fenugreek (*Trigonella foenum-graecum* L.) genotypes for fresh weight, seed productivity, symbiotic activity, callus formation and accumulation of steroids. Euphytica, 88(2): 129-138.
7. Khawari, K. M., Gulbitti- Onarici, S., Cocue, S., Erisen, S., Sancak, C. and Ozcan, S. (2004). In vitro crown galls induced by Agrobacterium tumefaciens strain A281 (pTiBo542) in *Trigonella foenumgraecum*. Biologia Plantarum, 48(3):441-444.
8. Oncina, R., Botia, J. M., Del Rio, J. A. and Ortune, A. (2000). Bioproduction of diosgenin in callus cultures of *Trigonella foenum-graecum* L. Food Chemistry,70(4):489-492.
9. Rezaeian, S. (2011). Assessment of Diosgenin Production by *Trigonella foenum-graecum* L. in vitro Condition. American Journal of Plant Physiology, 6(5): 261-268.
10. Luo, J. P. and Jia, J. F., (1998). Callus induction and plant regeneration from hypocotyl explants of the forage legume *Astragalus adsurgens*. Plant Cell Reports, 17(6-7): 567-570.
11. Zhang, Z. Y., Coyen, D. P. and Mitra, A. (1997). Factors affecting Agrobacterium-mediated transformation of common bean. Journal of the American Society for Horticultural Science, 122: 3, 300-305.
12. Barna, K.S. and Wakhlu, A. K. (1993). Somatic embryogenesis and plant regeneration from callus cultures of chickpea (*Cicer arietinum* L.). Plant Cell Rep., 12(9):521-4.
13. Lu, D.Y., Davey, M.R. and Cocking, E.C., (1982). Somatic embryogenesis from mesophyll protoplasts of Trigonella corniculata (leguminosae). Plant Cell Reports, 1(6): 278-280.

14. Huda, S., Islam, R., Bari and, M. A. and Asaduzzaman, M. (2000). Shoot differentiation from cotyledon derived callus of Chickpea (Cicer arietinum L.). Plant Tissue Cult., 13(1) : 53-59.
15. Hoori, F.; Ehsanpour, A.A.; Mostajeran, A. (2007). Comparison of somatic embryogenesis in *Medicago sativa* and *Medicago truncatula*. Pakistan Journal of Biological Sciences, 10 (3):481-485.
16. Pierik. 1979. In vitro culture of higher plants. Bibliography.Ponsen en looyen, Wageningen, the Netherlands,1-149.
17. Elnour, E. M. M., Lamias, M. and Saeed, E. D. (2013). *In vitro* Callus induction of Fenugreek (*Trigonellafoenum-graecum* L.) Using Different Media with Different Auxins Concentrations. Agric. Biol. J. N. Am., 2013,4(3): 243-251.
18. Faisal, M., Siddique, I. and Anis, M. (2006). In vitro rapid regeneration of plants from nodal explants of *Mucuna pruriens*- a valuable medicinal plant. Annals of Applied Biology, 148(1): 1-16.

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