Effect of Growth Hormone 2,4-D on Some Callus Traits of Different Faba Bean (*Vicia Faba* L.) Cultivars

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Abstract: In order to further increase the callus induction frequency of faba bean (*Vicia fab* L.), the effect of growth hormone 2,4-D on this process was investigated in this study. Different concentrations of 2,4-D (1, 2, 3 and 4 mg L^{-1}) were added to callus induction medium . Cotyledons explants collected from 14-21 days old seedling of two different cultivars, Giza 2 and Giza 843, were treated with previously growth hormone. The results indicated that the singinifant values of callus induction, callus fresh weight and friability of callus at $p \leq 0.001$ were observed between the different concentrations of 2,4-D . Callus induction efficiency varied from 72% at 4 mg L^{-1} to 97%at 2 mg L^{-1} depending on the 2,4-D concentrations. The highest value (0.714 g) of callus fresh weight was detected at 4 mg L^{-1} 2,4-D in Giza 843. It could be concluded that, in two cultivars of faba bean, the effect of different concentration of 2,4-D were more effect on friable callus in order to 4<3<2<1 mg L-1 of 2,4-D.
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Key Words: Faba bean, growth hormone, 2,4-Dichlorophenoxyacetic acid, callus Induction, callus fresh weight, friable callus

Abbreviations: (2,4-D) 2,4-Dichlorophenoxyacetic acid; (MS) Murashige and Skoog medium (Coty.) Cotyledons. (ADW) Autoclaved Distilled Water

1.Introduction

Legumes are the third largest family of dicotyledons leguminosae and it have high nutrional value **(Srinath *et al*., 2005).** This family include soybean, pea, faba bean, chickpea and common bean **(Shimaa *et al*., 2008).** The faba bean (*Vicia faba* L.) is an important grain legume because its high protein content and its used for human consumption and animal feeding.

The genetic improvement of faba bean against environment stress and to improve its nutritional value by conventional breeding has been used **(Shimaa *et al*., 2008).** More recently, biotechnology technologies have been used to improve many species more efficiently, whereby useful traits have been introduced from a broader range sources within an economically viable time frame **(Takahoshi and Takamizo, 2012).** Genetic transfer technology requires an efficient and suitable regeneration system either by directly from the excised tissue or indirectly after formation of callus **(Kuchuk, 2001).**

An efficient plant regeneration protocol through callus induction is a prerequisite for biotechnology breeding of economically important crops like faba bean and soybean **(Muthukrishman *et al.,* 2014).** Therefore, the success of callus and tissue culture research depends on upon reliable callus culture. In case of faba bean there have been several reports related to tissue culture conditions for callus induction **(Khalaf and Hattori, 2000; Kamal, 2009)**. Callus cultures of faba bean were obtained from hypocotyl explants **(Mitchell and Gildow, 1975)**, cotyledons **(Cionini *et al*., 1987; Edyta *et al*., 2012). Griga *et al.,* (1987)** was the first obtain embryogenic calluses from *Vicia faba*.

In the recent years, 2,4-D has been successfully employed to improve callus induction and callus morphogenesis in tissue culture and the problem of maintaining viable callus from Vicia faba cultivars has been discussed in a limited number of studies **(Edyta *et al*., 2012).** The frequencies of callus induction in tissue culture of faba bean are influenced by many factors such as: culture conditions, culture media composition, explant source and genotype **(Zaman *et al.,* 2010).** Also, **Khatun *et al.,* (2003)** indicated that genotypes, nutrient composition and hormone supplementation are regarded to be the major sources of variation in *In-vitro* culture.

The present studies describes the procedures for establishment callus of two different cultivars of faba bean and investigate the effect of different concentrations of 2,4-D on callus induction, callus fresh weight and friable callus using cotyledons explant.

2.Material and Methods

The experiment was performed during the period from September 2013 to January 2014 at Tissue culture Laboratory, Biological Science Department, Faculty of Science, KAU.

Two different faba bean cultivars (Giza 2 and Giza 843) supplied from the Crop Research Institute-Agriculture Research Center-Ministry of Agriculture-Cairo-Egypt were used in this experiment. Faba healthy grains were first washed by autoclaved distilled water three times, soaked in 70% ethanol for 3 minutes, then washed thoroughly with autoclaved distilled water. Grains were transferred into 20% Clorox, a commercial bleach contained 1% sodium hypochlorite plus 5 drops of tween-20 as a wetting agent to make the sterile agent in good contact with all surface of plant tissue. Sterilized grains were rinsed three times in ADW for complete removal of Clorox under a septic conditions in laminar air-flow hood.

Sterilized grains were then cultured on MS **(Murashige and Skoog, 1962)** medium 4.4 g L^{-1} MS and subsequently solidified by adding 8 g L^{-1} Agar. The pH of this medium was adjust at 5.7 and then autoclaved at 121ºC at pressure of 15 psi for 25 minutes. Culture were kept in an incubator at constant temperature of 25±1 ºC in darkness to encourage the germination. After one week the culture were exposed to 16 hours photoperiods and 3000 Lux provided by cool white fluorescent lamps. Germinated grains 14-21 days were used for the isolation of cotyledons. Cotyledons were dissected and separated from the seedling by a simple surgical treatment under aseptic conditions. Cotyledons were cut with scalpel blade into small spices (1.0 cm) in length with eliminate the midrib. The explant were horizontally placed in Petri dishes filled with callus induction medium containing agar solidified basal MS medium supplemented with 100 g L^{-1} Myo-inositol, 1.00 mg L^{-1} thiamine-HCL, 30 g L Sucrose and different concentration of 2,4-D (1, 2, 3 and 4 mg L^{-1}). The medium was autoclaved as described before. Each treatment had three replication and at least 25 explants. Four weeks after they cultured in the dark at 25±1 ºC, Frequency of callus induction (%) Was Measured by divide the number of inoculated embryo on the number of callus formation, Callus fresh weight (gm) and Frequency of friable callus (%) was measured by divide the number of friable callus on the number of callus formation.

The data were analyzed using ANOVA and the mean ± SE values of results are presented. Significant different between the treatments were analyzed using t test with Costat Software.

3. Results and Discussions

In the present study, different concentrations of 2, 4-D (i.e., from 1mg L^{-1} to 4 mg L^{-1}) were experimented to find out the best concentration for successful callus induction of two different cultivars of faba bean named Giza2 and Giza 843. Callus Tissue were initiated from cotyledons explants and started to growth after seven days from most of the explants with different compact shape and color **(Plate 1). Rao and Chopra (1987) and Srinath *et al.*, (2005)** indicated that the different in the time required for callus initiation and callus induction are varied depends on the genotypes, type of explants and media compositions. Also, calli color were determined visually and it was observed that , the color of the callus was creamish, creamy-yellow and brownish under 1, 2, 3 and 4 mg L^{-1} of 2,4-D, respectively. **Zaman *et al.* (2010)** concluded that, if 2,4-D concentration increase more than the optimum value, then they would influence negatively on the compactness and color of callus. The analysis of variance for the callus induction, callus fresh weight, friable and non friable callus showed highly significant value among the different treatments for all studied traits, while non significant differences were recorded between the genotypes for the previously traits **(Table 1).**

Table 1. Analysis of variance for effect of different concentrations of growth hormone 2,4-D callus induction, callus fresh weight and friability of callus of two cultivars of *Vicia faba*

SOV	df	MS			
		Callus induction	Callus fresh weight	friable callus	Non-friable callus
Genotypes	1	0.0001^{ns}	0.003^{ns}	1626.544^{ns}	0.009^{ns}
Treatments	3	0.077***	0.162***	6642.3***	14924.8***
Error	19	0.001	0.002	603.3	0.06
Total	24				

SOV: Source of variance, MS: Mean Square, df: degree of freedom and *, ** ,*** significant at 5%, 1% and, 0.1% probability level, respectively.

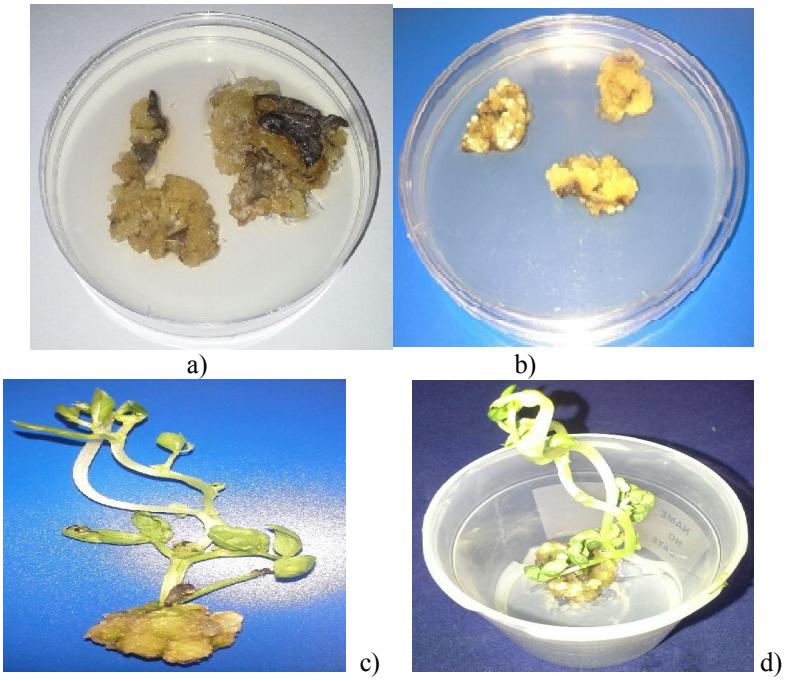

Plate 1. Callus induction and regeneration from cotyledon explants of faba bean (*Vicia faba* L.) a) yellow-brownish friable callus b) brownish friable callus and c&d) shoot regeneration obtained from callus (data not presented).

The frequency of callus induction after four weeks for two faba bean cultivars were ranged from 72 to 97%, the cultivar Giza 2 had the lowest and highest frequencies for callus induction under 4 mg L^{-1} and 2 mg L^{-1} of 2,4-D. callus induction was increased when concentration of 2,4-D was enhanced from 2 to 3 mg L^{-1} . With further decrease (1 mg L^{-1}) or increase (4 mg L^{-1}) in 2,4-D the callus induction was decreased **(Figure 1).** Most of these callus were able to differentiation to shoot and root **(Plate 1),** data not presented. Our results are in agreement with **Umer *et al.*, (2009) ,** they indicted that callus induction was increased significantly when concentration of 2,4-D increased from 2 mg L^{-1} to 3 mg L^{-1}, while with further increasing of 2,4-D concentration the callus induction was decreased. **Gosal and Baiaj (1979)** reported that 2.0 mg L^{-1} 2, 4-D which gave the best response in callus induction cotyledons, hypocotyls and roots of chickpea as explants and this was similar to our finding. 2,4-D has been successfully used by many other researchers to regenerate soybean plants **(Kumari *et al.*, 2006; Loganathan *et al.*, 2010; Saram *et al.*, 2003;)** although concentration varied widely. Also, **Ebony *et al.*, (2010)** abundant amounts of calli were obtained either from the cotyledons in the media modified with 2,4-D or from cotyledons with 2,4-D and NAA combined. Also, **Martins and Sondahil (1984)** observed that , the high level of 2,4-D inhibited the callus induction frequency. Callus induction media have been optimized by various researchers at varying concentrations of 2,4-D like and this difference in results in all these cases may rightly be regarded to difference in varieties, sources of explants and tissue culture conditions **Mehmooda *et al.*, (2010).**

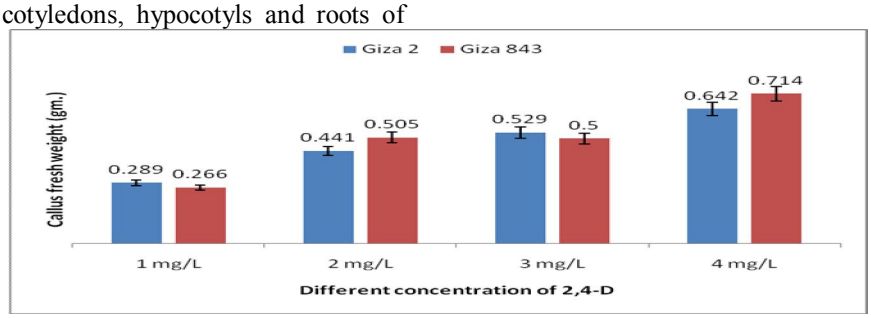

Fig 1. Effect of different concentrations of 2,4-D on callus induction (%) of two cultivars of faba bean after 4 weeks from culture.

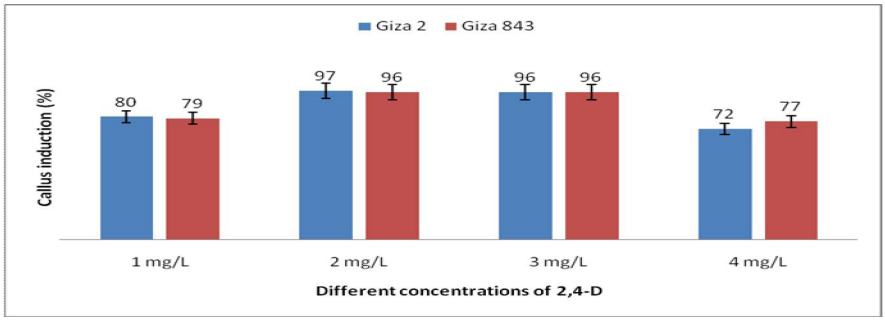

Fig 2. Effect of different concentrations of 2,4-D on callus fresh weight of two cultivars of Faba bean after 4 weeks from culture..

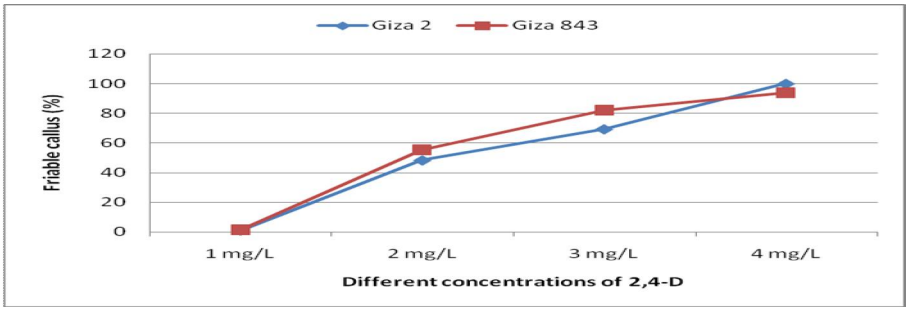

Fig 3. Effect of different concentrations of 2,4-D on percentage of friable callus of two cultivars of faba bean after 4 weeks from culture.

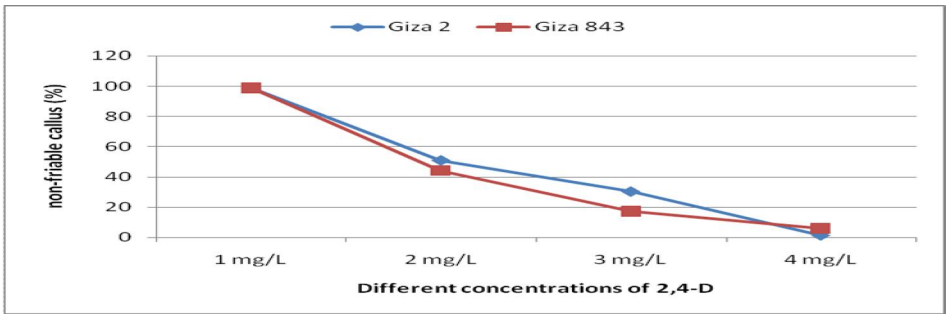

Fig 4. Effect of different concentrations of 2,4-D on percentage of non-friable callus of two cultivars of faba bean after 4 weeks from culture.

The average fresh weight of calli after four weeks was measured for two cultivars under study. Data illustrated in **figure (2),** showed that there were not differences in the callus fresh weight between the tested cultivars. On the other hand, 2,4-D at 4 mg L^{-1} provided better value of callus fresh weight (0.714 g) than other treatments. The lowest callus fresh weight (0.266 and 0.289) was observed under 1 mg L^{-1} of 2,4-D in Giza 843 and Giza 2, respectively. **Ebony *et al.* (2010)** observed that callus growth was faster than the callus produced at lower concentration of 2,4-D. our results are supported by **Umer *et al.* (2009),** which found that, callus fresh weight for four cultivars of wheat was positive correlation with the increasing in the concentrations of 2,4-D, where the highest value of callus fresh weight (0.62 and 0.65 g) were recorded under 4 and 5 mg L^{-1} 2,4-D for Inqilab and Tatora cultivars, respectively. but in the other hand, our results are not in agreement with **Srinath *et al.* (2005) ,**they detected that higher concentrations of 2,4-D at (13 µM/L) reduced the callus fresh and dry weight in *V. radiate*.

In the same time, after four weeks from cotyledons culture, callus induction showed that the degree of friability between the treatments for callus ranged from friable and non friable and intermediate **(Figures 3 and 4).** The friable callus which was characterized by easily divided into smaller cluster of call was obtained most frequently from cotyledons of Giza 2 (100%) and Giza 843 (94%) under 4 mg L^{-1} 2,4-D, while non friable callus was obtained most frequently from cotyledons explants of the same cultivars under 1 mg L^{-1}. From these results it could be concluded that culturing cotyledons faba bean on MS

medium consistently produced on equivalent or grater proportion of friable, rapidly growing callus cultures, and the relative friability of these cultures was often maximum on MS medium. Callus induction frequency was often maximum when the relative friable callus was minimum.

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References

Cionin, P.S., Bennici, A., and D'amato, F. (1978). Nuclear cytology of callus induction and development *in vitro*. I. Callus from *Vicia faba* cotyledons. ***Protoplasma***, 96: 101–112.

Ebony, Y., LaShonda, S. and Muhammad, A . (2010). Callus Induction and Organogenesis in Soybean [*Glycine max* (L.) Merr.] cv. Pyramid from Mature Cotyledons and Embryos. ***The Open Plant Science Journal, 4:*** **18-21**

Edyta, A., Liona, C. and Izaela, I. (2012).indirect organogenesis of faba bean (*Vicia faba* L. Minor) ***Acta Biological Cracoviensia Series Botanica***, 54: 102–108.

Gosal, S.S. and Bajaj, Y.P. (1979). Establishment of callus tissue culture and the induction of organogenesis in some grain Legume. ***Crop improvement,*** 6:154-160.

Griga, M., Kubalakova, M., and Tejklova, E. (1987) .Somatic embryogenesis in *Vicia faba* L. ***Plant Cell Tiss. Org. Cult.*** *9*, 167–171.

Kamal, M. A. H. M., Al Munsur, M. A. Z., Hossain, M. S. and Begum, S. (2009). Comparative studies of callus induction and plant regeneration from mature embryos in rice mutants. ***J. Bangladesh Agril. Univ.*** 7(1): 39–45

Khalafalla, M., and Hattori, K. (2000). Ethylene inhibitors enhance *in vitro* root formation on faba bean shoots regenerated on medium containing thidiazuron. ***Plant Growth Regul.*** *32*, 59–63.

Khatun, M., Ali, M.H. and Desamero, N.V. (2003). Effect of Genotype and Culture Media on Callus Induction and Plant Regeneration from Mature Seed Scutellum Culture in Rice. ***Plant Tissue Cult,*** 13: 99-107.

Kuchuk, N. (2001). Biotechnology. In: Carbohydrates in Grain Legume Seeds, C.L. Hedly, ed. (Wallingford, UK: CABI Publishing), pp. 145–207.

Kumari BDR, Settu A, Sujatha G. (2006). Somatic embryogenesis and plant regeneration in soybean. ***Indian J. Biotechnol*** , 243-245.

Loganathan M, Maruthasalam S, Shiu YL, Lien WC, Hsu WH, Lei PF, Yu CW, Lin CH. (2010). Regeneration of soybean (*Glycine max* L. Merrill) through direct somatic embryogenesis from the immature embryonic shoot tip. ***In Vitro*** **Cell Dev Bio – Plant**, 46: 265-273.

Martins, I.S. and M.R. Sondahl. 1984. Early stages of somatic embryo differentiation from callus cells of bean (*Phaseolus vulqaris* L) grown in liquid medium. ***J. Plant Physiol***. 117:97-103.

Mehmood, M., Rahmatullah, Q., Ghulam, M., Umer, R., Sabahat, N., Khalid, M., Shoukat, A. and Muhammad, R. (2010). Rimary callus induction, somatic embryogenesis and regeneration studies in selected elite wheat varieties from Pakistan ***Pak. J. Bot.,*** 42: 3957-3965.

Mitchell, J.P., and Gildow, F.E. (1975). The initiation and maintenance of *Vicia faba* tissue cultures. ***Physiologia Plantarum***, 34: 250–253.

Murashige, T., and Skoog, F. (1962) . A revised medium for rapid growth and bioassays with tobacco tissue cultures. ***Physiol. Plant.*** *15*, 473–497.

Muthukrishnan, A., Kondeti, S., Jeevaraj, T. and Andy, G. (2014). Optimized shoot regeneration for Indian soybean: the influence of exogenous polyamines. ***Plant Cell Tiss Organ Cult***, 117:305–309.

Rao, R.G. and Cahopra, V.L. (1987). Genotypic and explant differences in callus initiation and maintenance in chickpea. ***Int. Chikpea Newslett***, 10-12.

Sairam RV, Franklin G, Hassel R. (2003). A study on the effect of genotypes, plant growth regulators and sugars in promoting plant regeneration *via* organogenesis from soybean cotyledonary nodal callus. ***Plant Cell Tissue Organ Cul*** , 75: 79-85

Shimaa, B., , Omer, A. , Osama, E., , David, A., and Hany, E. (2008). Establishment of the Regeneration System for *Vicia faba* L. ***Curr. Issues Mol. Biol.*** 11: 47–54.

Srinath, R., Patil, R. and Kaviraj, C. (2005). Callus induction and organogenesis from various explants in *Vigna radiate* (L.) Wilczek. ***Indian J. Biotechnol.*** 4:556-560.

Takahoshni, W. and Takomizo, T. 2012. Molecular breeding of grasses by transgenic approaches for biofuels production ' In Transgenic Plants-Advances and Limitation', ed Y.O., Cafici (Pijeka, Croutia: In Tech), 91-116.

Umer, R., Shaukat, A., Ghulam, M., Najma, A., M. Shahid, M. (2009). Establishment of an efficient callus induction and plant regeneration system in Pakistani wheat (*Triticum aestivum)* cultivars . ***Electronic Journal of Biotechnology***, 12:1-12.

Zaman, M.A., Manjur, A.B.M.K., Ahmed, M. and Islam, M.M. (2010). Effect of 2,4-D on Callus Induction and Subsequent Morphogenesis in Mature Chickpea (*Cicer arietinum* L.) Embryo Culture. *In:* Role of Biotechnology in Food Security and Climate Change. Islam AS, Haque MM, Sarker RH and Hoque MI (Eds). Proc. Sixth Intl. Plant Tissue Cult. & Biotech. Conf., December 3-5, 2010, Bangladesh Assoc. Plant Tissue Cult. & Biotech. Dhaka, Bangladesh. pp. 53-58.