

## Effect of Plant Growth Regulators and Explant Type upon Cell Dedifferentiation and Callus Induction in Chickpea (*Cicer arietinum* L.)

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### Abstract

Chickpea (*Cicer arietinum* L.) is one of the most important sources of plant-based proteins. Somaclonal variation is a method for increasing the variation. The most common factors affecting somaclonal variation are explant types and growth regulators, in which the culture is established. The aim of the current research was to investigate the effect of the different growth regulators and explants sources on the induction of callus in chickpea (Bivanij cultivar). The callus induction experiment was conducted in MS medium supplemented by different concentrations of 2, 4-D (0, 1, 2 and 4 mg/L) plus 0.1 mg/L BAP in five explants (embryo, seed, root, hypocotyls and cotyledon). The evaluated traits include days to callus induction, callus induction percentage and callus growth rate. The results showed that the best response of callus induction occurs in the medium supplemented by 2 mg/L 2, 4-D. The shortest time for callus induction was belong to embryo explant in the medium supplemented by 0.1 mg/L BAP + 1 mg/L 2,4-D (4.25 days) and the longest time for callus induction was belong to cotyledon explant in the medium supplemented by 0.1mg/L BAP + 2 mg/L 2,4-D (14.25 days). The highest callus growth rate was belong to embryo in the medium supplemented by 1 mg/L 2, 4-D (0.1775 mm diameter/d) and the lowest was belong to cotyledon (0.02 mm diameter/d) in 4 mg/L 2, 4-D, and seed (0.01mm diameter/d) in 1 mg/L 2, 4-D.

**Keywords:** Growth Regulators, Chickpea, Callus, Tissue Culture

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### Introduction

Chickpea (*Cicer arietinum*), a legume of the Fabaceae family, stands second as for occupied area (about 10 million ha) in the area under cultivation and third in production (about 7 million tons) among the cultivated pulses in the world. Various characteristics of chickpea made it the most cultivated pulse crop and the most appreciated protein source among vegetarians all over the world. It is able to drive more than 70% of nitrogen symbiotic dinitrogen fixation; which makes it a capable crop for "alternative agriculture" that is now attracting substantial attention in the industrialized world [1-2].

There are several biotic and abiotic stresses which limit chickpea cultivation. In general, the major constraints to chickpea production in Iran are wheat and barley-based cropping system, lack of high-yielding varieties, susceptibility to blight and pod borer insects, etc. [2].

Crop breeding is important for ensuring food security by developing new varieties that are higher-yielding, resistant to pests and diseases, drought-resistant or regionally adapted to different environments and growing conditions [3].

Different types of breeding methods are employed in plant breeding since the last century. However, recent advances in the field of plant tissue culture have brought about new emerging technologies for plant breeding. The potential value of cell, tissue, organ, anther, microspore and embryo

culture for using in crop improvement has been reported [3-6].

The role of plant tissue culture in genetic engineering is important. Several researchers have described the in vitro callus induction of *C. arietinum* [7-12].

The frequency of callus induction and plant regeneration in tissue culture of chickpea is influenced by many factors, such as culture medium composition, explant source, genotype, environment, etc. Among them, the explant type, nutrient composition, and hormone supplementation are regarded to be the major sources of variation in in-vitro culture [13-17].

The present study was undertaken to investigate the effect of different concentrations of plant growth regulators and explant type on callus induction in chickpea.

### Materials and Methods

This study was conducted in Medicinal Plants Tissue Culture Lab., Agriculture and Natural Resources Campus, Razi University during 2013.

Chickpea (Bivanij cultivar), that is known as a high yield and sensitive to blight disease.

The mature chickpea seeds were surface-sterilized with sodium hypochlorite for 10 min. The sterilized seeds were rinsed in sterilized water for three times and then, were cultured on basal MS medium. The culture was incubated in phytotron at 25°C under a 16/8 hour light/dark cycle.





After that, some explants, such as hypocotyls and cotyledon, etc., were isolated. The embryo explants were isolated directly from sterilized seeds. The callus induction experiments were carried out for comparison of different growth regulators and various explants of chickpea (Bivanij cultivar). The callus induction experiment was conducted in MS medium supplemented by different concentrations of 2, 4-D (0, 1, 2 and 4 mg/L) plus 0.1 mL BAP in five explants (embryo, seed, root, hypocotyl and cotyledon). The interaction effects for 2, 4-D concentrations in explant have been numerated as shown in Table 1. The experiment was conducted as factorial based on completely randomized design (CRD) with four replications. The evaluated traits include days to callus induction (DCI), callus induction percentage (CIP) and callus growth rate (CGR). For CGR evaluation the callus diameters were recorded after 14, 21 and 28 days.

#### Statistical analysis

Specific analyses and the results are noted in the appropriate figure and table footnotes and text. Mean comparison was performed using Duncan's Multiple Range test at 0.05 probability level after ANOVA. Statistical analyses were done using Excel 2010, SPSS Ver. 19 and SAS Ver. 9.1 software.

**Table 1.** Number codes for 2, 4-D concentrations in explant interaction effects in callus induction experiment in chickpea (Bivanij cultivar).

Number Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Explant Type	R	R	R	R	C	C	C	C	H	H	H	H	E	E	E	E	S	S	S	S
2,4-D (mg/L)	0	1	2	4	0	1	2	4	0	1	2	4	0	1	2	4	0	1	2	4

Where R: root, C: cotyledon, H: hypocotyl, E: embryo and S: seed

#### Results

After incubation of cultured seeds, the stock plants achieved (Fig. 1).

#### Analysis of variance and mean comparison in the callus induction experiment

The analysis of variance showed that there are significant differences among plant growth regulators (PGRs) levels, explant types and their interactions for callus induction percentage (CIP), days to callus induction (DCI) and callus growth rate (CGR) (Table 2).

As the interaction effects are significant, and then the main effects are not considered. Since the number of interactions is abundant, they are shown as numbers codes as following. Duncan's multiple range test showed that interaction effects numbers (Table 2) 4, 7, 10, 12, 14 and 15 had the highest callus induction percentage (100%) and 1, 5, 9, 13, and 17 (2, 4-D free media) don't produce callus (Fig. 2 & 3). In the all explants (except root) in 2 mg/L 2, 4-D, callus induction percentage were 100%. Also hypocotyl and root explants in 4 mg/L 2, 4-D showed 100% callus induction percentage. Then 2 and 4 mg/L 2, 4-D is suggested for callus induction in chickpea.

Mean comparison results for days to callus induction (DCI) indicated that treatments No. 14 and 7 showed the lowest (4.25 d) and highest (14.25 d) values respectively (Fig. 4). Treatments No. 1, 5, 9, 13 and 17 (are marked

with stars in Figure 4) had not callus induction and they has not been considered for DCI treat. Mean comparison results for callus growth rate (CGR) indicated that treatment No. 14 showed the highest (0.1775 mm diameter/d) and treatments No. 8 and 18 showed the lowest (0.02 and 0.01mm diameter/d respectively) (Fig. 5).

**Table 2.** Mean squares for effect of growth regulators (PGRs), explant types and their interactions for callus induction percentage (CIP), days to callus induction (DCI) and callus growth rate (CGR).

SOV	DF	Mean Squares		
		CIP	DCI	CGR
Explant (E)	4	0.385**	72.26**	0.0093**
PGRs	3	3.39**	395.11**	0.070**
E × PGRs	12	0.24**	24.01**	0.005**
Error	60	0.010	3.21	0.0032
CV		17.24	21.27	20.6

\*\* Significant differences ( $p < 0.01$ )

SOV: source of variations, DF: degree of freedom and CV: Coefficient of variations.



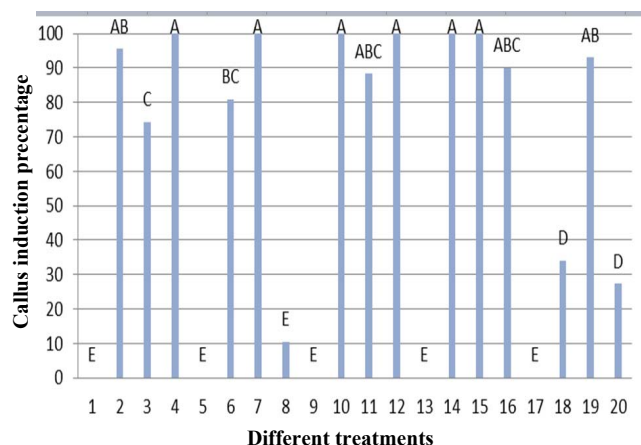
**Figure 1.** The serialized stock plant for explant preparation in order to callus induction experiment.



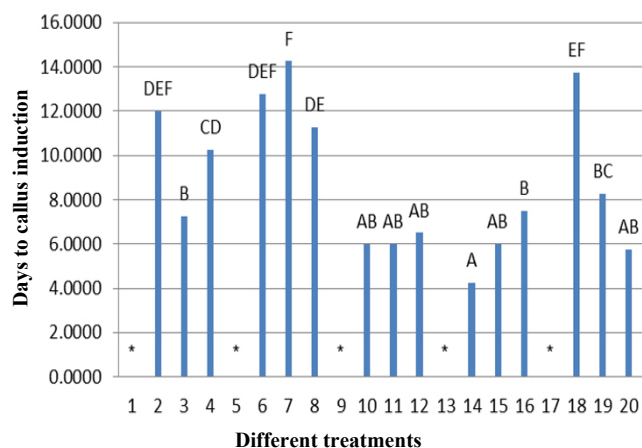
**Figure 2.** Callus induction in chickpea seed explant.

Also treatments No. 1, 5, 9, 13 and 17 (are marked with stars in figure 4) had not callus induction and they has not been considered for DCI treat.

This diagram indicates that the calli derived embryos show higher growth rate than other explants. The hypocotyl explants is placed in the next ranking. Overall CGR was low in the seed explant.



**Figure 3.** Callus induction percentage in 20 different interaction (PGRs × explant) effects in chickpea (Bivanij cultivar).



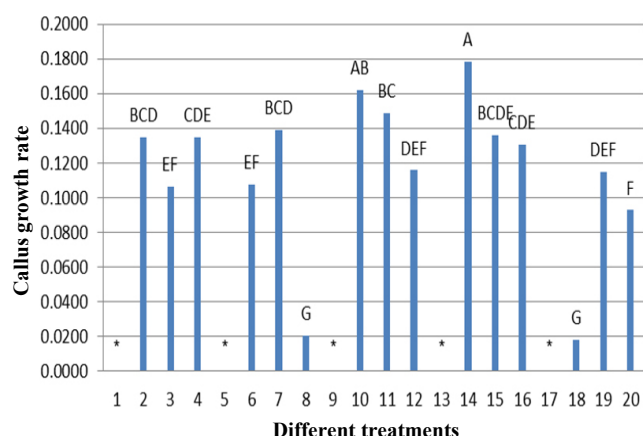
**Figure 4.** Days to callus induction (DCI) in 20 different interaction (PGRs × explant) effects in chickpea (Bivanij cultivar).

### Discussion

After recording of data related to callus indices, the statistical analysis of data including analysis of variance and mean comparison were carried out. When the interaction effects are significant, the main effects are not considered. Results showed that there are significant differences among plant growth regulators levels and explant types for callus induction percentage.

Gosal and Bajaj (1979) [18] in establishment of callus tissue cultures in chickpea reported that 2 mg/L 2, 4-D is the best concentration for callus induction in chickpea.

They used cotyledon, hypocotyl and root explants. Our results are in agreement with theirs.



**Figure 5.** Callus growth rate (CGR) in 20 different interaction (PGRs × explant) effects in chickpea (Bivanij cultivar).

Saleem and coworkers (2011) [19] investigated the callus induction in indigenous chick pea (*Cicer arietinum* L.) cultivars KK-1 and Hassan-2Kon MS and Bmedia containing different combinations and concentrations of growth regulators. For KK-1 cultivar, the maximum callus frequency (71 and 97%) followed by (65 and 96%) were observed on 4 mg/L 2, 4-D + 5 μM BAP in MS and 4 mg/L 2, 4-D in Bmedia, respectively after two and four weeks of culture. In Hassan-2K, the highest callus % (68 and 96) were recorded on MS + 4 mg/L 2,4-D + 0.50 mg/L NAA after two and four weeks of culture, respectively [19].

The explants in the control medium became swollen and finally signs of callus formation were observed in the following week, though with a low frequency of callus induction. In general, this was a wound reaction where the mitosis was induced in the cells from the cut surface leading to callus induction [20].

Kaberi and coworkers (2013) studied on callus induction via epicotyls, hypocotyls and shoot tip culture in chickpea. They reported the highest (100%) callusing response was obtained in MS + 2.0 mg/L 2,4-D + 0.5 mg/L NAA after three weeks of culture [21].

### Conclusion

The studied traits include days to callus induction, callus induction percentage and callus growth rate. The mostly medium induced the callus formation and the best response was in medium supplemented by 2 mg/L 2, 4-D. The lowest days to callus induction was belong to embryo explant in medium supplemented by 0.1mg/l BAP+1mg/l 2, 4-D. The highest callus growth rate was belong to embryo in medium supplemented by 1 mg/L 2, 4-D.

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