Biological Forum – An International Journal **7**(2): 970-974(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Evaluation of chickpea genotypes for callus induction using mature embryo culture

Azar Garosi*, Ezatollah Farshadfar and Mohammad Mahdi Jowkar****
**Department of Agronomy and Plant Breeding, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran*
,Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran*

(Corresponding author: Ezatollah Farshadfar)
(Received 28 August, 2015, Accepted 29 November, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Various attributes of chickpea made it the most cultivated pulse crop, and the most appreciated protein source among vegetarians all over the world. Efficient plant regeneration from cultured cells and tissues is required for the successful application of biotechnology in crop improvement. Therefore, this study was performed in order to evaluate the reaction of 11 chickpea genotypes to callus induction. Studied traits were: callus growth, callus water content, percentage of callus induction, callus diameter, callus fresh weight and dry weight. The results of analysis of variance showed significant differences (P<0.01) among the genotypes for callus diameter, percentage of callus induction, callus fresh weight and dry weight. Mean comparison indicated that the most desirable genotypes for callus induction were genotypes 5 and 8 and the weakest genotypes were 6 and 7. Based on the traits investigated, cluster analysis was done. The genotypes were classified in four categories. The results of the correlation analysis exhibited that the highest correlation between diameter and dry weight.

Keywords: Chickpea, callus induction, mature embryos

INTRODUCTION

Chickpea (*Cicer arietinum* L.), a member of grain legume group, is favoured all over the world for its high seed protein content. Chickpea is the most important legume in Iran and includes nearly 84% of the food legume with 17-24% protein, 41-51% carbohydrates, high percentage of other mineral nutrients and unsaturated linoleic andoleic acid (Farshadfar and Farshadfar, 2008). The tissue culture method is a novel approach, and the main idea is that cultivated cells are used as the selection units rather than whole plants (Butenko and Kuchku, 1979). The insertion of in vitro tissue culture techniques in a breeding program offers considerable opportunities for genetic improvement of plants by saving space and time required by conventional methods (Ortiz,1998). The utilisation of biotechnology in plant breeding is largely dependent on callus induction and subsequent plant regeneration from various explant sources. The success in this process is affected predominantly by genotypes and the type of explant material (Ozgen *et al.*,1996; Ozgen *et al.*, 1998).

Callus is used for most of these transformation methods such as particle gun (McCabe *et al.*, 1998) and agro-bacterium tumefaciens-mediated transformation (Stiekema *et al.*, 1988) as well as initiation of cell culture. A callus from an explant tissue occurs as a result of dramatic changes in the appearance and metabolism of the cells (Aitchison *et al.*, 1978).

The frequencies of callus induction and plant regeneration in tissue culture of chickpea are influenced by many factors: culture medium composition, explant source, genotype and environment etc. Among them the genotype, nutrient composition and hormone supplementation are regarded to be the major sources of variation in vitro culture (Khanna and Raina, 1998 and Khatun *et al.*, 2003). Mature embryos which are readily available at all times are the least frequently used explant sources because of their low frequency of callus induction. However, some new techniques such as the endosperm-supported callus induction method have been successfully used in callus induction from mature embryo cultures (Ozgen *et al.*, 1998).

Successful development of an embryo depends on many factors. As with most other processes, the plant genotype greatly influences success. Embryos of some species are easier to grow in culture than are others, and differences sometimes occur between closely related cultivars (Collins and Grosser, 1984; Rangan, 1984). According to Pierik (1989), there are in principle two types of embryo culture: culture of immature embryo and mature embryos.

Mature embryos are excised from ripe seeds and cultured mainly to avoid inhibition in the seed for germination. This type of culture is relatively easy as embryo requires simple nutrient medium containing mineral salts, sugar and agar for growth and development.

The aim of this study was to evaluate the behavior of chickpea genotypes on callus induction from mature embryos under normal condition.

MATERIALS AND METHODES

The present study was conducted to evaluate the response of chickpea (*Cicer arietinum* L.) genotypes to callus induction from mature embryos. A completely randomized design with 6 replications was used in the tissue culture laboratory, Islamic Azad University, Kermanshah, Iran during 2013-2014. For mature embryo culture, embryos were aseptically dissected from the seeds with scaple and forceps and placed on MS medium (Murashige and Skoog, 1962). Six mature embryos were placed in every petri dish. The petri dishes transferred to dark growth chamber with a temperature of 25°C and the samples were kept for 4 weeks. The names of genotypes studied are listed in Table 1.

Table 1: The names of genotypes studied.

Code	Name
1	FLIP-82-150C
2	FLIP-GG-26C
3	FLIP-82-245
4	HASHEM
5	FLIP-00-40C
6	FLIP-00-6C
7	FLIP-82-115
8	ARMAN
9	S95-181
10	S95-349

A. Studied traits

Percentage of Callus Induction (PCI): PCI was evaluated 4 weeks (suitable for sub-culturing) after embryo culture in Petri dishes as: (Arzani and Mirodjagh, 1999) (number of seeds producing callus)/(number of seeds plated in Petri dishes).

B. Callus growth rate (CGR)

CGR (mm/day) of cultured embryos on MS medium were measured at 7, 14, 21 and 28 days, respectively after transferring calli to medium. CGR was calculated using the following formulas (Compton, 1994):

CGR1 = d7/7, CGR2 = d14 /7, CGR3 = d21/7, CGR4 = d28/7

CGR = (CGR1+ CGR2 + CGR3 + CGR4) / 4

where d7, d14, d21, d28, respectively were diameter of callus in days 7, 14, 21 and 28, respectively. Diameter of callus was calculated as:

Diameter of callus = DC = % length × width

C. Relative Water Content (RWC)

Callus samples of known fresh weight were dried in an oven set at 700C for 24 h and RWC was calculated by following formula (Errabi *et al.,* 2006):

$$RWC = [(FW-DW)/DW] \times 100$$

where, FW and DW are the callus fresh and dry weights, respectively.

D. Statistical analysis

The results of this study were analyzed by statistical software SPSS and MSTATC. The means were compared by Duncan multiple range test at 1% probability level.

RESULTS AND DISCUSSION

A. Analysis of variance and mean comparison

The results of Analysis of variance (Table 2) showed significant differences ($P<0.01$) among the genotypes for callus diameter, percentage of callus induction (PCI), callus fresh weight and callus dry weight indicating the presence of genetic variability and different responses of genotypes to callus induction. There was no significant difference in genotypes for callus growth rate, callus relative water content. Comparison of means characteristics was performed using Duncan, s multiple range test. Researchers from Callus growth rate (CGR) are referred as an important criterion in the evaluation of genotypes callusing and the ability to respond to tissue culture (Ozgen *et al.,* 1996). According to the researchers, high growth rate of some genotypes in vitro, are similar to genetic mechanism (epistasis and dominance) of these traits (Fennell *et al.,* 1996). Comparing genotypes with the ability of better respond to the culture medium for regeneration and gene transfer is better. According to the results of mean comparison (Table 3), the highest callus growth (CGR) rate belonged to genotype 5 and genotype 3 revealed the lowest CGR. The highest relative water content (RWC) of callus was attributed to the accessions 8, 9, and 5 respectively, and genotype 10 exhibited the least RWC. Genotype 5 had the highest callus diameter (CD) followed by genotypes 1 and 8. The least callus diameter was related to genotypes 6 and 10. The largest callus fresh weight (FW) was related to genotypes 5, 2 and 8, respectively and the least value of this attribute belonged to genotypes 6 and 7.

Table 2: Analysis of variance for the traits investigated using mature embryos.

S.O.V	DF	CD	FW	DW	PCI	RWC	CGR
Genotype	9	0.10316**	0.00992963**	0.00049926**	193.666667	28.11527^{ns}	0.00003548^{ns}
Error	50	0.01698**	0.00171333	0.000138	0	16.704471	0.00001906

^{ns}, * and **: Non significant, significant at the 5% and 1% probability levels, respectively

Table 3: The results of mean comparison for studied traits.

Genotype	CD	FW	DW	PCI	RWC	CGR
1	0.809^{ab}	0.173^{abc}	0.012^{b}	100^{a}	10.25 ^{a}	0.005 ^{b}
2	0.777^{a}	0.222^{a}	0.017^{ab}	100^{a}	13.556 ^{a}	0.006 ^{ab}
3	0.728^{abc}	0.202^{ab}	0.027^{a}	100^{a}	11.033 ^{a}	0.002 ^{ab}
4	0.759^{abc}	0.190 ^{c}	0.032 ^{b}	100^{a}	11.667 ^{a}	0.007 ^{ab}
5	0.933^{ab}	0.272 ^{a}	0.030^{ab}	100^{a}	14.417 ^{a}	0.011^{a}
6	0.508^{ab}	0.147^{ab}	0.013 ^{b}	83^{c}	11.083 ^{a}	0.008 ^{b}
7	0.666^{bc}	0.143^{bc}	0.012 ^{b}	100^{a}	11.833 ^{a}	0.004 ^{b}
8	0.876^{bc}	0.215^{abc}	0.032^{ab}	100^{a}	15.833 ^{a}	0.007 ^{b}
9	0.682^{bc}	0.173^{abc}	0.012 ^{b}	92^{b}	14.500 ^{a}	0.004 ^{b}
10	0.564^{c}	0.147^{bc}	0.012^{ab}	100^{a}	9.000 ^{a}	0.005 ^{b}

Table 4: Ranking and selection of the best groups of chickpea based on studied traits.

Traits	Group 1	Group 2	Group 3	Group 4
RWC	4	3	1	2
CGR	3	2	1	1
PCI	2	1	1	3
CD	3	2	1	4
FW	2	3	1	4
DW	4	3	1	2

Genotypes 4, 5 and 8 in terms of callus dry weight (DW) had the highest quantity and the least value of this attribute was related to genotype 1, 6, 7 and 9, respectively. Different responses of accessions for the traits investigated can be attributed to the effects of genotype and environment on the seed size. It seems that in breeding programs genotypes 5 and 8 are useful for mature embryos followed by genotypes 2 and 4. This difference is induced by individual and interactive effects of two factors: Genotype and environment These factors are decisive for chickpea callogenic potential expression (Arora and Chawla, 2008; Khan *et al.*, 2011). Callogenesis rate varied significantly with the genotype tested. Khan *et al.* (2011) reported difference in the capacity of callogenesis expression for two different indigenous chickpea genotypes, KK1 and Hassan 2K even when subjected to identical in vitro culture conditions. The recalcitrant nature observed in some genotypes may be due to their physiological characteristics (Sani and Mustapha, 2010), specially endogenous hormones levels, or to a genetic inability. Genotype effect on callogenesis is reported as well for chickpea (Zare and Bagheri, 2011 and Khan *et al.*, 2011).

B. Cluster analysis

Cluster analysis or clustering is the task of grouping a set of objects in such a way that objects in the same group are more similar (in some sense or another) to each other than to those in other groups.

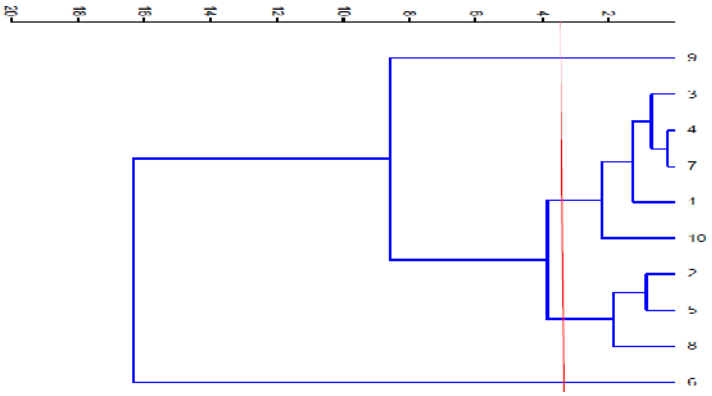

Fig. 1. Cluster analysis of chickpea genotypes for the traits investigated.

Results of cluster analysis based studied traits showed that genotypes were placed in four categories (Fig. 1). Based on this analysis genotypes 1,4,7 and 10 were located in a group and genotypes 2, 5 and 8, as well as genotypes 6 and 9 were each in separate groups. Genotype 6 for all studied traits, had almost the lowest average.

C. Ranking method

Based on the studied traits, after averaging the varieties within each group, the ranking is used to determine the best group. The best group is the group with the lowest score. Results indicated that the best groups for studied traits are group 3 and 2 respectively.

D. Correlation coefficient analysis

Results of the correlation analysis (Table 5), showed that Most of the traits had significant high correlation with each other. For example a positive and significant correlation was observed between traits of callus diameter and fresh weight, dry weight, percentage of callus induction and callus water content.

Table 5. Correlation between studied traits in non-stress condition.

	CD	DW	FW	PCI	RWC	CGR
CD	1					
DW	0.84965**	1				
FW	0.65995**	0.7155**	1			
PCI	0.62027**	0.39649*	0.38647*	1		
RWC	0.61356**	0.63014**	0.45406**	0.031792	1	
CGR	-0.07063	0.12176	0.13981	-0.556**	0.16368	1

^{ns}, * and **: Non significant, significant at the 5% and 1% probability levels, respectively

High correlation between callus diameter and fresh weight (0.84) indicated that increase of callus diameter also increases the amount of water of callus. The correlation coefficient between relative growth rate of callus and most of the traits is not significant. Percentage of callus induction was the only character that revealed significant high correlation with callus growth.

REFERENCES

Abdelsamad, AOE., El - Sayed, Ibrahim F. (2007). *Journal of Appllied Science Research*, **3**(11): 1589-1599.

Aitchison, PA., MacLeod, AJ., Yeoman, M. (1978). Growth patterns in tissue (callus) cultures. In HE Street, ed, Plant Tissue and Cell Culture, Blackwell Sci. Pub. Oxford, pp. 267-306.

Arora, A., Chawla, SH. (2005). Organogenic plant regeneration via callus induction on chickpea (*Cicer arietinum*) role of genotypes, growth regulators and explants. *Indian Journal of Biotechnology*, **4**: 251-256.

Arzani, A., Mirodjagh, S. (1999). Response of durum wheat cultivars to immature embryo culture, callus induction and in vitro salt stress. *Plant Cell Tissue Organ Culture*, **58**: 67-72.

Butenko, R.G., Kuchko, A.A. (1979). Physiological Aspects of Procurement, Cultivation, and Hybridization of Isolated Potato Protoplasts. *Soviet Plant Physiology*, **26**: 901-909.

Chen, JJ., Yue, RQ., Xu, HX., Chen, XJ. (2006). Study on plant regeneration of wheat mature embryos under endosperm supported culture. *Agricultural Science in China*, **5**(8): 572-578.

Compton ME, (1994). Statistical methods suitable for the analysis of plant tissue culture data. *Plant Cell Tissue Organ Culture*, **37**: 217-242.

Collins, G.B., Grosser, JW. (1984). Culture of embryos, p. 241-257. In: I.K. Vasil (ed.). Cell culture and somatic cell genetics of plants.vol. 1. Laboratory procedures and their applications. Academic, New York.

Errabi, T., C.B. Gandonou, M. Essalmani, J. Abrini, M. Idaomar, N. Skali-Senhagi. (2006). Growth, praline and ion accumulation in sugarcane callus caltures under drought-induced osmotic stress and its subsequent relief. *African. Journal of Biotechnology*, **5**(16): 1488-1493.

Farshadfar M., Farshadfar, E. (2008). Genetic variability and path analysis of chickpea (*Cicer arietinum* L.) land races and lines. *Journal of Applied Science*, **8**: 3951-3956 .

Fennell, S., Bohorova, N., M. Ginkel, M. V. crossa, I. Hoisington D. (1996). Plant regeneration from immature embryos of 48 elite CLMMYT bread wheat. *Theoretical and Applied Genetics*, **92**: 163-169.

Khan, S., Ahmad, F., Ali, F., Khan, H., Khan, A., Swati, ZA. (2011). Callus induction via different growth regulators from cotyledon explants of indigenous chickpea (*Cicer arietinum* L.) cultivars KK-1 and Hassan-2K. *African Journal of Biotechnology*, **10**(40): 7825-7830.

Khanna, HK., Rain,a SK. (1998).Genotype × culture media interaction response of three indica rice cultivars. *Plant Cell, Tissue and Organ Culture*, **52**: 145-153.

Khatun, M., Ali, MH., Desamero, NV) 2003). Effect of genotype and culture media on callus induction and plant regeneration from mature seed scutellum culture in rice. *Plant Tissue Culture*, **13**(2): 99-107.

McCabe, DE., Swain, WF., Martinell, BJ., Christou, P. (1988) . Stable transformation of soybean (*Glycine max*) by particle acceleration. *Biotechnology*, **6**: 923-926.

Ozgen, M., T , Ret, M., Ozcan, S., Sancak, C. (1996) .Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. *Plant Breeding*, **115**: 455-458.

Ozgen, M., T.ret, M., Altynok, S., Sancak, C. (1998). Efficient callus induction and plant regeneration from mature embryoculture of winter wheat (*Triticum aestivum* L.) genotypes. *Plant Cell Reports*, **18**: 331-335.

Pierik, R.L.M. (1989). In Vitro culture of higher plants. Martinus Nijhoff Publishers, Dordrecht.

Ortiz, R. (1998). Critical role of plant biotechnology for the genetic improvement of food crops- perspective for the next millenuim. *Electronic Journal of Biotechnology* at http://www.ejb.ucv.cl/content/vol1/issue3/full/7/

Rangan, T.S. (1984). Culture of ovules, p. 227-231. In: I.K. Vasil (ed.). Cell culture and somatic cell genetics of plants. vol. **1**. Laboratory procedures and their applications. Academic, New York.

Sani, L., Mustapha, Y. (2010). Effect of genotype and 2, 4- d concentration on callogenesis in sugarcane (*Saccharum* spp. hybrids). *Bayero Journal of Pure and Applied Sciences*, **3**(1): 238 – 240

Stiekema, WJ., Heidekamp, F., Louwerse, JD., Verhoeven, HA., Dijkhuis, P.(1988). Introduction of foreign genes into potato cultivars Bintje and Desiree using an *Agrobacterium tumefaciens* binary vector. *Plant Cell Reports*, **7**: 47-50.

Zare, M., Bagheri, AR. (2011). Efficient protocol for break impasses of regeneration via callus for 20 genotypes of chickpea. *International Journal of Plant Production*, **4**(2): 115-128.