DOI: http://dx.doi.org/10.22092/cbj.2012.100433

Genetic variation in drought tolerance in chickpea (*Cicer arietinum* L.) genotypes

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Received: July 2012
Accepted: September 2012

ABSTRACT

Kanouni, H., M. R. Shahab, M. Imtiaz, and M. Khalili. 2012. Genetic varaition in drought tolerance in chickpea (*Cicer arietinum* L.) genotypes. **Crop Breeding Journal 2(2): 133-138.**

Drought is one on the most important factors limiting the productivity of spring chickpea in drylands of Iran. Sixty genotypes of chickpea from ICARDA's germplasm accessions, as well as one drought susceptible check (ILC 3279), were sown in spring of 2010 at two locations, Sanandaj and Maragheh, in the western highlands of Iran for one year. The experiment in each location was laid out in a randomized complete block design with two replications. The results of the analysis of variance for seed yield, 100-seed weight, pods per plant, plant height and days to maturity indicated that genotypic differences were significant. Seed yield ranged from 266 kg/ha (FLIP06-58C) to 1020 kg/ha (FLIP 06-60C). The phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) and the environmental coefficient of variation (ECV) for all traits. The PCV was highest for drought tolerance score (44.54%), followed by plant vigor (32.24%), seed yield (28.47%) and pods per plant (27.59%). Similarly, the GCV was highest for drought tolerance (39.27%), followed by plant vigor, seed yield and pods per plant. The GCV and PCV were lowest for days to maturity, followed by days to flowering and 100-seed weight. Heritability of days to maturity, days to flowering and drought tolerance was greater than the heritability of the other traits. Positive significant ($P < 0.05$) relationships were found between seed yield per plant and traits pods per plant, 100-seed weight and plant height. The genotypic path coefficient analysis based on seed yield per plant as a dependent variable revealed that drought tolerance score, 100-seed weight, plant height and pods per plant exhibited high positive direct effects. Vigor, days to maturity and 100-seed weight showed the highest direct influence. Therefore, this research suggests that drought tolerance score and pod per plant can be good selection criteria for improving seed yield per plant in chickpea for drought stress environments.

Keywords: abiotic stresses, chickpea, *Cicer arietinum* L., genetic diversity, seed yield

INTRODUCTION

Chickpea, an ancient crop, is important in both developed and developing nations (Yadav *et al*., 2007). It is the third most important food legume crop worldwide and the most important food legume in Iran. The total area sown to chickpea is about 700,000 ha in Iran, which ranks fourth in the world after India, Pakistan and Turkey. Chickpea production and yield in Iran are 350,000 t and 500 kg ha^{-1}, respectively (FAO, 2001).

Drought is the most important abiotic stress limiting chickpea production (Saxena *et al*., 1993). On the other hand, chickpea is considered to be the most drought tolerant cool-season food legume crop because it has a long taproot that can extract water from the lower depths of the soil profile. Chickpea requires only 6-10 inches of rainfall and/or irrigation water during the growing season and thus is well suited to dryland or limited-irrigation production. However, exposure of chickpea plants to terminal drought is one of the major constraints to increasing productivity. Therefore, development of early maturing cultivars with early growth vigor may help chickpea varieties utilize the available soil moisture more efficiently and produce higher yields (Kumar and van Rheenen, 2000).

In the last decade, the main breeding strategy used to cope with terminal drought in chickpea was selecting for drought escape by reducing crop duration and securing seed yield before soil water was depleted. This strategy was successful in increasing yield stability and resulted in the release of early maturing varieties (Kashiwagi *et al*., 2007; Sabaghpour *et al*., 2006).

Knowledge of the relative magnitude of various genetic parameters for seed yield and yield components is essential for an efficient breeding program. Genetic variation among traits is important for breeding and selecting desirable types. On the other hand, an analysis of the correlation between seed yield and related characters is essential for determining appropriate selection criteria; path coefficient analysis helps to determine the direct effect of traits and their indirect effects on other traits. Genetic variability, broad-sense heritability and genetic advance parameters have been estimated (Arshad *et al*., 2002; Yücel *et al*., 2006), and phenotypic correlation coefficients between seed yield and yield-determining characters have been analyzed in chickpea (Khorgade *et al*., 1985; Farshadfar and Farshadfar, 2008; Sidramappa *et al*., 2008).

The objective of the present study was to determine the extent of genetic variability, broad-sense heritability, genetic advance and genetic relationships between seed yield and yield component characters of 60 chickpea genotypes. In addition to phenotypic correlations, genetic correlations among traits were also estimated to devise suitable selection criteria for further yield improvement.

MATERIALS AND METHODS

In this investigation 60 Kabuli chickpea genotypes obtained from International Center for Agricultural Research in the Dry Areas (ICARDA), along with one drought susceptible check (ILC 3279) originating from Syria, were studied (Table 1). The trial was conducted at two experiment stations belonging to the Dryland Agricultural Research Institute (DARI) of Iran: the first at Maragheh (latitude: 37° 23′ N., longitude: 46° 14′ E., and 1477 meters above sea level) and the second at Saral, Sanandaj (latitude: 35° 40′ N., longitude: 47° 07′ E., and 2120 meters above sea level), in northwestern and western Iran, respectively. The experiment was sown during the spring of 2010 using a randomized complete block design (RCBD) with two replications under rainfed conditions. Each line was sown in one row, 2 m in length, with 30 cm between adjacent lines. The susceptible check was repeated after every two test entries, to be evaluated more precisely. The land was fallow in the previous year and 50 kg ha^{-1} of N fertilizer was applied before sowing.

Plots were managed following recommended practices for land preparation, fertilization, pest and weed control, but were planted three weeks later than the normal sowing date, to subject the plants to drought conditions. Five randomly selected plants were taken from each plot at each location for data assessment. Different plant traits were measured, including days to flowering (DFLR), days to maturity (DMAT), early plant vigor (VIGO), pods per plant (PD/PL), plant height (PHT), 100-seed weight (100SW) and seed yield (SYLD). Visual estimates were made of yield potential: 1= very good; 2= good; 3= average; 4= poor and 5= very poor. Drought tolerance was evaluated visually at maturity using a drought tolerance score (DTS) on a 1-9 scale (Singh *et al*., 1997): 1= free, very good pod setting; 2= highly tolerant, 91-95% pod setting; 3= tolerant, 81-90% pod setting; 4= moderately tolerant, 71-80% pod setting; 5= intermediate, 51-70% pod setting; 6= moderately susceptible, 31-50% pod setting; 7= susceptible, 11-30% pod setting; 8= highly susceptible, late flowering, lack of early plant vigor, 1-10% pod setting; and 9= plants dead, no pod setting.

Data were analyzed according to the RCBD over locations. Correlation coefficients were calculated with the MSTATC program to determine the relationships between the tested traits and seed yield per plant. Path coefficient analysis was performed by examining SYLD as a dependent variable for major contributors to SYLD with PATHSAS, a SAS computer program, as described by Cramer *et al*. (1999).

Simple and combined analyses of variance were performed for each trait measured in the experiments. Based on the analysis of variance, phenotypic and genotypic variances, phenotypic and genotypic coefficients of variation, broad-sense heritability, genetic advance, genetic advance expressed as a percentage of the mean, and phenotypic and genotypic correlations between yield and some related traits were estimated by the multivariate restricted maximum likelihood estimation method (REML) using the SAS Proc MIXED procedure as described by Holland (2006).

D= Designated; U= Undesignated.

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance revealed significant differences among lines for days from sowing to maturity, number of pods per plant, plant height, 100-seed weight and seed yield, and among locations for all studied traits except vigor and number of pods per plant (Table 2). This indicates the existence of a high degree of genetic variability in the germplasm which could be exploited in breeding programs (Table 1). Genotype × location interactions were significant for DMAT, PHT, 100SW and SYLD, indicating that differences among mean values of genotypes vary with location Based on results, average seed yield differed among test entries. Genotypes FLIP 06-60C produced the highest seed yields (1020 kg/ha), while FLIP 06-58C had the lowest seed yields (266 kg/ha), even lower than that of the drought susceptible check cultivar (Table 2).

Table 1. Combined analysis of variance across locations for studied traits of chickpea genotypes in western Iran, spring 2010.

		Mean squares							
S.O.V.	df	Days to flowering	Days to maturity	Drought tolerance score	Vigor	No. of pods plant^{-1}	Plant height	100-seed weight	Seed yield
Location (L)	1	62443.00**	92274.00**	266.77*	48.72	0.097	555.74*	211.95**	5662.30*
Replication/L	2	12.84	11.82	6.98	4.47	6.68	13.51	34.22	128.31
Genotype (G)	60	3.65	12.13**	2.19	0.62	7.95*	18.97**	28.59**	239.69*
G × L	60	2.61	14.28**	1.76	0.71	0.13	11.13**	20.81*	230.77*
Error	120	3.52	2.97	1.84	0.63	5.19	3.87	13.91	156.73
R^2		0.98	0.99	0.71	0.64	0.97	0.83	0.66	0.63
Mean		53.13	104.14	3.04	2.46	8.26	25.33	32.88	642.96

* and **: Significant at the 5% and 1% probability levels, respectively.

Table 2. Name, pedigree, FAO status (FAO), drought tolerance score (DTS) and average seed yield (SYLD) of studied chickpea genotypes.

Entry No.	Name	Pedigree	FAO	DTS	SYLD (kg/ha)
1	FLIP02-04C	X99TH 6/FLIP91-14CX FLIP90-19C	U	3	454
2	FLIP02-47C	X98TH118/(FLIP87-38CXILC4339XS95159)XS96114	U	2	554
3	FLIP03-22C	X99TH 62/FLIP93-2C X FLIP94-115C	U	1	729
4	FLIP03-27C	X98TH86/[(ILC267XFLIP89-4C)XHB-1]XS95345	U	1	900
5	FLIP03-50C	X99TH 62/FLIP93-2C X FLIP94-115C	U	2	608
6	FLIP03-99C	X00TH 49/FLIP98-52CXFLIP98-10C	U	1	654
7	FLIP05-17C	X2001TH 38/(FLIP98-52CXFLIP98-7C)XSEL15042	U	2	625
8	FLIP05-19C	X2001TH 171/UZ-7332XSEL85314	U	1	637
9	FLIP05-43C	X2000TH 39/FLIP98-29CXS99001	U	2	704
10	FLIP05-57C	X2001TH 83/S15063XFLIP97-22C	U	3	579
11	FLIP05-88C	X2000TH 31/FLIP98-29CXS99093.	U	3	591
12	FLIP05-162C	X2001TH 61/(Turkesh2Xselter85530)XFLIP98-47C	U	1	675
13	FLIP05-169C	X2001TH 73/(sozlaniiz-304Xselter85581)XFLIP98-47C	U	3	575
14	FLIP05-170C	X2001TH 73/(sozlaniiz-304Xselter85581)XFLIP98-47C	U	3	708
15	FLIP05-183C	X2000TH 39/FLIP98-29CXS99001.	U	1	791
16	FLIP06-1C	X2002TH 5/FLIP98-130C X FLIP97-219C	U	1	858
17	FLIP06-2C	X2002TH 5/FLIP98-130C X FLIP97-219C	U	1	812
18	FLIP06-6C	X2002TH 7/S00762 X FLIP98-023C	U	3	483
19	FLIP06-7C	X2002TH 7/S00762 X FLIP98-023C	U	3	679
20	FLIP06-8C	X2002TH 7/S00762 X FLIP98-023C	U	1	737
21	FLIP06-10C	X2002TH 8/S00787 X FLIP98-028C	U	2	550
22	FLIP06-11C	X2002TH 10/S00835 X FLIP98-079C	U	1	600
23	FLIP06-12C	X2002TH 17/FLIP98-38C X FLIP98-053C	U	1	816
24	FLIP06-18C	X2002TH 21/S00787 X FLIP97-261C	U	1	800
25	FLIP06-20C	X2002TH 21/S00787 X FLIP97-261C	U	1	766
26	FLIP06-22C	X2002TH 21/S00787 X FLIP97-261C	U	3	441
27	FLIP06-25C	X2002TH 22/S00790 X FLIP97-281C	U	2	633
28	FLIP06-27C	X2002TH 22/S00790 X FLIP97-281C	U	4	379
29	FLIP06-28C	X2002TH 23/S00835 X FLIP98-053C	U	3	441
30	FLIP06-29C	X2002TH 23/S00835 X FLIP98-053C	U	3	562
31	FLIP06-32C	X2002TH 24/S99439 X FLIP98-130C	U	1	858
32	FLIP06-35C	X2002TH 28/FLIP98-28C X FLIP98-079C	U	3	750
33	FLIP06-36C	X2002TH 28/FLIP98-28C X FLIP98-079C	U	3	625
34	FLIP06-38C	X2002TH 28/FLIP98-28C X FLIP98-079C	U	4	562
35	FLIP06-50C	X2002TH 53/FLIP98-38C X FLIP98-048C	U	1	987
36	FLIP06-52C	X2002TH 54/FLIP98-130C X FLIP98-121C	U	1	875
37	FLIP06-53C	X2002TH 55/S00754 X FLIP98-175C	U	3	529
38	FLIP06-57C	X2002TH 76/S99858 X FLIP97-026C	U	2	787
39	FLIP06-58C	X2002TH 76/S99858 X FLIP97-026C	U	6	266
40	FLIP06-60C	X2002TH 76/S99858 X FLIP97-026C	U	1	1020
41	FLIP06-61C	X2002TH 78/S00704 X FLIP97-149C	U	3	566
42	FLIP06-62C	X2002TH 78/S00704 X FLIP97-149C	U	1	645
43	FLIP06-68C	X2002TH 89/S00878 X FLIP97-81C	U	3	450
44	FLIP06-71C	X2002TH 91/S99858 X FLIP98-28C	U	2	479
45	FLIP06-73C	X2002TH 91/S99858 X FLIP98-28C	U	3	691
46	FLIP06-80C	X2002TH 109/FLIP98-130CXreti.sel01th1214	U	3	591
47	FLIP06-85C	X2002TH 114/S00835 X echi.sel01th 12186.	U	1	833
48	FLIP06-89C	X2002TH 118/(FLIP98-64CXFLIP98-12CXSel99TER85448)X FLIP97-026C	U	3	608
49	FLIP06-91C	X2002TH 118/(FLIP98-64CXFLIP98-12CXSel99TER85448)X FLIP97-026C	U	3	604
50	FLIP06-95C	X2002TH 119/(FLIP98-64CXFLIP98-47CXSel99ter85488) X FLIP98-022C	U	2	658
51	FLIP06-103C	X2002TH 122/(S98588XS99093XS99358)X FLIP98-175C	U	2	637
52	FLIP06-108C	X2002TH 125/(S98588XS99442XSel99ter85488)X L.market-1.	U	1	895
53	FLIP06-112C	X2002TH 128/(ILWC81XS85530)XFLIP97-149C	U	4	345
54	FLIP06-133C	X2002TH 139/(ILWC181XS85581)XFLIP97-81C	U	1	800
55	FLIP06-134C	X2002TH 139/(ILWC181XS85581)XFLIP97-81C	U	3	545
56	FLIP06-144C	X2001TH 85/S15042XFLIP97-25C	U	3	533
57	FLIP06-146C	X2002TH 65/S00706 X FLIP97-131C	U	4	395
58	FLIP06-161C	X98TH58/(Malik1XILC7795XFLIP94-92C)XS96233.	U	1	879
59	FLIP87-59C	X85TH274/ILC3843XFLIP82-130C (drought tolerant check)	D	3	537
60	FLIP97-116C	X94TH11/FLIP90-132CXS91345 (drought susceptible check)	U	1	650
61	ILC 3279	Susceptible check	D	8	280
	LSD=0.05			3.01	208

Genetic variation

Genetic and phenotypic variances (σ^2g and σ^2p), genetic, phenotypic and environmental coefficients of variation (GCV and PCV), broad-sense heritability (h^2) and genetic advance (GA) as a percentage of the mean were calculated for various traits (Table 3). There were differences between PCV and GCV for almost all traits. Drought tolerance score and early plant vigor had the highest GCV and PCV, followed by seed yield and number of pods per plant. Days to flowering and days to maturity showed the lowest values. Higher phenotypic coefficient of variance (PCV) values were found for most measured traits, indicating that the expression of these traits is highly influenced by the environment.

Broad-sense heritability estimates ranged from 28.19 to 98.99% with the highest values obtained for days to flowering, days to maturity, drought tolerance score and seed yield, and the lowest value for number of pods per plant. Heritability combined with genetic advance is more useful than heritability alone for estimating the selection effects.

Heritability and genetic advance were highest for seed yield and days to maturity, followed by plant height. Similar findings were reported by Yücel *et al*. (2006). For all studied traits, PCV values were higher than GCV values, indicating the influence of environment on the expression of the traits. Similar results have been reported by Güler *et al*. (2001) and Arshad *et al*. (2002). This suggests that crop improvement, in terms of these traits, may be possible by simple selection, given that high heritability coupled with high genotypic variation reveals the presence of additive gene effects (Yücel *et al*., 2006).

Table 3. Genetic variance (σ^2g), phenotypic variance (σ^2p), broad-sense heritability (h^2), genetic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), environmental coefficient of variation (ECV), genetic advance (GA), genetic advance as a percentage of the mean (GAM) for yield and yield components of chickpea under drought conditions in western Iran, 2010.

Traits	Range	SE(±)	σ^2g	σ^2p	h^2 (%)	GCV	PCV	ECV	GA	GAM
Days to flowering	52- 77	1.03	253.25	260.37	98.99	2.28	3.53	1.63	3.89	7.32
Days to maturity	103.5- 127	1.26	376.24	387.82	98.94	1.11	1.68	0.89	7.09	6.80
Drought tolerance score	3- 8	0.11	1.43	3.04	77.72	39.27	44.54	12.54	2.37	77.71
Vigor	3- 5	0.06	0.48	0.87	76.01	25.03	32.24	10.38	1.23	40.41
No. pods plant^{-1}	8- 13	0.14	1.42	4.61	28.19	23.45	27.59	12.04	1.64	19.85
Plant height (cm)	25- 37	0.22	8.07	11.74	58.25	6.92	7.71	3.31	5.23	20.65
100-seed weight (g)	33.5- 42.5	0.29	7.46	19.97	43.97	5.51	11.43	4.44	4.84	14.72
Seed yield (g m^2)	42.85- 108.80	0.92	140.33	208.29	61.99	21.69	28.47	9.99	19.35	44.02

Correlations among traits

The association of yield with other traits was estimated by phenotypic and genotypic correlation coefficients. Seed yield exhibited significant positive phenotypic and genotypic correlations with PHT and 100 SW (Table 4). These results suggest that any increase in such traits would bring about gains in seed yield. These results are in agreement with those reported by Güler *et al*. (2001) and Sidramappa *et al*. (2008). On the other hand, significant negative relationships were found between seed yield and traits DFRL and DMAT. In this case, it may be suitable to select short duration lines for increasing seed yield and escaping late drought.

The high negative correlations between drought tolerance score and traits DFRL and DMAT indicate that cultivars with short life duration contribute to drought tolerance. These results were in accordance with the findings of Sabaghpour *et al*. (2006).

Path coefficient analysis

Path coefficients in genetic and phenotypic terms were partitioned into direct and indirect effects by using seed yield as a dependent variable. Direct and indirect effects are given in Table 5. In this analysis, the magnitude of direct effects shows that seed yield primarily depends on days to maturity and early plant vigor. Despite the positive significant correlation between 100-seed weight and seed yield, this trait had a low direct effect on yield. However, 100-seed weight contributed negatively through days to maturity, indicating the disadvantage of selecting on the basis of correlation studies alone.

Path coefficient analysis of seed yield indicated that drought tolerance score exerted the greatest phenotypic direct effect. This trait made major contributions to seed yield, and hence could enhance the success of chickpea breeding in the western highlands of Iran.

Although the direct effect of pods per plant was small, the indirect effect of this trait via DTS was remarkable. These results were in accordance with those reported by Farshadfar and Farshadfar (2008). The high indirect contribution of days to maturity to SYLD via DTS implies that earliness is very important in drought prone environments. Similar findings were reported by Saxena *et al*. (1993).

As a conclusion, in this study seed yield ranged from 1020 to 280 kg/ha, with FLIP 06-60C and ILC 3279 (the susceptible check) showing highest and lowest seed yield, respectively. This study

Table 4. Genetic and phenotypic† correlation coefficients among eight traits of chickpea genotypes measured under drought condi

Traits	Days to flowering	Days to maturity	Drought tolerance score	Vigor	No. of pods plant^{-1}	Plant h
Days to flowering		0.998**	-0.849**	-0.721**	0.036	-0.8
Days to maturity	0.995**		-0.844**	-0.709**	0.034	-0.8
Drought tolerance score	-0.759**	-0.755**		0.685**	0.167	0.5
Vigor	-0.628**	-0.619**	0.620**		-0.132	0.7
No. of pods plant^{-1}	0.017	0.006	0.080	-0.173		-0.3
Plant height (cm)	-0.665**	-0.672**	0.411**	0.461**	-0.086	
100-seed weight (g)	-0.337**	-0.330**	0.094	0.218	0.073	0.2
Seed yield (g m-^{1})	-0.482**	-0.485**	0.114	0.027	0.048	0.3

*** and **: Significant at the 5% and 1% probability levels, respectively.**

†Genetic and phenotypic correlation coefficients are above and below diagonal, respectively.

Table 5. The direct, indirect and percentage contribution of various traits to seed yield per plant of chickpea grown under droug

		Indirect effects				
Traits	Direct effect	Days to flowering	Days to maturity	Drought tolerance score	Vigor	No. of pods pl
Days to flowering	0.139[a]		0.272	0.070	0.331	0.085
	0.024[b]		-0.011	0.606	0.009	0.001
Days to maturity	-0.383	0.095		-0.086	0.086	0.072
	-0.024	0.011		-0.658	0.006	0.001
Drought tolerance score	-0.098	0.098	0.338		0.408	0.085
	-0.979	0.015	-0.016		0.010	0.001
Vigor	0.484	0.095	0.207	0.083		0.047
	0.014	0.015	-0.010	0.793		0.001
No. pods plant^{-1}	0.172	0.069	0.161	0.048	0.132	
	0.004	0.005	-0.006	0.324	0.001	
Plant height (cm)	0.006	0.018	-0.136	0.003	0.018	0.087
	0.007	-0.001	0.002	0.006	0.001	0.001
100-seed weight (g)	0.279	0.033	-0.218	0.061	0.126	0.105
	0.011	0.001	-0.009	0.475	0.002	0.002

[a] Genetic path coefficient; [b] Phenotypic path coefficient.

suggests that efficient selection for chickpea yield improvement under dry conditions should be based on both high pods per plant and vigor, as well as low drought tolerance score.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dryland Agricultural Research Institute (DARI), Iran, for financing this study and for providing the facilities for conducting this investigation. They also wish to thank Arsalan Behzadi for his technical assistance in field data collection.

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