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MULTIVARIATE ANALYSIS AND DROUGHT STRESS TOLERANCE INDICES IN CHICKPEA (*Cicer arietinum* L.) UNDER DIFFERENT IRRIGATION REGIMES

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ABSTRACT

In order to evaluate the association between genetic parameters and quantitative drought resistance criteria in chickpea, ten genotypes were evaluated under irrigated and rain-fed field environments. Significant difference was reported among all the measured traits under both environmental conditions. Phenotypic coefficient variation (PCV) ranged from 8.74% (for Proline) to 19.70% (for number of seeds per plant) in irrigated environment while it ranged from 9.90% (for proline) to 19.70% (for 100-seed weight) for rainfed condition. The GCV values were the lowest (8.40%) for chlorophyll content and highest (30.71%) for number of seeds per plant and the lowest (14.73%) for plant height and highest (50.39%) for 100-seed weight in irrigated and rain-fed environmental conditions respectively. High heritability values observed in 100-seed weight, number of seeds per plant and seed yield in both environments and also high heritability for physiological traits in rain-fed environment indicating that these traits are controlled mainly by additive genes and that selection of such traits may be effective for improving seed yield in both environments. There was a positive significant correlation between YSI and YI with seed yield, MP and GMP in both environments, suggesting that these parameters are able to discriminate superior group of genotypes under both environments. Results of factor analysis showed that two and three factors explained 80.34% and 89.66% of the total variance caused in the characters in irrigated and Rain-fed environment, respectively. High heritability for most of the morphological and physiological traits in both environments indicated that additive gene effects are important in determining these traits and selection based on these traits could be very useful in chickpea breeding program to introduce drought resistance chickpea genotypes for rain-fed climates.

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1 Introduction

Chickpea (*Cicer arietinum* L.) is an important legume crop under arid and semi-arid conditions and plays a vital role in the economy of the farming communities resides in these areas. Expanding the improved chickpea cultivars and evolution of these genotypes with narrow genetic bases, making them vulnerable to biotic and biotic stresses and it caused low and unsuitable yield productivity in most chickpea cultivation area (Talebi & Fayyaz, 2012). Drought is a major abiotic stress which responsible for production losses (Khan et al., 2007). According to Mafakheri et al. (2011), this stress is considered as one of the most important limiting factors for chickpea growth and production. Information on the relative importance of various yield components to seed yield is useful for producers to manage the crop by focusing on key yield components. Different adaptive responses like physiological and biochemical processes, canopy architects, antioxidant and osmotic adjustments have been reported in plants against drought stress (Duan et al., 2007). Any traits in plant that related to seed yield has its own genetic system and depending to heritability and nature of each trait, different environmental effects for yield components has been reported (Mohammadi & Talebi, 2015). Therefore, separation of heritable and non-heritable components is necessary. This separation should be based on its genotypic and phenotypic coefficient of variation, heritability and genetic gain (Kahrizi & Mohammadi, 2009; Maniee et al., 2009). Information on the relative importance of various yield components to seed yield is useful for producers to manage the crop by focusing on key yield components. This type of information is also useful for chickpea breeders to establish selection criteria in genetic manipulation (Talebi & Karami, 2011). The main goal in plant breeding is looking and selection the genotypes with high seed yield and quality. Seed yield is complex trait that directly or indirectly associated with other morphological and physiological traits. Selection based on seed yield and its components should be based on genotypic variance and the proportion of the genetic gain and heritability for each trait (Biçer & Sakar, 2008; Talebi & Karami, 2013).

Therefore, the present study was conducted to assess genetic variability and heritability of morphological and physiological traits in different chickpea cultivars constructed to well-watered and rain-fed conditions in order to select the appropriate genotype(s) that are suited to different environments.

2 Materials and Methods

2.1 Plant materials and experiment procedure

Ten chickpea (*Cicer arietinum* L.) accessions were chosen for the study of seed yield, morphological characters and physiological attributes under irrigated and non-irrigated conditions. Field study was conducted in completely

randomized design with three replication under both irrigated and rainfed conditions. Each plot contained four rows (4 meter long) with 0.35m distances between rows to rows. Irrigated plot received five supplemented irrigation during flowering and pod-filing stages, while rainfed plots did not receive any supplemented irrigation. For morphological traits characterization, randomly 10 plants from each experimental plot for estimating the number of pods per plant, plant height, number of seeds per plant, weight of one-hundred seed and seed yield per plant. Further, Leaf relative water content was measured by the formula given by Turner, 1981.

$$RWC = \frac{\text{leaf fresh weight} - \text{leaf dry weight}}{\text{leaf turgid weight} - \text{leaf dry weight}}$$

Here leaf turgid weight was measured as the weight of leaf after soaking in water for 12 h at room temperature and dry weight measured after drying the leaf in 85 °C for 48 h.

2.2 Measurement of Physiological Parameters

At flowering stage (60 days after sowing), fresh leaves sample were collected from the selected plants from each plot and 0.5 g leaf sample from each plot digested in 3% (W/V) aqueous sulpho-salicylic acid and the proline content was measured as absorbance of fraction with toluene aspired from liquid phase in 520 nm by spectrophotometer (Shimadzu UV-VIS 1201). For assessment of leaf chlorophyll content, 0.1 mg of fresh leaf soaked in 80% acetone and homogenized solution were used for measure total leaf chlorophyll content with the help of spectrophotometer at 663 and 645 nm wave length (Bates et al., 1973).

2.3 Data analysis

All measured data were subjected to variance analysis and mean comparisons using Duncan's test ($P=0.05$) by SAS software. Pearson correlation between traits and factor analysis were measured using STATISTICA software. Genotypic variance (GV), phenotypic variance (PV), environmental variance (EV) and broad-sense heritability (h^2) of measured traits were estimated by using formula given by Singh & Chaudhary (1979).

$$EV = MSE$$

$$GV = \frac{MSG - MSE}{r}$$

$$PV = \frac{EV + GV}{r}$$

Where, MSE and MSG are the values of mean square for error and genotypes in variance analysis, respectively. Broad-sense heritability was estimated using the following formula:

Table 1 Mean squares for morphological and physiological traits in 10 chickpea genotypes evaluated in irrigated and rainfed environments.

		Mean square									
Environment	SOV	PP	SP	Y(g/p)	SW	PH	CHL	RWC	Na ⁺	PRO	K ⁺
Irrigated	Rep	2	77.23*	0.52ns	4.33**	0.127ns	5.96*	3.8ns	1.43ns	5.66ns	0.273ns
	G	9	139.6**	189.3**	14.85**	65.3**	16.06**	785.7**	364.7**	1630.2**	353.01**
	Error	18	15.7	13.71	0.25	0.175	1	7.43	1.3	16.95	0.415
	Cv%		15.52	14.86	6.8	1.44	3.75	3.93	2.93	2.65	1.39
Rain-fed	Rep	2	13.16*	0.29ns	3.11*	7.88	3.43ns	1.53ns	714.85*	163.57*	0.304ns
	G	9	9.99**	49.07**	15.18**	177.9**	61.27*	100.12**	321.28*	3147.24**	385.77**
	Error	18	2.72	1.27	0.318	9.23	18.84	1.04	106.15	16.1	0.4144
	Cv%		16.87	9.78	12.52	8	15.21	5	19.06	2.11	1.68

[*, ** significant at $P = 0.05$ and 0.01 , respectively. PH=plant height; SP=number of seeds per plant; Y=seed yield (gram/plant); SW=100-seed weight; PRO=proline content; CHL=leaf chlorophyll content; RWC=leaf relative water content; Na⁺=leaf sodium content; K⁺=leaf potassium content].

$$h^2 = \frac{GV}{PV}$$

Genotypic, phenotypic and environmental coefficients of variation were measured using the following formulas:

$$GCV = \frac{\sqrt{GV}}{\bar{U}} \times 100$$

$$ECV = \frac{\sqrt{EV}}{\bar{U}} \times 100$$

$$PCV = \frac{\sqrt{PV}}{\bar{U}} \times 100$$

Where, $\bar{U}$ is the mean value of each measured traits.

In addition, quantitative selection criteria including stress susceptibility index (SSI; Fischer & Maurer, 1978), stress tolerance index (STI; Fernandez, 1992), tolerance (TOL; Rosielle & Hamblin, 1981), mean productivity (MP; Rosielle & Hamblin, 1981) and geometric mean productivity (GMP; Fernandez, 1992) were estimated using grain yield under irrigated (Yp) and drought stress (Ys) conditions. The drought tolerance of chickpea genotypes was evaluated based on these criteria.

3 Results and Discussion

3.1 Variance analysis and genetic parameters

Results of variance analysis showed significant differences ($P < 0.01$) for all measured morphological and physiological characters. These differences indicated high diversity for measured traits between genotypes. The experimental coefficient of variation (CV) varied from 1.39 to 15.52 and 1.68 to 16.87 in irrigated and rain-fed environments respectively (Table 1).

Plant Breeder success in selecting genotypes possessing higher seed yield and favorable morphological traits depends on the existence and exploitation of genetic variability and high heritability for seed yield and its components. Genotypic, phenotypic and environmental coefficients of variation for all measured traits are presented in Table 2. Lowest and highest value of PCV observed for proline (8.74%) and number of seeds per plant (19.70%) for proline content ranged from 8.74% to 19.70% in irrigated conditions and 9.90% for proline content to 31.39 for 100-seed weight rain-fed environment, respectively. The GCV values were the lowest (8.40%) for chlorophyll content and highest (30.71%) for number of seeds per plant in irrigated conditions while it was the lowest (14.73%) for plant height and highest (50.39%) for 100-seed weight for rain-fed environmental conditions. Broad sense heritability (h^2) were comparatively high for all measured traits in irrigated environment and ranged from 2.17 for

number of pods per plant to 2.99 for K^+ content and 1.21 for RWC to 2.99 for K^+ content in both irrigated and rain-fed environments, respectively (Table 2). These results indicated that measured traits in this study in both irrigated and rain-fed environments can be used for improving seed yield in chickpea. Effective selection in chickpea breeding for traits with higher heritability observed in this study are in accordance with the findings of Zali et al. (2011), Talebi &

Rokhzadi (2013) and Mohammadi & Talebi (2015). According to Several characters like number of pods per plant, 100-seed weight, seed yield, RWC and leaf K^+ content in both environments showed high degree of GCV in chickpea, these findings are in accordance with the findings of Zali et al. (2011) and Mohammadi & Talebi (2015), this information providing sufficient scope for phenotypic selection.

Table 2 Genetic parameters for different morphological and physiological traits in chickpea genotypes in irrigated and rain-fed environments.

		EV	GV	PV	GCV	ECV	PCV	Hi ²
Irrigated Environment	PP	15.7	41.3	19	25.18	15.53	17.08	2.17
	SP	13.71	58.53	24.08	30.71	14.86	19.70	2.43
	Y	0.25	4.86	1.7	29.55	6.70	17.48	2.86
	SW	4.46	16.86	7.1	14.15	7.28	9.18	2.37
	PH	0.17	21.7	7.29	16.02	1.42	9.28	2.98
	CHL	1	5.02	2	8.40	3.75	5.30	2.51
	RWC	7.43	259.42	88.95	23.23	3.93	13.60	2.92
	Na ⁺	1.3	121.13	40.81	28.21	2.92	16.38	2.97
	PRO	16.95	537.75	184.9	14.90	2.65	8.74	2.91
	K ⁺	0.41	117.53	39.31	23.40	1.38	13.54	2.99
Rain-fed Environment	PP	2.72	2.42	1.71	15.96	16.92	13.41	1.42
	SP	1.27	15.93	5.73	34.59	9.77	20.74	2.78
	Y	0.318	4.95	1.75	49.44	12.53	29.40	2.83
	SW	9.23	56.22	21.81	50.39	20.42	31.39	2.58
	PH	18.84	14.14	10.99	14.73	17.00	12.99	1.29
	CHL	1.04	33.02	11.35	28.21	5.01	16.54	2.91
	RWC	106.15	71.71	59.28	15.67	19.07	14.25	1.21
	Na ⁺	0.59	125.6	42.06	34.95	2.40	20.22	2.99
	PRO	16.1	1043.7	353.26	17.02	2.11	9.90	2.95
	K ⁺	0.41	128.45	42.95	29.56	1.67	17.09	2.99

[PH=plant height; SP=number of seeds per plant; Y=seed yield (gram/plant); SW=100-seed weight; PH=plant height; CHL=leaf chlorophyll content; RWC=leaf relative water content; Na⁺= leaf sodium content; PRO=leaf proline content; K⁺= leaf potassium content].

Table 3 Drought stress indices in chickpea based of seed yield in irrigated (YP) and rain-fed (YS) environments.

Genotype	YP	YS	MP	GMP	TOL	STI	YSI	YI
Hashem	6.36	3.10	4.73	4.44	3.26	0.06	0.49	0.69
ILC482	4.22	2.92	3.57	3.51	1.30	0.02	0.69	0.65
Flip51-87C	7.97	4.90	6.44	6.25	3.07	0.06	0.61	1.09
Arman	7.76	3.35	5.56	5.10	4.41	0.08	0.43	0.74
Flip2005-3C	8.52	3.58	6.05	5.52	4.94	0.09	0.42	0.79
Flip2005-7C	8.90	6.88	7.89	7.83	2.02	0.04	0.77	1.53
ILC3279	4.71	1.97	3.34	3.05	2.74	0.05	0.42	0.44
Flip2005-1C	10.84	8.04	9.44	9.34	2.80	0.05	0.74	1.79
ILC263	5.42	2.57	4.00	3.73	2.85	0.05	0.47	0.57
Flip2005-5C	9.93	7.73	8.83	8.76	2.20	0.04	0.78	1.72

Table 4 Correlation coefficients between Yp, Ys and drought tolerance indices.

	YP	YS	MP	GMP	TOL	STI	YSI	YI
YP	1.00							
YS	0.88**	1.00						
MP	0.97**	0.97**	1.00					
GMP	0.95**	0.98**	1**	1.00				
TOL	0.22	-0.26	-0.02	-0.09	1.00			
STI	0.22	-0.26	-0.02	-0.09	1**	1.00		
YSI	0.50*	0.83**	0.69**	0.73**	-0.71**	-0.71**	1.00	
YI	0.88**	1**	0.97**	0.98**	-0.26	-0.26	0.83**	1.00

3.2 Drought tolerance indices

The genotypes showed significant differences in seed yield and other traits under both environmental conditions. Seed yield, morphological traits and most of physiological parameters under irrigated conditions were decreases in rain-fed environment. Thus, indirect selection for a drought-prone environment based on the results of optimum conditions which will not be efficient (Talebi et al., 2009; Talebi & Karami 2011). In present study, resistance indices were calculated on the basis of genotypes (Table 3). To determine the most desirable drought tolerance criteria, the correlation coefficient between Yp, Ys and other quantitative indices of drought tolerance were calculated (Table 4). A positive correlation was reported between TOL and yield under normal condition (Yp) and a negative correlation was reported between TOL and yield under stress (Ys) (Table 4) suggest that selection based on TOL will results in reduced yield under well-watered conditions. Similar results were reported in several crops like wheat (Talebi et al., 2009), chickpea (Talebi et al., 2011) and barley (Rizza et al., 2004).

The results of study revealed that there were positive and significant correlations among Yp and Ys with MP, GMP and STI were better predictors of Yp and Ys than TOL and SSI. The genotypes Hashem, Arman and Flip 2005-3c with high yield under stress produced a lower yield under non-stress condition and showed the highest STI value (Table 3). STI showed negative correlation with seed yield in both environments (Table 4), suggesting that these parameters could not be efficient parameter for selection superior chickpea genotypes in both environments. Further, significant positive correlation was reported between YSI and YI with seed yield in both environmental conditions and MP and GMP (Table 4), suggesting that these parameters are able to discriminate tolerant group of genotypes under both environments. As STI, GMP and MP were able to identify cultivars producing high yield in both conditions, therefore, these parameters are useful indicators for chickpea breeding under drought stress zones. This finding is in agreement with Talebi et al. (2011) and Talebi & Karami (2011).

Table 5 Factor analysis for physiological and agro-morphological parameters in chickpea subjected to irrigated and rainfed conditions.

	Irrigated		Rain-fed		
	Factor1	Factor2	Factor1	Factor2	Factor3
Pods per Plant	0.76	-0.60	-0.63	-0.62	-0.09
Seeds per Plant	-0.53	0.84	0.90	0.22	-0.13
Yield (gr/plant)	0.75	-0.40	0.84	0.50	-0.10
100-Seed Weight	-0.54	0.74	-0.45	0.81	-0.08
Plant Height	-0.60	0.64	0.16	-0.51	0.84
Chlorophyll	-0.53	0.56	-0.42	-0.61	0.63
RWC	0.89	0.17	0.78	-0.35	-0.16
Na+	0.90	-0.01	0.95	0.10	-0.02
Proline	0.80	0.00	0.97	-0.10	-0.06
K+	0.94	-0.03	0.92	-0.15	0.12
Eigen Value	2.179	1.18	3.11	2.42	1.25
% of variance explained	55.02	25.32	56.36	21.45	11.84
Cumulative variance	55.02	80.34	56.36	77.81	89.66

3.3 Factor analysis

Factor analysis combined variables that were correlated into two and three factors in order of the amount of variance in both irrigated and rain-fed environments, respectively (Table 5). In irrigated environment, two factors explained 80.34% of the total variance caused in the characters (Table 5). The first factor could explain 55.02% of the total variation. Factor 1 comprised number of pods per plant, seed yield, RWC, Proline, Na⁺ and K⁺. These have direct and indirect positive effects on the various growth traits of chickpea. Therefore, this factor can be called the detector yield factors and physiological parameters that related to osmotic adjustment in plant. This result is in agreement with previous studies on chickpea (Talebi and Karami, 2011) and common bean (Sadeghi et al., 2011). Second factor through 100-seed weight and seeds per plant as most important characters showed 25.32% of variation (Table 5). Therefore, this factor can also be the detector yield components factors. These results suggested that the selection for high yield chickpea genotypes under optimal condition may be improved by increasing the weight of 100-seed, number of pods per plant, and more partitioning the soluble contents in leaves like Na⁺, K⁺ and chlorophyll due to increasing the photosynthesis and consequently increasing the potential yield. In rain-fed environments, three factors explained 89.66% of the total variance caused in the characters (Table 5). The first factor could explain 56.36% of the total variation. These factor comprised number of seeds per plant, seed yield, RWC, Proline, Na⁺ and K⁺. Like as irrigated environment, these factors can also called the detector yield components factors and osmotic adjustment regulator factor. Second and third factors explained 21.45% and 11.84% of variations for 100-seed weight and plant height, respectively (Table 5). These results suggested that the selection for high yield chickpea genotypes under rain-fed condition may be improved by increasing the plant height, 100-seed weight and more partitioning the soluble contents in leaves to increasing the RWC and consequently increasing the potential yield. These findings are in agreement with Yucel et al., (2006), Ali et al., (2009) and Talebi & Karami (2011).

Overall these results revealed a wide range of variability for different morphological and physiological traits in both environmental conditions with high heritability for important yield traits hence selection is effective for these traits. Further study on gene action is needed for recommendation of selection of high yield in chickpea in different environmental conditions. Genetic analysis of most of the quantitative traits in chickpea showed additive and non-additive components of genetic variances in relative to inheritance of quantitative traits (Karami & Talebi, 2013). Broad-sense heritability for seed yield components characters in chickpea has been reported ranged from 11 to 87% and between quantitative traits in chickpea low heritability for seed yield and plant height due to high effects of these environmental conditions on these traits (Tambal et al., 2000), therefore, early generation selection based on these traits in chickpea could not be useful (Tilahun et al., 2015). According to Ashraf & Harris (2004) and Zali et

al. (2011) selection based on heritability alone is not very useful in breeding program. High heritability for number of seeds per plant, 100-seed weight, seed yield and RWC in both environments indicated that additive gene effects are important in determining these traits. Crop improvement for the traits is assumed to be possible by simple selection, due to high heritability coupled with high genotypic variation and additive gene effects (Noor et al., 2003; Karami & Talebi, 2013).

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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