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Morpho-physiological and biochemical alternation responses in different chickpea (*Cicer arietinum* L.) genotypes under two constructing water regimes

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Abstract

The experiment was conducted to assess the differential morpho-physiological response to stimulated water deficit and to determine the relationship between some of these morphological and physiological traits and yield components of ten chickpea genotypes grown in field under irrigated and rain-fed conditions. Variance analysis of the data showed that the environment was a significant source of variation for all measured characters and genotypes showed significant differences for all measured traits in both environments. In well-watered condition, the highest correlation was belonged to number of seeds per plant and number of pods per plant ($P<0.01$). The seed yield had highly significant positive correlation with number of seeds per plant ($P<0.01$) and number of pods per plant ($P<0.01$). Also, seed yield showed positive significant ($P<0.05$) correlation with RWC, Na+ and K+ uptake. In water deficit condition high significant positive correlation were observed between grain yields with physiological traits, while in irrigated environment the correlation between grain yields with proline accumulation was not significant. In general, the results suggested that a chickpea cultivar, for increased yield under irrigated conditions, should have maximum number of seeds and pods per plant and under stress conditions should have maximum number of seeds and pods per plant and also keep the high level of RWC, chlorophyll and proline content in their leaves. Thus, identifying these traits as selection criteria in chickpea breeding program may be useful for breeders to introduce suitable drought resistant chickpea cultivars for arid regions.

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Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crop with a total annual global production of 9.7 M tones from 11.5 M ha (FAO, 2009). Among the abiotic stress factors, drought stress problem is relatively important in chickpea. The major chickpea cultivation areas are almost completely in the arid and semiarid zones of the world. In these areas, chickpea is continuously exposed to increasing drought and high temperatures during flowering and maturity stages (Toker *et al.*, 2007a; Talebi *et al.*, 2013) due to insufficient and irregular rainfall. Under mentioned conditions, chickpea faces two types of drought, terminal (soil moisture content is continuously decreased towards the end of the growing season) and intermittent drought (soil moisture depends on precipitation but rainfall is irregular and also insufficient) (Toker *et al.,* 2007b; Talebi *et al.,* 2011). So, in vegetative and reproductive growth phases, plants are subjected to intermittent and terminal drought stresses, respectively (Ganjeali *et al.,* 2005). In Iran, chickpea generally grown under rainfed conditions either on stored soil moisture in subtropical environments with summer-dominant rainfall or on current rainfall in winter-dominant mediterranean-type environments. In both environments, un-irrigated chickpea suffers yield penalties from terminal drought (Yadav *et al.,* 2006). It is recognized that plants under stress have developed various physiological and biochemical adaptive responses. These include water status, pigment content, osmotic adjustment and photosynthetic activity (Dhanda *et al.,* 2004; Serraj *et al.,* 2004; Benjamin and Nielsen 2006; Praba *et al.,* 2009).

Sodium (Na^+) and potassium (K^+) plays a vital role in many physiological processes such as membrane structure and stomatal function, cell division and cell wall synthesis, which influence growth and responses to environmental stresses. Moreover, it plays a significant role in osmoregulation. Under drought stress, potassium (K^+) increases the plant's drought resistance through its functions in stomatal regulation, osmoregulation, energy status, and charge balance (Martinez *et al.,* 2007).

The alternation of protein synthesis or degradation is one of the fundamental metabolic processes that may influence water stress tolerance (Jiang and Huang 2002; Najafi *et al.,* 2010; Kottapalli *et al.,* 2009). Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, by affecting cholorophyll components and by damaging the photosynthetic apparatus (Iturbe-Ormaetxe *et al.,* 1998). Ommen *et al.* (1999) reported that leaf chlorophyll content decreases as a result of drought stress. The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995). The accumulation of osmolytes may ensure the maintenance of the structural integrity of membranes. There are some evidences that plants are more tolerant to water deficit when water is withheld under conditions that favor osmotic adjustment (Moinuddin and Khanna-Chopra, 2004; Talebi *et al.,* 2013). Proline is one of the osmolytes, which increase faster than other amino acids in plants under water deficit stress and help the plants to maintain cell turgor (Zhao *et al.*, 2008). Thus, proline accumulation can be used as a criterion for drought resistance assessment of varieties (Gunes *et al.*, 2008). In this sense, the interactions between different morphological and physiological attributes with drought stress in chickpea investigated under greenhouse or controlled conditions (Gunes *et al.*, 2005; Zhao *et al.*, 2008, Moinuddin and Khanna-Chopra, 2004), but it seems that more studies for revealing these factors under field conditions should be carried. Therefore, keeping in view the important of chickpea production in rain-fed zones in Iran, the present study was initiated to examine and compare the morpho-physiological and biochemical attributes of drought tolerant and non-tolerant chickpea genotypes under field conditions.

Materials and methods

Site description and plant material

The experiment was carried out in 2011-2012 at the research farm of Sanandaj Islamic Azad University ((35◦11_ lat. N; 46◦59_ long. E,1400 m above sea level). Sanandaj is located in north-west of Iran and has a mean annual temperature 12 °C and annual rainfall of 512 mm. pattern of monthly rainfall (mm) and temperature (°C) during the crop season is presented in Fig 1. Ten Kabuli chickpea (*Cicer arietinum* L.) accessions were chosen for the study based on their reputed differences in yield performance under irrigated and non-irrigated conditions. Seeds of these varieties were obtained from the International Centre for Agricultural Research in the Kurdistan, Sanandaj and Sararood Dryland Agricultural Research Institute, Kermanshah, Iran.

Experiment procedure

The experiment was carried out in randomized complete block design with three replications, in two environments (Irrigated and rain fed). Seeds were hand drilled and each genotype was sown in four rows of 3.0 m, with row to row distance of 0.25 m. Sowing was performed in 30 February for all treatments. Plants in rain-fed plots did not receive any water except rainfall during the experiment. In irrigated plots, six supplement irrigations (once per week) were applied during flowering and grain-filling period. The reference evapotranspiration for each month within growing season in region as daily mean (mm/day) was fallow as: 1.8 at March, 2.88 at April, 5.11 at May and 5.14 at June.

Yield components traits measurement

Five plants were randomly chosen from each plot to measure the number of seeds per plant, number of pods per plant, Plant height, 100-seed weight and plant yield (g/plant). Leaf relative water content (RWC) was determined according to the methods of Turner (1981), based on the following equation: RWC = (FW– DW)/ (SW – DW) × 100, where FW is leaf fresh weight, DW is dry weight of leaves after drying at 85 °C for 3 days, and SW is the turgid weight of leaves after soaking in water for 4 h at room temperature (approximately 20 °C). Half of the third (from the top) fully expanded leaf was used.

Physio-chemical traits measurement

Assessments of proline and chlorophyll contents were performed at 65 days (flowering) after the onset of the experiment. Proline was extracted from a sample of 0.5 g fresh leaf material samples in 3% (w/v) aqueous sulphosalycylic acid and estimated using the ninhydrin reagent according to the method of Bates *et al.* (1973). The absorbance of fraction with toluene aspired from liquid phase was read at a wave length of 520 nm. Proline concentration was determined using a calibration curve and expressed as μ mol proline g^{-1} FW. Chlorophyll content was determined in 80% acetone extract. After centrifugation (20 000 g, 20 min) the absorbance was read spectrophotometrically at 663 and 645 nm. Total chlorophyll was calculated according to Arnon (1949). Potassium and Sodium were determined by atomic absorption (Shimadzu UV-VIS 1201).

Data analysis

Data were subjected to analysis of variance (ANOVA), and means were compared using Duncan's range test at $P = 0.05$. Pearson correlations were calculated between measured traits in both environments. All calculations were performed with the help of the SAS software, version 9.1.

Results

Variance analysis

Combined analysis of variance of the data (Table 1) showed that the environment was a significant source of variation for all measured characters. Two-way interaction of environment × genotypes was significant ($P<0.01$) for all measured traits. Stress intensity was estimated to be 0.58, indication high water deficit stress. The result of variance in irrigated and rain-fed environments (Table 2) indicated that genotypes differences was significant ($P<0.01$) for all measured traits in both environments.

Mean comparisons

Yield components and physio-chemical attributes were calculated for all genotypes in both environments are presented in Table 3. The responses of genotypes at each of the two conditions were different. All the measured traits except leaf prolin content under water-stress conditions were lower than those under non-stress conditions.

Table 1. Combined analysis of variance for morphological and physiological traits of chickpea genotypes.

		Mean square									
S.O.V	df	PP	SP	Y(g/p)	SW	PH	CHL	RWC	Prolin	Na^+	K^+
Environmnet (E)	1	3716.8**	2681.6**	131.4**	1196.9**	4.64ns	596.7**	3515.6**	1809**	723.6**	954.49**
Error(R/P)	4	5.19ns	0.405ns	0.20ns	4.22ns	1.78ns	3.75*	59.32ns	11.2**	1.17ns	0.288ns
Genotype (G)	9	80.3**	170.1**	28.3**	114.4**	31.56**	285.6**	918.9**	444.5**	695.08**	727.4**
G × E	9	69.3**	68.25**	1.73**	118.56**	95.02**	30.6**	188.13**	333**	46.96**	11.4**
Error (R× G/E)	36	9.21	7.49	0.288	6.84	9.5	1.02	56.8	16.2	0.948	0.415
CV %		17.19	15.01	8.97	7.81	10.7	4.3	12.2	2.35	2.74	1.52

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

Table 2. analysis of variance for morphological and physiological traits in chickpea genotypes in Irrigated and rain-fed conditions.

			Mean square								
Environment	SOV		PP	SP	Y(g/p)	SW	PH	CHL	RWC	Na^+	Prolin	K^+
Irrigated	Rep	2	77.23*	0.52ns	4.33**	0.57ns	0.127ns	5.96*	3.8ns	1.43ns	5.66ns	0.273ns
	G	9	139.6**	189.3**	14.85**	55.04**	65.3**	16.06**	785.7**	364.7**	1630.2**	353.01**
	Error	18	15.7	13.71	0.25	4.46	0.175	1	7.43	1.3	16.95	0.415
	Cv%		15.52	14.86	6.8	7.28	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*	0.92ns	163.57*	0.304ns
	G	9	9.99**	49.07**	15.18**	177.9**	61.27*	100.12**	321.28*	377.39**	3147.24**	385.77**
	Error	18	2.72	1.27	0.318	9.23	18.84	1.04	106.15	0.59	16.1	0.4144
	Cv%		16.87	9.78	12.52	8	15.21	5	19.06	2.39	2.11	1.68

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

Drought stress reduced the seed yield of all genotypes. Yield reduction (YR) of genotypes varied from 41% to 78% (Figure 2). The results indicated the presence of a considerable amount of genotypic variation among the chickpea accessions under drought stress condition. Genotypes Arman, Flip2005-3C and ILC3279 showed lower yield reduction than the average yield reduction (61.4%). Surprisingly, most of the genotypes showed more than 50% yield reduction under drought stress. Flip2005-1C, Flip2005-5C and Flip2005-7C showed higher grain yield and its component (Number of pods/plant, seeds/plant and seed weight) in both environment. Also, the RWC, total chlorophyll content, Na^+ and K^+ uptake were decreased in water stress environments compare to irrigated environments. In most of the genotypes prolin content was accumulated higher in water-stress environment.

Correlation coefficient analysis

The coefficient correlation between seed yield, yield components and physiological characters both irrigated and water deficit environments are presented in Table 4.

In well-watered condition, the highest correlation was belonged to number of seeds per plant and number of pods per plant (0.927**). The seed yield had highly significant positive correlation with number of seeds per plant (0.778**) and number of pods per plant (0.812**). Also, seed yield showed positive significant

($P<0.05$) correlation with RWC, Na^+ and K^+ uptake. High positive significant correlation observed between physiological traits in irrigated environment. In water deficit condition, the highest positive correlation (0.906^{**}) belonged to seed weight with grain yield per plant. Surprisingly, in water deficit condition high significant positive correlation were observed between grain yields with physiological traits, while in irrigated environment the correlation between grain yields with proline accumulation was not significant. In water deficit environment high positive significant ($P<0.01$) correlation observed between physiological characters. These results indicated that selection for grain yield based on these traits (both morphological and physiological) might be possible and more effective in both environments.

Table 3. Mean comparisons of various morphological and physiological traits in chickpea under irrigated and rain-fed conditions.

Environment	Genotype	PP	SP	Y(g/p)	SW	PH	CHL	RWC	Na^+	Prolin	K^+
Irrigated	Hashem	23.16bcd	21.16cd	6.36e	30.00bc	30.01d	28.03d	51.9f	24.18g	174.12a	42.89d
	ILC482	22.33cd	17.00d	4.22g	30.80b	30.11d	39.23a	81.03b	48.26b	166.9ab	53.38c
	Flip51-87C	30.33ab	30.17ab	7.97d	26.28cd	25.73e	29.32d	84.9b	45.99c	174.47a	57.35a
	Arman	27.83abc	30.66ab	7.76d	25.33d	25.32ef	14.00g	63.23d	33.01e	163.57bc	39.94e
	Flip2005-3C	29.83ab	26.5bc	8.52cd	32.04b	32.39c	24.53ef	75.30c	38.35d	143.7d	42.96d
	Flip2005-7C	21.33cde	16.99d	8.90c	37.49a	37.77a	37.12b	83.70b	48.38b	170.67ab	54.57b
	ILC3279	17.33de	18.50d	4.71fg	25.06d	24.73fg	13.75g	40.87g	27.32f	116.87e	32.60f
	Flip2005-1C	34.99a	35.0a	10.84a	32.62b	34.62b	23.33f	89.80a	51.61a	170.06ab	57.58a
	ILC263	14.66e	16.16d	5.42f	26.53cd	25.77e	25.5e	56.97e	24.12g	111.87e	27.14g
	Flip2005-5C	33.49a	36.99a	9.93b	24.11d	24.43g	31.94c	65.73d	48.94b	159.00c	54.86b
Rain-fed											
	Hashem	11.00ab	13.21cd	3.10d	10.20f	31.67ab	23.13b	57.60a	30.77d	190.53b	38.99e
	ILC482	12.00ab	9.17e	2.92de	11.02e	31.00ab	26.80a	62.26a	38.63c	213.07a	48.05b
	Flip51-87C	12.37a	12.70d	4.90c	14.20dc	29.33ab	26.37a	57.13a	39.13c	217.20a	50.34a
	Arman	8.87bcd	9.42e	3.35d	13.70de	36.33a	12.98d	58.05a	21.13f	179.17c	31.02g
	Flip2005-3C	6.62d	9.51e	3.58d	13.25de	23.33bc	18.70c	54.56a	24.38e	171.13d	36.82f
	Flip2005-7C	9.75abcd	15.76b	6.88b	17.63bc	20.33c	27.36a	60.10a	41.11b	218.13a	48.06b
	ILC3279	7.50cd	5.12f	1.97e	15.6cd	26.00bc	12.63d	27.41b	20.34f	139.90e	23.17h
	Flip2005-1C	9.75abcd	18.03a	8.04a	18.65b	30.00ab	13.92d	59.10a	48.34a	219.60a	44.13d
	ILC263	9.37abcd	7.55e	2.57de	12.92de	30.00ab	22.78b	45.80a	15.25g	135.80e	17.14i
	Flip2005-5C	10.62abc	14.96bc	7.73ab	21.7a	27.33bc	19.04c	58.30a	41.63b	214.07a	45.77c

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

Table 4. Simple correlation coefficient between morphological and physiological traits in chickpea genotypes under irrigated and rain-fed conditions.

Environment	PP	SP	Y(g/p)	SW	PH	CHL	RWC	Na^+	Prolin	K^+
Irrigated										
	1									
	0.927^{**}	1								
	0.812^{**}	0.778^{**}	1							
	0.009	-0.299	0.258	1						
	0.120	-0.174	0.373	0.988^{**}	1					
	0.031	-0.208	0.045	0.493	0.431	1				
	0.567^{*}	0.319	0.550^{*}	0.574^{*}	0.597^{*}	0.545	1			
	0.649^{*}	0.459	0.591^{*}	0.382	0.427	0.556^{*}	0.860^{**}	1		
	0.609^{*}	0.399	0.464	0.386	0.413	0.461	0.627^{*}	0.598^{*}	1	
	0.706^{**}	0.490	0.593^{*}	0.390	0.433	0.597^{*}	0.809^{**}	0.914^{**}	0.835^{**}	1

Rain-fed	PP	SP	Y(g/p)	SW	PH	CHL	RWC	Na^+	Prolin	K^+
	1									
	0.405	1								
	0.240	0.906^{**}	1							
	-0.106	0.444	0.779^{**}	1						
	0.338	-0.149	-0.292	-0.431	1					
	0.640^{*}	0.183	0.024	-0.224	-0.262	1				
	0.545^{*}	0.683^{*}	0.513^{*}	0.064	0.167	0.448	1			
	0.577^{*}	0.853^{**}	0.819^{**}	0.475	-0.185	0.292	0.624	1		
	0.651^{*}	0.823^{**}	0.739^{**}	0.358	-0.057	0.389	0.804^{**}	0.940^{**}	1	
	0.611^{*}	0.719^{**}	0.633^{*}	0.304	-0.187	0.475	0.764^{**}	0.897^{**}	0.966^{**}	1

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

Discussion

In different crops, as well as in chickpea differential genotypic response to drought stress as results of variation in their morphological and physiological alternation was reported (Gunes *et al.*, 2006; Talebi and Karami 2011; Talebi *et al.,* 2013). It has been established that drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion growth (Kusaka *et al.*, 2005; Shao *et al.*, 2008). The yield response to drought stress of chickpea is given in Table 3. The yield and other yield related traits of all ten varieties of chickpea were affected by drought stress. Flip2005-1C, Flip2005-5C and Flip2005-7C showed higher grain yield and its component (Number of pods/plant, seeds/plant and seed weight) in both environment. The physiological changes observed could be the result of deleterious effect of water deficit on important metabolic processes as well as responses of various defense mechanisms adapted by the plant under drought stress. Our study focused on changes in grain yield, yield components, RWC and leaf chemical compositions in chickpea under water deficit stress. All of the characters except leaf proline were decreased as a result of water deficit. In general, relative water content (RWC) and total chlorophyll content significantly decreased in all genotypes under drought stress, but these reductions in tolerant genotypes in less. The results are agreement with previous studies described a significant decrease of chlorophyll content caused by water deficit in *Triticum aestivum* (Nyachiro *et al.*, 2001) and chickpea (Talebi *et al.,* 2013) cultivars. Variety differences in proline content or interactions between variety and drought treatment were significant. The proline content of the leaf, however, increased at water deficit environment in all varieties of chickpea (Table 3). The proline content depends on plant age, leaf age, leaf position or leaf part (Chiang and Dandekar, 1995). Under water deficit environment, drought stress increased proline content, this increasing roles as an osmotic compatible and adjust osmotic potential which resulted in drought stress avoidance in chickpea (Mafakheri *et al.,* 2011). Prolin accumulation is believed to play adaptive roles in plant stress tolerance (Verbruggen and Hermans, 2008). Accumulation of proline has been advocated as a parameter of selection for stress tolerance (Yancy *et al.*, 1982; Jaleel *et al.,* 2007). Decreasing water availability under drought generally results in reduced total nutrient uptake and frequently reduces the concentrations of mineral nutrients in crops (Gunes *et al.*, 2006; Baligar *et al.*, 2001). In present study, different chickpea genotypes showed varied response with respect to nutrient uptake in normal and stress conditions. Drought stress significantly reduced Na and increase K uptake of genotypes. Drought stress affects the growth, dry mater and harvestable yield in a number of plant species, but the tolerance of any species to this menace varies remarkably.

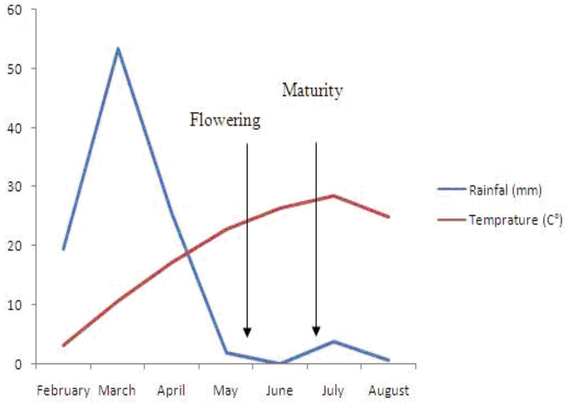

Fig. 1. Pattern of monthly rainfall and temperature amounts recorded during the crop season 2012.

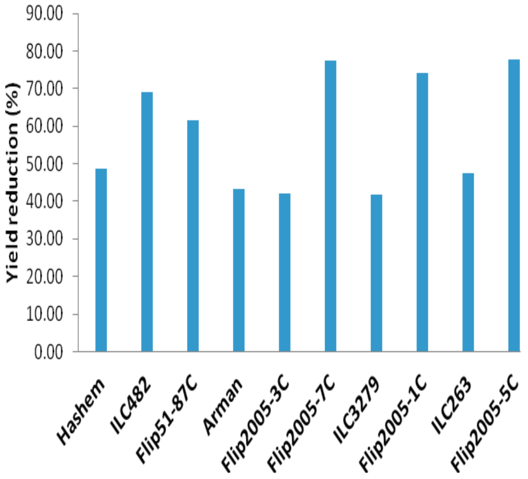

Fig. 2. yield reduction (%) of chickpea genotypes under rain-fed conditions.

In addition to other factors, changes in photosynthetic pigments are of paramount importance to drought tolerance. Of the two photosynthetic pigments classes, proline and chlorophyll show multifarious roles in drought tolerance including light harvesting and protection from oxidative damage caused by drought. Thus, increased contents specifically of proline are important for stress tolerance. These experiments indicate that high grain yield, number of pods and seeds per plant, seed weight, proline and chlorophyll contents could be used as a selection criterion for higher yield in irrigated and stresses chickpea genotypes. Based on these relations and yield data in stress and non-stress environment, Flip2005-1C, Flip2005-3C, Flip2005-7C and Flip51-87C which exhibited the highest grain yield in both irrigation and rain-fed conditions, were the most drought-resistant genotypes.

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