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Evaluation of physiologic and metabolic indicators of drought resistance in chickpea

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Abstract

In order to evaluate drought tolerant genotypes in chickpea using agronomic, physiologic and molecular indicators a factorial experiment with completely randomized design (CRD) was conducted under water stress and non-stress conditions. Statistical analysis indicated significant differences for prolin content (PC), total chlorophyll content (TCC), chlorophyll a (Cha), chlorophyll b (Chb) and soluble sugar (SS) expressing genetic variability and possibility of selecting resistant genotypes under drought stress condition. Mean comparison classified the genotypes for yield and drought tolerance criteria in different groups. Maximum stress yield (Ys) and yield potential (Yp) was attributed to accession 8. According to 3 dimensional plot between stress tolerance index (STI) and multiple selection index (MSI) the most drought tolerant genotype with high Yp and Ys was identified as number 8 (X96TH41K4) (group A). Multiple selection index which discriminate drought tolerant genotypes based on all physiologic and metabolic indices introduced the most drought tolerant genotype as number 8.

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Introduction

Chickpea (*Cicer arietinum* L.), one of the most important grain-legume crop, is grown in more than 45 countries, mostly in arid and semiarid zones (Kumar and Abbo, 2001). It's the second important legume in the world with 12.1 million ha under cultivation and with 11.1 million tonnes produced annually (FAOSTAT, 2012.). Drought, cold and salinity are the major abiotic stresses affecting chickpea in order of importance (Croser *et al.,* 2003).It has been estimated that 70% of the crop yield loss can be attributed to abiotic stresses, especially drought (Bray *et al.*, 2000). Drought is a meteorological term and an environmental event, defined as a water stress due to lack or insufficient rainfall and/or inadequate water supply (Toker *et al.*, 2007).The seriousness of drought stress depends on its timing, duration and intensity (Serraj *et al.*, 2004). Worldwide, 90% of chickpea is grown under rain fed conditions (Kumar and Abbo, 2001) where the terminal drought stress during the chickpea reproductive phase results heavy yield losses (Sharma, 2004).

Deleterious responses to drought can include reduction of growth, decrease in chlorophyll, increase in hydrogen peroxide, which causes lipid peroxidation and consequently membrane injury (Mukherjee and Choudhuri, 1983).

It is recognized that resistent plants under water stress conditions develope various physiological and biochemical responses of adaptive nature. These include changes of water use efficiency, pigment content, osmotic adjustment and photosynthetic activity (Dhanda *et al.*, 2004; Serraj *et al.*, 2004; Benjamin and Nielsen, 2006; Kalefetoğlu and Ekmekçi, 2009; Praba *et al.*, 2009). These mechanisms play a key role in preventing membrane disintegration and provide tolerance against drought and cellular dehydration (Hanson and Hitz, 1982; Bohnert and Jensen, 1996; Mahajan and Tuteja, 2005). High relative water content (RWC) and low excised-leaf water loss (rate of water loss, RWL) are associated with drought resistance, and these parameters have also been proposed as more valuable indicators of plant water status in comparison to other water potential parameters under drought stress (Keles and Oncel, 2004).

Photosynthetic pigments play an important role in light harvesting and dissipation of excess energy. It is known that the content of both chlorophyll a and b changes under drought stress (Farooq *et al.*, 2009). Carotenoids participate in energy dissipation and can aid plant resistance against drought stress (Gunes *et al.*, 2008). The above parameters have been used as screening techniques separately in different crops, but their relative efficiency has not been evaluated. As a major crop, wheat has gained special attention with respect to morphological and physiological characters and traits affecting drought tolerance, but there is not enough information for chickpea about the relevant parameters and their relationships with drought tolerance indices among chickpea cultivars.

As mechanisms of responses to drought stress varies with genotypes and growth stages of individual plants (Ashraf and Harris, 2004), it would be much more valuable if biochemical indicators could be specified for individual crop species. Knowledge on interrelationships among various physiological responses to dehydration can offer insight for developing usefull strategies to improve drought stress tolerance in chickpea. The measurement of each of these variables is demanding in terms of time and resources. The identification of suitable plant characters for screening large numbers of genotypes, in a short time at critical stages of crop growth, with the aim of selecting drought tolerant cultivars, remains a major challenge to the plant breeder. The objectives of the present investigation were (i) to determine the magnitude of genetic diversity in metabolic and physiological traits related to drought tolerance in chickpea genotypes (ii) to explore relationships among potentially useful traits to be used in breeding programs for drought tolerance and (iii) to discriminate drought tolerant chickpea genotypes.

Materials and methods

In order to evaluate drought tolerance of chickpea genotypes using agronomic, physiologic and metabolic criteria 9 genotypes of chickpea (*cicer arientinum* L.) (1-Bivanich, 2-Filp-00-6c, 3-X95TH69, 4-Filp-00-40c, 5-X94TH154, 6-Hashem, 7-filp-82-245, 8-X96TH41K4, 9-S96085) were used.

Field experiment

In the field, each experimental plot consisted of 3 rows of 2 meter in length with 60 cm distances between the rows and 10 cm distances between the shrubs. The seeds were planted in a randomized completely block design with 3 replications under water stress and non-stress conditions in College of Agriculture, Razi University, Kermanshah, Iran. In the stress experiment water stress was imposed before anthesis, while in the non-stress experiment irrigation was done until harvesting. During the experiment weeds were controlled by hand and for facing pod borer worm of chickpea the poisons *Phenon* and *Alrite* were sprayed. At harvest time (July) central shrubs of rows from each plot of each experiment were harvested and seed yield (yield potential = Yp and stress yield = Ys) of all shrubs were measured by digital balance.

Laboratory experiment

In the laboratory conditions 5 sterilized seeds by *Captan* fungicide with ratio of 2.5% were planted in the vases in College of Science, Razi University, Kermanshah, Iran. The vases were filled with soil in the ratio of 2, 1 and 1 soil, homos and sand, respectively. The experiment was conducted in a completely randomized design (CRD) factorial design with two factors (stress and genotype). In the non-stress site for each genotype 3 vases (replication) and in the stress site for each genotype 5 vases (replication) were used. Each 3 days the vases were irrigated until 15 days after seeding and the first harvest was performed 16 days after seeding. The harvested samples were provided from leaves and they were put in the oven at 70°C for 48-72 hours. Then until day 10 (for the second harvest) the vases under stress were not irrigated two times. For day 20th (the third harvest) and day 30th (the fourth harvest) the control vases were irrigated completely but the vases under stress were received around ¼ rate of received water by vases under irrigation. After imposing stress condition the following characters were measured.

(i) Proline content (PC)

The leaves were sampled from stress experiment and washed then oven dried in 80°C for 3 days. Dry weight of the samples were fixed and powdered.The PC was determined according to the method of Bates *et al.* (1973). Plant material of (0.1 g) was ground after anthesis stage with 10 ml of 5% sulfosalicylic acid. The homogenate was filtered and 1 ml of glacial acetic acid and 1 ml of acid ninhydrin reagent were added to a1 ml of filtrate. Then the mixture was shaken by hand and incubated in boiling water bath for 1 h. After that, it was transferred to ice bath and warmed to room temperature. 2 ml toluene was added to the mixture and the upper toluene layer was measured at 520 nm using Bausch and Lomb UV spectrophotometer 70.

(ii) Soluble sugars (SS)

Soluble sugars were measured using Phenol-sulfuric acid method (Dubios *et al.*, 1956; Kennedy, 1987). It is based on acidic hydrolysis of the soluble sugars and creating furfural combination that produces colorful complex with phenol. Then 0.1 g of sample arrived to the volume of 15 ml with alcohol 70%. The resulted mixture was maintained in the refrigerator and mingled everyday. After a week 2 ml of sample with 1 ml of phenol 5% was mixed. Then 5 ml of tick sulfuric acid was added. Half hour later absorbing solutions were read at wavelengths of 485 nm.

(iii) Chlorophyll content

0.2 g of dry weight of chickpea leaves was tested in zero, 10^{th}, 20^{th} and 30^{th} days of stress and controll. Dry weight of leaves was powdered in 2 or 3 cc of sodium dodecyl sulfate 5%. Then the volume arrived to 8 ml by distilled water. Obtained materials were transferred to a centrifuge pipe and were centrifuged in 80% speed of (100000 cycles) for 3 minutes. Then

spectrophotometer pipe was filled by the above part and its absorption was read in wavelengths of 663 and 645 nm (Ashraf *et al.*, 1994). Chlorophyll density in expressed juice can be calculated by the following formulas:

Chlorophyll a (Ch.a) (mg/ml) : $0.0127A_{663}$ - $0.00269A_{645}$

Chlorophyll b (Ch.b) (mg/ml) : $0.0229A_{645}$ - $0.00468A_{663}$

Total chlorophyll (T.ch.) (mg/ml) : $0.0202A_{645}$ + $0.00802A_{663}$

(iv) Multiple selection index (MSI)

In order to calculate multiple selection index the above mentioned characters were first standardizes and then added (Farshadfar *et al.*, 2004):

MSI= Ch.a + Ch.b + T.ch.+ Na +K + Protein + Sugar + Prolin.

(v) Yield based drought resistance indices

Mean productivity (MP)

Mean productivity was calculated by the formula of (Rosielle and Hambelen, 1981) as:

$$MP = \frac{Y_s + Y_p}{2}$$

Geometric mean productivity (GMP)

Geometric mean productivity was calculated by the formula of (Fernandez, 1992) as:

$$GMP = \sqrt{(y_s)(Y_p)}$$

Stress tolerance index (STI)

Stress tolerance index was calculated by the formula of (Fernandez, 1992) as:

$$STI = \left(\frac{Y_p}{\overline{Y}_p}\right)\left(\frac{Y_s}{\overline{Y}_s}\right)\left(\frac{\overline{Y}_s}{\overline{Y}_p}\right) = \frac{(Y_p)(Y_s)}{(\overline{Y}_p)^2}$$

Statistical analysis

Variance analysis, mean comparison (Duncan، s test), cluster analysis and correlation analysis were done MSTAT-C, SPSS and NTSYS softwares.

Results and discussion

The results of analysis of variance for yield indicated significant difference between the genotypes for Yp, but no significant difference was observed for Ys (Table 1). As F-test in the analysis of variance can only detect large differences between the genotypes, therefore non-significancy in the table of analysis of variance does not mean no significant difference between the genotypes for the character Ys that is why mean comparisons classified the genotypes for Ys in different groups (Farshadfar *et al.*, 2008).

Table 1. Analysis of variance for seed yield under stress and non-stress conditions.

		Mean squares	
S.O.V.	d.f.	Y_S	Y_P
Replication	2	4217.20	586.25
Genotype	8	9526.53	3721.31*
Error	16	4413.18	1043.25
C.V.%	-	23.13	28.27

*: Significant at 0.05 probability level.

Table 2. Mean comparisons and drought tolerance indices for the genotypes investigated.

Genotypes	$Y_{S\ (g/m2)}$	$Y_{P\ (g/m2)}$	STI	MP	GMP
1	114.333bc	296.100ab	0.41	205.217	183.532
2	91.667bc	241.233b	0.23	166.405	139.187
3	150.667ab	249.133b	0.45	199.900	191.529
4	107.000bc	277.300b	0.36	192.150	171.438
5	114.00bc	266.267b	0.36	190.133	168.504
6	66.333c	265.000b	0.20	165.667	130.061
7	113.167bc	352.933ab	0.49	233.050	199.465
8	184.667a	403.500a	0.90	294.083	271.048
9	86.333c	233.467b	0.25	159.900	141.819

Genotypes with common letter (s) have no significant differences at 0.05 probability level.

Mean comparison classified the genotypes for yield and drought tolerance criteria in different groups (Table 2). Maximum Ys and Yp was attributed to accession 8, while the least Ys and Yp was related to genotypes 6 and 9 respectively. Based on the indices STI, MP and GMP genotype 8 revealed the highest amount therefore this genotype is identified as drought tolerant, while genotype 6 with minimum amount as drought sensitive, therefore they can be used for the genetic analysis and QTLs mapping using hybridization programs. Vrious authors used STI, MP and GMP for sceening drought tolerant genotypes in crop plants (Farshadfar *et al.*, 2013; Golabadi *et al.*, 2006; Fernandez, 1992; Farshadfar and Sutka, 2002).

Table 3. Variance analysis of physiologic and metabolic traits under drought stress condition.

		Mean squares				
S.O.V.	d.f.	Prolin	Sugar	T.Ch.	Ch.a	Ch.b
Day	3	1078618.52**	32097.96**	235.37**	83.37**	65.09**
Genotype	8	375997.52**	7073.03**	66.72**	33.24**	10.47**
Genotype × day	24	152766.85**	2475.03**	32.50**	17.15**	3.45**
Error	144	173.60	70.86	2.44	1.39	0.32
C.V.%	-	4.70	12.81	7.23	7.71	8.98

**: Significant at 0.01 probability levels.

Screening drought tolerant genotypes based on three-dimensional plot

Three-dimensional plot based on Ys, Yp and STI (Fig. 1) divided X-Y surface into 4 parts (A, B, C and D). Group A exhibited genotypes with high yield in stress and non-stress environments group B showed genotypes with high yield in non-stress environment, group C revealed genotypes with high yield in stress environment and group D genotypes with low yield in both environments. According to Fig. 1 The most suitable genotype with high Yp, Ys and STI (drought tolerant) was identified number 8 (group A). Three dimensional plot was used for discriminating drought tolerant genotypes by various researchers (Fernandez, 1992; Farshadfar *et al.*, 2012).

Table 4. Mean comparisons of genotypes for the characters studied under drought stress condition.

Genotypes	Prolin	Sugar	T.Ch.	Ch.a	Ch.b	MSI
1	525.4a	57.51e	17.22e	12.44e	4.78e	6.41
2	115.2i	107.7a	22.36abc	16.61a	5.76d	6.35
3	412.1b	71.54c	20.81d	14.44d	6.36c	6.54
4	191.3g	46.49f	22.80ab	15.97ab	6.82ab	6.15
5	147.0h	45.53f	23.38a	16.31a	7.06a	6.15
6	245.9e	64.70d	22.78ab	16.32a	6.46bc	6.44
7	286.5d	62.05de	21.71cd	15.48bc	6.23c	6.64
8	393.9c	59.01e	22.12bc	14.99cd	7.13a	6.80
9	203.8f	76.94b	21.63cd	15.08cd	6.53bc	6.31

Means with common letter(s) have no significant difference at 0.05 probability level.

Evaluation of physiologic and metabolic traits under drought stress condition

Analysis of variance using factorial experiment with two factors genotype and measuring processe (day) stress condition indicated highly significant differences for all measured traits and day of measuring (Table 3), indicating the presence of genetic variability and possible screening drought

tolerant genotypes based on physiologic and metabolic criteria of drought tolerant. As can be seen in table with regard to the evaluated traits between genotypes and measuring processes there is significant difference. Interaction between measuring processe and genotypes was also highly significant expressing the effect day on the measuring traits.

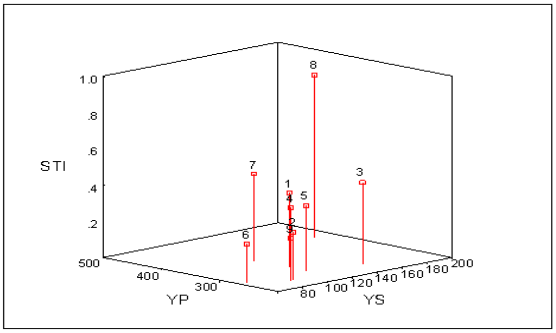

Fig. 1. Three-dimensional diagram of Y_P, Y_S and STI

Mean comparison of physiologic and metabolic traits under drought stress condition indicated that maximum amount of PC, SS, T. ch, Ch. a and Ch. b were related to genotype 1 (Bivanich), 2, 5, 2 and 8, respectively, whrease minimum amount of PC, SS, T. ch, Ch. a and Ch. b were attributed to genotypes 5, 4, 3, 1 and 1 (Table 4).

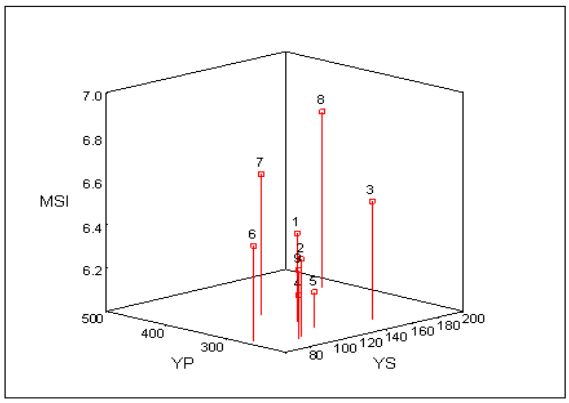

Fig. 2. Three-dimensional diagram based on MSI, Yp and Ys.

Screening drought tolerant genotypes based on MSI

Multiple selection index which discriminate drought tolerant genotypes bsed on all physiologic and metabolic indicators ispresented in Table 4. According to MSI the most drought tolerant genotype was identified as number 8. Base on the three-dimensional plot (Fig. 2) between MSI, Yp and Ys genotype 8 was distinguished as the most drought tolerant with high Yp and Ys. MSI was used for identification of QTLs controlling drought tolerance criteria in rye using wheat-rye disomic addition lines (Farshadfar *et al.*, 2003).

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