

UNCOVERING THE BIOLOGICAL AND AGRONOMIC STABILITY OF CHICKPEA (*CICER ARIETINUM* L.) GENOTYPES AGAINST SODIUM CHLORIDE STRESS

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Abstract

Irrigation practices have imposed the salinity stress in cultivated areas which resultantly inhibiting the crop productivity. Chickpea is also being grown under versatile environmental conditions across the region. Stability of the genotypic performance across different environments is indicator of tolerance against subjected stress. In current research experiment, agronomic and biological stability of chickpea genotypes was evaluated against different artificial saline environments. GGE biplot analysis was used for identification of stable performance of chickpea genotypes based on morphometric, physiological and biochemical markers. Chickpea genotypes were found to be negatively affected by salinity stress at early growth stages but magnitude of responses was different across the genotypes which have indicated the differential levels of genotype into environment interactions. Genotype 6009 was found biologically stable for chlorophyll contents, relative water contents and growth related parameters while genotype 6003 was biologically stable for relative water contents, Na⁺ concentration, K⁺ concentration and growth related traits. Venhar-2000, Bital-98 and Noor-2009 showed agronomic stability and recommended for further progression, manipulation and improvisation against salinity stress for better productivity.

Key words: Stability, GGE biplot, Multi-environment, Chickpea, Salinity.

Introduction

Irrigation practices in agriculture were begun 2500 years ago and their use was extensively increased in last 30 years. World agricultural productivity substantially increased by irrigation practices but resultantly salinization in agricultural land also increased with evident yield losses. It is estimated that about one third of the cultivatable land is affected by salinity. Out of salt affected lands about 37% is saline, sodic or waterlogged. Typically, irrigation water which is applied at rate of 1.0 to 1.5 m annually contains 0.1- 4 kgm⁻³ of salt. Thus, 1 to 60 metric tons of salt per hectare is applied to crop lands annually so, salinization is unavoidably linked with irrigation. Indus Basin, Pakistan is one of the most salt degraded region in the world (United Nations University Press Release, 2014). In Pakistan, about 5.727 million hectares is salt affected out of 19.82 million hectares cultivated area (FAOSTAT, 2013). High quantities of soluble salts in saline soils affect plant growth at different stages and create production differences among crops and variation in ion composition in various plant parts (Hamdy *et al.*, 2000). Salinity stress induce various problems like suppression in seed germination, seedling growth and yield of crops. In arid and semi-arid regions, the use of poor quality saline water for crop production is often unavoidable due to the shortage of water. Increase of salinity above threshold level is associated with decline in yield (Kafi & Goldani, 2001).

Chickpea (*Cicer arietinum* L.) mostly is salt-sensitive crop, and extensive reduction in yield is observed under excessive application of NaCl (Shaheenuzzamn, 2014). The effects of salinity on chickpea are wide ranging like delayed and reduced seed germination, and suppressed vegetative growth (Shaheenuzzamn, 2014). The process of soil salinization and the preponderance of saline water sources, pointing to future reliance on salt resistant crops (Hamdy *et al.*, 2000). Leading chickpea producing

countries are India, Turkey, Pakistan and Iran. Chickpea ranks fifth among legumes worldwide on the basis of production after soybean, groundnuts, beans and peas and third most widely grown grain legume (Cokkizgin, 2012). The genetic diversity in the available chickpea germplasm can lead to evolve the salt tolerant genotypes through screening and breeding. The genetic variation among the genotypes at different levels of salinity could be estimated to find the genotype by environment interactions (G×E). Differences in genotypic responses and tolerance level against different stresses are attributed to consistent or stable performance across different environments. Sensitivity analysis is also synonymously used for stability analysis. Stability is further classified into two types i.e. biological or static stability and agronomic or dynamic stability (Becker, 1981). Genotype whose phenotype showed little deviation across different environments is known as biological stability, analogous to genetic homeostasis. Responses parallel to mean trend in particular environment or no change in genotypic rank with higher mean is described as agronomic stability. Stability of the genotype is determined by genotype into environment interaction (GEI) as lack of GEI is the indicator of stability. Different biometrical strategies like variance component approach, regression approach, gene action, genetic correlation approach (Freeman, 1973; Hill, 1975), additive main effects and multiplicative interaction (AMMI; Gauch, 1992) and genotype, genotype into environment (GGE) interaction are being used for estimation of GEI and genotypic stability. GGE biplot is most preferred and appropriate method for analysis of stability due to its inner product property and *which won where* feature (Yan & Kang, 2003). This research experiment was planned to find out the biological and agronomic stability of the chickpea genotypes to study the GEI and to find the stable genotypes against diverse environments of sodium chloride salt.

Materials and Methods

Description of experiment: This experiment was conducted in greenhouse of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Total 20 different chickpea genotypes namely 6022, Venhar-2000, 6003, PB-2000, 6015, 6029, 6035, 6016, 6024, 7040, 6009, 3008, 6017, 6030, 6013, 1012, Noor-2009, 6014, 6011 and Bital-98 were collected from the Pulses Research Group, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Selection of these 20 chickpea genotypes for experiment was based on the availability, popularity, commonality, accessibility for researchers and farmers, representativeness of germplasm and coverage of cultivated area. Seeds of subjected genotypes were sown in polythene bags ($22 \times 15 \text{ cm}^2$) filled with clay loamy soil. Seeds were irrigated in polythene bags with water of EC 0.48 dSm^{-1} . Germination started after 4-7 days of sowing with temperature $30 \pm 1^\circ\text{C}$ and day light of 11.63 hours. Two weeks after germination, uniform seedlings were uprooted to transplant into hydroponic culture. Experiment comprised of four different NaCl environments; So: normal environment (0.48 dSm^{-1}), S1: 40 mM NaCl (4 dSm^{-1}), S2: 80 mM NaCl (8 dSm^{-1}), and S3: 120 mM NaCl (12 dSm^{-1}) salt solutions. There was conflict between the previous reports of salinity responses in chickpea as it was described as sensitive to moderately tolerant (Al-Mutawa, 2003; Vadez *et al.*, 2007; Flowers *et al.*, 2010; Hirich *et al.*, 2014). This conflict was attributed to the differences in the experimental conditions, genetic background of genotypes, parameters studied, protocol used for estimation of parameters, duration of the experiment, growth stage of treatment application, growth stage for response estimation, geographical origin of study material and meteorological conditions of the experimental site. So, instead of strictly adhering to any previously recommended pattern of salinity treatments, wide range of salinity treatments from normal water treatment to extremely high level of salt stress (12 dSm^{-1}) for chickpea were used in current study to visualize the responses of targeted genotypes. The experiment was conducted under triplicated factorial treatment structure in completely randomized design (CRD) having two factors (genotypes and salinity levels) with four treatment levels (So, S1, S2 and S3). Hydroponic culture was maintained by using Hoagland solution prepared by following the Hoagland & Arnon (1950). Amount of NaCl salt required for developing desired EC levels were calculated by following Haider & Ghafoor (1992). Plants were harvested 40 days after transplantation. Chickpea genotypes were evaluated based on the following traits; leaf temperature ($^\circ\text{C}$), chlorophyll contents ($\mu\text{g}/\text{cm}^2$), relative water contents (%), Na^+ ion concentration (ppm), K^+ ion concentration (ppm), Na^+/K^+ ratio, plant biomass (g), root length (cm), shoot length (cm) and root/Shoot ratio. Leaf temperature was recorded with the help of infrared thermometer (RAYRPM30CFTRG®). Leaf chlorophyll contents were measured by using chlorophyll meter (atLEAFplus). Relative water contents (RWC; %) were calculated by following Barr & Weatherley (1962).

$$\text{RWC} = \frac{\text{Fresh weight-Dry weight}}{\text{Saturated weight-Dry weight}} \times 100$$

Na^+ and K^+ ions concentration (ppm) of the leaf samples was estimated by using Hald, (1947) method with Sherwood-410 flame photometer.

Statistical analysis: Variation among genotypes was estimated by analysis of variance (ANOVA) following Steel *et al.*, (1997) and means were compared using Tukey's test (Sneath & Sokal, 1973) by using computer based statistical software Statistix8.1. The collected data for morphometric, physiological and biochemical traits were analyzed by GGE-biplot analysis (Yan *et al.*, 2000). GGE biplot has multiple advantages over other biometrical techniques like performance of different genotypes in particular environment, performance of a given genotype in different environments, comparison of different genotypes, *which won where* investigations for multi-environment data, genotype evaluation based on mean value and evaluation of genotypic stability can be determined by this biplot analysis (Yan and Kang, 2003). GGE biplot analysis was carried out by using GenStat 15th edition statistical software. For testing n genotypes in m environments with $n \times m$ interaction is denoted as Y . Y is subjected to singular value decomposition (SVD) to develop k principle components. Each principle component comprised of three key parts; (I) λ called singular value, (II) ξ_i known as set of genotypic eigen vectors and (III) η_j is set of environment eigen vectors. GGE biplot model for evaluation of n genotypes under m environments is as following (Yan & Kang, 2003);

$$Y_{ij} = \sum_{l=1}^k \lambda_l \xi_{il} \eta_{lj}$$

whereas:

$i : 1 \dots n, j = 1 \dots m, k = \min(m, n)$,

$Y_{ij} : \text{yield of genotype } i \text{ in location } j$,

$l : \text{the rank of a principal component, } l = 1 \dots k$,

$\lambda_l : \text{singular value of } l\text{th principal component, with } \lambda_1 > \lambda_2 > \dots > \lambda_k$. The square of λ_l is the sum of squares explained by the l th principal component.

$\eta_{lj} : \text{the eigenvector or singular vector of location } j \text{ for the } l\text{th principal component, and}$

$\xi_{il} : \text{the eigenvector or singular vector of genotype } i \text{ for the } l\text{th principal component.}$

Results

Analysis of variance for factorial treatment structure:

As current research experiment was performed by following completely randomized design under factorial treatment structure so, corresponding analysis of variance was performed. Genotype and treatment factors were significantly different for leaf temperature ($^\circ\text{C}$), chlorophyll contents ($\mu\text{g}/\text{cm}^2$), relative water contents (%), Na^+ ion concentration (ppm), K^+ ion concentration (ppm), Na^+/K^+ ratio, plant biomass (g), root length (cm), shoot length (cm) and root shoot ratio. Interaction of genotype and treatments was also significant for all studied traits which showed the variable response of different genotypes for different traits under different saline environment (Table 1).

Tukey's post-hoc test: Tukey's test was performed as post-ANOVA test for treatment mean comparison. Genotype mean comparison was also performed but results were not comprehensive to draw remarkable conclusion about genotypic response as responses were variable across the treatments and traits. So, genotype mean comparison was not entertained in current study and replaced by most accurate and precise statistical GGE Biplot analysis to draw comprehensive conclusions. Treatment mean comparison based on all genotypes showed that chlorophyll contents ($\mu\text{g}/\text{cm}^2$), relative water contents (%), K^+ ion concentration (ppm), plant biomass (g), root length (cm), and shoot length (cm) decreased with increase of salinity stress. Whereas, leaf temperature ($^{\circ}\text{C}$), Na^+ ion concentration (ppm), Na^+/K^+ ratio and root/shoot ratio increased with increase of saline conditions (Table 2). Treatment mean comparison is helpful only to depict the generalized trend of genotypic responses against different saline environments.

GGE Biplot analysis: GGE biplot analysis was done for each trait separately based on all of four saline environments. GGE biplot graph depicted the different unique features which were not elaborated by other biometrical tools. Genotypes are scattered across the graph and environments were represented as vectors which were named as environment vectors (EVs). EVs for each environment were of different color to discriminate the environments from each other. EVs were labeled with mean values of corresponding trait for which biplot was drawn and corresponding environment was also labelled. Each EV carried an arrow which represented direction of above average value for studied traits for that particular environment. Angle between the EVs showed the similarity of responses of environment for subjected trait. GGE biplot representing more than 70% variability was considered most appropriate for retrieval of useful information and variability percentages were presented in parenthesis on GGE biplot axis. GGE biplot for leaf temperature depicted 87.97% of total genetic variability of the subjected data (Fig. 1). GGE biplot for leaf temperature based on 4 different saline environments showed that EV for S1 and S3 were overlapping which showed that these environments have equivalent discrimination power. Genotypes 6014 and 6011 were having highest mean leaf temperature for S1 and S3 environments whereas, genotypes Venhar-2000, 6015,

6003 and 7040 were having lowest mean leaf temperature. Discrimination ability of so environment was slightly different from S1 and S3 as there was little acute angle between these EVs. Genotypes showed extensively different responses at S2 environment due to right angle alignment of vector over the other EVs. Genotypes 3008 and 6009 showed highest mean leaf temperature at S2 environment. However, genotypes present at the origin of GGE biplot graph were called most stable genotypes as these were irresponsive to environmental effects. Under diverse environmental conditions most stable genotypes were selected which could sustain their performance across multiple environments (Fig. 1).

GGE biplot for chlorophyll contents showed 87.62% of total variability of whole variation for chlorophyll. So, S1 and S3 environments were showing similar responses and discriminativeness for genotypes. Genotypes 6030, 7040 and 6015 were having higher mean chlorophyll content whereas, 6014 and 1012 were having least mean values. S2 environment was distinctive from others due to right angled allocation. Genotype 6009 was located at origin of graph which showed its irresponsive behavior (Fig. 2). GGE biplot for relative water contents was depicting the 90.31% of total genetic variability among data. Angle between the environment vectors was prominent which showed that all of these environments have different discrimination ability for genotypes based on relative water contents. So and S3 were closely resembling in responses. Genotypes Venhar-2000, 6015, 7040, 6024, Noor-2009 were having higher mean values at So, S1 and S3. Genotypes 1012, 6011 and 6014 were having lower mean values and located opposite to the environment vector arrow (Fig. 3). Genotypes 6022, 6003 and 6009 were having consistent RWC irrespective to saline environments (Fig. 3). GGE biplot for Na ions was based on 98.36% of total variation in data. Environment vectors were having no similarity in their response as there was significant angle between them. Most of the genotypes were clustered across the S3 vector. Genotype 6003 has consistent response across the environments whereas; genotypes Bital-98, 6014, 1012, 6013 and 6017 were having higher Na ions generally for all environments and specifically for S3 environment. Genotypes 6015, Venhar-2000, 6024, 7040, 6030 and Noor-2009 were having lower Na ion contents in all subjected environments (Fig. 4).

Table 1. Analysis of variance for different traits of chickpea genotypes.

SOV	DF	Leaf temp. ($^{\circ}\text{C}$)	Chlorophyll contents ($\mu\text{g}/\text{cm}^2$)	RWC (%)	Na^+ ions (ppm)	K^+ ions (ppm)	Na^+/K^+ ratio	Plant biomass (g)	Root length (cm)	Shoot length (cm)	root/shoot ratio
Genotypes	19	3.56**	23.8**	98.18**	2865.2**	12026.5**	1.05**	31.78**	19.83**	22.58**	0.05**
Treatments	3	178.05**	20857.6**	6268.77**	68127.8**	75640.6**	15.59**	4285.49**	2755.25**	1484.52**	0.83**
G×T	57	0.53*	5.8*	10.89**	206.1**	243.0**	0.14**	6.04**	3.01**	4.26**	0.04**
Error	160	0.37	2.0	1.55	24.7	74.6	0.0012	0.27	0.56	1.21	0.00151

*= Significant at 1% Probability level, **= Significant at 5% Probability level

Table 2. Treatment mean comparison for different traits of chickpea genotypes.

Treatment	Leaf temp. ($^{\circ}\text{C}$)	Chlorophyll ($\mu\text{g}/\text{cm}^2$)	RWC (%)	Na^+ ions (ppm)	K^+ ions (ppm)	Na^+/K^+	Plant biomass (g)	Root length (cm)	Shoot length (cm)	Root/shoot
So	16.58(C)	60.19(A)	45.77(A)	49.72(D)	180.14(A)	0.29(D)	21.60(A)	29.59(A)	36.90(A)	0.515(C)
S1	16.72(C)	47.42(B)	42.56(B)	67.49(C)	155.19(B)	0.47(C)	19.40(B)	23.12(B)	35.76(B)	0.655(B)
S2	19.23(B)	29.09(C)	31.99(C)	99.13(B)	122.03(C)	0.89(B)	8.97(C)	19.49(C)	31.17(C)	0.677(B)
S3	19.94(A)	18.38(D)	23.39(D)	125.69(A)	99.76(D)	1.44(A)	3.84(D)	13.39(D)	25.99(D)	0.803(A)

Environment Interaction (GEI) was also significant for all studied chickpea traits. Analysis of variance for factorial treatment structure indicated the existence of GEI which directed to further analyze the data to explore the nature of interaction. Significance of GEI for current study indicated that data should be further subjected to robust biometrical analysis like stability analysis. Effectiveness of factorial treatment structures for partitioning the total variation into different components like genotype, environmental difference and their joint effects was described by Fisher, (1926) and further supported by large number of researchers (Aslam *et al.*, 2013; Naveed *et al.*, 2014; Maqbool *et al.*, 2015; Aslam *et al.*, 2015).

Salinity stress has inhibitory effects on crop productivity and performance. Chickpea genotypes were affected by different levels of saline environments. Mean comparison test was performed to evaluate the general trend of performance for studied traits based on mean value of all genotypes. Leaf temperature increased with increasing level of salinity stress as mean value at So was 16.58°C and at S3 was 19.94°C. This parallel incline of leaf temperature is previously attributed with stomatal closure and other metabolic protective measures under prevalence of salinity stress (Heidari-Sharif-Abad, 2006; Taiz & Zeiger, 2006). Chlorophyll contents of studied chickpea genotypes were reduced with increasing level of salinity stress as mean value for So treatment was 60.19µg/cm² and for S3 was 18.38µg/cm². Different researchers attributed the reasons for decline of chlorophyll contents to be decline in osmotic potential and stomatal closure (Jamil *et al.*, 2007; Sivritepe, 2010). Decline in chlorophyll fluorescence is also associated with accumulation of sodium ions. Visual observations indicated the yellowing of chickpea leaves which is also indicator of chlorophyll degradation due to accumulation of sodium salts, reduction of stomatal conductance, increase of leaf temperature and reduction of osmotic potential of leaves imposed by salinity stress (Jamil *et al.*, 2007; Sivritepe, 2010). Relative water contents (RWC) of chickpea leaves reduced by increasing the level of salinity stress as mean values for So were 45.77% and at S3 were 23.33%. Decline of RWC is indicator that sodium salt induced the water deficiency in the plants and lead to dehydration in chickpea plants. Dehydration symptoms were visually evident as leaves were showing the evident wilting even these were grown in aquaculture. Dehydration and RWC reduction due to salinity stress were also reported by several researchers (Navarro *et al.*, 2003; Suárez & Medina, 2008).

Concentration of sodium ions increased in leaves with increase in the level of sodium salt stress as means were 49.72 ppm for So and 125.69 ppm for S3. Potassium ions reduced with increase in the sodium stress. Na⁺/K⁺ also increased with increase in the level of salt stress. Qin *et al.*, (2010) reported the increase of Na⁺ upto 16 times than normal conditions and K⁺ ions reduced upto 30% under salinity stress. It is also reported that Na⁺ uptake increased for osmotic adjustment under prevailing salts stress which resultantly induced the ionic imbalance and ionic toxicity (Yildirim *et al.*, 2009). Numerous toxic effects are attributed to Na toxicity like metabolic, biochemical and osmotic problems which are also evident

in current study in the form of declined growth, RWC, chlorophyll contents, and increased leaf temperature (Bonilla *et al.*, 2004). Potassium is one of the main plant macronutrient and has key functioning in osmoregulation, stomatal behavior, cell expansion, enzyme activity, membrane polarization and neutralization of negatively charged ions. Concentration of potassium ions reduced in chickpea with increasing salinity stress as chickpea genotypes at So has 180.14ppm and at S₃ has 99ppm K⁺ ions. Increased Na⁺ and reduced K⁺ leads to the substitution of potassium by sodium in plants. Substitution and competence for enzymatic binding site leads to negative effects on cellular functions (Yildirim *et al.*, 2009). Blumwald *et al.*, (2000) reported that among nutrient uptake, Na⁺ uptake is more favored than K⁺ and under salinity stress Na⁺ are more accumulated in plants than K⁺ ions as in accordance with current study. Biomass, root length and shoot length of chickpea plants reduced with increasing level of NaCl salt stress. Decline in root length, shoot length and plant biomass also attributed to NaCl toxicity which has reduced the photosynthesis, increased leaf temperature, effects on enzyme activity, protein synthesis, carbohydrate synthesis, cell division and cell elongation (Jamil *et al.*, 2007; Taffouo *et al.*, 2009; Kapoor & Srivastava, 2010).

All the studied genotypes were not equivalently responsive against different levels of salt stress. Increase of Na⁺, leaf temperature and reduction of root and shoot lengths, biomass, K⁺, RWC were not following same trend for all genotypes which showed that genotypic differences are prevailing. Genotypic differences are attributed to differences in tolerance level of genotypes and interaction with environment. Genotypic differences against different stresses are reported for several crops by researchers (Jamil *et al.*, 2007; Taffouo *et al.*, 2009; Kapoor & Srivastava, 2010; Aslam *et al.*, 2013; Naveed *et al.*, 2014; Aslam *et al.*, 2015; Maqbool *et al.*, 2015). Identification of statically stable and desirably stable genotypes is the key success for breeders due to irresponsive or desirably responsive nature of genotypes. AMMI biplot and GGE biplot are used for stability analysis across multi-environments (Yan & Kang, 2003). GGE biplot is preferably used in current research for evaluation of stability and responsiveness of genotypes across multiple environments. Genotypic stability is very critical feature of varietal performance which provides the information about consistent outcome across multiple environments. Under salinity stress, consistent and higher rank performance is indicator of tolerance against stressful environment. Genotype 6009 performed statically consistent (static stability) and irresponsive for chlorophyll contents, relative water contents and growth related parameters which showed that this genotype rendered these parameters unaltered under varying levels of salinity stress. Genotype 6003 given unaltered response for relative water contents, Na⁺ concentration, K⁺ concentration and subjected growth related traits which showed highly tolerant response of this genotype against extensive varying saline environments. However, consistent performance is only preferred if it is associated with desirable mean value either higher or lower.

Genotypes Venhar-2000, Bital-98, and Noor-2009 showed dynamically stable performance across multiple variable saline environments as these retained higher ranks. Stability with higher mean value for desirable traits (chlorophyll contents, RWC, K⁺, growth related traits) and stability with lower mean value for undesirable or harmful traits (i.e. higher leaf temperature and Na⁺ ions) is indicator of suitable performance. Genotype into environment interaction exploitation for evaluation of stability using GGE biplot analysis is also carried out by Yan & Rajcan, 2002; Yan & Kang, 2003; Yan & Tinker, 2005; Maqbool *et al.*, 2015. Conclusively genotypes Venhar-2000, Bital-98, and Noor-2009 are dynamically stable and recommended for further investigations at molecular levels and for manipulation in field level as these retained their ranks across diverse saline environments with higher means.

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