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Mitigation of Drought Effects by Nitrogen Foliar Spray in Chickpea (*Cicer arietinum* L.)

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Abstract

Legumes, as we know, have an edge of nodule formation over other crops to fix atmospheric nitrogen and its uptake which, otherwise, would not be so easily available. But drought affects nodules by reducing their activity and ultimately reducing yield. So, there is a need to compensate this loss of nitrogen by different means. Therefore, a study was planned where different chickpea genotypes were subject to different water treatments and nitrogen spray. Three treatments i.e., irrigation at pod formation (T1), no irrigation at pod formation (T2), and no irrigation plus two foliar sprays of 1% nitrogenous fertilizer at the initiation of flowering and pod filling stage (T3) were applied to fifty genotypes sown in two-factor factorial under RCBD. Treatment mean comparison showed that primary branches, secondary branches, days to flowering were non-significantly different in T1 (irrigated) and T3 (2 nitrogen spray and no irrigation) while other traits were significantly different for all treatments. Radar diagram showed that grain yield for T1 and T3 were very closer to each other which showed that nitrogen spray was able mitigate or supplement the inhibitory effects of drought on grain yield. Direct Principle component analysis on yield data proved to be ineffective for diversity analysis due to representation of least variability (25% only) by PCA biplot. Drought tolerance indices based PCA biplot showed 80% cumulative variability for PC1 and PC2 so, we preferred it for diversity analysis. Ca-7046, Ca-950131, Ca-4004, CH 7 and Ca-6003 were found to be tolerant to drought stress and responsive to nitrogen spray. Ca-7027, Ca-5006, Ca-7012, Ca-7050, Ca-6013 and Ca-6011 were drought susceptible and least responsive to nitrogen spray. Hence, it was concluded that foliar application increases tolerance against drought but responses were different genotypically.

Key Words: Drought tolerance indices, PCA, radar diagram, yield and yield components

Introduction

Globally chickpea (*Cicer arietinum* L.) is the third most important legume crop after dry bean (*Phaseolus vulgaris* L.) and peas (*Pisum sativum* L.) with a wide distribution across tropics, subtropics and temperate regions (Singh, 2006). It contained about 38-59% carbohydrates, 20-22% protein, 3% fiber and 4.8-5.5% oil (Miao *et al*., 2009). The Ten top chickpea producing countries in order of importance are India, Australia, Pakistan, Turkey, Myanmar, Ethiopia, Iran, Spain, Canada and Mexico; out of which Pakistan accounts for 8.7% of the total global chickpea production and ranked in second position (FAOSTAT, 2012). Chickpea is important source of dietary protein for the predominantly vegetarian population of Subcontinent (Viveros *et al.*, 2001). In Pakistan chickpea (*Cicer arietinum* L.) is one of the most important Rabi legume crop and plays a dominant role in rainfed agricultural areas of the country. It was cultivated on 0.98 Mha with production of 0.67 Mtons (Economics Survey of Pakistan, 2013). The major areas under the chickpea cultivation are Thal region that consists of districts Bhakhar, Mianwali, Leyyah, Khushab and parts of Jhang. It is also grown in Attock, Rawalpindi, Jehlum and Chakwal districts. Chickpea is the major source of livelihood of the rural population. In Pakistan on an average, Punjab contributed about 88% of this production (Economics Survey of Pakistan, 2013).

Yield of chickpea per unit area in Pakistan is very low as compared to other leading chickpea growing countries of the world. In Pakistan, chickpea is mostly grown under rainfed areas and sometimes long break in rain result in scarcity of water that adversely affects the yield (Mushtaq *et al*., 2013). In Mediterranean climatic regions it is sown in autumn or spring and grown during the cool wet months of winter and spring (Kumar and Abbo, 2001). In both environments, chickpea crop is exposed to drought during pod set and seed filling stages. Additionally, crop exposed to drought at flowering stage cause the

inhibition of pod setting and grain filling followed by inhibited yield (Reza *et al.*, 2013).

Nitrogen is very vulnerable to be lost from soil but legume have the benefit of nodulation which ensured the provision of nitrogen to plants. Under drought stress, nodules are effected through reduction of their nodule dry weight, nitrogen fixation ability and nitrogenase activity. Damage to bacteroid membranes, damage to infected tissue, vacuolation of host cell, loss of pribacteroid membrane, deterioration of hot cell cytoplasm and senescence of bacteroids are caused by drought stress which further cause the inhibitory effects on yield of legume crop (Ramos *et al.*, 2003; Ashraf and Iram, 2005; Onuh and Donald, 2009). Foliar application of nitrogen during drought conditions is the most attractive and cost-effective option which might be useful for the plant health (Bahr, 2007). At flowering stage, it increased yield and seed protein contents. Thus supply to chickpea plants with supplementary nitrogen showed the beneficial effect on enhancing growth, increasing seed yield and seed protein (Palta *et al.*, 2005).

This research experiment was planned for testing of hypothesis that inhibitory effects of drought stress on chickpea can be encountered or supplemented by foliar spray of nitrogen as nitrogen spray was found to be promotive for growth and development.

Materials and Methods

Experimental Site

The present experiment was conducted in the research area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan, during Rabi season 2013-14. Faisalabad is located in central Punjab, Pakistan and climatic conditions of central Punjab are dry semi-arid. Latitude of Faisalabad is 31.43^{o}N, longitude 73.1^{o}E, elevation is 184.5 m from sea level. Rainfall occurred during summer monsoon and winter bears very little rain. Field which was used for experiment was under the rotation of different pulses and maize. Data for climatic factors of Faisalabad were collected from data base entitles "Climate-Data.Org" (http://en.climate-data.org/location; Table-1).

Experiment Description

The experimental material comprised of 50 chickpea genotypes was sown in three sets following the two factor factorial randomized complete block design with three replications each. Each genotype was sown by keeping plant-to-plant distance of 15cm and row-to-row distance were kept 30cm. All agronomic practices were followed uniformly. Experiment was splitted into three subunits. Out of these three, one subunit was treated as normal while rest of two sets were treated under drought stress conditions from which one was treated with 1% solution of urea for the foliar nitrogen application at flowering stage and pod filling stage. Rainfall was common factor in all of three treatments.

Three treatments were applied as; Irrigation at pod formation (T1), No irrigation at pod formation (T2), No irrigation and two foliar sprays of 1% nitrogenous fertilizer at the initiation of flowering and pod filling stage (T3).

Data were recorded on individual plant as well as per unit basis at appropriate time for the following traits; Number of primary branches per plant (PB), Number of secondary branches per plant (SB), Days taken to flowering (D-F), Plant height (PH), Plant weight (PW), Number of pods per plant (PpP), Number of grains per pod (GpP), 100-Grain weight (100GW), Grain yield per plant (GY). These parameters were measured in SI units.

Statistical Analysis

Analysis of Variance

Collected data were analyzed statistically using Fisher' analysis of variance (ANOVA) for significance of treatment effects and genotypes (Steel *et al.*, 1997). The treatment means were compared with Turkey's HSD (Honestly significant difference) test to evaluate the effectiveness of treatments. Same alphabets were assigned to non-significantly different treatment means and different alphabets were assigned significantly different treatment means. Radar diagram was made for treatment mean comparison across three treatments. In radar diagram distance from the origin is directly linked with higher grain yield.

Principal Component Analysis (PCA)

Principal component analysis (PCA) was used for analysis of multivariate data of fifty chickpea accessions across three different treatments. Principle component analysis (PCA) is multivariate analysis which is used to analyze the variables which are correlated. Observed variance is analyzed in PCA. PCA is pure mathematical technique and it explained variance. PCA is an independent technique and preferred for prediction. It is data reduction tool which efficiently reduce the large data set into smaller manageable variables which can be subjected to subsequent analysis. If first few components have most of variance of raw data then these few components are helpful for further data analysis. PCA can draw large number of variables on two dimensional plot which otherwise becomes difficult to deal. Recognition of outliers and clustering of accessions becomes easier with the help of PCA (Chatfield and Collins, 1990; Johnson and Wichem, 1996). Effect of environmental factors like;

temperature, rainfall, humidity and soil moisture contents were determined on mustard planting which showed that November is most suitable period for mustard planting (Mandal *et al.,* 2008). Genetic diversity in the germplasm could be studied with the help of multivariate PCA (Johnson and Wichern, 1988). Data of Interrelated quantitative dependent variables is analyzed by PCA (Abdi and Williams, 2010).

Drought tolerance indices:

Yield of chickpea genotypes under three different nitrogen and water treatments was subjected to drought tolerance indices. Following drought tolerance indices were used evaluate the performance of chickpea yield; Mean Productivity (MP; Rosielle and Hambling, 1981), Geometric Mean Productivity (GMP; Fernandez, 1992), % change (Choukan *et al.*, 2006), Tolerance index (TOL; Rosielle and Hambling, 1981), Yield Stability Index (YSI; Bouslama and Schapaugh, 1984), Yield Index (YI; Gavuzzi *et al.,* 1997), Stress Tolerance Index (STI; Fernandez, 1992), Stress Susceptibility Index (SSI; Fischer and Maurer, 1978), Relative Drought Index (RDI; Farshadfar and Elyasi, 2012), relative decrease in yield (RDY; Farshadfar and Elyasi, 2012). Drought tolerance indices were measured by following ways; (1) T1 is considered as normal treatment and T2 is considered as stress environment and assigned the subscript $_{(T1+T2)}$, (2) T1 was considered as normal and T3 was considered as stress environment and assigned subscript $_{(T1+T3)}$. Association of drought tolerance indices with yield under three subjected treatments was also studied by using correlation studies. PCA based biplot analysis for drought tolerance indices was used to categorized genotypes into four characteristics groups. Genotypes performs better under normal and stress conditions (Group A), genotypes perform better under normal and poor under stress conditions (Group B), genotypes perform relatively better under stress and poor under normal (Group C), genotypes perform poor under normal and stress condition (Group D). Above mentioned drought tolerance indices are capable of separately recognizing these four distinct groups so, these indices were used in current studies.

Results

Analysis of Variance and Treatment Mean Comparison:

Data of yield and yield components for 50 chickpea genotypes were subjected to two factor factorial analysis of variance. Genotypes and treatments were given equal importance so, two factor factorial analysis of variance was most appropriate. Genotypic, treatment and their interactions had significant effects for PB, SB, D-F, PH, PW, PpP, GpP, 100GW and GY (Table-2). Tukey HSD all pairwise mean comparison test for treatments was carried out to evaluate the effectiveness of treatments. Same alphabets were assigned to non-significantly different treatment means and different alphabets were assigned significantly different treatment means. PB, SB and D-F had non-significantly different mean values for T1 and T3 but significantly different for T2. Treatment means under T1 and T3 were higher than T2 for PB, SB and D-F. T3 had highest mean value for PH, PW, PpP, GpP, 100GW and GY followed by T1 and T2 (Table-3). Standard error for treatment means, critical value for treatment mean comparison and range for treatment means was also presented in Table (3). Radar diagram showed the responses of chickpea genotypes to water and nitrogen treatments. Ca-6013, Ca-1219, Ca-5002, Ca-2050, CH7, Ca-4004, Ca-950131 and Ca-7046 had high mean yield per plant across the subjected treatments whereas, Ca-5006, Ca-7027, Ca-7012, Ca-7050 and Aug-812 had lowest mean yield per plant across the imposed treatments (Figure-1). Noor2009, PB2008, Ca-7002, Ca-1013, Ca-928 and Ca-7027 had highest yield under T2 relative to other treatments (Figure-1).

Principal Component Analysis:

Principal component analysis transformed the raw data (nine variables for three treatments) into twenty seven principle components (PCs). PC1 had highest eigenvalue 3.77 and total ten PCs (PC1, PC2, PC3, PC4, PC5, PC6, PC7, PC8, PC9 and PC10) had more than 1 eigenvalue. These ten PCs described 76.74% cumulative variability whereas, rest of seventeen PCs described 23.26% variability (Figure-2). PC1 and PC2 for PCA were most important as these had highest value for variability.

First ten PCs were named depending on the highly contributing variable i.e. PC1 is named as seed size component as eigenvector value for 100GW(T1), 100GW(T2) and 100GW(T3) was highest in this PC (Table). PC2, PC4 and PC6 were named as yield components as different yield traits (YpP, PpP and GpP) had highest eigenvector values in these PCs. PC3, PC4, PC7, PC9 and PC10 were named as plant structural components as different structural traits (PW, PB, SB and PH) for these PCs had highest eigenvector values. PC5 and PC8 were named as phonological components as phonological traits (D-F) had highest eigenvector values for these PCs (Table-4).

Trait based PCA scatter plot showed the discrimination power of traits depending on their length. Shortest vector length for GpP(T1), PW(T3), PW(T2), PW(T1), GpP(T3), D-F(T3) and GpP(T2) showed their discrimination power is negligible.

Longest vector length for 100GW(T1), 100GW(T2), 100GW(T3), PH(T2), D-F(T1), D-F(T2), YpP(T3) and YpP(T1) showed that they have strong discrimination power for differentiation of genotypes (Figure-3). Correlation between vectors was diverse as shown by 360º scattering of traits on biplot. Such diverse scattering was due to diverse background of treatments under which these parameters were measured. Diverse treatments diversified the traits and their correlations which made the PCA least effective as strong and significant association between traits is prerequisite for effectiveness of PCA for diversity analysis and data reduction (Figure-3).

PCA showed that, Ca-6011, Ca-7002, Ca-7021, Pb-2008, Noor 2009 and Bitall 98 most variability whereas, Aug-98, Ca-950131 and Ca-7041 had least variability on the basis of studied traits and treatments (Figure-4). But preferential selection of these genotypes for further incorporation in any breeding program is not recommended because these were depicting only 25% of the total variability in data (Figure-4). So, for proper selection of genotypes for being better under drought stress and highly responsiveness to nitrogen spray, we used drought tolerance indices based PCA which was described in very next section.

Drought Tolerance Indices:

Among numerous indices we used selectively, MP, GMP, %change, TOL, YSI, YI, STI, SSI, RDI and RDY. Association of drought tolerance indices with mean yield under different water and nitrogen treatments was studied before their PCA and biplot analysis. T1 and T2 had significant moderate positive association whereas, T1 and T3 had significant strong positive correlation. T1 had significant strong positive correlation with $MP_{(T1+T2)}$, $MP_{(T1+T3)}$, $YI_{(T1)}$, $YI_{(T3)}$, $STI_{(T1+T2)}$, $STI_{(T1+T3)}$, $GMP_{(T1+T2)}$ and $GMP_{(T1+T3)}$ whereas, T1 had negative correlation with $TOL_{(T1+T3)}$, $YSI_{(T1+T2)}$, $YSI_{(T1+T3)}$, $SSI_{(T1+T3)}$, $RDI_{(T1+T2)}$ and $RDI_{(T1+T3)}$. T2 had negative correlation with $\%_{(T1+T2)}$, $TOL_{(T1+T2)}$, $YSI_{(T1+T3)}$, $SSI_{(T1+T2)}$, $SSI_{(T1+T3)}$, $RDI_{(T1+T3)}$, $RDY_{(T1+T2)}$ whereas, T2 had positive correlation with $\%_{(T1+T3)}$, $MP_{(T1+T2)}$, $MP_{(T1+T3)}$, $YSI_{(T1+T2)}$, $YI_{(T1)}$, $YI_{(T2)}$, $YI_{(T3)}$, $STI_{(T1+T2)}$, $STI_{(T1+T3)}$, $GMP_{(T1+T2)}$, $GMP_{(T1+T3)}$, $RDI_{(T1+T2)}$, $RDY_{(T1+T3)}$. T3 had significant positive correlation with $MP_{(T1+T2)}$, $MP_{(T1+T3)}$, $TOL_{(T1+T2)}$, $YI_{(T1)}$, $YI_{(T2)}$, $YI_{(T3)}$, $STI_{(T1+T2)}$, $STI_{(T1+T3)}$, $GMP_{(T1+T2)}$, $GMP_{(T1+T3)}$ whereas, T3 had negative correlation with $\%_{(T1+T3)}$, $TOL_{(T1+T3)}$, $YSI_{(T1+T2)}$, $RDI_{(T1+T2)}$, $RDY_{(T1+T3)}$. Magnitude of correlation of drought tolerance indices with T1 and T3 were more similar or closer relative to T2 (Table-5).

Principle component analysis transformed the drought tolerance indices into nine different principle components. Among these nine principle components, only three principle components had eigen value greater than one which depicted that these three were conducive for further study. T1, T2, T3, $\%_{(T1+T3)}$, $MP_{(T1+T2)}$, $MP_{(T1+T3)}$, $TOL_{(T1+T3)}$, $YSI_{(T1+T2)}$, $YI_{(T1)}$, $YI_{(T2)}$, $YI_{(T3)}$, $STI_{(T1+T2)}$, $STI_{(T1+T3)}$, $GMP_{(T1+T2)}$, $GMP_{(T1+T3)}$, $RDI_{(T1+T2)}$, $RDY_{(T1+T3)}$ were positively contributing in PC1 whereas, others were negatively contributing. T1, T3, $\%_{(T1+T2)}$, $MP_{(T1+T2)}$, $MP_{(T1+T3)}$, $TOL_{(T1+T2)}$, $YSI_{(T1+T3)}$, $YI_{(T1)}$, $YI_{(T3)}$, $STI_{(T1+T2)}$, $STI_{(T1+T3)}$, $GMP_{(T1+T2)}$, $GMP_{(T1+T3)}$, $SSI_{(T1+T2)}$, $SSI_{(T1+T3)}$, $RDI_{(T1+T3)}$, $RDY_{(T1+T2)}$ were positively contributing in the variability of PC2 whereas, other indices were negatively contributing. T1, $\%_{(T1+T2)}$, $\%_{(T1+T3)}$, $TOL_{(T1+T2)}$, $TOL_{(T1+T3)}$, YI(T1), $STI_{(T1+T3)}$, $GMP_{(T1+T3)}$, $SSI_{(T1+T2)}$, $RDY_{(T1+T2)}$, $RDY_{(T1+T3)}$ were positively contributing in the variability of the of PC3 whereas, other indices had negative contribution in PC3 variability (Table-6). Cumulative variability contributed by PC1, PC2 and PC3 was 99.9% and individual variability was 47.77%, 32.41% and 19.92% respectively. Combined variability contributed by PC1 and PC2 was 80% which was significant for the study of biplot and assortation of genotypes so, biplot was designed by using PC1 and PC2 (Figure-5). Two types of biplots were made, vector biplot which described the scattering pattern of traits and genotype biplot showed the variability and responsiveness of genotypes.

Drought tolerance indices were categorized into different groups by PCA based biplot. $Y1_{(T3)}$, T_3, $GMP_{(T1+T3)}$, $MP_{(T1+T3)}$, $STI_{(T1+T3)}$, $YI_{(T1)}$ and T_1 were categorized in Group1. Group2 comprised of $MP_{(T1+T3)}$, $STI_{(T1+T2)}$, $GMP_{(T1+T2)}$, $Y1_{(T2)}$ and T_2. Group3 consisted of $RDI_{(T1+T3)}$, $\%_{(T1+T3)}$, $YSI_{(T1+T2)}$, $RDI_{(T1+T2)}$ and $TOL_{(T1+T3)}$. Group4 comprised of $TOL_{(T1+T2)}$, $RDY_{(T1+T2)}$, $\%_{(T1+T2)}$, $SSI_{(T1+T2)}$, $RDI_{(T1+T3)}$, $YSI_{(T1+T3)}$ and $SSI_{(T1+T3)}$ (Figure-6). Genotypes of chickpea were also categorized into four different groups. Ca-7046, Ca-950131, Ca-4004, Ca-2050, CH7 and Ca-5002 were better performer genotypes in Group-1. AUG810, Ca-1028, Ca-7041, Ca-3020 and Ca-66101 were prominent in Group-2. PB91, Ca-6054 and Bittal98 prominent in Group-3. Ca-7027, Ca-5006, Ca-7012 and Ca-7050 were prominent in Group-4 (Figure-7).

Discussion

Biotic and abiotic stresses are gigantic barrier in productivity of agricultural crops (Aslam *et al.*, 2013a, b, c; Naveed *et al.*, 2013; Aslam *et al.*, 2014; Aslam *et al*., 2015a,b,c; Maqbool *et al*., 2015a,b; Aslam *et al*., 2016; Maqbool *et al*., 2016). Drought is most devastating abiotic stress which is

seriously hindering the performance of many crop species across the globe at different growth stages. Agricultural crops are more effected by drought stress than any other stress which disturbed the growth, development, phenology, morphology and numerous molecular processes (Reddy *et al.,* 2004; Gunes *et al.,* 2008). Effects of drought stress on yield and yield components of numerous crops have been reported by numerous researchers (Reddy *et al.,* 2004; Gunes *et al.,* 2008; Talebi *et al.,* 2013). Chickpea is mainly grown on marginal lands in Pakistan where it is facing the problem of terminal drought stress and yield alongwith its numerous components is perturbed seriously so, we focused on yield and its components for evaluation of chickpea responsiveness. Structural traits of chickpea i.e. plant height, number of primary branches, number of secondary branches, plant weight; phenological traits i.e. days to flowering; yield and its components i.e. pods per plant, grain per pod, 100 grain weight and grain yield were subjected to study under drought stress. All of the studied chickpea genotypes showed significant differences for structural, phenological, yield and yield associated traits under subjected treatments. Significant differences due to different drought treatments were also previously reported in chickpea and other crops (Ahmad *et al.,* 2003; Islam *et al.,* 2008; Gunes *et al.,* 2008).

To evaluate the effectiveness of these three treatments on the performance of chickpea genotypes, we conducted the treatment mean comparison over genotypes. T1 and T3 were non-significant for primary branches, secondary branches and days to flowering. Plant height and plant weight were significantly higher in T3 than T1 and T2. These results showed that application of nitrogen foliar spray had increased the vegetative growth of chickpea by improving the structural traits. Application of nitrogen foliar spray alone or in combination with multiplex micronutrients was reported to increase the vegetative growth of chickpea (Ganga *et al.,* 2014). Yield and yield components were increased in response of T1 relative to T3 and T2. This showed that application of irrigation at podding stage has improved the pod formation and subsequently increased the grain yield Application of nitrogen foliar spray at flowering showed that it had promoted the vegetative growth by redirecting the photoassimilate translocation towards vegetative parts rather than grains. Asghari *et el.* (2010) reported that drought stress disturbed the yield of chickpea in more complex way because in additionally it also impaired the legume-Rhizobium symbiosis which is not happened in other crops. Nodule formation, growth and development of nodules and fixation by nodules have been effected badly by drought stress resultantly nitrogen deficiency caused the much more adverse effects (Ashraf and Iram, 2005; Onuh and Donald, 2009). Soil application of nitrogen fertilizer was found to be negligible effective for improvement of chickpea growth because nitrogen use efficiency is also dependent on water availability (Aliloo *et al.,* 2012).

Due to water deficiency, nutrient uptake is also reduced whereas, nitrogen is most important micronutrient. It was reported that late or terminal growth stages are more severely affected by nutrient deficiency imposed by drought stress relative to early growth stages (Gunes *et al.,* 2006). Foliar application of nitrogen, supplemented the nitrogen uptake deficiency which resulted the improvement of chickpea yield performance under limited availability. Nitrogen deficiency is also reported the global growth limiting factor for yield of crops (Fuzhong *et al.,* 2008; Salvagiotti *et al.,* 2008). Nitrogen is structural component of proteins, nucleic acid and chlorophyll which are essential components for the survival of plant. Nitrogen application improved the chickpea performance through regulation of protein biosynthesis, nucleic acid linked processes, protoplast formation, chlorophyll synthesis, leaf area, cell size and photosynthetic activity. As it was previously reported the involvement of nitrogen in above mentioned plant linked essential processes (Caliskan, *et al.,* 2008; Dordas and Sioulas, 2008; Waraich *et al.,* 2011).

Namvar *et al.* (2013) applied the nitrogen fertilizer and rhizobium inoculum in different treatment combinations. They observed improvement in relative water contents, cell membrane stability, leaf area index, chlorophyll contents, grain protein contents, plant height, number of primary branches, number of secondary branches, pod per plant, grains per plant, economical and biological yield due to inorganic nitrogen and bio-fertilizer. They applied the nitrogenous fertilizer in soil whereas, in current study we applied through foliar spray which also was proved to be effective for improved performance under limited water availability.

In current study, withholding of irrigation at pod formation resulted the subsequent reduction in architectural traits, yield and yield components of chickpea. Duration of pod development was reduced which reduced the pod size and subsequently reduced yield. Pod abortion was also increased in case of water stress which was also cause of reduction of yield when irrigation was withhold at pod formation (Leport *et al.,* 2006).

Magnitude of inhibition for subjected traits is not same in all genotypes, some genotypes showed higher level of susceptibility whereas, others showed higher level of resistance. Differences in relative

performance are best evaluated by using relative indices. Numerous researchers have used drought tolerance indices for evaluation of different crops under drought stress (Choukan *et al.,* 2006; Farshadfar and Elyasi, 2012; Maqbool *et al.,* 2015). Drought tolerance indices were used for evaluation of relative responsiveness of chickpea genotypes to drought and nitrogenous foliar spray. Mean Productivity (MP; Rosielle and Hambling, 1981), Geometric Mean Productivity (GMP; Fernandez, 1992), % change (Choukan *et al.,* 2006), Tolerance index (TOL; Rosielle and Hambling, 1981), Yield Index (YI; Gavuzzi *et al.,* 1997), Stress Susceptibility Index (SSI; Fischer and Maurer, 1978), Yield Stability Index (YSI; Bouslama and Schapaugh, 1984), Stress Tolerance Index (STI; Fernandez, 1992), Relative Drought Index (RDI; Farshadfar and Elyasi, 2012), relative decrease in yield (RDY; Farshadfar and Elyasi, 2012) were extensively used by numerous researchers in many crops for evaluation of drought responsiveness. These drought tolerance indices categorized the chickpea genotypes into four different groups. Ca-7046, Ca-950131, Ca-4004, Ca-2050, CH7 and Ca-5002 were comprised in Group-1. Characteristics features of genotypes in Group-1 are that these have relatively better performance under normal and stressful conditions so, these genotypes can be used as parent for hybridization and can also be grown under uncertain conditions regarding water availability. AUG810, Ca-7041, Ca-3020, Ca-1028 and Ca-66101 performed relatively better under normal condition but poor under stressful conditions (Group-2) so, these genotypes must be subjected to cultivation when proper water availability is ensured. Ca-6054, PB91 and Bittal98 performed relatively better under stressful conditions (Group-3). Ca-7027, Ca-7012, Ca-5006 and Ca-7050 performed poor under normal and stressful conditions so, these accessions can only be used as parent in hybridization program.

It is concluded from the research that performance of chickpea genotypes is seriously dependent on the water availability. Withholding of irrigation at pod formation caused the significant reduction in yield. Foliar application of nitrogen fertilization proved to be effective for amelioration of drought severity through maintaining different molecular and physiological processes which resultantly harbored higher grain yield. Biplot based grouping of chickpea genotypes proved to be effective for categorization of chickpea genotypes into distinct groups which sorted the genotypes which can be used as parent in hybridization and which can be grown under certain or uncertain water availability.

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Table-1: Climate Data for Experimental Site

	2013					2014				
Month	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
Average high °C	37.3	36.5	33.1	27.1	21.5	20.1	22.8	27.8	34.1	39.1
mean °C	32.1	30	24.9	19.1	13.8	12.3	15.3	20.5	26.2	31
Average low °C	27.1	23.6	16.8	11.2	6.1	4.5	7.8	13.2	18.4	22.9
Precipitation (mm)	90	34	6	3	9	15	16	22	15	14

Table-2: Mean squares under Two Factor Factorial Analysis of Variance

SOV	df	PB	SB	D-F	PH	PW	PpP	GpP	100GW	GY
Blocks (B)	2	0.325	1.845	1.059	5.45	0.07	17.34	0.016	3.99	4.38
Genotypes (G)	49	0.55**	0.654**	6.719**	18.71**	11.99**	16.19**	0.367**	8.93**	44.53**
Treatments (T)	2	45.82**	67.55**	430.67**	3085.16	6251.59	5749.05	52.596	1057.71	2565.90
G×T	98	0.269**	0.444**	10.37**	12.21	7.75	4.95	0.187	2.64	8.60
Error	298	0.348	0.357	7.336	8.47	8.65	7.55	0.313	2.85	4.03

PB: Number of primary branches per plant **SB**: Number of Secondary branches per plant **D-F**: Days taken to flowering
PH: Plant height **PW**: Plant weight **PpP**: Number of pods per plant
GpP: Number of grains per pod **100-GW**: 1000-Grain weight **GY**: Grain yield per plant

Table-3: Treatment Mean Comparison of Chickpea traits using Tukey HSD mean comparison test

Treatments	PB	SB	D-F	PH	PW	PpP	GpP	100GW	GY
T1	3.37(A)	6.77(A)	119.62(A)	59.06(B)	65.25(B)	31.11(A)	2.65(A)	28.85(A)	118.5(A)
T2	2.51(B)	5.61(B)	116.41(B)	53.81(C)	55.95(C)	23.03(C)	1.49(C)	22.81(C)	98.0(C)
T3	3.53(A)	6.77(A)	118.96(A)	62.85(A)	68.35(A)	30.20(B)	2.28(B)	26.11(B)	115.3(B)
Std. Error	0.0681	0.0690	0.3127	0.3360	0.3395	0.3173	0.0646	0.1948	2.2317
Crit. value	0.1596	0.1618	0.7329	0.7874	0.7957	0.7436	0.1514	0.4566	3.5431
Range(T1)	1.39	1.76	7.73	7.99	7.65	9.37	1.33	3.99	7.63
Range(T2)	1.49	1.23	7.97	8.15	11.38	5.23	1	4.42	5.99
Range(T3)	1.53	1.33	6.74	7.54	5.61	16.49	1.33	5.67	9.34

PB: Number of primary branches per plant **SB**: Number of Secondary branches per plant **D-F**: Days taken to flowering
PH: Plant height **PW**: Plant weight **PpP**: Number of pods per plant
GpP: Number of grains per pod **100-GW**: 1000-Grain weight **GY**: Grain yield per plant
T1: Irrigation at pod formation **T2**: No Irrigation at pod formation
T3: No irrigation at pod formation but two nitrogenous sprays (1st at flowering and 2nd at pod formation)
Std. Error: Standard error for comparison **Crit. Value**: Critical value for comparison

Figure-2: Scree Plot for Principle Component analysis

Table-4: Eigenvector values for chickpea traits under three different treatments for ten Principle components of PCA

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
PB(T1)	-0.165	-0.016	-0.211	0.261	-0.199	-0.034	-0.095	0.058	0.234	**-0.468**
PB(T2)	-0.034	0.174	-0.221	0.185	-0.140	0.140	**-0.300**	0.239	0.200	0.083
PB(T3)	-0.140	0.147	-0.224	0.169	-0.093	0.179	-0.296	0.233	-0.007	-0.018
SB(T1)	0.199	0.095	0.237	-0.252	0.083	0.020	-0.083	0.161	-0.025	-0.062
SB(T2)	0.217	0.127	-0.052	-0.192	-0.129	0.289	**0.350**	0.119	-0.200	0.037
SB(T3)	0.109	0.141	-0.042	-0.030	-0.175	**0.402**	0.239	0.206	-0.192	0.185
D-F(T1)	-0.286	0.134	0.009	-0.165	0.249	-0.236	0.211	0.102	0.194	-0.149
D-F(T2)	-0.229	0.123	-0.083	-0.145	**0.366**	-0.044	0.185	**0.371**	0.089	-0.125
D-F(T3)	-0.080	-0.044	-0.195	-0.182	0.294	0.007	-0.141	**0.490**	-0.092	0.207
PH(T1)	-0.162	**-0.315**	0.195	0.239	-0.142	-0.099	0.119	0.187	-0.019	**0.311**
PH(T2)	-0.160	**-0.314**	0.285	0.142	-0.137	-0.104	0.149	0.178	0.152	0.086
PH(T3)	-0.114	-0.161	0.182	0.097	-0.251	-0.148	0.100	**0.438**	-0.173	-0.115
PW(T1)	0.144	0.043	**0.432**	0.028	0.219	-0.082	-0.191	-0.040	0.002	-0.088
PW(T2)	0.074	0.015	0.248	-0.085	0.186	0.180	**-0.351**	0.124	**0.347**	0.132
PW(T3)	0.056	0.034	-0.053	-0.166	-0.177	-0.145	0.278	-0.023	**0.558**	**0.392**
PpP(T1)	-0.010	**0.310**	-0.194	**0.338**	-0.005	-0.206	0.186	-0.025	-0.096	0.041
PpP(T2)	0.181	0.268	-0.092	0.138	0.114	**-0.308**	0.013	-0.033	0.045	0.273
PpP(T3)	-0.091	0.205	0.119	0.264	**0.320**	0.112	**0.354**	-0.078	0.052	-0.162
GpP(T1)	0.017	0.068	**0.309**	**0.311**	0.067	0.060	-0.042	0.063	-0.187	-0.025
GpP(T2)	-0.101	0.052	0.079	**0.353**	0.235	0.237	-0.084	-0.118	0.050	**0.331**
GpP(T3)	-0.037	-0.022	0.126	0.151	0.110	**0.476**	0.245	0.028	0.241	-0.133
100GW(T1)	**0.431**	-0.017	0.079	-0.009	-0.022	-0.063	0.007	0.189	0.064	-0.299
100GW(T2)	**0.321**	0.116	0.006	0.225	0.058	-0.292	0.059	0.211	-0.048	0.001
100GW(T3)	**0.399**	0.140	0.045	0.168	-0.133	-0.002	0.025	0.171	0.280	-0.032
YpP(T1)	-0.207	**0.358**	0.247	-0.148	-0.284	0.002	-0.026	-0.013	0.083	-0.065
YpP(T2)	-0.188	**0.366**	0.208	-0.030	-0.065	-0.127	-0.094	0.040	-0.279	0.174
YpP(T3)	-0.198	**0.358**	0.240	-0.125	-0.292	0.016	-0.047	-0.017	0.101	-0.047

Table-5: Correlation of Drought Tolerance Indices with Grain Yield under three studied treatments

	T1	T2	T3	%(T1+T2)	%(T1+T3)	MP(T1+T2)	MP(T1+T3)	TOL(T1+T2)	TOL(T1+T3)	YSI(T1+T2)	YSI(T1+T3)	YI(T1)
T1	**1.00**	**0.61**	**0.95**	0.02	0.26	**0.89**	**0.99**	**0.34**	-0.08	-0.02	-0.26	**1.00**
T2		**1.00**	**0.51**	**-0.77**	**0.37**	**0.91**	**0.57**	**-0.53**	0.17	**0.77**	**-0.37**	**0.61**
T3			**1.00**	0.11	-0.06	**0.80**	**0.99**	**0.41**	**-0.40**	-0.11	0.06	**0.95**
	YI(T2)	**YI(T3)**	**STI(T1+T2)**	**STI(T1+T3)**	**GMP(T1+T2)**	**GMP(T1+T3)**	**SSI(T1+T2)**	**SSI(T1+T3)**	**RDI(T1+T2)**	**RDI(T1+T3)**	**RDY(T1+T2)**	**RDY(T1+T3)**
T1	**0.61**	**0.95**	**0.85**	**0.98**	**0.85**	**0.99**	0.02	-0.26	-0.02	-0.26	0.02	0.26
T2	**1.00**	**0.51**	**0.94**	**0.56**	**0.94**	**0.57**	**-0.77**	**-0.37**	**0.77**	**-0.37**	**-0.77**	**0.37**
T3	**0.51**	**1.00**	**0.76**	**0.99**	**0.75**	**0.99**	0.11	0.06	-0.11	0.06	0.11	-0.06

Bold valued showed the significance of correlation at 5% significance level.

Table-6: Eigenvector contribution of Grain Yield and Drought Tolerance Indices under three studied treatments for PC1, PC2 and PC3

	T1	**T2**	**T3**	**%(T1+T2)**	**%(T1+T3)**	**MP(T1+T2)**	**MP(T1+T3)**	**TOL(T1+T2)**	**TOL(T1+T3)**	**YSI(T1+T2)**	**YSI(T1+T3)**	**YI(T1)**
PC1	0.24	**0.28**	0.20	-0.16	0.15	**0.29**	0.22	-0.07	0.07	0.16	-0.15	0.24
PC2	0.20	-0.07	**0.26**	**0.25**	-0.16	0.06	0.24	**0.31**	-0.24	**-0.25**	0.16	0.20
PC3	0.06	-0.12	-0.05	0.21	**0.34**	-0.04	0.00	0.21	**0.33**	-0.21	**-0.34**	0.06
	YI(T2)	**YI(T3)**	**STI(T1+T2)**	**STI(T1+T3)**	**GMP(T1+T2)**	**GMP(T1+T3)**	**SSI(T1+T2)**	**SSI(T1+T3)**	**RDI(T1+T2)**	**RDI(T1+T3)**	**RDY(T1+T2)**	**RDY(T1+T3)**
PC1	**0.28**	0.20	**0.29**	0.22	**0.29**	0.22	-0.16	-0.15	0.16	-0.15	-0.16	0.15
PC2	-0.07	**0.26**	0.04	0.24	0.04	0.23	**0.25**	0.16	**-0.25**	0.16	**0.25**	-0.16
PC3	-0.12	-0.05	-0.05	0.01	-0.05	0.01	0.21	**-0.34**	-0.21	**-0.34**	0.21	**0.34**

Bold valued showed the significance of correlation at 5% significance level.

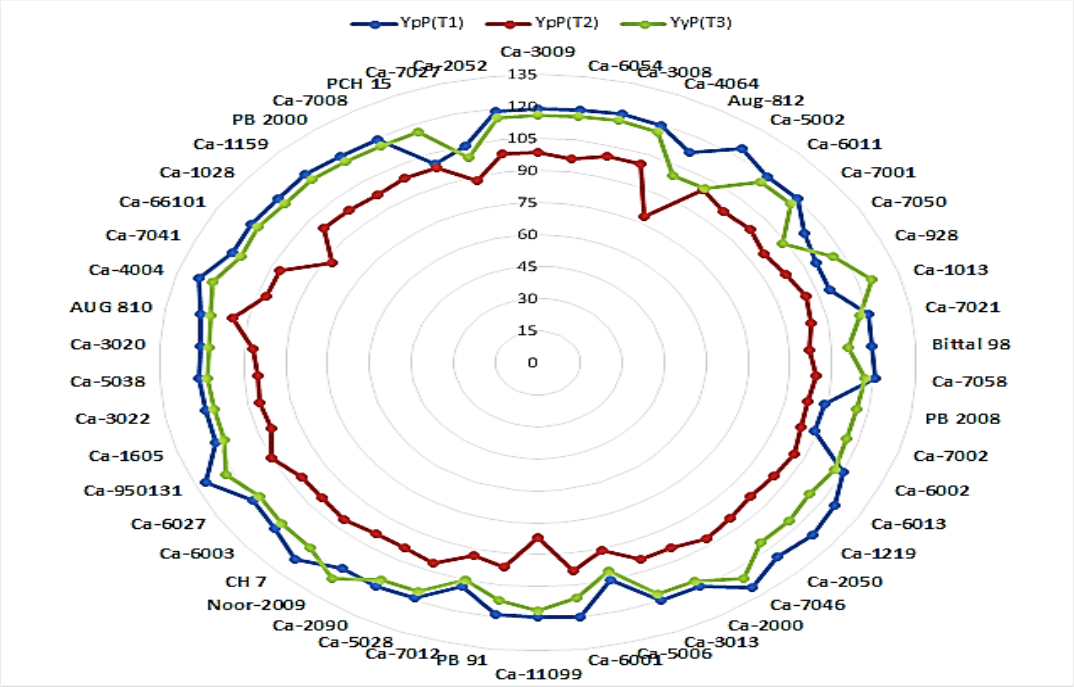

Figure-1: Radar diagram for treatment mean comparison of grain yield for 50 genotypes. Distance of line from the origin is directly associated with mean yield of genotypes.

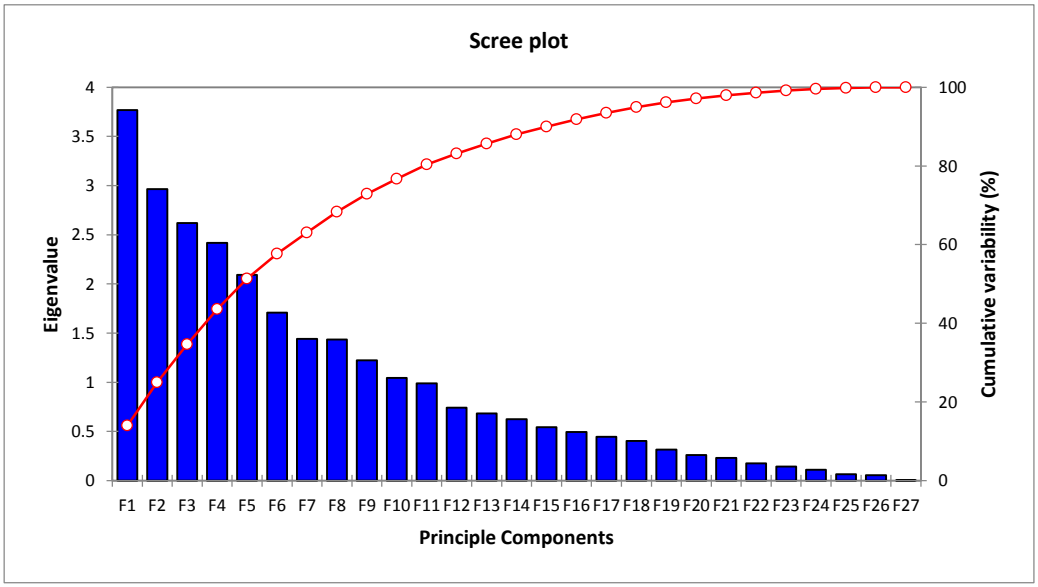

Figure-2: Scree Plot for Principle Component analysis

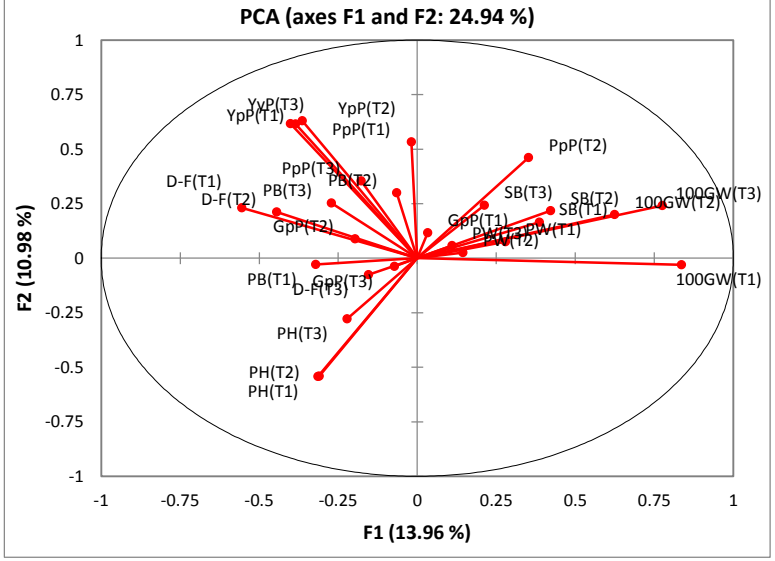

Figure-3: PCA biplot for chickpea traits

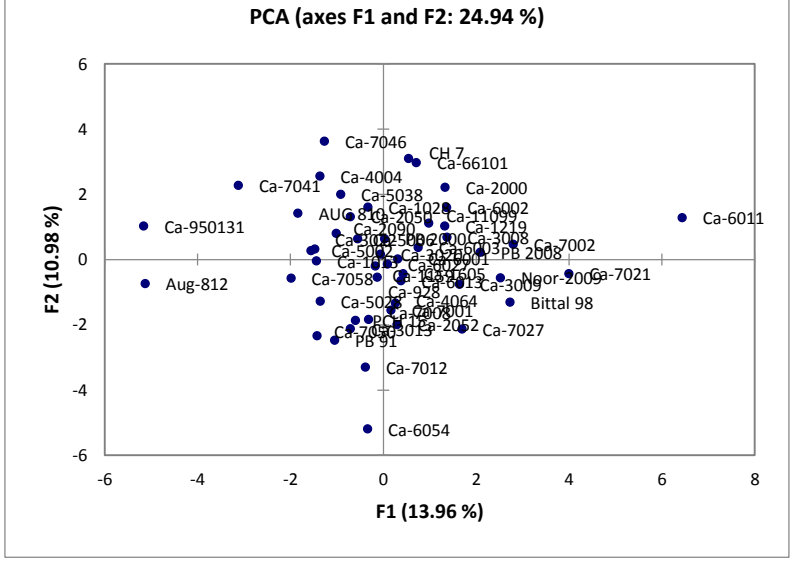

Figure-4: PCA biplot for chickpea genotypes

Figure-5: Scree Plot for Drought Tolerance Indices Based PCA

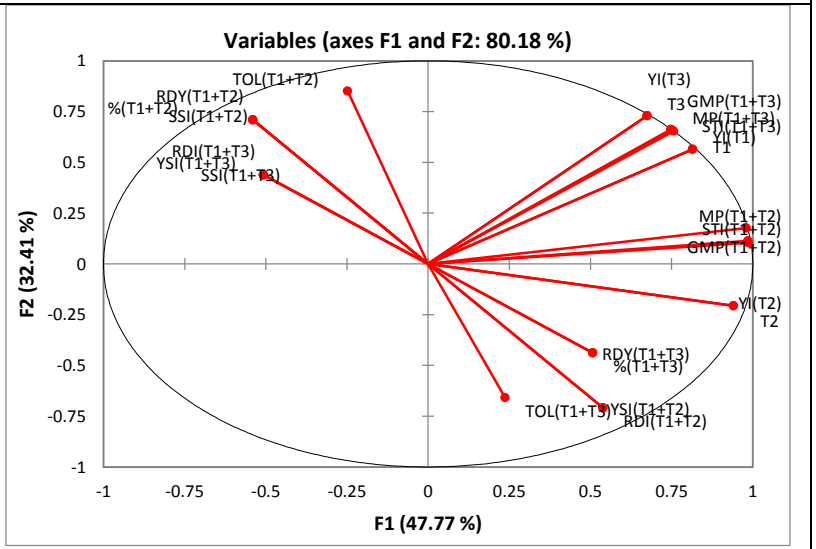

Figure-6: Drought Tolerance Indices based PCA biplot for chickpea Traits

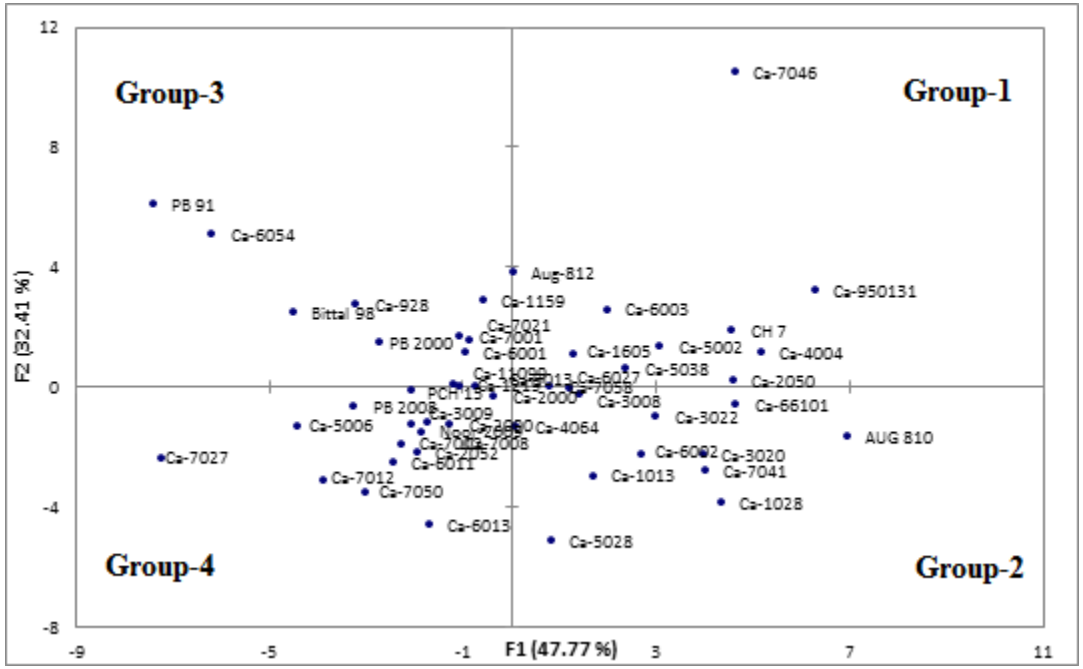

Figure-7: Drought Tolerance Indices based PCA biplot for chickpea Genotypes