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Morphological characterization and grouping of chickpea (*Cicer arietinum*) genotypes for drought tolerance

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ABSTRACT

Fifty genotypes of chickpea (*Cicer arietinum* L.) were evaluated to estimate for drought tolerance as well as genetic variability among the selected chickpea genotypes for quantitative characters under normal and drought conditions. A significant variation was observed for control and drought treatments for most of the investigated characters. Under moisture stress treatment, there was sufficient decrease in the mean of most of the characters under study. The two way analysis of variance was carried out for all the characters for irrigated and under drought. The mean sum of square was highly significant for all the characters under study indicating significant variability in the materials. The analysis of variance for irrigated and drought conditions revealed that the differences among the genotypes were significant. The mean sum of square was highly significant for all the characters. Pusa 1103 and Pusa 362 were the most tolerant along with ICC 4958 which is a well-known donor for the drought tolerance.

Key words: Abiotic stress, Chickpea, Drought tolerance, Genetic variability, Genotypes

Chickpea (*Cicer arietinum* L.; Family: Fabaceae) is a self-pollinated, diploid (2n=16), cool season pulse crop with a genome size of ~738 Mb and an estimated 28 269 genes (Varshney *et al*. 2013). Chickpea is grown in about 52 countries including north and east Africa, west and south Asia, Australia and south Europe (Ganjeali *et al*. 2011). Globally chickpea growing area is recorded as 13.5 million ha (Mha) with an annual production of 13.1 million tonnes (MT) (FAOSTAT 2015). Climate change has a global impact on the productivity of both irrigated and rainfed agriculture (Mall *et al*. 2006). Biotic and abiotic stresses are the major constraints of plant due to climatic changes, which potentially devastating effect on the yield of the crop. Chickpea incurs heavy yield losses up to 40-50% due to drought as it is largely grown under rainfed restricted irrigated conditions on residual soil moisture (Toker and Cagirgan 1998, Leport *et al*. 1999). The narrow genetic base among cultivated chickpea accessions due to repeatedly using of some varieties is limiting genetic improvement of chickpea through breeding efforts (Kumar *et al*. 2015). Exploring the extent of natural variation among cultivated chickpea accessions for drought tolerance is important to develop pre-breeding and breeding strategies for chickpea. The reaction of plants toward the drought stress varies upon the intensity and duration of stress as well as the plant species and the stage of growth (Parameshwarappa and Salimath 2008). For the present investigation, 50 chickpea genotypes consisting released varieties, pre- released advance breeding lines, germplasm collections, land races and wild derivatives lines of both desi and kabuli types were used for this study.

MATERIALS AND METHODS

Fifty chickpea genotypes consisting of released varieties, pre released advance breeding lines, germplasm collections, land races and wild derivatives lines of both *desi* and *kabuli* types were evaluated to estimate drought tolerance as well as genetic variability among the selected chickpea genotypes for morphological characters under normal and drought conditions (Table 1).

The field experiment was carried out in a randomized block design with two replications at the farm of Indian Agricultural Research Institute, New Delhi**.** Each genotype was grown in four rows of 2 m length with 45 cm spacing between rows and 10 cm within the rows**.** The established agronomic practices were followed during the crop season for proper crop growth**.** The crop was maintained free from weeds, diseases, and pest by applying appropriate plant protection methods**.** Observations were recorded on six parameters, viz**.** days to flowering **(**DTF**)**, days to maturity **(**DTM**)**, 100 seed weight, yield/plant **(**g**)**, relative water content **(**RWC**)** and membrane stability index **(**MSI**)** **(**Blum and Ebercon 1981). Mean values of the samples from

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Table 1 List of chickpea genotype used in the present investigation

Variety	Type	Variety	Type
CSG 8962	Desi	IG 5856	Kabuli
JG 62	Desi	IG 5857	Kabuli
Pusa 1103	Desi	IG 5884	Kabuli
AVARODHI	Desi	IG 5894	Kabuli
BGD 112	Desi green	IG 5906	Kabuli
ANNEGIRI	Desi	ILC0 (Italy)	Kabuli
SBD 377	Desi	ILC 10768	Desi
Pusa 362	Desi	ILC0 (Czech Rep.)	Desi
ICC 1882	Desi	ILC0 (Syria)	Desi
ICC 4958	Desi	ILC 8666	Kabuli
Pusa 547	Desi	ILC0 (Latvia)	Desi
Pusa 72	Desi	ILC 1312	Kabuli
ICCV 97119	Desi	IG 5855	Kabuli
ICCV 06101	Desi	IG 5867	Kabuli
ICCV 00104	Desi	IG 5890	Kabuli
ICCV 10111	Desi	IG 5895	Kabuli
L 550	Kabuli	IG 5896	Kabuli
ICCV 10	Desi	IG 5904	Kabuli
ICCV 2	Kabuli	IG 5980	Kabuli
GOKCEE	Kabuli	IG 5982	Kabuli
ICCV 92337	Kabuli	IG 5985	Kabuli
ICCV 10316	Kabuli	IG 6000	Kabuli
ICCV 03302	Kabuli	GLW 91	Desi
ICCV 01318	Kabuli	GLW 69	Desi
IG 5844a	Kabuli	GLW 36	Desi

each replication were subjected to statistical analysis using CROP-STAT (version 8.5) statistical package. Pearson's correlation matrix among the traits under control and rainfed condition was generated by employing GenSTAT version 16.1. The factorial and clusters analysis based on drought stress morphological traits was done by using DARwin 5 software 5.0.158.

RESULTS AND DISCUSSION

Comparative performance of genotypes in normal and drought conditions

The mean performance for different characters in normal and drought environments revealed that wide range of estimates for characters under study (Table 2).

Days to 50% flowering varied from 57-103 days in normal condition and 49-87 days in drought stress, the mean was 78 and 68 with standard error of 2.32 and 1.79 for normal and stress, coefficient of variation was 20.97 and 18.77 in normal and stress conditions. There was 13.97% reduction observed in stress condition for the days to flowering. Days to maturity varied from 124-136 days in normal condition and 112-121 days in drought stress, the mean was 134 and 116 days with standard error of 0.32 and 0.43 for normal and stress, coefficient of variation was 1.68 and 2.61 in normal and stress conditions. There was 13.57% reduction observed in stress condition for the days to maturity. Plant height varied from 44-75 cm in normal condition and 37-65 cm in drought stress, the mean was 57 cm and 50 cm with standard error of 0.97 and 0.76 for normal and stress, coefficient of variation was 11.95 and 10.76 in normal and stress conditions. There was 12.09% reduction observed in stress condition for the plant height. Pods/plant varied from 21-56 pods in normal condition and 11-44 pods in drought stress, the mean was 38 and 24 pods with standard error of 1.31 and 1.06 for normal and stress, coefficient of variation was 24.49 and 1.31 in normal and stress conditions. There was 36.63% reduction observed in stress condition for the pods/plant. Seeds/pod varied from 1-2 seed in normal condition and 1-2 seed in drought stress also with the mean 2 and 2 seed with standard error of 0.07 and 0.07 for normal and stress, coefficient of variation were 30.93 and 30.93 in normal and stress conditions. There was 0.00% reduction observed in stress condition for the seeds/pods.

Table 2 Range, mean, standard error, coefficient of variation and percentage decrease in normal and rainfed condition

Traits	Normal			Drought stress			Percent decrease (%)
	Mean±SE	CV (%)	Range	Mean±SE	CV (%)	Range	
Days to 50% flowering	78 ± 2.32	20.97	57-103	68±1.79	18.77	49-87	13.97
Days to maturity	134±0.32	1.68	124-136	116±0.43	2.61	112-121	13.57
Plant height (cm)	57±0.97	11.95	44-75	50±0.76	10.76	37-65	12.09
Pods/Plant	38±1.31	24.49	21-56	24±1.06	30.79	11-44	36.63
Seed/Pod	2±0.07	30.93	1-2	2±0.07	30.93	1-2	0.00
Relative water content	67±1.46	15.48	52-83	66±1.67	17.79	51-90	0.35
Membrane stability index	60±1.67	19.60	41-78	59±1.78	21.21	40-80	1.08
Yield	79±3.09	27.59	40-139	52±2.44	33.39	24-85	34.41

Table 3 Two way ANOVA for morphological traits and yield under control and rainfed condition

Source of variation	Mean sum of square							
	DTF	MSI	DTM	PH	PPP	RWC	SPP	YLD
Genotype	1211.98**	878.8**	24.694**	205.50**	289.48**	713.7**	1.46939	2067.93**
Treatment	8769.61**	37.45**	25208.33**	3508.92**	13981.01**	8.00**	0	56115.36**
Genotype treatment	70.02**	15.3**	17.81**	23.24**	138.20**	23.3**	0	258.82**
Residual	1.85	0.08	1.874	0.4436	1.672	0.08	0	1.599

* and ** is the significance at 5% and 1% respectively; DTF, Days to 50% flowering; MSI, Membrane stability index; DTM, days to maturity; PH, Plant height; PPP, pods/plant; RWC, relative water content; SPP, seeds/pod; YLD, plant yield.

Relative water content varied from 52-83 in normal condition and 51-90 in drought stress, the mean was 67 and 66 with standard error of 1.46 and 1.67 for normal and stress, coefficient of variation was 15.48 and 17.79 in normal and stress conditions. There was 0.35% reduction observed in stress condition for the relative water content. Membrane stability index varied from 41-78 in normal condition and 40-80 in drought stress, the mean was 60 and 59 with standard error of 1.67 and 1.78 for normal and stress, coefficient of variation was 19.60 and 21.21 in normal and stress conditions. There was 1.08% reduction observed in stress condition for the membrane stability index. Yield varied from 40-139 g in normal condition and 24-85 g. in drought stress, the mean 79 and 52 g. with standard error of 3.09 and 2.44 for normal and stress, coefficient of variation was 27.59 and 33.39 in normal and stress conditions. There was 34.41% reduction observed in stress condition for the yield.

The two way analysis of variance was carried out for all the characters for irrigated and under drought. The mean sum of square is highly significant for all the characters under study indicating significant variability in the materials. The analysis of variance for irrigated and drought conditions revealed that the differences among the genotypes were significant. The mean sum of square was highly significant for all the characters (Table 3).

Date of flowering was positively and significantly correlated with date of maturity (r=0.417), MSI was negatively correlated with DTF (r=-0.006), plant height positively and significantly correlated with date of maturity (r=0.47), pods per plant were positively and significantly correlated with date of maturity (r=0.602), RWC was positively and significantly correlated with MSI (r=0.912), SPP is showed significant but negative correlation with days to 50% flowering (-0.265) that means if days to flowering increases seeds/pod will reduce or it gives an adverse effect to the seeds formation because late flowering will delay the reproductive stage and reduce the seeds. Yield was positively and significantly correlated with DTM (r=0.549), MSI (0.585), PH (0.265) PPP (0.588) and RWC (0.590) (Table 4).

All the morphological data was analysed and euclidean distances were calculated for the stress condition the genotypes grouped as per the characters. The two distinct groups (A and B) were formed (Fig 1). Tolerant lines are in the group A and the susceptible lines are in the group B. The tolerant group was further divided into two groups, viz. highly tolerant and moderately tolerant according to their different morphological characters. Pusa 1103 and Pusa 362 the most tolerant along with ICC 4958 which is a well-known donor for the drought tolerance. Similarly the susceptible group was further divided into two groups, viz. highly susceptible to moderately susceptible according to their different morphological characters. All the susceptible lines formed a distinct group. IG 5844, IG 5985, ICC 1882, ILC 132 and SBD 377 were the most susceptible genotypes.

A data matrix plot based on the morphological characters was subjected to Principle component analysis (PCoA) for estimating genetic differentiation among 50 genotypes of chickpea. The scatter plot based on these components disclosed a pattern of mainly two groups which distinctively separated the tolerant and susceptible genotypes. The plot showed that the tolerant genotypes made a group with ICC 4958 which is a known drought tolerance genotype and susceptible genotypes falls in group along with SBD 377 a known susceptible genotype for drought tolerance. Most of the genotypes were scattered between tolerance and susceptible genotypes. Distribution of genotypes according to geographical origin was lacking in the matrix plot of fifty genotypes (Fig 2).

The acclimatization of plants to drought stress conditions is dynamic and complex, which involves hundreds of genes and their interactions with different environmental factors throughout the plant development (Kumari *et al*. 2009). Chickpea incurs heavy yield losses due to terminal heat and drought as it is largely grown under rainfed restricted irrigated conditions on residual soil moisture. The narrow genetic base of cultivated chickpea accessions is limiting due to the excessive use of favourable characters of limited genotypes for the genetic improvement of chickpea through breeding efforts. Exploring the extent of natural variation among cultivated chickpea accessions for drought tolerance is important to develop pre-breeding and breeding strategies for chickpea (Kumar *et al*. 2015). Therefore it is important to understand the molecular responses as well as phenotypic characters in plants under stress for the improvement of plant biomass or yield. It is also important to identify the suitable phenotypes that respond to the drought stress appearing either simultaneously or sequentially, under field

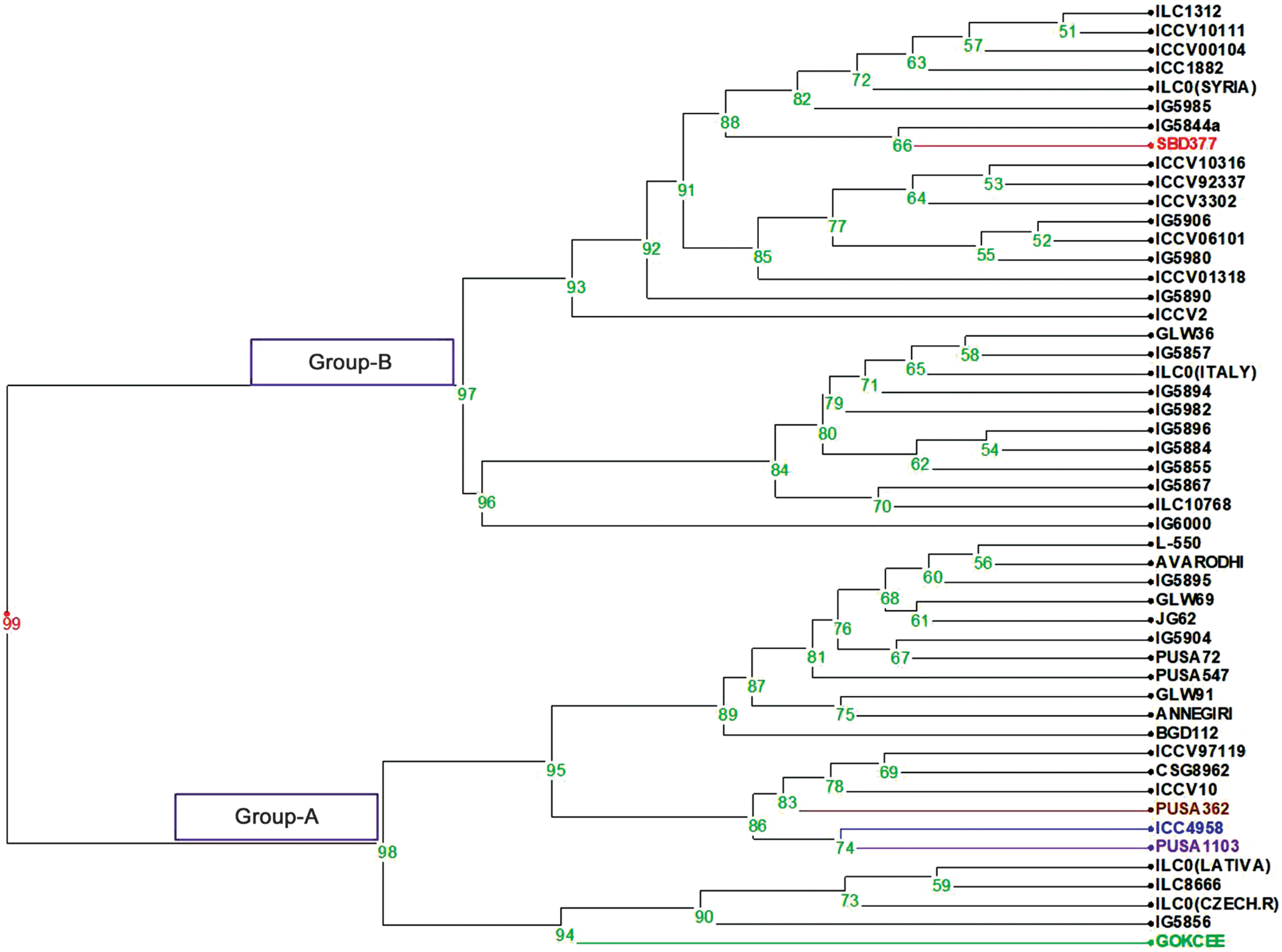

Fig 1 Dendogram generated from an unweighted pair group method analysis (UPGMA) cluster analysis based on all the stressed morphological characters for drought. First two clusters form Group A showing all tolerant to moderate tolerant genotypes.

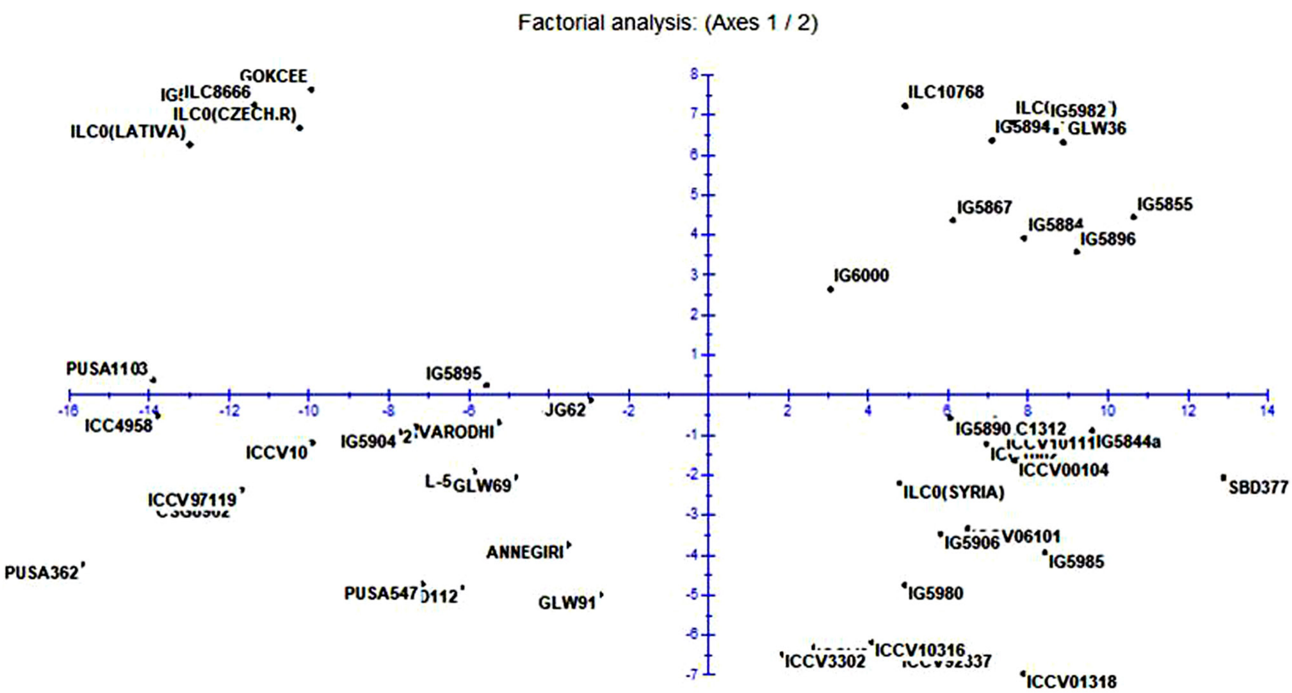

Fig 2 Representation of the 1–2 plane of factorial analysis based on drought stress morphological traits for fifty chickpea genotypes.

conditions. Severity of drought stress can be easily estimated by morphological features and physiological processes of plants during its growth and development like days to 50% flowering, days to maturity, plant height, pods/plant, seed per pod, relative water content (RWC), membrane stability index (MSI), yield (Toker and Cagirgan 1998, Jaleel *et al*. 2009). The effect of these morphological and physiological changes in response to drought stress can be used to identify drought tolerant genotypes for better productivity under abiotic stress (Nam *et al*. 2001, Martinez *et al*. 2007).

Earlier studies have indicated that the chickpea from Indian subcontinent had a narrow genetic pool (Bharadwaj *et al*. 2011a) which is limiting the genetic improvement of chickpea through conventional breeding efforts. Further, it has been observed that the gene pool from west Asia and north Africa was quite diverse from those of the Indian subcontinent (Bharadwaj *et al*. 2011b). Drought, heat and salinity are three of the most important abiotic stresses that alter plant water status and severely limit plant growth and development.

Fifty genotypes of chickpea were evaluated to estimate for drought tolerance as well as genetic variability among the selected chickpea genotypes for quantitative characters under normal and drought conditions. A significant variation was observed for control and drought treatments for most of the investigated characters. Significant variation in their mean sum of squares was shown by all the characters under study, indicating the presence of sufficient diversity as inferred from *F*-test. Such a variable population would aid in identification of genotypes based on their performance. Such identified genotypes having traits of interest can be used for recombination and advancement of generations (Bharadwaj *et al*. 2011b). Variation in drought tolerance is dependent on genotype and its ability to withstand stress through various mechanisms. Remarkable variation in morphological, physiological and phenological characters and yield and its components in chickpea as also reported by Kumar *et al*. (2015). Genetic study of drought-tolerance parameters is prerequisite for breeders in selection of desired genotypes.

Under moisture stress treatment, there was sufficient decrease in the mean of most the characters under study. The two way analysis of variance was carried out for all the characters for irrigated and under drought. The mean sum of square leas highly significant for all the characters under study indicating significant variability in the materials. The analysis of variance for irrigated and drought conditions revealed that the differences among the genotypes were significant. The mean sum of square was highly significant for all the characters. RWC was introduced as a best criterion and used instead of plant water potential as RWC referring to its relation with cell volume, accurately can indicate the balance between absorbed water by plant and consumed through transpiration. In wheat, Schonfeld *et al*. (1985) showed that the cultivars having high RWC are more resistant against drought stress. Ramos *et al*. (2003) identified significant differences in RWC in bean leaves and the values were lesser in drought conditions than normal. Earlier researchers also reported that characters like membrane thermo stability, canopy temperature depression, filled pods/plant and number of seeds/plant to be highly affected by drought conditions (Leport *et al.* 1999). Reduction in biomass or biological yield, days to 50% flowering and seed yield are the consequences of impaired biological processes at cellular, molecular and organ level under terminal drought conditions. Under drought stress, the photosynthetic process is affected especially during pod filling stages when demand for assimilates is the greatest (Krishnamurthy *et al*. 2011). When growth resources are limited by drought conditions, the reduction in number of filled pods may be due to high terminal drought affecting the pod filling. This current study clearly demonstrated the presence of considerable amount of genetic variability for drought tolerant traits among both the released and pre-released chickpea genotypes. Decreases in biomass and seed yield are the major concerns in terms of compromised biological processes under drought-stressed conditions at different levels (organ, cellular and molecular level). Drought tolerance is a complex trait influenced by several factors including days to flowering and maturity, yield, shoot biomass production, early shoot growth vigour, root length density, total transpiration, root: shoot ratio and transpiration efficiency. Based on the superior performance for different parameters genotypes, which showed least reduction in yield under stress conditions, have immense potential to be used as reliable donor for terminal drought tolerance (Supriya *et al*. 2018). Besides, it is also noteworthy that different genotypes possess different physiological mechanism to cope up the effect of terminal drought stress. These genotypes provide ample opportunities to breeder to combine them together in developing the drought tolerant genotypes.

Pusa 1103, and Pusa 362, were the most tolerant along with ICC 4958 which is a well-known donor for the drought tolerance. The outcome of the study suggests that genotypes Pusa 1103, and Pusa 362 which may be utilized for genetic improvement programs through hybridization and as direct introduction desire traits in chickpea.

Conclusion

Plant genetic resources comprise obsolete varieties, landraces and crop wild relatives. These are the reservoirs of natural genetic variation but the reluctance of the breeder to use exotic germplasm has restricted the introgressive hybridization of useful variation present in the exotic germplasm. So it is important to utilize the genetic resources of chickpea conserved across gene bank globally, by employing various genomic tools to broaden genetic base for sustainable chickpea production. There is an urgent need to identify genotypes tolerant to drought.

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