



RESEARCH ARTICLE

Evaluation and identification of drought tolerant wild annual *Cicer* accessions for enhancing genetic gains towards chickpea improvement

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Abstract

Chickpea is an annual food legume crop widely grown under rainfed environment where drought stress occur frequently limiting crop production. In the present study, 39 wild annual *Cicer* accessions belonging to five species viz. *Cicer reticulatum* Ladizinsky, *C. echinospermum* P.H. Davis, *C. judaicum* Boiss., *C. pinnatifidum* Jarb. & Spach, and *C. yamashitae* Kitam along with two check varieties of cultivated chickpea namely, ICC 4958 (drought tolerant) and BG 1053 (drought susceptible), were screened for drought tolerance under controlled conditions. The wild *Cicer* accessions exhibited significant variations for 14 agro-physiological traits based on analysis of variance and boxplot. The hierarchical clustering using Ward's function and principal component analysis clearly indicated the genetic diversity present in the wild *Cicer* species. Among the wild species, *C. judaicum* and *C. reticulatum* accessions were mainly found tolerant for physiological and agronomical traits respectively. Donors for multiple traits associated with drought tolerance were identified namely, ILWC 20, ILWC 38 (*C. judaicum*); ILWC 46, ILWC 219 (*C. reticulatum*) and ILWC 214 (*C. yamashitae*). The identified promising *Cicer* accessions would be useful in developing chickpea varieties with enhanced resilience to low moisture condition by broadening the genetic base and introgression of desired genes.

Keywords: *Cicer* species, drought, germplasm screening, MSI, NDVI, RWC

Introduction

Cultivated chickpea (*Cicer arietinum* L.) ranks second most important pulse crop after dry bean globally in terms of production as well as acreage (FAOSTAT, 2020). It plays a pivotal role in human nutrition and food security of resource poor people (Jukanti et al. 2012; Wallace et al. 2016). It contains 20–25% protein and 2–3 times higher iron and zinc content than wheat crop. Over 65% (10.9 million ha) of global chickpea cultivated area lies in India followed by Pakistan (0.94 million ha), Turkey (0.51 million ha) and Iran (0.51 million ha) (FAOSTAT, 2020). The current world mean yield of chickpea is about 1.01 tons ha⁻¹, however, the estimated potential yield of chickpea under optimum growing conditions is 6 tons ha⁻¹ (Thudi et al. 2016). Despite efforts of several dedicated national and international institutes, major breakthrough in yield enhancement could not be realized. Although, over 98000 *ex-situ* chickpea germplasm collections are conserved in more than 30 gene banks and are being utilized in characterization and evaluation to identify new trait specific sources (Chandora et al. 2020), however no breakthrough in chickpea productivity has been realized. In fact, there is increase in its average productivity

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over the last 50–60 years, but the increase has been very marginal and also slow.

The major constraint for breeding high yielding chickpea cultivars is the narrow genetic base of cultivated gene pool (Abbo et al. 2003; Nguen et al. 2004) which is mainly due to single domestication event and self-pollination nature of chickpea. Some other factors include drastic loss of ecotypes due to biotic and abiotic stresses, shift from winter to spring season, replacement of locally evolving landraces by recently developed high yielding elite varieties and limited distribution and utilization of wild progenitors (Abbo et al. 2003; Croser et al. 2003; Varshney et al. 2013). As a result, cultivated chickpea improvement programs rely on limited variability available within the cultivated genepool. Therefore, use of wild species for broadening the genetic base is warranted. The primary gene pool of chickpea consists of domesticated chickpea, *C. arietinum*, and the immediate progenitor, *C. reticulatum*, which are easily crossable. The *C. echinospermum* represents a secondary gene pool and is crossable with cultivated chickpea, but with reduced pollen fertility. The tertiary gene pool consists of other 6 annual and 34 perennial species having poor crossing compatibility with cultivated chickpea and requiring advanced approaches for gene transfer (Ladizinsky 1998). The systematic evaluation, characterization, and utilization of wild species-specific targeted genes for broadening the genetic base of chickpea cultivars and sustainable yield gain under biotic and abiotic stresses, are the emergent and immediate requirements. Broadening of the genetic base is now necessary and useful and it is well recognized in all crops mainly in chickpeas and other pulse crops. Inter-specific hybridization has also led to the development and release of chickpea cultivars in India (Bhardwaj et al. 2011) for example, Pusa 1103 developed by Indian Agricultural Research Institute was tolerant to root disease, Pant Gram 4 (PG 065) by Govind Ballabh Pant University Agriculture and Technology was having semi erect plant type, tolerance to wilt, BGM and dry root rot (Anonymous 2017).

Among abiotic stresses, severe drought stress can decrease chickpea yield by 50% (Sabaghpour et al. 2006) due to disruption of key physiological and biochemical processes in plants (Chaves et al. 2009; Pinheiro and Chaves. 2011). In general, chickpea crop suffers from “terminal drought” during the reproductive phase (Leport et al. 1999; Siddique et al. 1999) on account of increased ABA level in plants, which impairs pod set and cause pod abscission thereby causing significant yield losses (Pang et al. 2017). Further, drought stress at vegetative stage has been also reported leading to reduced growth and biomass (Siddique et al. 1999). Wild *Cicer* species belonging to primary and secondary gene pool are increasingly being utilized to broaden the genetic base of cultivated chickpea, in trait identification under abiotic stresses (Van der Maesen and Pundir 1984; Singh et al.

1990; 1995; Toker et al. 2007; Canci and Toker 2009) and introgression in cultivated chickpea germplasm (Chandora et al. 2020; Singh et al. 2021).

In addition, perennial wild species of chickpea namely; *C. anatolicum*, *C. microphyllum*, *C. montbretii*, *C. oxydon* and *C. songaricum* were also compared with annual species *C. echinospermum*, *C. pinnatifidum* and *C. reticulatum*, and cultivated chickpeas for resistance to drought (Toker et al. 2007). Under Indian condition, Singh et al. 2014 evaluated annual wild species of chickpea for agro-morphological traits, biotic stresses and cold stress, however, drought stress evaluation for this germplasm is being reported in the present study. Thus, evaluation of wild *Cicer* germplasm for various drought tolerance traits would help in tapping the unexplored variability and identification of donor germplasm along with development of climate resilient varieties. Therefore, the present study was conducted with the aim of assessing annual wild *Cicer* species for drought tolerance under controlled conditions to identify the promising donors for drought tolerance associated traits.

Materials and methods

Experimental material

The experimental material for the study included a total of 39 wild annual *Cicer* accessions belonging to five species namely, *C. reticulatum* (8), *C. judaicum* (18), *C. pinnatifidum* (11), *C. echinospermum* (1) and *C. yamashitae* (1) procured from International Centre for Agricultural Research in Dry Areas (ICARDA), Aleppo, Syria (Table 1). The experiment was conducted for screening against drought stress under controlled conditions (rainout shelter) at ICAR-National Bureau of Plant Genetic Resources Experimental Farm, New Delhi located between 28°35 N, 70°18 E, altitude, 226 m above mean sea level (amsl) during *rabi* 2014-15. The experiment was conducted in Randomized Block Design with three replications and two environments, *i.e.*, irrigated (non-stress) and non-irrigated (stress). Two popular check varieties *viz.*, ICC4958 (drought tolerant) and BG1053 (drought susceptible) were also grown for comparing the genotypes. Each accession was grown in a single row plot of 1 m length with row to row spacing of 30 cm. Seeds were sown manually at 2 cm depth maintaining plant to plant distance of 10 cm. In irrigated plot, two irrigations were applied for normal growth, first at pre-reproductive phase and second during flowering to pod setting stage, whereas in drought environment, irrigation was not applied to impose drought stress throughout the cropping season.

Phenotyping for agro-physiological traits

Observations were recorded for ten agronomical traits which included canopy diameter (CD), days to 50% flowering (DF), days to maturity (DM), plant height (PH, cm), numbers of primary branches per plant (NBP), number of pods per plant (PPP), number of seeds per plant (SSP), biomass per plant

Table 1. Wild annual *Cicer* species evaluated for drought tolerance (NSE: Non-stress experiment; SE: Stress experiment)

S no.	Accessions	Species	Origin	PCA Rank (NSE)	PCA Rank (SE)
1	BG 1053	<i>C. arietinum</i>	India	25	22
2	ICC 4958	<i>C. arietinum</i>	India	17	15
3	IG 135444	<i>C. pinnatifidum</i>	Syria	26	23
4	IG 135852	<i>C. judaicum</i>	Jordon	4	5
5	IG 136820	<i>C. pinnatifidum</i>	Syria	5	7
6	ILWC 20	<i>C. judaicum</i>	Lebanon	3	3
7	ILWC 207	<i>C. judaicum</i>	Syria	7	8
8	ILWC 21	<i>C. reticulatum</i>	Turkey	38	39
9	ILWC 211	<i>C. judaicum</i>	-	12	25
10	ILWC 214	<i>C. yamashitae</i>	Afghanistan	2	2
11	ILWC 216	<i>C. reticulatum</i>	Turkey	37	40
12	ILWC 219	<i>C. reticulatum</i>	Turkey	13	19
13	ILWC 22	<i>C. pinnatifidum</i>	Syria	21	21
14	ILWC 223	<i>C. judaicum</i>	Ethiopia	9	10
15	ILWC 225	<i>C. pinnatifidum</i>	-	16	16
16	ILWC 226	<i>C. pinnatifidum</i>	Turkey	15	14
17	ILWC 23	<i>C. pinnatifidum</i>	Syria	11	17
18	ILWC 233	<i>C. reticulatum</i>	Turkey	8	12
19	ILWC 237	<i>C. reticulatum</i>	Turkey	31	35
20	ILWC 246	<i>C. echinospermum</i>	-	41	41
21	ILWC 250	<i>C. pinnatifidum</i>	Turkey	40	37
22	ILWC 251	<i>C. pinnatifidum</i>	-	39	28
23	ILWC 256	<i>C. judaicum</i>	Jordon	24	24
24	ILWC 257	<i>C. reticulatum</i>	Turkey	33	36
25	ILWC 263	<i>C. pinnatifidum</i>	Syria	34	34
26	ILWC 273	<i>C. judaicum</i>	Lebanon	19	20
27	ILWC 274	<i>C. judaicum</i>	Lebanon	29	30
28	ILWC 275	<i>C. judaicum</i>	Lebanon	30	29
29	ILWC 283	<i>C. judaicum</i>	Syria	10	13
30	ILWC 30	<i>C. judaicum</i>	Israel	6	4
31	ILWC 31	<i>C. judaicum</i>	Jordon	22	18
32	ILWC 36	<i>C. reticulatum</i>	Turkey	35	38
33	ILWC 38	<i>C. judaicum</i>	Lebanon	18	11
34	ILWC 4	<i>C. judaicum</i>	Lebanon	32	27
35	ILWC 41	<i>C. judaicum</i>	Syria	27	6
36	ILWC 45	<i>C. judaicum</i>	Syria	20	9
37	ILWC 46	<i>C. reticulatum</i>	Turkey	1	1
38	ILWC 48	<i>C. judaicum</i>	Syria	36	33
39	ILWC 49	<i>C. pinnatifidum</i>	Syria	23	31
40	ILWC 50	<i>C. judaicum</i>	Syria	14	26
41	ILWC 51	<i>C. pinnatifidum</i>	Turkey	28	32

– = Not known

(BM, g), seed yield/plant (SY, g) and 100-seed weight (SW, g) along with four physiological parameters. Five plants were randomly selected from each accession for the assessment of the agronomical and yield related traits. Observation for normalized difference vegetation index (NDVI) and canopy temperature was taken thrice after 50% flowering at 10 days interval (Pask et al. 2019). Membrane stability index (MSI) was calculated by taking the electrical conductivity of leaf leachates in double distilled water at 40° and 100°C following the method of Sairam (1994). The relative water

content (RWC) was estimated in fresh leaf samples following the method given by Barrs and Weatherley (1962). Stress intensity was calculated as per the formula given by Fisher and Maurer (1978): $SI = 1 - (\bar{Y}_s / \bar{Y}_p)$ whereas SI is stress intensity and \bar{Y}_s and \bar{Y}_p are the means of all genotypes under stress and non-stress conditions, respectively. Following that, drought susceptibility index (DSI), $DSI = [1 - (Y_s/Y_p)]/SI$ was calculated where Y_s = mean of the genotype in stress environment and Y_p = mean of the genotype under non stress environment.

Statistical analysis

Statistical data analysis was done using IBM SPSS version 20 and SAS-JMP 14 software. Analysis of variance (ANOVA) was performed following the general linear model (GLM). Correlation coefficients (Pearson's) were calculated by multivariate analysis for normal (non-stressed) as well as drought (stressed) conditions. Boxplot was drawn for each species separately for all the traits to compare genotype performance under both conditions. Hierarchical cluster analysis was performed using Euclidean distance matrix following Ward's minimum variance method for genotype grouping. Principal component analysis (PCA) was carried out on the basis of phenotypic correlation matrix of the 14 agro-physiological traits. Each principal component was calculated by taking a linear combination of an Eigen vector of the correlation matrix with a variable. The 2D scatter plot for first two PCs was also drawn to understand relationship between the variables. Loading scores and principal component scores were also calculated. PCA ranking value was used for assessing stress tolerance of different genotypes. The ranking value for each chickpea accession was computed according to modified formula given by Liu et al. (2015) as: $\text{PCA Ranking value} = (\text{contribution of PC1 (\%)} \times \text{PC1}) + (\text{contribution of PC2 (\%)} \times \text{PC2}) + (\text{contribution of PC3 (\%)} \times \text{PC3}) + (\text{contribution of PC4 (\%)} \times \text{PC4})$. The top-ranking accessions were considered tolerant and bottom ranking were as susceptible.

Results

Genetic variability in wild annual *Cicer* species

The analysis of variance revealed significant differences among genotypes for all agro-physiological traits under stress as well as non-stress conditions (Supplementary Table S1). The analysis also showed significant genotype \times treatment interactions for all the traits except seeds per plant, NDVI and MSI. Significant replication sum of squares for some traits were taken care by the averaging effect of treatments variables. The summary statistics including mean, range, standard error, skewness and kurtosis is presented in Supplementary Table S2. The number of total pods per plant ranged from 4 to 109.67 under normal condition but declined by 2.6% due to drought stress and ranged from 4 to 89.67. Likewise, seeds per plant ranged from 5.67 to 169 under non-stress condition and 4.33 to 135.67 under drought stress. Biomass per plant ranged from 2.62 to 26.73g (non-stress) and 2.18 to 23.39g (stress). Seed yield per plant ranged from 0.12 to 7.87g (non-stress) and 0.10 to 6.53g (stress) and seed weight ranged from 1.0 to 22.82 (non-stress) and 0.80 to 20.47 (stress). Maximum percent mean reduction was observed for biomass with 18.8% reduction followed by number of primary branches (18.4%), MSI (17.8%), RWC (14.8%) and seed yield (11.4%). Either negligible or less reduction was observed for DF, DM and PH.

Boxplot analysis also showed variation for accessions of different species for different traits under stress and non-stress conditions (Supplementary Fig. S1). Reduction in measured values of variables under stress condition was also recorded. In comparison to the cultivated genotypes, wild *Cicer* genotypes required more time to flower and maturity whereas, plant height was less in many accessions except for few genotypes of *C. judaicum* and *C. pinnatifidum*. The canopy diameter was relatively more in *C. judaicum* in comparison to cultivated and other wild annual species. However, no. of primary branches, no. of pods per plant and no. of seeds per plant were highest in *C. yamashitae*. Biomass and seed yield of *C. reticulatum* were comparable to cultivated species and their seed weight was less than cultivated checks and more than other species. Regarding physiological parameters, *C. yamashitae* had comparable NDVI to cultivated species. Moreover, *C. judaicum* had cooler canopies, high MSI and RWC. Based on mean performance of different traits, top five superior genotypes were selected under stress condition and donors for multiple traits were identified namely ILWC 20 for SPP, PPP, MSI and CT; ILWC 46 for SPP, PPP, SY and BM; ILWC 38 for PH, CT and MSI; ILWC 214 for DM, NBP, PPP and SPP, and ILWC 219 for NBP, SY, SW (Table 2). Based on drought susceptibility index nature of tolerance of these genotypes were also mentioned. Here, genotypes with DSI value less than 1.0 were tolerant and those with more than 1.0 were susceptible. Further the tolerant genotypes were categorized into two groups; highly tolerant (HT) with DSI value less than 0.55 and moderately tolerant (MT) with values between 0.55 and 1.0. Similarly, susceptible genotypes were categorized into two groups; highly susceptible (HS) with DSI value more than 1.5 and moderately susceptible (MS) with values between 1.05 and 1.5 (Sareen et al. 2020).

Multivariate analysis

The correlation coefficient between all the studied traits was also analyzed under stress and non-stress conditions and the results are presented in Fig. 1. A highly significant positive correlation was observed between NDVI and SY (0.568*), PPP and SPP (0.977**), PPP and SY (0.735**), SPP and SY (0.662**), SY and SW (0.619**) and significant negative correlation was observed between DF and SY (-0.529*), SW and DF (-0.723**) under stress condition. Under non-stress condition, high and significant correlation was observed between NDVI and PH (0.457**), CT and SW (0.412*), PPP and SPP (0.953**), PPP and SY (0.738**), SPP and SY (0.625**), SY and SW (0.529**) and significant negative correlation was observed between CT and CD (-0.529*), SW and DF (-0.766**). Seed yield was positively correlated with NDVI (0.270), PPP (0.738), SPP (0.625) and BM (0.424) under non-stress condition and NDVI (0.568), PPP (0.735), SPP (0.662) and BM (0.437) under stress condition.

Two-way hierarchical clustering using Euclidean distance

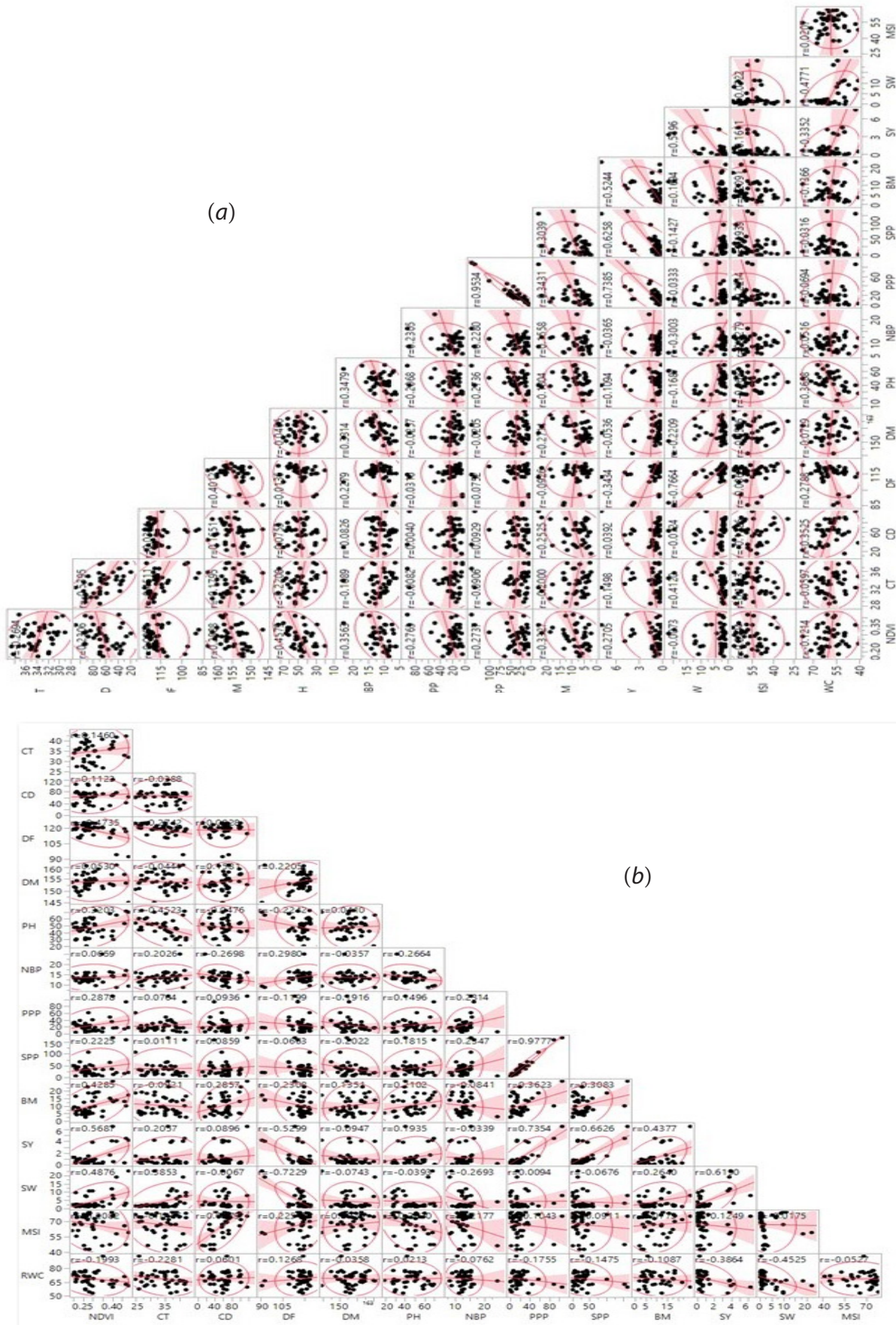


Fig. 1. Scatter plot matrix showing correlation between 14 agro-physiological traits of wild chickpea genotypes under (a) non-stress and (b) stress conditions

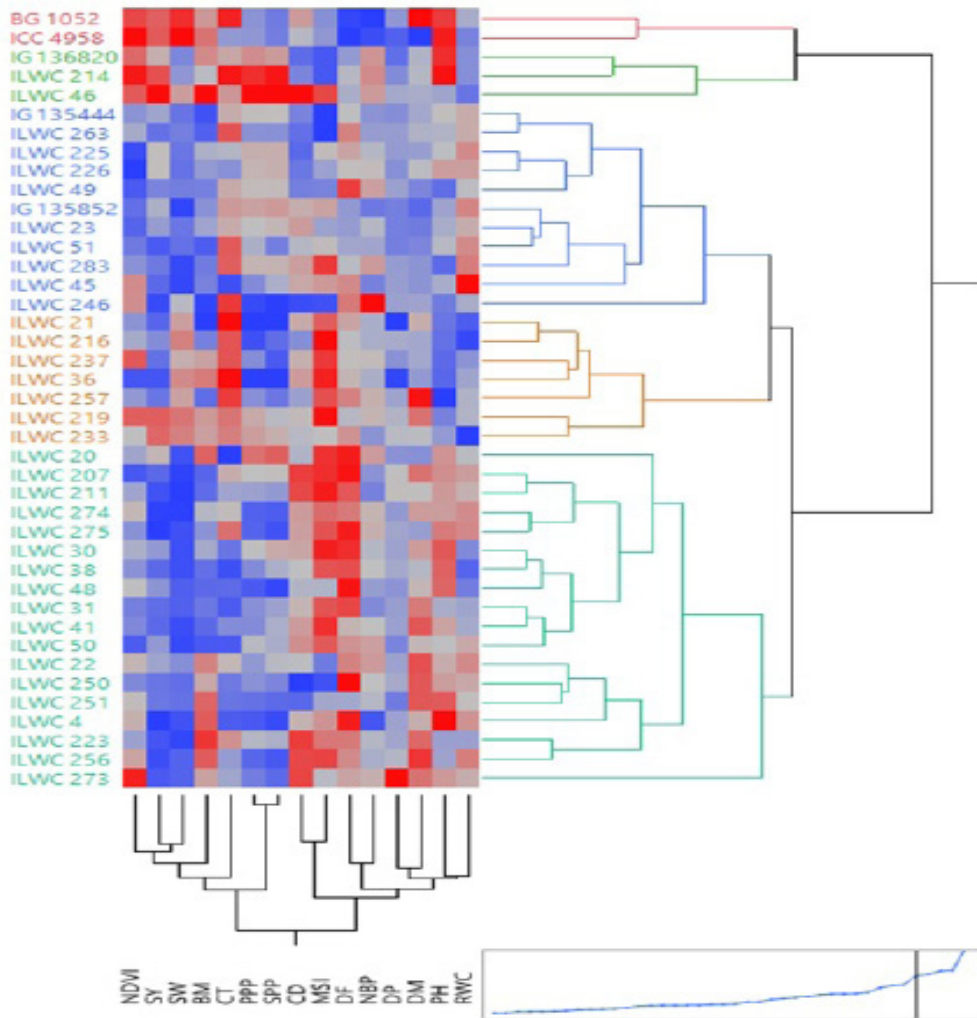


Fig. 2. Hierarchical cluster analysis depicting genetic relationship among chickpea genotypes using Euclidean distance and Ward method based on agro-physiological characters under non-stress evaluation

depicted genetic relationship among wild chickpea genotypes based on agro-physiological characters under non-stress evaluation which classified all the accessions into five groups and demonstrated the trait variability for each accession (Fig. 2). Cluster I included two accessions (BG 1053 and ICC 4958); cluster II had three accessions (IG 136820, ILWC 214, ILWC 46); cluster III grouped together IG 135852, IG 135444, ILWC 225, ILWC 226, ILWC 23, ILWC 246, ILWC 263, ILWC 283, ILWC 45, ILWC 49, ILWC 51; cluster IV included ILWC 21, ILWC 216, ILWC 219, ILWC 233, ILWC 237, ILWC 257, ILWC 36; and cluster V grouped ILWC 20, ILWC 207, ILWC 211, ILWC 22, ILWC 223, ILWC 250, ILWC 251, ILWC 256, ILWC 273, ILWC 274, ILWC 275, ILWC 30, ILWC 31, ILWC 38, ILWC 4, ILWC 41, ILWC 48, ILWC 50 (Fig. 2). The data was visualized by combining heatmap with HCA for exploring the complex relationships between multiple parameters.

The PCA based on correlation was used to reduce the dimension of data set where major share of variance (68.99 and 66.89%) was explained by first four components

under non-stress and stress conditions, respectively (Supplementary Table S3). Under non-stress condition, the first component (PC1) accounted was loaded on DF, BM, SY, SW, NDVI, CT in positive direction and CD and RWC in negative direction whereas PC2 was loaded on CD, SPP, BM and SY. PC3 was related to DM and RWC and PC4 for DM and MSI. Under stress condition, major contributions on the first component (PC1) were recorded by DF, PPP, BM, SY, SW, NDVI in positive direction and CD and RWC in negative direction whereas PC2 was loaded on CD, SPP, BM and SY. PC3 was related to PH, SW and MSI and PC4 for DF and PPP. The 2D bi-plots were also generated

based on first two PCs to depict the accessions' scores as well as relationships among variables (Fig. 3). Further, based on PCA ranking, the stable genotypes with high mean performance under non-stress and stress conditions were identified such as, ILWC 46, *C. reticulatum*; ILWC214, *C. yamashitae*; IG 136820, *C. pinnatifidum*, IG 135852, *C. judaicum*; ILWC 20, *C. judaicum*; ILWC 207, *C. judaicum*; ILWC 223, *C. judaicum*; ILWC 223, *C. judaicum*; ILWC 30, *C. judaicum*, ILWC 219, *C. reticulatum* (Table 1).

Discussion

Despite extensive research work on chickpea for last several decades, traditional breeding approaches could not produce cultivars with large impact on chickpea yield and production. This was mainly due to the narrow genetic base of cultivated chickpea germplasm caused by early domestication process (Abbo et al. 2003) and even revealed by molecular markers (Choudhary et al. 2013; Nguyen et al. 2004). As a result, the chickpea cultivation is adversely affected by spread of

Table 2. Superior accessions (top five) identified under stress condition for traits studied

Traits	Genotypes (Species)		Traits	Genotypes (Species)	
Days to 50% flowering	ICC 4958MT	<i>C. arietinum</i>	Biomass per plant(g)	ILWC 46 MT	<i>C. reticulatum</i>
	BG 1053 HS	<i>C. arietinum</i>		ILWC 223 MT	<i>C. judaicum</i>
	ILWC 21 MS	<i>C. reticulatum</i>		ILWC 256 MT	<i>C. judaicum</i>
	ILWC 36 MS	<i>C. reticulatum</i>		ILWC 251 HT	<i>C. pinnatifidum</i>
	IG 136820 MS	<i>C. pinnatifidum</i>		ICC 4958 HT	<i>C. arietinum</i>
Days to maturity	ICC 4958 MT	<i>C. arietinum</i>	Seed yield per plant(g)	ILWC 46 MT	<i>C. reticulatum</i>
	BG 1053 HS	<i>C. arietinum</i>		ILWC 233 HT	<i>C. reticulatum</i>
	ILWC 257 MS	<i>C. reticulatum</i>		ILWC 214 HT	<i>C. yamashitae</i>
	ILWC 36 MS	<i>C. reticulatum</i>		ILWC 219 HT	<i>C. reticulatum</i>
	ILWC 214 MS	<i>C. yamashitae</i>		ICC 4958 HT	<i>C. arietinum</i>
Plant height(cm)	BG 1053 MT	<i>C. arietinum</i>	100-seed weight(g)	ICC 4958 HT	<i>C. arietinum</i>
	ILWC 251 HT	<i>C. pinnatifidum</i>		BG 1053 MS	<i>C. arietinum</i>
	ICC 4958 HT	<i>C. arietinum</i>		ILWC 219 HT	<i>C. reticulatum</i>
	ILWC 38 HT	<i>C. judaicum</i>		ILWC 216 MS	<i>C. reticulatum</i>
	ILWC 48 HT	<i>C. judaicum</i>		ILWC 233 HT	<i>C. reticulatum</i>
Canopy diameter(cm)	ILWC 223 MT	<i>C. judaicum</i>	Normalized Difference Vegetation Index	ILWC 273 MT	<i>C. judaicum</i>
	ILWC 207 MT	<i>C. judaicum</i>		ILWC 45 HT	<i>C. judaicum</i>
	ILWC 273 MT	<i>C. judaicum</i>		IG 136820 MS	<i>C. pinnatifidum</i>
	ILWC 50 HT	<i>C. judaicum</i>		ILWC 237 MS	<i>C. reticulatum</i>
	ILWC 256 HT	<i>C. judaicum</i>		ILWC 4 HT	<i>C. judaicum</i>
Numbers of primary branches per plant	ILWC 50 HT	<i>C. judaicum</i>	Canopy temperature	ILWC 20 HT	<i>C. judaicum</i>
	ILWC 214 HT	<i>C. yamashitae</i>		ILWC 273MS	<i>C. judaicum</i>
	ILWC 273 HT	<i>C. judaicum</i>		ILWC 256MS	<i>C. judaicum</i>
	ILWC 219 HT	<i>C. reticulatum</i>		ILWC 38 HT	<i>C. judaicum</i>
	ILWC 226 HT	<i>C. pinnatifidum</i>		ILWC 45 MT	<i>C. judaicum</i>
Number of pods per plant	ILWC 46 MT	<i>C. reticulatum</i>	Membrane stability (%)	ILWC 251 HT	<i>C. pinnatifidum</i>
	ILWC 214 HT	<i>C. yamashitae</i>		ILWC 257 HT	<i>C. reticulatum</i>
	ILWC 20 MT	<i>C. judaicum</i>		ILWC 38 HT	<i>C. judaicum</i>
	IG 136820 HT	<i>C. pinnatifidum</i>		ILWC 20 HT	<i>C. judaicum</i>
	ILWC 233 HT	<i>C. reticulatum</i>		ILWC 31 MT	<i>C. judaicum</i>
Number of seeds per plant	ILWC 46 MS	<i>C. reticulatum</i>	Relative water content (%)	ILWC 250 HT	<i>C. pinnatifidum</i>
	ILWC 214MS	<i>C. yamashitae</i>		IG 135444 HT	<i>C. pinnatifidum</i>
	ILWC 20 HT	<i>C. judaicum</i>		ILWC 263 HT	<i>C. pinnatifidum</i>
	IG 135852 HT	<i>C. pinnatifidum</i>		ILWC 48 HT	<i>C. judaicum</i>
	IG 136820 MS	<i>C. pinnatifidum</i>		ILWC 4 HT	<i>C. judaicum</i>

MS = Moderately susceptible; HS = Highly susceptible; MT = Moderately tolerant; HT= Highly tolerant based on Drought Susceptibility Index.

several devastating diseases, like *Fusarium* wilt, *Ascochyta* blight, root rot, etc. and abiotic stresses, like terminal drought, heat and cold. Several screening studies indicated that there is scarcity of resistant donors in cultivated chickpea germplasm for devastating diseases like ascochyta blight, botrytis grey mold, dry root rot, etc. (Pande et al. 2006ab; Sharma et al. 2015; Gayacharan et al. 2020). Similarly, abiotic stresses, particularly terminal drought, heat, cold and salinity stress have become a major challenge in chickpea cultivation. The exploitation of natural genetic variation across various gene pools is important for improving abiotic stress tolerance, including drought. Considerable genetic variability for drought stress tolerance in chickpea has been reported for various morpho-physiological and grain yield-related parameters under different moisture levels (Toker et al. 2007; Krishnamurthy et al. 2010; Jha et al. 2014; Pang et al. 2017). Several chickpea genotypes with superior yield

performance have been identified in cultivated and wild chickpea using field based screening techniques (Singh and Ocampo 1997; Toker and Cagirgan 1998; Canci and Toker 2009). Krishnamurthy et al. 2010 observed significant genetic variability for various phenological and yield-related traits under water stress by using stress tolerance indices and principal component-based analysis. Wild species of chickpea such as *C. anatolicum*, *C. microphyllum*, *C. songaricum*, *C. pinnatifidum*, *C. reticulatum* were considered as an important reservoir for drought tolerance (Toker et al. 2007, Toker et al. 2009). However, wild *Cicer* germplasm still remains underutilized for the trait discovery and identification of trait-specific donor genotypes. Therefore, utilization of wild *Cicer* species particularly from primary and secondary gene pools which are known to have higher genetic diversity than the cultigens (Penmetsa et al. 2016) will have a greater impact on chickpea improvement.

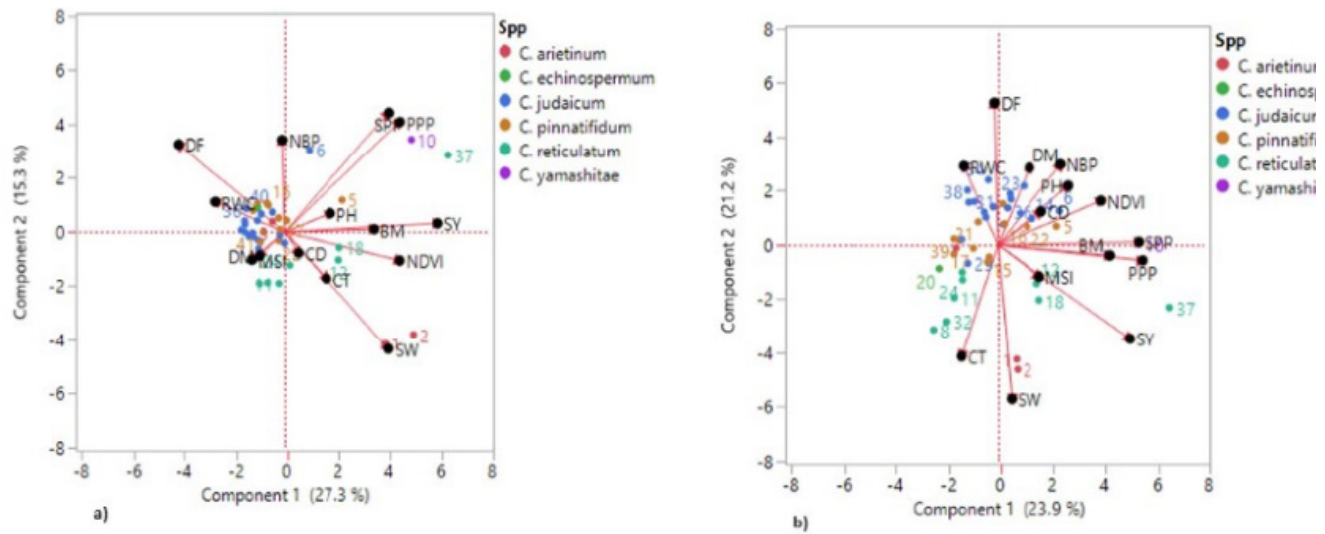


Fig. 3. Principal component Scatter-plot based on agro-physiological traits a) Non-stress evaluation; b) Stress evaluation [DF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), CD = Canopy diameter (cm), NBP = Numbers of primary branches per plant, PPP = Number of pods per plant, SPP = Number of seeds per plant, BM = Biomass per plant(g), SY = Seed yield per plant (g) and SW = 100-seed weight (g), NDVI = Normalized Difference Vegetation Index, CT = Canopy temperature, MSI = Membrane stability (%) and RWC = Relative water content (%)]

The wild species in this study revealed substantial variability for drought tolerance which may be attributed to their morphological distinctness and varying response to low moisture stress conditions. The low stress intensity or less effect of drought stress observed by genotypes for different agro-physiological traits revealed inherent tolerance of wild species against drought stress. Positive association of NDVI with key traits revealed that this can be a good indirect measure for biomass, seed yield and seed weight. Clustering of genotypes into five classes based on observations recorded under irrigated condition depicted sufficient diversity which was congruent with tolerance and susceptible nature of genotypes. PCA biplots showed the relative contributions of the variables and clear distribution of accessions under both the conditions. The PCA and HCA revealed the high level of genetic variations existing in the wild chickpea collection owing to distinct behavior of species and explain the traits contributing for this diversity. On the basis of PCA rankings, the tolerant accessions (ILWC 46, *C. reticulatum*; ILWC 214, *C. yamashitae*; IG 136820, *C. pinnatifidum*, IG 135852, *C. judaicum*; ILWC 20, *C. judaicum*; ILWC 207, *C. judaicum*; ILWC 223, *C. judaicum*; ILWC 223, *C. judaicum*; ILWC 30, *C. judaicum*, ILWC219, *C. reticulatum*) were selected. Other multiple trait donors (ILWC 20, ILWC 38, ILWC 46, ILWC 214, ILWC 219) under stress evaluation were also identified which could be highly useful in chickpea pre-breeding programs to improve the tolerance against drought. Here, genotypes with tolerance nature meant mean performance of genotypes were stable under both condition and genotypes with susceptible nature meant although under stress condition, these genotypes are relatively better but has reduction in comparison to

their optimum value. Although, many of these accessions belonging to secondary and tertiary gene pool which may produce shriveled seeds with reduced germination of crossed seeds in *Cicer* as reported earlier by Ahmad et al. (1988). Therefore, specialized techniques of gene transfer may be employed such as application of growth hormones, using mentor pollen technique, embryo rescue, etc. (Mallikarjuna and Jadhav 2008; Pratap et al. 2021). However, successful interspecific crosses between *C. arietinum* and *C. judaicum*, *C. arietinum* and *C. cuneatum*, *C. arietinum* and *C. pinnatifidum*, and *C. arietinum* and *C. bijugum* have been realized. Subsequently, wide hybridization was attempted between *C. arietinum* and *C. echinospermum* by various workers (Pundir and Mengesha 1995; van Dorrestein et al. 1998); they had also attempted crosses involving *C. arietinum*, *C. bijugum* and *C. judaicum* but with partial success. The programme on utilization of wild species of *Cicer* was initiated long back to improve chickpea for higher productivity (Yadav et al. 2002). In this endeavour, Pusa 1103 was the first chickpea variety possessing wilt, root rot, and bruchids resistance developed through interspecific hybridization utilizing wild species, *Cicer reticulatum* released in 2005 (Yadav et al. 2007). Recently, a new genotype of chickpea (*Cicer arietinum* L.), PBG8 derived conventionally from an interspecific cross, *Cicer arietinum* x *C. judaicum* (Singh et al. 2022) has been released for general cultivation.

Due to the use of *in-vitro* technique, success has been made in achieving hybrids between *C. arietinum* and *C. bijugum* and *C. arietinum* and *C. judaicum*. Badami et al. (1997) also reported successful hybridization between *C. arietinum* and *C. pinnatifidum* using embryo rescue technique.

Successful introgression of useful genes into cultivated chickpea from these crosses has shown the transferability even from the cross-incompatible wild *Cicer* species. Hence, these results will be of greater use in order to identify superior accessions for improving various component traits of drought tolerance in cultivated chickpea utilizing wild species in genetic enhancement and pre-breeding programs.

Supplementary material

Supplementary Tables S1, S2 and S3 and Supplementary Fig. S1 are provided online www.isgpb.org.

Authors' Contributions

Conceptualization of research (JK, GC, SM); Designing of the experiments (JK, MS); Contribution of experimental materials (MS, AS); Execution of field/lab experiments and data collection (JK, SM, TPS); Analysis of data and interpretation (JK); Preparation of the manuscript (GC, MS, NM, AK).

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Supplementary Table S1. Analysis of variance of 14 agro-physiological traits studied for drought tolerance

Source	df	DF	DM	PH	CD	NBP	PPP	SPP
GEN	40	373.32**	56.52**	924.40**	3140.07**	32.33**	2445.00**	6577.39**
REP	2	9.76	4.24	4.86**	8.29**	20.29	22.93	288.08*
TRT	1	76.19**	57.38**	26.22**	303.80**	398.55**	126.26**	373.92*
GEN × TRT	40	39.53**	9.50**	0.95**	92.85**	22.29**	189.52**	117.94
Error	80	1.53	4.37	0.19	0.41	6.55	20.71	83.38
Total	246							
Source	df	BM	SY	SW	NDVI	CT	MSI	RWC
GEN	40	175.11**	18.50**	149.38**	0.09**	88.34**	351.16**	246.74**
REP	2	6.63**	0.72	0.12	0.01	0.09	5.85*	5.52
TRT	1	241.10**	1.27**	1.84**	0.07**	110.42**	8755.13**	6406.93**
GEN × TRT	40	15.89**	0.87**	0.56**	0.01	23.64**	293.09	95.69**
Error	80	1.95	0.38	0.10	0.01	0.27	2.70	18.43
Total	246							

[DF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), CD = Canopy diameter (cm), NBP = Numbers of primary branches per plant, PPP = Number of pods per plant, SPP = Number of seeds per plant, BM = Biomass per plant(g), SY = Seed yield per plant (g) and SW = 100-seed weight (g), NDVI = Normalized Difference Vegetation Index, CT = Canopy temperature, MSI = Membrane stability (%) and RWC = Relative water content (%)]

*Significant at 5% level

** Significant at 1% level.

Supplementary Table S2. Summary statistics of studied traits under normal(non-stress) and drought (stress) conditions along with stress intensity

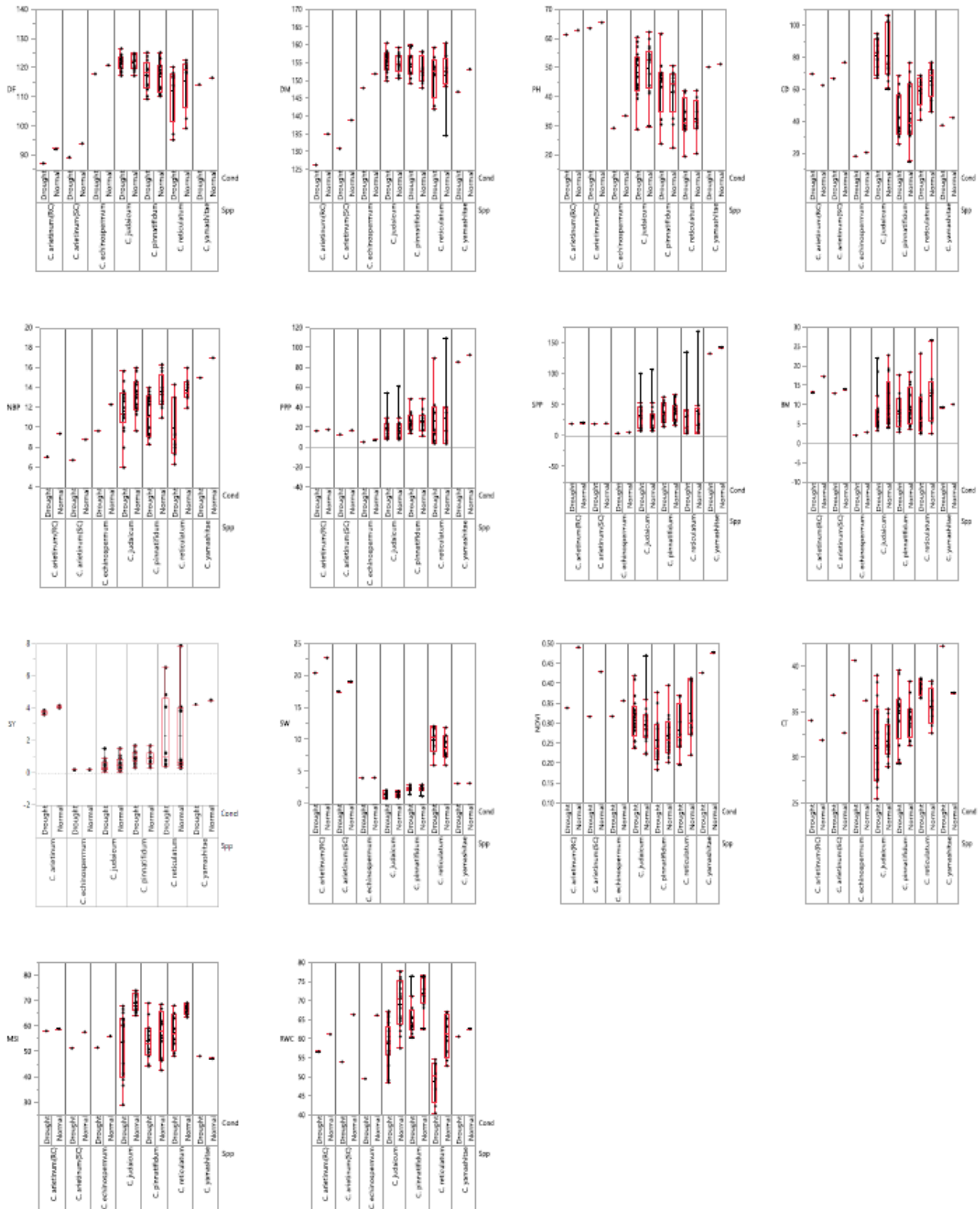
	DF	DM	PH	CD	NBP	PPP	SPP	BM	SY	SW	NDVI	CT	MSI	RWC
Normal														
Mean	118.25	153.96	48.13	65.78	13.67	24.79	37.68	10.49	1.28	4.44	0.31	34.79	66.78	68.67
Standard Error	1.09	0.54	1.99	3.81	0.41	3.33	5.51	0.94	0.23	0.76	0.01	0.78	1.74	1.20
Standard Deviation	6.97	3.43	12.77	24.42	2.62	21.34	35.28	6.00	1.48	4.89	0.08	4.98	11.13	7.70
Kurtosis	6.88	0.03	-0.56	-0.15	7.84	7.51	7.13	-0.11	3.98	5.80	0.16	-1.04	-0.38	1.55
Skewness	-2.39	-0.03	0.00	0.05	1.81	2.54	2.53	0.81	2.14	2.36	0.96	-0.20	-0.90	0.44
Minimum	92.24	134.67	20.67	15.25	8.80	3.67	5.67	2.62	0.12	1.00	0.20	25.51	42.81	53.07
Maximum	125.33	160.67	65.60	106.40	17.00	109.67	169.00	26.73	7.87	22.82	0.49	42.27	79.32	93.63
Drought														
Mean	116.99	152.93	47.99	63.69	11.30	24.02	34.67	8.53	1.14	4.24	0.30	33.73	54.92	58.59
Standard Error	1.40	0.50	1.94	3.49	0.51	3.00	4.88	0.82	0.26	0.79	0.01	0.39	1.49	1.19
Standard Deviation	8.94	3.20	12.43	22.35	3.27	19.20	31.24	5.25	1.66	5.08	0.06	2.50	9.52	7.63
Kurtosis	3.54	-0.87	-0.52	-0.80	2.72	6.05	4.94	1.07	5.88	4.02	-0.63	-0.75	-0.02	0.10
Skewness	-1.98	0.25	-0.08	-0.16	1.04	2.28	2.07	1.22	2.39	2.08	0.25	0.06	-0.66	-0.23
Minimum	87.33	126.42	19.67	18.67	6.00	4.00	4.33	2.18	0.1	0.80	0.18	28.99	29.24	40.62
Maximum	126.67	160.67	63.58	95.00	15.67	89.67	135.67	23.39	6.53	20.47	0.43	38.49	69.25	76.60
Stress Intensity(D)	0.009	0.007	0.003	0.031	0.184	0.026	0.066	0.188	0.114	0.044	0.032	0.030	0.178	0.148

[DF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), CD = Canopy diameter (cm), NBP = Numbers of primary branches per plant, PPP = Number of pods per plant, SPP = Number of seeds per plant, BM = Biomass per plant(g), SY = Seed yield per plant (g) and SW = 100-seed weight (g), NDVI = Normalized Difference Vegetation Index, CT = Canopy temperature, MSI = Membrane stability (%) and RWC = Relative water content (%)]

Supplementary Table S3. Loading matrix of 14 traits towards principal components under normal(non-stress) and drought (stress) conditions

Traits	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Normal (non-stress) condition							
DF	0.71	-0.17	0.02	-0.17	0.32	0.27	-0.28
DM	0.26	-0.27	-0.51	0.55	0.03	0.29	0.26
PH	0.09	-0.12	0.78	0.37	-0.09	0.23	0.00
CD	-0.65	0.51	0.15	0.22	0.26	-0.18	-0.05
NBP	-0.15	-0.14	0.30	0.03	0.81	0.03	0.43
PPP	0.28	0.11	0.25	-0.80	0.08	-0.17	0.02
SPP	-0.02	0.54	-0.46	0.32	0.36	0.16	-0.37
BM	0.71	0.65	0.08	0.15	-0.12	-0.01	0.16
SY	0.64	0.70	0.10	0.12	-0.15	-0.05	0.17
SW	0.55	0.02	0.45	-0.05	0.26	0.19	-0.26
NDVI	0.94	0.05	0.03	0.08	-0.04	-0.08	0.13
CT	0.64	-0.68	-0.15	0.10	-0.03	-0.10	-0.05
MSI	-0.20	-0.16	0.68	0.54	-0.17	-0.15	-0.15
RWC	-0.42	0.17	0.15	-0.32	-0.26	0.72	0.12
variance explained (%)	27.26	42.52	56.78	68.99	77.36	83.76	88.40
Drought (stress) condition							
Traits	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
DF	0.60	0.25	-0.22	0.40	0.19	0.00	-0.20
DM	-0.21	-0.63	0.42	0.02	0.38	-0.19	0.03
PH	0.26	0.19	-0.74	-0.05	-0.42	0.12	-0.12
CD	-0.02	0.81	0.06	-0.40	0.05	-0.06	0.08
NBP	0.19	0.44	-0.36	-0.17	0.62	-0.10	0.28
PPP	0.41	0.34	0.23	0.71	-0.19	0.03	0.02
SPP	0.37	0.46	0.14	0.22	0.46	-0.23	-0.33
BM	0.85	-0.09	0.36	-0.29	-0.15	-0.16	-0.02
SY	0.82	0.02	0.34	-0.28	-0.24	-0.15	-0.06
SW	0.65	-0.06	-0.27	0.05	0.18	0.34	0.44
NDVI	0.77	-0.53	0.02	-0.06	-0.04	-0.10	0.20
CT	0.08	-0.87	-0.24	0.25	0.12	-0.02	0.05
MSI	0.25	-0.18	0.25	-0.23	0.27	0.76	-0.34
RWC	-0.20	0.45	0.63	0.23	-0.12	0.26	0.37
Variance explained (%)	23.86	45.05	57.99	66.89	75.48	82.25	87.50

[DF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), CD = Canopy diameter (cm), NBP = Numbers of primary branches per plant, PPP = Number of pods per plant, SPP = Number of seeds per plant, BM = Biomass per plant(g), SY = Seed yield per plant (g) and SW = 100-seed weight (g), NDVI = Normalized Difference Vegetation Index, CT = Canopy temperature, MSI = Membrane stability (%) and RWC = Relative water content (%)].



Supplementary Fig. S1. Boxplot showing species wise variation in agro-physiological traits under stress and non-stress condition [DF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), CD = Canopy diameter (cm), NBP = Numbers of primary branches per plant, PPP = Number of pods per plant, SPP = Number of seeds per plant, BM = Biomass per plant(g), SY = Seed yield per plant (g) and SW = 100-seed weight (g), NDVI = Normalized Difference Vegetation Index, CT = Canopy temperature, MSI = Membrane stability (%) and RWC = Relative water content (%)]