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Full Research Article

Genetic Variation and Diversity Analysis of Chickpea Genotypes based on Quantitative Traits under High Temperature Stress

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Abstract

Sixty two chickpea genotypes were investigated to capture the existing genetic variability for heat stress tolerance. Genotypes revealed significant wide genetic variation for eight different quantitative and morpho-physiological traits. Considering phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), membrane stability trait exhibited highest PCV (64.75%) and GCV (56.3%). While, highest broad sense heritability (h^2_B) was recorded for membrane stability (75%), followed by pods/plant (53%) and days to maturity (46%). Importantly, positive correlation between days to 50% flowering and plant height, pods/plant and plant height, pods /plant and membrane stability were recorded. Similarly, days to maturity and membrane stability showed high positive correlation. Cluster analysis based on the given 8 traits grouped all the 62 genotypes into eight distinct clusters. Cluster 2 and 8 contained 12 genotypes each. The first three principal components of 8 traits contributed 60.8% of the total existing variability present in the given genotypes. Most important traits contributing diversity in PC_1 were days to maturity and membrane stability, where as, pods/plant and plant height in PC_2 and plot yield and leaf area index (LAI) in PC_3. Additionally, the following two genotypes Katila (232.5 gm), Vaibhav (222.5 gm) were showed higher plot yield than check ICC92944 (heat stress tolerant) (192.5 gm) under heat stress. Results from the given study would be useful for to design heat stress tolerant improved chickpea cultivar.

1. Introduction

Human-led emission of greenhouse gases coupled with rapid industrialization and other related activities have resulted in increase in global temperature continuously over the past decades (McKersie, 2015). Considering this, it is predicted that global climate will evidence an increase of 2-4 °C at the end of 21st century (IPCC, 2007). Therefore, to feed the burgeoning human population worldwide under the current scenario of global climate change, serious attention is needed to develop improved crop cultivars. To this end,increasing incidences of high temperature is becoming a global threat to crop production, resulting in global food security at great risk (Lobell and Field, 2007; Teixeira et al., 2013). Noticeable phenological changes are evidenced in autumn and spring grown plant species (Ibanez et al., 2010; Wolkovich et al., 2012). Consequently, examination of crop genetic variability is of great interest to plant breeder community to combat against increasing incidences of heat stress (HS) (Jha et al., 2014a). Chickpea ranks second most important pulse crop next to common bean in terms of production across the world (Varshney et al., 2013). Southern and South-Eastern Asia contribute 80% of the global chickpea production (Gaur et al., 2012). Global production of chickpea is recorded to be 13.1 million tons from 13.5 Mha area (FAO, 2013). India remains the top producer of chickpea across the globe (FAO, 2013). It serves an important pulse crop offering important dietary protein to the vegetarians. However, it faces many biotic and abiotic stresses resulting in significant yield loss across the globe (Ryan, 1997). Among the abiotic stress, terminal heat stress is garnering serious attention in perspective of global warming, causing change in chickpea phenology and resulting in significant yield loss in tropical and subtropical regions worldwide (Krishnamurthy et al., 2011; Jha et al., 2014b). In India, chickpea is mostly cultivated in *rabi* season but recently, witness of practices of chickpea cultivation from cool season of Northern India to warm climate of Central and Southern India has also been recorded (Kuldeep et al., 2015). Likewise, change in chickpea cultivation from cooler region to warmer region has been evidenced across the chickpeagrowing region in Asia (Krishnamurthy et al., 2013). Consequently, chickpea faces terminal heat stress causing negative impact on crop yield. The reproductive stage is most vulnerable stage for heat stress in chickpea (Summer field et al., 1984; Wang et al., 2006, Devasirvatham et al., 2012a) resulting in yield loss. To this end, progress in breeding for terminal heat stress in chickpea remains limited. Few genotypes viz., ICC1205 and ICC92944 (Devasirvatham et al., 2013; Devasirvatham et al., 2012b) are reported to be heat stress tolerance. Therefore, to stabilize chickpea production andyield, a thorough assessment of genotypic variability and concerned different morpho-physiological traits of chickpea germplasm, facilitating enhanced terminal heat stress tolerance is a prerequisite. Taken togetherthe present study was aimed to capture the existing genotypic variability coupled with phenotypic trait components present in the 62 genotypes to tailor improved heat stress tolerance in chickpea.

2. Materials and Methods

2.1. Experimental location, design and plant material

The present study was conducted at ICAR-Indian Institute of Pulses Research (IIPR), Kanpur, India during the crop season 2014. A total of 62 genotypes (Table 1). including three checks ICC4958, ICCV 92944 and RSG888 were undertaken. In order to evaluate the genetic variability under terminal heat stress, the given genotype sets were sown in randomized block design (RBD) with two replications. The genotypes were sown in second week of January month under late sown condition to face terminal heat stress. The average weekly temperature recorded during the crop growth period from January 2014 to April 2014 is given in (Figure 1). The maximum day temperature was noted to be above 35 °C during pod formation and grain filling stage. Notably, temperature above 35 °C is detrimental to pollen development, and pod formation in chickpea (Devasirvatham et al., 2012a, 2013). Considering spacing, row length was kept 3m and spacing between row to row was kept to 30 cm and plant to plant was 10 cm. The data were recorded from 5 randomly selected plants for each genotype. The considered traits are days to 50% flowering, plant height, days to maturity, 100 seed weight, leaf area index (LAI), cell membrane stability (CMS), pods plant^{-1} and plot yield. Electrolyte leakage serves as an important criterion for measuring stability of cell membrane under stress condition in plant (Sullivan,1972; Blum and Ebercon, 1979). Membrane stability is denoted by membrane injury Index (MII) postulated by Blum and Ebrecon (1979). MII = (C1/C2)×100. Where C_1 denotes electrolyte measured at 40 °C and C_2 denotes electrolyte measured at 80 °C. In this study cell membrane stability (CMS) trait has been studied as important parameter for assessing heat stress tolerance in the given genotypes.

2.2. Statistical analysis

The genetic parameters viz., mean, genotypic variance, phenotypic variance, broad sense heritability and genetic advance (GA) were estimated by applying formula followed by Burton and Devane, (1953). Further, genotypic correlation coefficient for the undertaken traits was calculated as per the method of Searle (1961). Whereas, significance of correlation coefficients was tested by comparing with 't' value at (n-2) d.f. (Snedecor and Cochran, 1967). Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) wereconducted to visualize all the data together. HCA was performed to identify the genotype groups having similar phenotypic constitution (Chen et al., 2014). To visualize the analyzed data as heat map with dendogram, "heatmap.2" function of R package was used.

3. Results and Discussion

3.1. Genetic variability

Significant genetic variation was observed for some important traits among the given 62 genotypes. Higher value of phenotypic variance than genotypic variance for the given traits was recorded. Likewise, phenotypic coefficient of variation for the given traits showed higher value than genotypic coefficient of variation. Highest PCV (64.75%) value was recorded for CMS, followed by 100 seed wt. (54.03%) and LAI (39.8%) given in (Table 2). Considering GCV, highest GCV (56.3%) value was noted for CMS. While, lowest GCV (1.83%) for days to 50% flowering was noted. Similarly a good deal of genetic variability for CMS trait was reported in various legume crops (Srinivasan et al., 1996). Importantly, high broad sense heritability (h^2_B) was recorded for CMS (75%). Earlier low to intermediate heritability of this trait was recorded in wheat, cow pea and in barley under heat stress (Ibrahim and Quick, 2001; Thiwa and Hall, 2004; Verma and Verma, 2011). Notably, heritability of pods plant^{-1} was recorded to be (53%) in current study. Similarly high heritability for pod yield was recorded in groundnut under drought stress (Songsri et al., 2008).While, the value of genetic advance (GA) calculated in this study ranged from 1% to 101% having highest 101% GA for CMS followed by pods plant^{-1} exhibiting 36.05% GA. Likewise, Mensah et al. (2006) recorded high genetic advance for pods/plant in groundnut under salinity stress.

3.2. Correlation analysis

Significant correlation coefficient among the various quantitative traits for 62 genotypes were estimated under terminal heat stress (Table 3). The result showed positive significant correlation between days to 50% flowering and

Table 1: Details of the chickpea genotypes used in the study

Genotype	Source	Type
Annegiri1	ARS, Gulbarga	RC
ICC5912	ICRISAT, Patancheru	Accession
JG74	JNKVV, Jabalpur	RC
RSG-143-1	Durgapura, Rajasthan	RC
ICC4958(CH)	ICRISAT, Patancheru	Selection
K850	CSAUA and T, Kanpur	RC
BDG72	IARI, New Delhi	RC
PG96006	MPKV, Rahuri	RC
ICC1205	ICRISAT, Patancheru	Accession
KWR108	CSAUA and T, Kanpur	RC
ICCV92944(CH)	ICRISAT, Patancheru	RC
PANTG 114	GBPUA and T, Pantnagar	RC
ICCV10	ICRISAT, Patancheru	RC
VIJAY	PDKV, Akola	RC
RSG888(CH)	Durgapura, Rajasthan	RC
KATILA	India	Landrace
ICC4567	ICRISAT, Patancheru	Accession
PUSA244	IARI, New Delhi	RC
BG1053	IARI, New Delhi	RC
PBG5	PAU, Ludhiana	RC
DCP-92-3	IIPR, Kanpur	RC
RSG991	RAU, Durgapura	RC
PUSA240	IARI, New Delhi	RC
ICC15614	ICRISAT, Patancheru	Accession
PUSA362	IARI, New Delhi	RC
ICC10685	ICRISAT, Patancheru	Accession
ICC8950	ICRISAT, Patancheru	Accession
VAIBHAV	IGKV, Raipur	RC
AVRODHI	CSAUA and T Kanpur	RC
PG5	MPKV, Rahuri	RC
RSG931	ARS, Durgapura	RC
ICC1356	ICRISAT, Patancheru	Accession
JG315	JNKVV, Jabalpur	RC
GCP-101	JAU, Junagadh	RC
GPF-2	PAU, Ludhiana	RC
PDG-3	PAU, Ludhiana	RC
Pusa-209	IARI, New Delhi	RC
Dig`vijay	MPKV, Rahuri	RC
GL-769	PAU, Ludhiana	RC
C-235	PAU, Ludhiana	RC
PDG-4	PAU, Ludhiana	RC
JG-11	ICRISAT, JNKVV, PKV	RC
JG-130	JNKVV, Jabalpur	RC
RSG-902	ARS, Durgapura, Rajasthan	RC
GNG-1488	Ganganagar, Rajasthan	RC
PBG-1	PAU, Ludhiana	RC
BGM-413	IARI, New Delhi	RC
CSG-8962	CSSRI, Karnal	RC
Pusa-1103	IARI, New Delhi	RC
Pusa261	IARI, New Delhi	RC
RSG-973	ARS, Durgapura, Rajasthan	RC
BG-256	IARI, New Delhi	RC
Pusa-547	IARI, New Delhi	RC
JGK-1	JNKVV, Jabalpur	RC
Pusa-372	IARI, New Delhi	RC
GNG1958	Sri Ganganagar, Rajasthan	RC
RSG-896	ARS, Durgapura, Rajasthan	RC
JAKI-9218	PDKV, Akola	RC
HC-1	CSSHAU, Hisar	RC
Pusa-72	IARI, New Delhi	RC
RSG-963	ARS, Durgapura, Rajasthan	RC
RSG-11	ARS, Durgapura, Rajasthan	RC

TC: Traditional cultivar; RC : Released cultivar

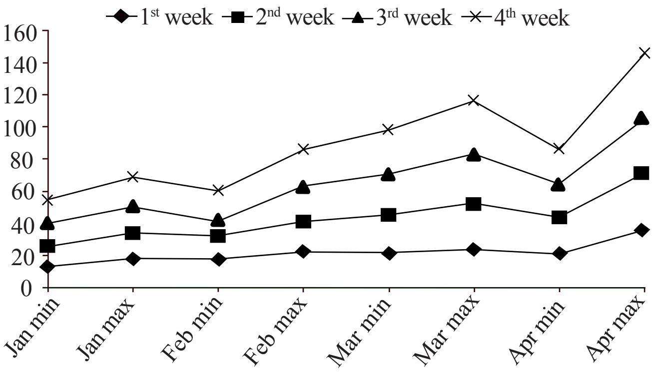

Figure 1: Mean minimum and maximum temperature in ^{0}C given in Y axis recorded from January to April end

plant height, pods plant^{-1} and plant height, and pods plant^{-1} and membrane stability. Similarly, days to maturity and CMS showed highly significant positive correlation. This result was in agreement with the result recorded by Verma and Verma (2005) in wheat under heat stress. While, days to 50% flowering and membrane stability, pods plant^{-1} and 100 seed weight revealed high negative significant correlation. Likewise, pods

Table 2: Estimation of genetic parameter for 8 different agro-morphological traits

Traits	RangeW	Mean	SEm±	σ2G	σ2P	GCV%	PCV%	h^2B%	GA%
50% FLO (days)	52-64	59.2	2.5	1.1	14.6	1.83	6.46	8	1.07
Plant height (cm)	28.1-47.6	33.8	2.6	2.3	16.1	4.51	11.8	14	3.52
MAT (days)	88-97	93.4	1.7	5.2	11.3	2.4	3.6	46	3.45
Leaf area index	0.5- 2.95	1.96	0.5	0.031	0.612	9.03	39.89	5	4.21
CMS (%)	11.3-78.7	41.41	9.2	544.9	719	56.36	64.75	75	101
100 Seed wt (g)	9-28.2	18.2	9.2	12.6	97.4	19.5	54.03	13	14.5
Pods Pt^{-1}	11-47	23.02	3.5	30.1	56.1	23.86	32.53	53	36.05
Plot yield (g)	42-232.5	147.71	6.4	162.1	2439.6	8.62	33.43	6	4.5

50% FLO: Days to 50% flowering; MAT: Days to maturity

Table 3: Correlation coefficients for different characters in 62 genotypes

	50% FLO	Plant height	MAT	Pods plant^{-1}	LAI	CMS	100 Seed wt.	Plot yield
50% FLO	1	0.289*	-0.236	0.019	0.158	-.565**	-0.173	0.092
Plant height		1	-0.114	0.261*	0.111	-260*	-0.081	-0.038
MAT			1	0.21	-0.091	0.580**	-0.188	-.307*
Pods Pt^{-1}				1	0.049	0.308*	-388**	-0.037
LAI					1	-0.103	-0.006	0.169
CMS						1	-0.065	-0.167
100 Seed wt.							1	0.101
Plot yield								1

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed tailed)

plant^{-1} and 100 seed weight showed high negative correlation in faba bean (Abdelmula and Abuanja, 2007). Importantly, plant height and membrane stability, plot yield and days to maturity showed significant negative correlation. Likewise, Kilic and Yagbasanlar (2010) reported days to maturity had significant negative correlation with drought susceptibility index in wheat and negative significant correlation with yield in lentil (Azizi-e-Chakherchaman et al., 2009).

3.3. *Principal Component Analysis (PCA)*

Existence of genotypic variability in 62 genotypes was further confirmed by PCA analysis. PCA analysis revealed that first three principal components contributed 60.8% of the total existing variability. Similarly, 77% of genetic variability explained by using PC$_1$, PC$_2$ and PC$_3$ (Jha and Shil, 2015). First three principal components explained about 28.1%, 18.4% and 14.2% of the variation obtained from eigenvector analysis given in (Table 4). Reflecting high degree of association among the traits analyzed. In the first PC, CMS (0.884), days to maturity (0.753) and pods plant^{-1} (0.276) had the greatest effect. Similarly, pods plant^{-1} (0.744), plant height (0.638) and 50% flowering (0.362) were important traits in PC$_2$. Likewise, days to 50% flowering was major trait accountable for diversity in PC$_2$ has been investigated (Jha and Shil, 2015). Equally important, in PC$_3$ plot yield (0.758) and LAI (0.486) had the

Table 4: Eigenvectors and eigenvalues of the first three principle components of 8 traits

Variable	PC$_1$	PC$_2$	PC$_3$
Eigen value	2.2526	1.4787	1.1369
Variation%	28.158	18.483	14.211
Cumulative%	28.158	46.641	60.852
50% Flowering	-0.6749	0.36295	-0.18035
Plant height	-0.35197	0.6386	-0.34726
MAT	0.75387	0.22155	-0.10654
Pods plant^{-1}	0.27602	0.74436	0.05067
LAI	-0.28221	0.28052	0.48651
CMS	0.88446	0.0642	0.18746
100 Seed wt	-0.19236	-0.49881	-0.34971
Plot yield	-0.3603	-0.0657	0.75875

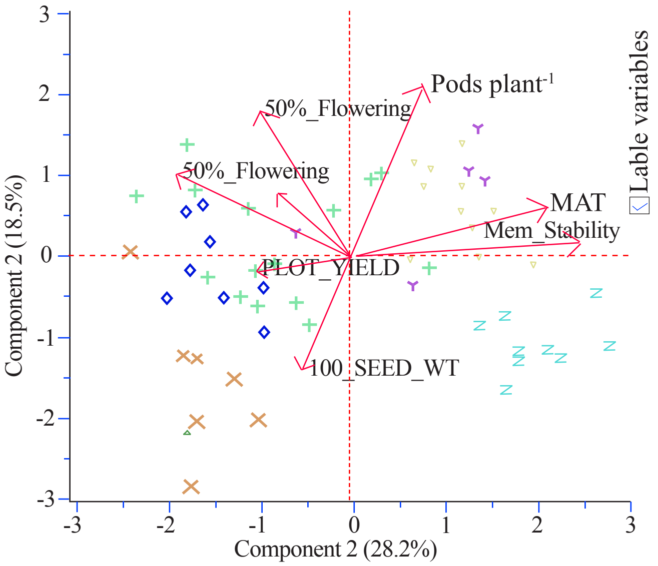

Figure 2: Biplot graphical display of measured traits based on PC_1 and PC_2 in chickpea

z denotes the genotypes belonging to cluster 8
+ denotes the genotypes belonging to cluster 2
x denotes the genotypes belonging to cluster 4
v denotes genotypes belonging to cluster 7
◊denotes genotypes belonging to cluster 3
Y denotes genotypes belonging to cluster 6

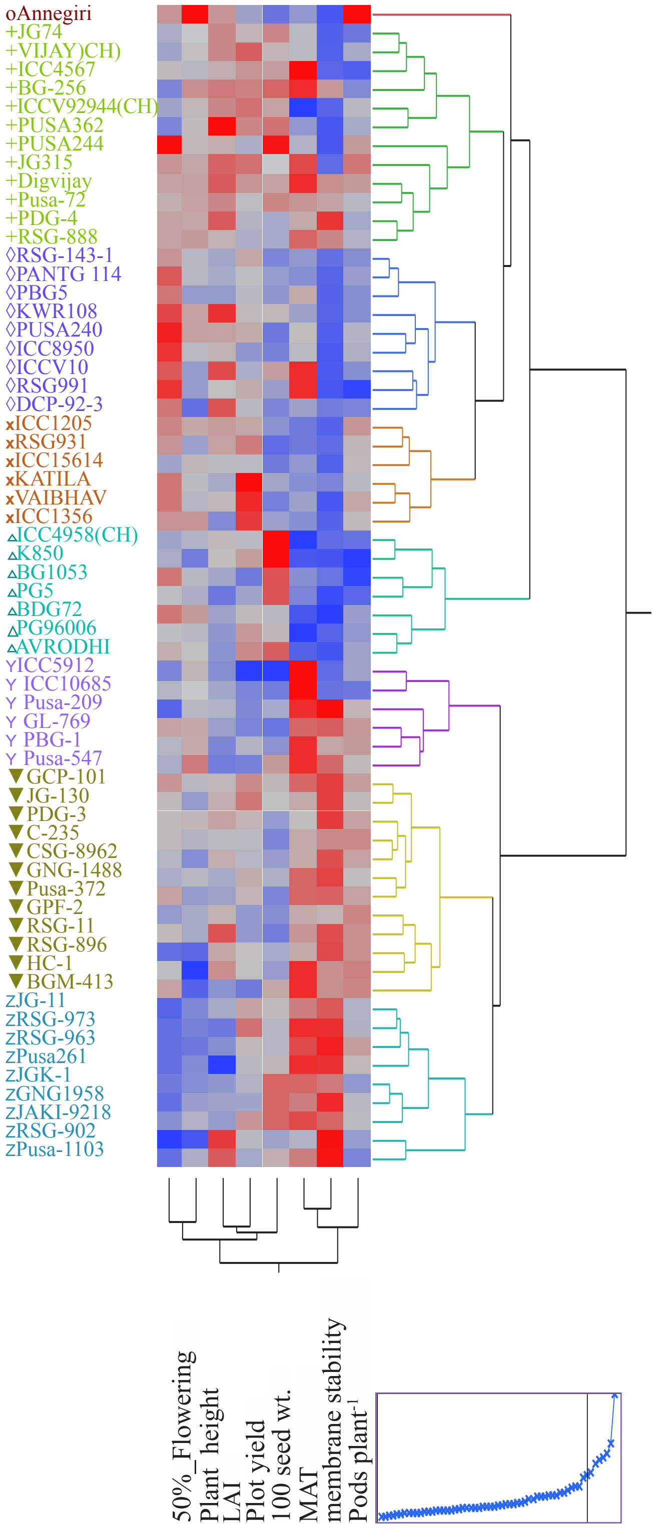

Figure 3: Biplot graphical display of measured traits based on PC_1 and PC_2 in chickpea

greatest effect. Similarly plot yield was major contributor for diversity in PC_3 has been recorded in chickpea under heat stress (Jha and Shil, 2015). Considering the importance of biplot in data analysis, the relationship between the yield contributing traits and the undertaken genotypes were depicted in biplot graph (Figure 2). Similar results were obtained in pea (Parihar et al., 2014).

3.4. *Cluster analysis*

In hierarchical cluster analysis all the sixty two genotypes were grouped into eight separate groups given in (Figure 3). The first cluster included only one genotype Annegiri. The second and seventh cluster contained 12 genotypes each. Whereas, the cluster 3 and cluster 8 included 9 genotypes each. Importantly, cluster 4 and 6 contained 6 genotypes each. Additionally, two way denodogramdepicted the grouping of genotypes based on yield contributing traits and vice versa. Moreover, three dimensional graph of 62 genotypes based on above mentioned traits (principal component analysis) was depicted in (Figure 4). Inter crossing among the genotypes from divergent clusters may broaden the genetic variability for yield and other desirable traits in chickpea. To this end, considering plot yield under heat stress. Katila (232.5 g), Vaibhav (222.5 g) genotypes exhibited superiority to check (tolerant) ICCV92944 (192.5 g). This result is in agreement with the result obtained by Jha and Shil (2015). While, Mishra and Babar (2014) selected the KAK2, JGK2, ICCV07311 and ICCV06301 genotypes showing lesser yield reduction on percentage basis under high temperature stress.

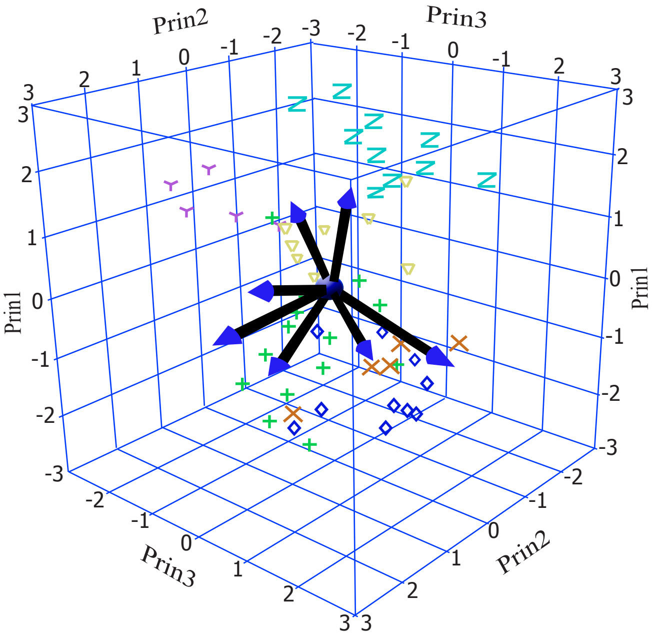

Figure 4: Principal component analysis of 62 chickpea genotypes based on 8 traits (Three dimensional graph)

4. Conclusion

The present study exhibited considerable genetic variability for morpho physiological traits for heat stress tolerance among 62 chickpea genotypes. High heritability of membrane stability, days to maturity and pods per planttraits can be exploited for transferring to offspring in chickpea crossing programme to reduce yield loss under heat stress. Furthermore, CMS and pods per planttraits exhibited high genetic advance, providing the opportunity to select superior genotypes and incorporate these traits into elite high yielding yet heat sensitive cultivars in chickpea.

5. Conflict of interest

The authors declare no conflict of interest.

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