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## Research Article

### Genetic diversity analysis for physiological, reproductive, biochemical and yield traits of blackgram (*Vigna mungo* L.) genotypes under high temperature stress

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#### Abstract

In the present study, 30 blackgram genotypes were evaluated for physiological, reproductive, biochemical and yield traits at flowering stage during summer, 2022 and 23. ANOVA showed highly significant genetic variability among the 30 blackgram genotypes for 20 heat tolerance traits studied indicating that significant amount of genetic variability among the genotypes. Higher PCV, GCV and heritability was recorded for seed yield per plant and number of pods per plant during both the years. Moreover, the results of cluster analysis for these heat tolerance traits revealed that the genotypes TBG-129, LBG-1015, PU-1804, TBG-104 and PU-31 were identified as promising genotypes under high temperature stress conditions during both the years. Principal component analysis showed that first three components showed 82.62 % of total variation during summer, 2022, whereas 77.06 % of the total variance was covered by the first two principal components during summer, 2023. The identification of genotypes displaying heat tolerance can be useful in future breeding programmes for the development of heat stress tolerant genotypes.

**Keywords:** Blackgram, Genetic diversity, High temperature stress, Principal component analysis

#### INTRODUCTION

Blackgram is an important short duration pulse crop which is rich in proteins, vitamins and minerals. Optimum temperature for proper growth and development of blackgram is 25-35°C. Temperature above 35°C leads to massive flower and pod drop resulting in low or no yield (Anitha *et al.*, 2015). In addition to this, high temperatures reduce the photosynthetic rate by decreasing the content of photosynthetic pigments. Heat stress also leads to the production and accumulation of reactive oxygen species (ROS) in the membranes of the cell wall. The

accumulated ROS disrupts the structural integrity of cell wall by altering the molar ratio of biomolecules and structure of biomembranes leading to lipid peroxidation and protein denaturation. So, membrane damage serves as a reliable indicator that can be effectively used to identify thermotolerant blackgram genotypes. Numerous reports have stated that cool canopies were associated with better yield output as a result of higher transpiration and photosynthesis (Pinto and Reynolds, 2015). Hence, understanding the physiological responses of blackgram

genotypes to high temperature stress is required to counteract the ill-effects of global warming in near future.

Plants accumulate a variety of low molecular weight compounds, secondary metabolites and several enzymatic antioxidant defense pathways to quench ROS in order to cope up with stressful conditions. Superoxide Dismutase (SOD) plays a role in the dismutation of  $O_2^-$  to  $H_2O_2$  and molecular oxygen. Different types of catalases and peroxidases are involved in quenching  $H_2O_2$  into water and oxygen molecule by oxidation, thereby protecting the cell from oxidative damage (Sharma *et al.*, 2023). However, the information regarding the impact of heat stress as well as response of this crop to heat stress during flowering stage is rarely available. Breeding efforts for temperature tolerance in blackgram are hampered due to less information on the traits governing the heat tolerance and the unavailability of tolerant blackgram genotypes. Hence, it is important to understand the genotypic variability and the physiological and biochemical mechanisms governing heat stress tolerance which can help in identifying heat tolerant blackgram genotypes that can yield better under climate change scenarios. Heat tolerant blackgram genotypes can bring more areas under cultivation during summer season as well as the cultivation can be extended to warmer locations also.

In this regard, cluster analysis and principal component analysis (PCA) are the reliable methods to assess genetic variability (Tiwari *et al.* 2020). Cluster analysis helps in grouping of genotypes on the basis of taking  $D^2$  values as the measure of generalized distance between two closely associated genotypes (Rao, 1952). Multivariate analysis with the  $D^2$  technique estimates the nature of genetic diversity in a given population with respect to several traits (Sarma *et al.*, 2021). PCA helps to pick up the component traits based on their relative importance contributing towards the total variability in a data set where PC1 describes the most variation, PC2 describes the second most variation and so on (Gupta and Khandelwal, 2022). Thus, the primary objective was to assess the genotypic variability for physiological, reproductive, biochemical and yield traits to identify the heat tolerant climate resilient blackgram genotypes.

## MATERIALS AND METHODS

Thirty blackgram genotypes were evaluated for high temperature stress tolerance at College Farm (15°54' N latitude and 80°47' E longitude), Agricultural College, Bapatla, Andhra Pradesh, India during summer, 2022 and 23. The experiment was conducted in randomized block design with two replications. The mean maximum temperature was 36.0 and 37.2 °C at flowering during summer, 2022 and 23, respectively (Fig. 1). Observations pertaining to physiological, reproductive and biochemical traits were recorded at flowering stage. The genotypes were evaluated for physiological [total chlorophyll, carotenoids, chlorophyll stability index (CSI) , membrane

injury index (MII) and canopy temperature depression (CTD)], reproductive [pollen viability (PV), pollen germination percentage (PGP) and flower to pod setting percentage (FPSP)], biochemical [superoxide radical (SOR), hydrogen peroxide (HPR), malondialdehyde (MDA), proline, superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APOX)] and yield [number of pods per plant (NPP), number of pod clusters per plant (NPCP), pod weight (PW) and seed yield per plant (SYP)] traits at reproductive stage during both the years.

The mean values of various physiological, reproductive, biochemical and yield traits were subjected to statistical analysis as per the Randomized Block Design (RBD) as described by Panse and Sukhatme (1985). Phenotypic coefficients of variation (PCV) and Genotypic coefficients of variation (GCV) were computed according to Burton and Devane (1953). Heritability in broad sense was calculated according to the formula suggested by Hanson *et al.* (1956). The mean data set over the years was subjected to diversity analysis using Mahalanobis  $D^2$  Analysis (Mahalanobis, 1936) and clustering was done by Tocher's method following Rao (1952). Further, PCA was carried out following Singh and Choudhury (1988). Both the analysis was carried out using INDOSTAT software.

## RESULTS AND DISCUSSION

The analysis of variance indicated significant variability with respect to all the 20 traits tested for heat tolerance in 30 blackgram genotypes. The individual year wise mean, range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and heritability during summer, 2022 and 23 are presented in the **Table 1**. GCV and PCV are simple measures for the assessment of genetic variability. These measures give an idea about the magnitude of variability present in a genetic population. The highest value for PCV and GCV was observed for SYP (59.9 and 59.5) followed by NPP (51.2 and 50.8) during summer, 2022 and SYP (59.9 and 59.6) followed by NPP (51.3 and 50.8) during summer, 2023, respectively. In the present study, it was recorded that PCV values were higher than GCV value for all the traits. This indicated more effect of environment on the traits under heat stress conditions. Results were significantly concurrence with Neeru and Singh (2017), Rajashree and Singh (2018) and Ramanuj *et al.*, (2018) in wheat and Mani and Deshpandae (2017) in maize. The presence of significant variations indicated the worth of the materials under study for further diversity and PCA analysis. Heritability gives basic information about the parameters which help breeders in effective selection. Heritability ranged from 33.6 to 98.8 during summer, 2022 and 29.6 to 98.8 during summer, 2023. During summer, 2022 higher heritability was recorded for SYP (98.8) followed by NPP (98.4), whereas during summer 2023, higher heritability was recorded for NPP (97.8) followed by SYP (95.7). Similar results were previously reported by Mani and Deshpandae (2017) in maize.

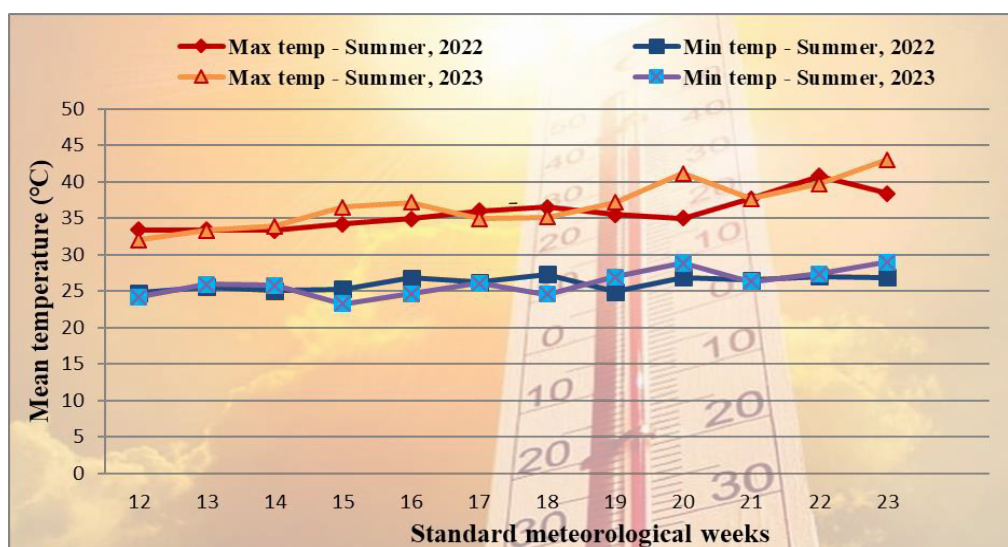


Fig. 1. Standard week wise mean temperatures during summer, 2022 and 23

Table 1. Mean, range, PCV, GCV and heritability for physiological, reproductive, biochemical and yield traits during summer, 2022 and 23

	Summer, 2022					Summer, 2023				
	Mean	Range	PCV	GCV	Heritability	Mean	Range	PCV	GCV	Heritability
Total Chlorophyll	1.03	0.76-1.25	11.1	8.7	60.9	0.9	0.6-1.1	11.3	9.5	71.2
Carotenoids	0.78	0.60-1.02	14.7	13.0	78.3	0.7	0.52-0.83	12	10.1	72.0
CSI	75.77	58.5-91.1	12.0	9.9	67.9	74.9	64.8-89.4	10	7.5	55.3
MII	30.28	23.5-38.4	12.3	10.2	68.9	31.0	26.6-64.8	9.9	7.8	61.1
CTD	1.38	(-0.33)-(-2.75)	40.6	39.9	96.9	-1.1	(+0.42)-(-2.25)	48.4	47.6	96.7
PV	73.42	58.2-83.5	7.6	4.4	33.6	64.5	49.2-74.7	8.5	4.6	29.6
PGP	62.52	49.6-75.6	8.6	6.0	47.8	56.8	45.1-66.3	9.4	6.9	53.0
FPSP	63.73	50.2-75.8	8.7	6.0	54.6	51.5	40.5-52.8	8.7	6	61.3
SOR	1.35	1.04-1.81	12.5	10.8	75.1	1.7	1.5-2.1	12.5	10.9	50.6
HPR	7.34	5.6-9.2	10.9	8.8	64.3	8.5	7.1-11.0	11	8.8	70.1
MDA	13.38	10.5-16.4	10.5	8.5	64.9	17.0	13.5-21.7	11	8.5	72.4
Proline	1.25	0.87-1.63	16.0	14.4	81.0	1.60	1.0-2.0	16	14.4	80.0
SOD	1.01	0.7-1.4	17.3	15.9	83.8	1.5	0.8-2.1	17.4	15.9	83.0
CAT	3.77	2.8-5.2	14.5	13.0	81.0	5.1	3.8-7.4	14.5	13	85.6
POX	14.63	11.2-20.9	17.3	16.0	85.4	19.9	13.4-26.6	17.3	16	85.9
APOX	35.08	26.3-42.8	10.8	8.3	58.8	39.3	29.3-49.5	10.8	8.3	69.5
NPCP	4.42	2.2-7.9	36.9	35.9	94.7	3.7	2.3-6.02	36.9	35.9	94.5
NPP	12.52	4.0-25.8	51.2	50.8	98.4	9.9	3.5-17.3	51.3	50.8	97.8
PW	4.14	1.5-6.7	41.7	41.2	97.5	3.2	1.9-5.2	41.7	41.2	92.6
SYP	2.52	0.8-5.4	59.9	59.5	98.8	2.0	1.1-3.2	59.6	59.9	95.7

Cluster analysis: Mahalanobis  $D^2$  analysis was used for clustering of genotypes. The 30 blackgram genotypes were grouped into six clusters, during summer, 2022. Out of six clusters formed, cluster I and II were largest comprising of maximum number of nine genotypes

followed by cluster III with eight genotypes, cluster IV with two genotypes and cluster V and VI with one genotype each (Table 2). The highest inter cluster distance was observed between cluster III and cluster IV (2901.55) followed by cluster II and III (2454.16) (Table 3).

**Table 2. Clustering pattern of blackgram genotypes grown under high temperature stress during summer, 2022 by Tocher's method**

S.No.	Cluster number	Total number of genotypes	Genotypes
1	I	9	VBN-8, PU-1801, LBG-1006, LBG-1010, LBG-752, PUSA B-58, LBG-932, LBG-645 and LBG-918
2	II	9	LBG-999, LBG-996, Tutimumu, LBG-904, LBG-989, LBG-997, LBG-1009, PU-1822 and OBG-48
3	III	8	TBG-141, LBG-995, PU-31, PU-1804, TBG-104, LBG-1015, LBG-1004 and TBG-129
4	IV	2	TBG-125 and LBG-1023
5	V	1	LBG-1016
6	VI	1	GBG-1

**Table 3. Average intra and inter-cluster distances ( $D^2$  values) among 6 clusters of blackgram genotypes during summer, 2022**

	I	II	III	IV	V	VI
I	83.19	344.92	1165.86	591.46	169.19	436.59
II		89.01	2454.16	310.55	739.95	1233.96
III			146.10	2901.55	648.73	324.45
IV				29.96	945.10	1739.66
V					0.00	169.85
VI						0.00

Hybridization between genotypes of cluster III with either IV or II will be useful to obtain maximum hybrid vigour and may result in desirable segregants. Kuldeep *et al.*, (2015) obtained 16 clusters from 100 chickpea genotypes grown under heat stress conditions.

Cluster III (TBG-141, LBG-995, PU-31, PU-1804, TBG-104, LBG-1015, LBG-1004 and TBG-129) showed higher cluster mean for all the physiological parameters such as total chlorophyll, carotenoids, CSI and CTD indicating the role of physiological mechanisms in conferring heat tolerance. Cluster III recorded higher cluster mean for reproductive characters such as PV, PGP and FPSP indicating heat tolerance. In addition to this, cluster III recorded higher cluster mean for biochemical characters such as proline, SOD, CAT, POX and APOX whereas, lower cluster mean for HPR and MDA indicating their strong antioxidant defence system which might have scavenged the free radicals and reduced the MDA content. Moreover, cluster III recorded higher cluster mean for yield traits such as NPCP, NPP, PW and SYP. Hence, the genotypes from cluster III can be considered as heat tolerant genotypes and can be recommended for future breeding programmes for developing thermotolerant genotypes.

Lowest cluster mean for total chlorophyll, carotenoids, CSI, CTD, PV, PGP, FPSP, proline, SOD, CAT, POX, APOX activity, NPCP, NPP, PW and SYP was recorded in cluster IV (TBG-125 and LBG-1023) indicating their susceptibility to high temperatures (Table 4). Whereas,

cluster IV recorded higher cluster mean for MII, SOR, HPR and MDA reflecting increase in the membrane damage due to lipid peroxidation leading to MDA accumulation, which might be the reason behind lower yield in these genotypes. The order of heat tolerance based on cluster means is cluster III > cluster IV > cluster I > cluster II > cluster V and cluster IV.

During summer, 2023 the 30 blackgram genotypes were grouped into five clusters (Table 5). Out of five clusters formed, cluster II was the largest comprising of 10 genotypes followed by cluster IV with 7 genotypes, cluster I with 5 and Cluster III and V were aggregated with 4 genotypes. The highest inter cluster distance was observed between cluster I and cluster V (3033.69) and cluster I and III (2065.90) which indicated that the genotypes included in these different clusters may give high heterotic response and thereby better segregants (Lal *et al.*, 2001) (Table 6).

Cluster I (TBG-129, LBG-1015, PU-31, TBG-104 and PU-1804) showed higher cluster mean for all the physiological parameters such as total chlorophyll, carotenoids and CSI indicating the role of physiological mechanisms in imparting heat tolerance at cellular level. Cluster III (LBG-1004, GBG-1, TBG-141 and LBG-995) recorded higher cluster mean for CTD and peroxidase activity. Cluster I recorded higher cluster mean for reproductive efficiency characters such as PV, PGP and FPSP which might be the reason behind higher yield. In addition to this, cluster I recorded higher cluster mean for biochemical characters

**Table 4. Cluster means of six clusters estimated by Tocher's method in blackgram genotypes during summer, 2022**

S.No.	Character	Cluster number					
		I	II	III	IV	V	VI
1	Total Chlorophyll	1.03	1.01	1.14	0.77	0.97	1.14
2	Carotenoids	0.76	0.75	0.91	0.61	0.69	0.88
3	CSI	75.58	69.30	86.39	59.11	76.70	83.38
4	MII	30.60	32.39	26.32	37.41	29.38	26.74
5	CTD	-1.39	-1.77	-0.74	-2.58	-1.18	-0.84
6	PV	72.64	71.10	78.78	64.89	72.14	76.05
7	PGP	61.60	59.81	68.37	52.92	64.24	66.09
8	FPSP	65.36	59.07	68.60	54.40	66.40	68.20
9	SOR	1.39	1.40	1.20	1.71	1.36	1.04
10	HPR	7.47	7.67	6.57	8.91	7.26	6.54
11	MDA	13.89	13.92	11.79	15.73	13.18	12.48
12	Proline	1.20	1.17	1.48	0.91	1.15	1.42
13	SOD	1.00	0.91	1.22	0.69	1.02	1.14
14	CAT	3.76	3.46	4.34	2.88	3.75	4.10
15	POX	13.32	13.44	17.96	11.59	13.38	18.09
16	APOX	33.81	34.28	38.87	28.13	35.00	37.50
17	NPCP	4.06	3.20	6.73	2.26	4.20	4.83
18	NPP	11.57	7.50	21.17	4.42	10.83	15.00
19	PW	4.08	2.43	6.39	1.86	4.74	6.10
20	SYP	2.21	1.14	4.72	0.83	2.86	3.38

**Table 5. Clustering pattern of blackgram genotypes grown under high temperature stress during summer, 2023 by Tocher's method**

S.No.	Cluster number	Total number of genotypes	Genotypes
1	I	5	TBG-129, LBG-1015, PU-31, TBG-104 and PU-1804
2	II	10	LBG-752, LBG-904, LBG-1009, LBG-989, OBG-48, LBG-918, LBG-645, LBG-999, Tutimumu and PU-1822.
3	III	4	LBG-1004, GBG-1, TBG-141 and LBG-995
4	IV	7	VBN-8, PU-1801, LBG-1006, LBG-1016, PUSA B-58, LBG-1010 and LBG-932
5	V	4	TBG-125, LBG-1023, LBG-996 and LBG-997

**Table 6. Average intra and inter-cluster distances ( $D^2$  values) among 5 clusters of blackgram genotypes during summer, 2023**

	I	II	III	IV	V
I	100.94	2065.90	300.49	1099.38	3033.69
II		109.89	1159.00	272.50	259.53
III			122.68	509.21	1962.21
IV				106.00	656.90
V					231.83

such as proline, SOD, CAT, POX and APOX whereas, lower cluster mean for SOR, HPR and MDA was recorded for cluster I indicating strong antioxidant defence which might have scavenged the free radicals and MDA.

Moreover, cluster I recorded higher cluster mean for yield traits such as NPCP, NPP, PW and SYP. Hence, the genotypes from cluster I can be considered as heat tolerant genotypes and can be recommended for future

**Table 7. Cluster means of five clusters estimated by Tocher's method in blackgram genotypes during summer, 2023**

S.No	Character	Cluster number				
		I	II	III	IV	V
1	Total Chlorophyll	1.00	0.85	0.97	0.87	0.79
2	Carotenoids	0.76	0.63	0.75	0.65	0.59
3	CSI	82.51	71.49	80.79	76.25	65.80
4	MII	27.62	31.58	29.18	31.25	35.50
5	CTD	-0.75	-1.22	-0.31	-1.12	-1.90
6	PV	69.70	62.18	68.83	63.33	61.64
7	PGP	63.51	55.01	60.97	56.28	49.67
8	FPSP	57.28	48.84	56.00	51.49	46.15
9	SOR	1.55	1.77	1.58	1.73	1.95
10	HPR	7.50	8.77	7.83	8.58	9.68
11	MDA	14.87	17.77	15.15	17.11	19.55
12	Proline	1.87	1.60	1.80	1.50	1.32
13	SOD	1.98	1.40	1.89	1.50	1.09
14	CAT	6.71	4.71	5.63	4.80	4.20
15	POX	24.07	18.37	24.22	19.57	14.98
16	APOX	46.45	37.83	42.19	37.96	33.38
17	NPCP	5.52	3.16	4.22	3.46	2.36
18	NPP	16.26	7.39	13.31	9.09	5.97
19	PW	4.86	2.51	3.90	3.24	2.08
20	SYP	3.05	1.55	2.76	1.88	1.34

breeding programmes for developing thermotolerant genotypes (Table 7).

Lowest cluster mean for total chlorophyll, carotenoids, CSI, CTD, PV, PGP, FPSP, proline, SOD, CAT, POX, APOX activity, NPCP, NPP, PW and SYP was recorded in cluster V (TBG-125, LBG-1023, LBG-996 and LBG-997) indicating susceptibility to high temperatures. Whereas, cluster V recorded higher cluster mean for MII, SOR content, HPR and MDA reflecting increase in the membrane damage due to lipid peroxidation leading to MDA accumulation which might be the reason behind lower yield. The order of heat tolerance based on cluster means is cluster I > cluster III > cluster IV > cluster II and cluster V. Similar method of cluster analysis was followed for the identification of terminal heat tolerant genotypes in wheat (Mohanty *et al.*, 2017) and drought tolerant genotypes in blackgram (Mohanlal *et al.*, 2018). The clustering analysis helped in grouping these genotypes into different groups which can be used in genetic enhancement program for further improvement for stress tolerance in black gram. Moreover, our results were in accordance with Gurumurthy *et al.*, 2019 in blackgram who performed cluster analysis based on physiological and biochemical traits under drought stress conditions.

**Principal Component Analysis:** Principal component analysis (PCA) was used to find out the relative contribution of the traits towards the total variability and to provide a

guide for selection of traits. In the present investigation, during summer, 2022 the first three principal components with eigen values more than one contributed 82.62 per cent towards the total variability. The results implied that first principal components with eigen value 13.50 contributed maximum towards variability (67.53 %). The characters that contributed maximum variance in the PC1 were CSI (0.266), CTD (0.261), proline (0.263), NPCP (0.262), SYP (0.248), total chlorophyll (0.237), FPSP (0.231), SOD (0.231), NPP (0.230), PW (0.233), carotenoids (0.215), PGP (0.177) and PV (0.143) indicating their significance in divergence. Many other traits *viz.*, SOR (-0.261), HPR (-0.240), MII (-0.233), APOX (-0.201), MDA (-0.051) and CAT (-0.044) exhibited negative loadings. The second principal component with eigen value 1.65 contributed 8.25 per cent contribution towards variability through positive character loadings of PGP (0.405), carotenoids (0.393), HPR (0.216), SOD (0.128), CSI (0.111), total chlorophyll (0.101), APOX (0.056), SOR (0.029) and proline (0.008). The other characters like pollen viability (-0.516), MDA (-0.387), FPSP (-0.307), CAT (-0.228), MII (-0.088), NPCP (-0.078), PW (-0.079), POX (-0.060), NPP (-0.038), CTD (-0.036) and SYP (-0.01) manifested negative character loadings.

The third principal component with eigen value 1.36 contributed 6.83 per cent towards variability through significant positive loadings of MDA (0.581), APOX (0.286), total chlorophyll (0.108), CTD (0.104), carotenoids

(0.088), FPSP (0.086), CSI (0.059), NPCP (0.023), SOD (0.019), NPP (0.019) and HPR (0.008). Many other traits viz., CAT (-0.571), PV (-0.251), PGP (-0.249), MII (-0.237), SYP (-0.110), PW (-0.055), proline (-0.049) and POX (-0.024) exhibited negative loadings.

The PCA thus identified that the maximum contributing traits towards the existing variability were MDA, PGP, carotenoids, CSI, CTD, proline, NPCP and SYP. It is a common practice to select one variable from each of the identified groupings. For this reason, CSI, CTD, proline and NPCP were the best choice for the first group, which had the largest loading from component one, PGP for the second and MDA for third group.

The genotypes numbered 4, 5, 6, 24, 13, 2, 12, 18, 16, 17, 20, 28 and 30 *i.e.*, TBG-129, PU-1804, LBG-1015, TBG-104, TBG-141, LBG-1004, GBG-1, PU-31, LBG-995, OBG-48, Tutimumu, TBG-125 and LBG-1023 were placed distantly from other genotypes in the 3D plot confirming the Tocher's clustering in which these genotypes were placed in four different clusters viz., cluster III (TBG-129, PU-1804, LBG-1015, TBG-104, TBG-141, LBG-1004, PU-31 and LBG-995), V (GBG-1) II (OBG-48 and Tutimumu) and IV (TBG-125 and LBG-1023). The genotype diversity can be exploited for generating transgressive segregants in crop improvement programmes (Fig. 2).

During summer, 2023 the first two principal components with eigen values more than one contributed 77.06 per cent towards the total variability. The results implied that first principal components with eigen value 12.84 contributed maximum towards variability (64.23 %). The

characters that contributed maximum variance in the PC1 were SOD (0.268), APOX (0.262), SYP (0.261), PGP (0.258), FPSP (0.254), PW (0.252), NPP (0.251), CTD (0.240), POX (0.242), CAT (0.229), NPCP (0.224), total chlorophyll (0.220), carotenoids (0.218), PV (0.177) CSI (0.138) and HPR (0.026) indicating their significance in divergence. Many other traits viz., MII (-0.229), MDA (-0.229), SOR (-0.228) and proline (-0.077) exhibited negative loadings. The second principal component with eigen value 2.56 contributed 12.82 per cent contribution towards variability through positive character loadings of proline (0.47), total chlorophyll (0.28), PV (0.241), CAT (0.230), PGP (0.151), MII (0.105) and FPSP (0.054). The other characters like HPR (-0.463), CSI (-0.425), MDA (-0.233), POX (-0.203), SOR (-0.140), PW (-0.105), CTD (-0.095), NPP (-0.071), SOD (-0.069), APOX (-0.047), carotenoids (-0.040), NPCP (-0.017) and SYP (-0.013) manifested negative character loadings.

The PCA thus identified that the maximum contributing traits towards the existing variability were proline, total chlorophyll, SOD, APOX, SYP, PGP, FPSP, NPP, PW, CTD and POX. It is a common practice to select one variable from each of the identified groupings. For this reason, SOD, APOX and SYP were the best choice for the first group, which had the largest loading from component one and proline for the second group.

A three dimensional (3D) scattered diagram was obtained using these principal component varieties. The genotypes numbered 4, 5, 6, 18, 24, 13, 12, 2, 16, 8, 27, 28 and 30 *i.e.*, TBG-129, PU-1804, LBG-1015, PU-31, TBG-104, TBG-141, GBG-1, LBG-1004, LBG-995, LBG-997, LBG-996, TBG-125 and LBG-1023 were placed distantly from

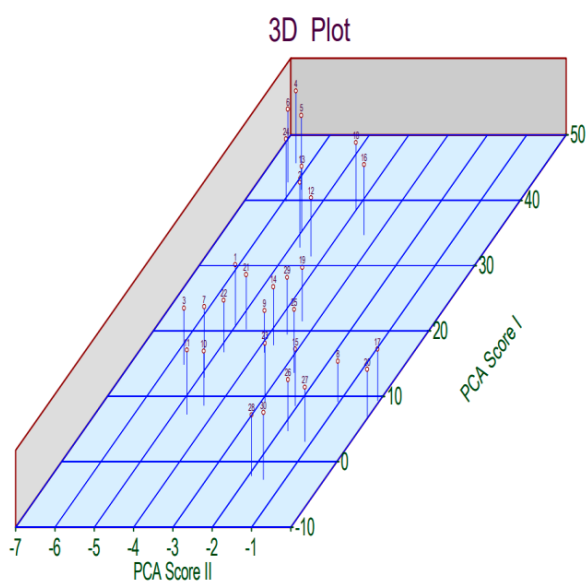


Fig. 2. Three dimensional (3D graph showing relative positions of 30 genotypes based on PCA scores) during summer, 2022

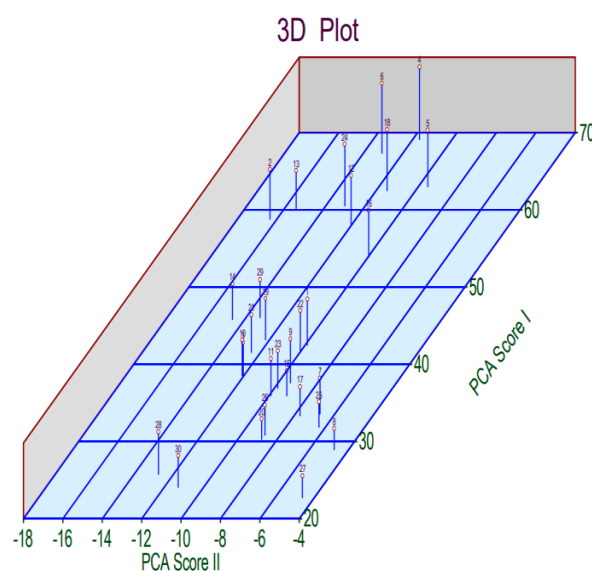


Fig. 3. Three dimensional (3D graph showing relative positions of 30 genotypes based on PCA scores) during summer, 2023

other genotypes in the 3D plot confirming the Tocher's clustering in which these genotypes were placed in three different clusters viz., cluster I (TBG-129, PU-1804, LBG-1015, PU-31 and TBG-104), III (TBG-141, GBG-1, LBG-1004 and LBG-995) and V (TBG-125 and LBG-1023) (Fig. 3). The PCA will increase the accessibility for the plant physiologist and plant breeders for discovering the number of plants to be assessed and characteristics that could be used to choose the tolerant genotypes. Similar findings were previously reported by Kushwah *et al.*, (2021) in blackgram and Mani and Deshpandae (2017) in maize.

From the above results, it can be concluded that the genotypes TBG-129, LBG-1015, PU-31, TBG-104 and PU-1804 were found to withstand high temperatures during both the years by up-regulating various physiological and biochemical pathways at cellular level. This up-regulation of physiological and biochemical mechanisms in response to high temperature stress might be the reason behind high yield in these genotypes. The genotypes TBG-125 and LBG-1023 recorded lower SYP indicating the susceptibility of these genotypes to high temperature stress during both the years. Moreover, the results of principal component analysis revealed that these genotypes were scattered relatively far away from other genotypes in this 3D plot, confirming the Tocher's clustering in which these genotypes were placed in three different clusters during summer, 2022 and 23. These genotypes can be exploited for generating transgressive segregants in crop improvement programmes. In addition to this, results have paved the way by identifying the key traits governing the heat tolerance which can be further used for screening heat tolerant genotypes. The genotypes should be evaluated across multilocations for confirming their tolerance to high temperature stress.

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