



Variation among Blackgram (*Vigna mungo* L.) Genotypes for Antioxidant Defence System and Yield under High Temperature Stress

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ABSTRACT

Background: Blackgram is a short duration pulse crop which is sensitive to high temperatures. Raising global temperatures are becoming a serious threat to the production of blackgram by altering biochemical processes at cellular level. Hence, the present investigation was carried out for better understanding of genotypic variability and the biochemical mechanisms governing heat stress tolerance which can help in identifying heat tolerant blackgram genotypes that can yield better under climate change scenarios.

Methods: Thirty blackgram genotypes selected from temperature induction response technique were evaluated for biochemical efficiency under natural high temperature conditions during summer, 2022 and 23. Field experiment was conducted at Agricultural College Farm, Acharya N.G Ranga Agricultural University, Agricultural College, Bapatla. Biochemical and yield parameters were recorded at flowering and the data were analyzed statistically and pooled.

Result: The results of the study revealed that the genotypes TBG-129, LBG-1015, PU-1804 and PU-31 recorded higher seed yield indicating their ability to withstand high temperatures by up-regulating various biochemical pathways involved in increased production of proline, carotenoids and antioxidant defense enzymes that might have scavenged the free radicals that were produced due to high temperature stress, thereby preventing lipid peroxidation. Lower seed yield was recorded in TBG-125 and LBG-1023 which might be due to oxidative damage caused by higher accumulation of free radicals and poor scavenging activity of antioxidant enzymes. Moreover, the results of correlation analysis revealed that all the biochemical traits such as proline, carotenoids and antioxidant defense enzymes showed positive association with seed yield except free radicals and malondialdehyde. The principal component analysis results revealed considerable variability among the traits accounting for 80.0%. The genotypes TBG-129, LBG-1015, PU-1804 and PU-31 can be used in breeding programmes for development of heat tolerant genotypes.

Key words: Antioxidant defense enzyme activity, Blackgram, High temperature stress, Principal component analysis.

INTRODUCTION

Blackgram is one of the important pulse crop which is rich in proteins, carbohydrates, vitamins and minerals. Often the productivity of blackgram is limited by environmental extremities such as heat stress, drought, salinity and heavy metal stress etc. In particular, the rising temperatures are raising apprehension regarding crop productivity and food security. Global air temperature is predicted to rise by 0.2°C per decade, which will lead to 1.8-4.0°C higher temperatures than the current level by 2100 (Hasanuzzaman *et al.*, 2013). Blackgram being a thermosensitive crop, its yield is sensitive to high temperature above 35°C. Under these circumstances, there is a need to screen blackgram genotypes for thermotolerance and identify the traits governing heat tolerance at cellular level.

High temperature stress causes production and accumulation of reactive oxygen species (ROS) resulting in oxidative stress. The accumulated ROS reacts with the lipids, proteins and nucleic acids leading to lipid peroxidation and DNA mutations (Van *et al.*, 2001). Lipid peroxidation is an indicator of membrane damage which can be detected by malondialdehyde (MDA) content. Plants respond to these damages by up-regulating various mechanisms involving in the production of osmolytes, secondary metabolites and

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antioxidant defense pathways in order to ensure their survival under stress conditions. Plants accumulate a variety of low molecular weight compounds in order to cope up with stressful conditions. Proline is one such low molecular weight compound which aids in avoiding stressful conditions by osmotic adjustment. In addition to this, it also plays a major role in ROS detoxification thereby protecting thylakoid membranes from photo-oxidative damage (Kishor *et al.*, 2005). Plants also increase the production of secondary metabolites which increase the survival under stressful conditions. Carotenoids are important secondary metabolite which acts as molecular antioxidants in cell by scavenging singlet oxygen (Knox and Dodge, 1985). Hence, the increase in levels of proline and carotenoids are of much importance in determining relative tolerance of genotypes to high temperature stress. Moreover, plants also activate several enzymatic antioxidant defense pathways to quench ROS. 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).

Genotypes vary in their ability to withstand high temperature stress. Principal component analysis was used to assess genetic variability in blackgram genotypes. However, the information regarding the role of antioxidant defense enzyme activity in conferring heat tolerance in blackgram genotypes under high temperature stress is rarely available. Hence, the present investigation was carried out to understand the genotypic variability and biochemical mechanisms underlying the high temperature tolerance in blackgram genotypes.

MATERIALS AND METHODS

Blackgram genotypes (30) were procured from AICRIP (pulses), Regional Agricultural Research Station (RARS), Lam, Guntur, Andhra Pradesh, India. The 27 tolerant and 3 susceptible genotypes selected from TIR technique were further assessed for biochemical and yield traits under natural high temperature conditions during summer 2022 and 23 at College Farm, Agricultural College, Bapatla, Acharya N.G Ranga Agricultural University. The weather parameters during cropping season were presented in (Fig 1). Observations such as free radicals *i.e.*, Superoxide radical (SOR), Hydrogen peroxide radical (HPR), Malondialdehyde (MDA), proline, carotenoids, Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POX) and Ascorbate peroxidase (APOX), Number of pods per plant (NPP), seed yield per plant (SYP) and Harvest Index (HI) were measured at 50% flowering during both the years as it is more sensitive to high temperature stress. The mean maximum temperature was 36.0 and 37.2° at flowering during summer, 2022 and 23, respectively.

Quantification of free radicals and lipid peroxidation

Superoxide free radical (SOR) was quantified by its capacity to reduce nitroblue tetrazolium chloride (NBT) by following

the method of Chaitanya and Naithani (1994). Hydrogen peroxide (HPR) was estimated by formation of titanium-hydroperoxide complex (Mukherjee and Choudhari 1983). The amount of MDA derived from unsaturated fatty acid peroxidation of membrane lipids was measured according to the method of Sese and Tobita (1998).

Proline and carotenoid content

The amount of proline was assayed according to Bates *et al.* (1973) and Carotenoid content was estimated by the following method of Arnon, (1949).

Antioxidant defense enzymes

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) using the method of Dhindsa *et al.* (1981). The extraction and assay of POX was carried out as per the method described by Putter (1974). Catalase (CAT) activity was determined spectrophotometrically by measuring the rate of H_2O_2 disappearance at 240 nm (Aebi, 1974). Ascorbate peroxidase (APOX) was assayed by the method as described by Nakano and Asada (1981).

Yield and yield attributes

Yield parameters such as number of pods per plant (NPP), seed yield per plant (SYP) and Harvest Index (HI) were recorded at harvest during both the years.

Statistical analysis

The data were analyzed statistically by following analysis of variance technique suggested by Panse and Sukhatme, (1985) for randomized block design (RBD). The statistical hypothesis of equalities of treatment means was tested by F-test at 1 to 5% per cent level of significance. The data collected on biochemical and yield traits were subjected to correlation analysis in OPSTAT and PCA was performed using R software.

RESULTS AND DISCUSSION

Superoxide radical content

Pooled data of two seasons revealed that SOR content varied significantly among the blackgram genotypes with mean values ranged from 1.90 and 1.30 g plant⁻¹. SOR content was lesser in TBG-129, GBG-1 (1.30 change in OD min⁻¹ g⁻¹ f.wt.) which was at par with PU-31 and TBG-104 (1.36 change in OD min⁻¹ g⁻¹ f.wt.) while, it was higher in TBG-125 (1.90 change in OD min⁻¹ g⁻¹ f.wt.) followed by LBG-1023 (1.89 change in OD min⁻¹ g⁻¹ f.wt.) (Fig 1). This decrease in SOR content in thermotolerant genotypes was due to increased SOD activity which might have scavenged the superoxide radicals. Similar findings of lower levels of SOR content in heat tolerant genotypes was previously reported by Jincy *et al.* (2022) in greengram, which support our current results.

Hydrogen peroxide content

Pooled data of two seasons revealed that there was significant variation among all the genotypes with respect

to HPR content. The mean values of HPR ranged from 6.55 and 10.08 $\mu\text{mol H}_2\text{O}_2\text{ g}^{-1}$. Lesser HPR content was recorded in TBG-129 (6.55 $\mu\text{mol H}_2\text{O}_2\text{ g}^{-1}$) which was at par with PU-1804 (6.58 $\mu\text{mol H}_2\text{O}_2\text{ g}^{-1}$), TBG-104 (7.11 $\mu\text{mol H}_2\text{O}_2\text{ g}^{-1}$) and LBG-1015 (7.13 $\mu\text{mol H}_2\text{O}_2\text{ g}^{-1}$) while, it was higher in TBG-125 (10.08 $\mu\text{mol H}_2\text{O}_2\text{ g}^{-1}$) followed by LBG-1023 (9.39 $\mu\text{mol H}_2\text{O}_2\text{ g}^{-1}$) (Fig 1). Similar findings of lower levels of HPR content in heat tolerant genotypes was previously reported by Almeselmani *et al.* (2009) in wheat and Jincy *et al.* (2022) in greengram, which support our current results. The increase in HPR content in the susceptible genotypes indicates the decrease in capacity of hydrogen peroxide scavenging system.

Malondialdehyde

Heat stress significantly affected the total chlorophyll content with mean values ranging from 12.26 to 19.07 $\mu\text{mol g}^{-1}$. MDA content was lower in TBG-129 (12.26 $\mu\text{mol g}^{-1}$) which was at par with LBG-995 (12.61 $\mu\text{mol g}^{-1}$) and PU-31 (12.88 $\mu\text{mol g}^{-1}$) while, it was higher in TBG-125 (19.07 $\mu\text{mol g}^{-1}$) followed by LBG-1023 (17.56 $\mu\text{mol g}^{-1}$) (Fig 1). Similar findings of lower levels of MDA in heat tolerant genotypes were previously reported by Jincy *et al.* (2022) in greengram and Sharma *et al.* (2023) in fieldpea. This reduction in MDA content of heat tolerant genotypes was due to increased activity of antioxidant defense enzymes which might have scavenged the ROS, thereby protecting cellular membranes from lipid peroxidation.

Proline

Proline content varied significantly among the genotypes with mean values of 0.99 and 1.77 $\mu\text{g g}^{-1}$ f.wt. Higher proline content was recorded in PU-1804 (1.77 $\mu\text{g g}^{-1}$) which was at par with TBG-129 (1.76 $\mu\text{g g}^{-1}$) and LBG-1015 (1.75 $\mu\text{g g}^{-1}$) while, it was lower in LBG-1023 (0.99 $\mu\text{g g}^{-1}$) followed by TBG-125 (0.99 $\mu\text{g g}^{-1}$) (Fig 2). Similar findings of increase

in proline content in the heat tolerant genotypes were previously reported by Jincy *et al.* (2022) in blackgram. Proline protects the plant cell from ROS damage by osmotic adjustment, ROS detoxification, membrane and enzyme stabilization (Divyaprasanth *et al.*, 2020).

Carotenoid content

Carotenoid content varied significantly among the genotypes with mean values of 0.59 and 0.89 mg g^{-1} f.wt. Higher carotenoid content was recorded in TBG-129 (0.89 mg g^{-1}) followed by LBG-1015, PU-31 (0.86 mg g^{-1}), LBG-995 (0.83 mg g^{-1}), GBG-1 (0.82 mg g^{-1}), PU-1804 and LBG-1004 (0.81 mg g^{-1}) whereas, lesser carotenoid content was recorded in TBG-125 (0.59 mg g^{-1}) followed by LBG-1023 (0.60 mg g^{-1}), LBG-752 (0.65 mg g^{-1}) and LBG-1016 (0.66 mg g^{-1}) (Fig 2). Carotenoids act as protectors of chloroplast pigments and membrane structure by quenching triplet chlorophyll and removing oxygen from excited chlorophyll oxygen complex (Young, 1991), thereby provide protection against damage due to high temperature stress. Our results are in accordance with the published reports of Sharma *et al.* (2023) in fieldpea.

Superoxide dismutase activity

Significant genetic variability was observed among the genotypes with respect to SOD activity. SOD activity at flowering ranged from 0.88 and 2.14 $\text{U. g}^{-1}\text{FW min}^{-1}$. Higher SOD activity was recorded in TBG-129 (2.14 $\text{U. g}^{-1}\text{FW min}^{-1}$) which was at par with GBG-1 (2.08 $\text{U. g}^{-1}\text{FW min}^{-1}$) and PU-1804 (1.96 $\text{U. g}^{-1}\text{FW min}^{-1}$) while, lower in LBG-1023 (0.88 $\text{U. g}^{-1}\text{FW min}^{-1}$) followed by LBG-996 (1.06 $\text{U. g}^{-1}\text{FW min}^{-1}$) and TBG-125 (1.10 $\text{U. g}^{-1}\text{FW min}^{-1}$) (Fig 3). SOD is considered as the most efficient anti-oxidant enzyme. It plays a key role in quenching active oxygen by working as a catalyst to carry out the dismutation of $\text{O}_2^{\cdot-}$ into H_2O_2 (Fu and Huang, 2001). Similar findings of increase in SOD activity

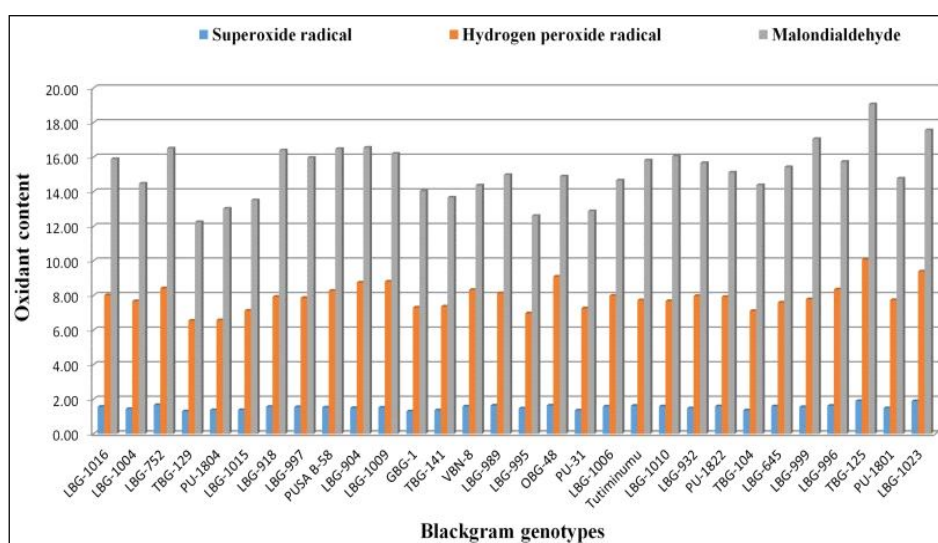


Fig 1: Effect of high temperature stress on oxidant content of blackgram genotypes (pooled data).

in the heat tolerant genotypes was previously reported by Almeselmani *et al.* (2009) in wheat and Sharma *et al.* (2023) in fieldpea.

Catalase activity

Catalase activity varied significantly among the genotypes with mean values of 3.78 and 5.12 $\mu\text{g H}_2\text{O}_2\text{ g}^{-1}\text{ min}^{-1}$. Higher catalase activity was recorded in TBG-129 (6.25 $\mu\text{g H}_2\text{O}_2\text{ g}^{-1}\text{ min}^{-1}$) followed by PU-1804 (5.75 $\mu\text{g H}_2\text{O}_2\text{ g}^{-1}\text{ min}^{-1}$) and LBG-1015 (5.63 $\mu\text{g H}_2\text{O}_2\text{ g}^{-1}\text{ min}^{-1}$) while, it was lower in TBG-125 (3.25 $\mu\text{g H}_2\text{O}_2\text{ g}^{-1}\text{ min}^{-1}$) which was at par with LBG-1023 (3.49 $\mu\text{g H}_2\text{O}_2\text{ g}^{-1}\text{ min}^{-1}$) (Fig 3). Lower catalase activity in TBG-125 and LBG-1023 might be due to heat stress induced inactivation of enzymes which resulted in accumulation of ROS leading to membrane damage. Similar findings were

previously reported by Almeselmani *et al.* (2009) in wheat who reported variations in heat stress-induced antioxidant enzyme activities between two wheat cultivars.

Peroxidase activity

Significant differences were observed among the genotypes with respect to POX activity. POX activity ranged from 12.62 and 23.78 $\text{U. g}^{-1}\text{ FW min}^{-1}$. Higher POX activity was recorded in TBG-129 (23.78 $\text{U. g}^{-1}\text{ FW min}^{-1}$) followed by GBG-1 (21.77 $\text{U. g}^{-1}\text{ FW min}^{-1}$), TBG-141 (21.47 $\text{U. g}^{-1}\text{ FW min}^{-1}$), LBG-1015 (21.35 $\text{U. g}^{-1}\text{ FW min}^{-1}$) and PU-1804 (20.44 $\text{U. g}^{-1}\text{ FW min}^{-1}$) while, it was lower in LBG-1023 (12.62 $\text{U. g}^{-1}\text{ FW min}^{-1}$) and TBG-125 (12.94 $\text{U. g}^{-1}\text{ FW min}^{-1}$) (Fig 4). POX is an enzyme that scavenges H_2O_2 generated by the dismutation of O_2^- catalyzed by SOD, hence its upregulation

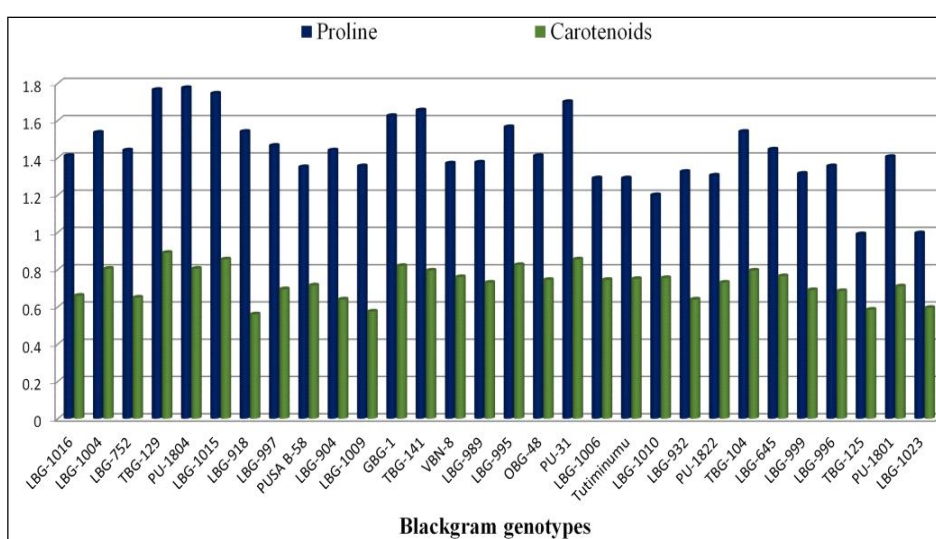


Fig 2: Effect of high temperature stress on proline and carotenoid content of blackgram genotypes (pooled data).

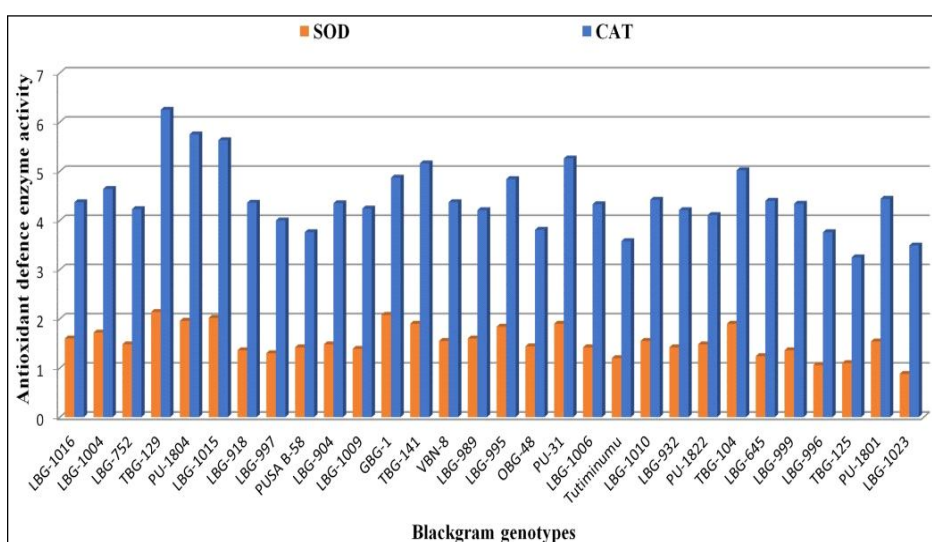


Fig 3: Effect of high temperature stress on SOD and CAT activity of blackgram genotypes (pooled data).

plays a significant role in ROS detoxification (Sharma *et al.*, 2023). Similar findings of increase in POX activity in the heat tolerant genotypes was previously reported by Almeselmani *et al.* (2009) in wheat and Sharma *et al.* (2023) in fieldpea.

Ascorbate peroxidase activity

Significant difference was observed among the genotypes with respect to APOX activity. APOX activity ranged from 31.13 and 45.38 U. g⁻¹ FW min⁻¹. Higher APOX activity was recorded in TBG-129 (45.38 μ mol) followed by pu-1804 (43.88 μ mol) and LBG-1015 (43.25 μ mol) while, lower in TBG-125 (27.75 μ mol) and LBG-1023 (31.13 μ mol) (Fig 4). The H₂O₂ scavenging enzyme, APOX, removes H₂O₂ efficiently, especially in the chloroplast where CAT is absent. Similar findings of increase in APOX activity in the heat tolerant genotypes was previously reported by Almeselmani *et al.* (2009) in wheat and Sharma *et al.* (2023) in fieldpea.

Yield and yield attributes

Pooled data of two seasons revealed that there was significant variation among all the genotypes with respect to all the yield and yield attributes. The NPP ranged from 3.8 to 21.5. NPP ranged from 3.8 to 21.5. The total NPP was higher in LBG-1015 (21.5) followed by PU-1804 (20.2), TBG-129 (19.9) and TBG-104 (19.8), whereas it was lower in TBG-125 (3.8) followed by LBG-1023 (4.7). The major reason for reduced yields due to heat stress was failure to set pods at high temperatures, especially by the heat sensitive genotypes. Our results agree with the published reports of Haritha (2020) who reported higher number of pods in thermotolerant genotypes.

SYP ranged from 1.0 to 4.3 g plant⁻¹. SYP was higher in LBG-1015 (4.3 g plant⁻¹) followed by PU-1804 and TBG-129 (4.1 g plant⁻¹) whereas, TBG-125 (1.0 g plant⁻¹) recorded lower SYP which was at par with LBG-999, LBG-1023

(1.1 g plant⁻¹). Reduction in seed yield of sensitive genotypes might be due to triggered flower abortion, pollen and ovule dysfunction which resulted in failure of fertilization, affecting seed filling and ultimately reduced the seed yield.

HI ranged from 15.7 to 17.3%. HI was higher in TBG-129 (30.3%) which was at par with LBG-1015 (28.2%) and PU-1804 whereas, it was lower in TBG-125 (15.7%) followed by LBG-1023 (17.3%). Higher HI of tolerant genotypes might be due to greater partitioning of photosynthates to sink. Similar results were previously reported by Devasirvatham *et al.* (2015) in chickpea.

Correlation analysis

Correlation studies among the biochemical and yield traits of blackgram genotypes grown under high temperature stress revealed vital results (Table 1). All the biochemical traits except SOR, HPR and MDA showed positive association with seed yield. Apart from this, SOR and HPR content showed a strong positive association with MDA content. This positive correlation of free radicals with MDA reflects the increase in the membrane damage due to lipid peroxidation leading to MDA accumulation. In addition to this, proline, carotenoids and all the antioxidant defense enzymes showed a strong negative correlation with free radicals. The occurrence of negative association of antioxidant defense enzymes with free radicals under heat stress conditions indicating the strong antioxidant defense activity, which might be the reason behind the higher seed yield in thermotolerant blackgram genotypes. Our results were in accordance with the published reports of Zafar *et al.* (2021) in cotton.

Principal component analysis

Principal component analysis was performed based on biochemical and yield traits of blackgram genotypes grown under heat stress environments. PCA analysis revealed

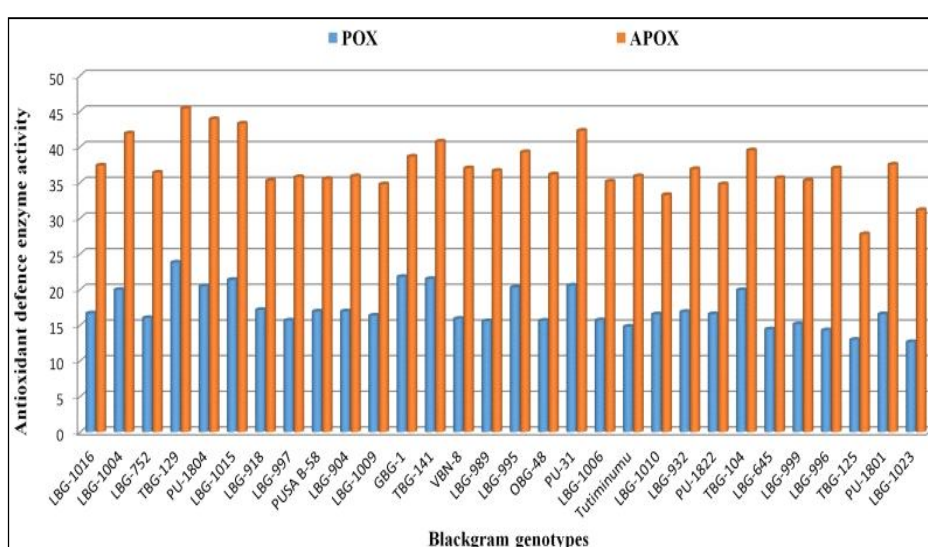


Fig 4: Effect of high temperature stress on POX and APOX activity of blackgram genotypes (pooled data).

that first principal component with eigen value more than 1 explained 80.0% of total variability. Biplots of investigated traits in blackgram genotypes under heat stress conditions (Fig 5). The biplots under heat stress conditions during both the years revealed that SYP showed a strong positive correlation with biochemical parameters such as proline, carotenoids, SOD, CAT, POX and APOX by possessing a small angle between the corresponding vectors of above traits during both the years. The seed yield also showed a significant negative correlation with SOR, HPR and MDA as there was a largest angle between the corresponding vectors of SYP, proline, carotenoid and all antioxidant

defense enzymes. In PCA of all 30 genotypes, TBG-129, LBG-1015, PU-1804 and PU-31 recorded higher proline, carotenoids, SOD, CAT, POX, APOX, NPP, SYP and HI indicating their tolerance to high temperature stress whereas, the genotypes TBG-125 and LBG-1023 recorded lower seed yield which might be due to more oxidative damage caused by the accumulation of free radicals and lower antioxidant defense enzyme activity during both the years. Moreover, these genotypes were placed distantly from other genotypes in the 2D plot. Our results are in accordance with the published reports of Sharma *et al.* (2023) in fieldpea.

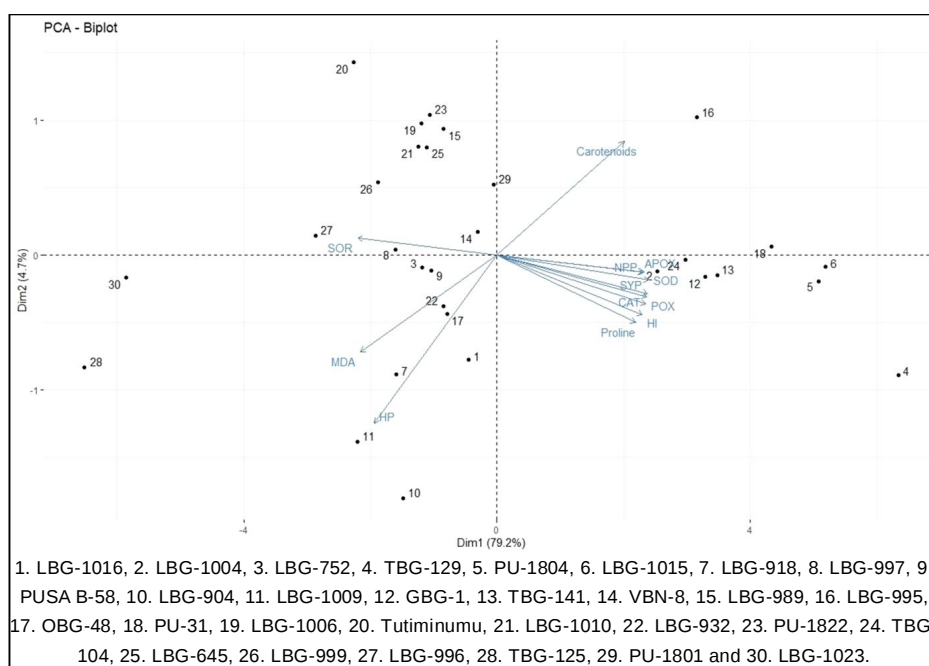


Fig 5: Biochemical and yield traits (pooled data) are represented in principal component analysis (PCA) biplot viz., Superoxide radical (SOR), Hydrogen peroxide radical (HPR), Malondialdehyde (MDA), proline, carotenoids, Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POX), Ascorbate peroxidase (APOX), Number of pods per plant (NPP), seed yield per plant (SYP) and Harvest Index (HI) genotypes.

Table 1: Correlation between biochemical and yield traits of blackgram genotypes grown under high temperature stress conditions (pooled data).

	SOR	HP	MDA	Proline	Carotenoids	SOD	CAT	POX	APOX	NPP	SYP	HI
SOR	1	0.667**	0.765**	-0.758**	-0.588**	-0.847**	-0.763**	-0.868**	-0.765**	-0.728**	-0.786**	-0.741**
HP		1	0.704**	-0.620**	-0.638**	-0.669**	-0.690**	-0.635**	-0.716**	-0.677**	-0.651**	-0.599**
MDA			1	-0.665**	-0.723**	-0.781**	-0.739**	-0.737**	-0.740**	-0.767**	-0.769**	-0.733**
Proline				1	0.616**	0.794**	0.824**	0.794**	0.820**	0.776**	0.768**	0.801**
Carotenoids					1	0.751**	0.691**	0.710**	0.703**	0.689**	0.724**	0.701**
SOD						1	0.885**	0.963**	0.831**	0.877**	0.894**	0.809**
CAT							1	0.833**	0.872**	0.894**	0.883**	0.894**
POX								1	0.827**	0.816**	0.902**	0.819**
APOX									1	0.801**	0.868**	0.879**
NPP										1	0.890**	0.804**
SYP											1	0.874**
HI												1

CONCLUSION

Genetic variability in various biochemical and yield traits was assessed over 2 years in 30 blackgram genotypes grown under heat stress conditions during reproductive stage. The results revealed that the genotypes TBG-129, LBG-1015, PU-1804 and PU-31 recorded higher SYP under high temperature stress conditions which might be due to increased accumulation of proline, carotenoids and antioxidant defence enzyme activity at cellular level that might have scavenged the free radicals thereby protecting cellular membranes from lipid peroxidation. SYP was lower in TBG-125 and LBG-1023 which was due to increased lipid peroxidation caused by higher accumulation of free radicals and poor antioxidant defense enzyme activity. Correlation analysis revealed that all the biochemical traits except SOR, HPR and MDA showed positive association with seed yield. Moreover, the results of principal component analysis revealed that the genotypes TBG-129, LBG-1015, PU-1804, PU-31, TBG-125 and LBG-1023 were placed distantly from other genotypes in the 2D plot. Hybridization between these diverse genotypes can be suggested.

Biochemical traits governing the heat tolerance in blackgram genotypes was thoroughly assessed in the present study. Further work can be focussed on identifying the genes responsible for up regulation of antioxidant defence enzyme under high temperature stress conditions. In addition to this, physiological and reproductive efficiency characteristics should be analyzed in these genotypes. The genotypes should be evaluated across multiple locations for confirming their tolerance.

Conflict of Interest

The authors declare that they have no conflict of interest.

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