



# Variation in Physiological Traits of Blackgram (*Vigna mungo* L.) Genotypes under High Temperature Stress

N. Pavithra<sup>1</sup>, K. Jayalalitha<sup>1</sup>, T. Sujatha<sup>1</sup>, N. Harisatyanarayana<sup>2</sup>, N. Jyothi Lakshmi<sup>3</sup>, V. Roja<sup>4</sup>

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## ABSTRACT

**Background:** Blackgram is one of the important short duration pulse crop which is sensitive to high temperatures. The rising global temperatures are threatening the yield of blackgram by altering the physiological processes at cellular level. Keeping this in view, the present investigation was carried out for better understanding of genotypic variability and the physiological 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 physiological efficiency under natural high temperature conditions during summer, 2022 and 2023. Field experiment was conducted at Agricultural College Farm, Acharya N.G. Ranga Agricultural University, Agricultural College, Bapatla. Physiological and yield parameters were recorded at flowering and the data were analyzed statistically and pooled.

**Result:** Significant genetic variability was observed among the blackgram genotypes with respect to all the physiological traits under high temperature stress conditions. Among the 30 genotypes tested for thermotolerance, the genotypes TBG-129, PU-1804, LBG-1015, PU-31 and TBG-104 were found to withstand high temperature stress at reproductive stage by maintaining higher total chlorophyll, carotenoids, chlorophyll stability index, total biomass production, cooler canopies resulting into higher canopy temperature depression, less membrane injury index values and higher yield. Correlation analysis revealed a strong positive correlation for all the physiological traits with seed yield except membrane injury index under heat stress conditions. The PCA results revealed considerable variability among the traits accounting for 82.65% of total variability. The genotypes TBG-129, PU-1804, LBG-1015 and TBG-104 can be potentially used as donors in the breeding programmes for the development of heat tolerant genotypes.

**Key words:** Blackgram, Genotypic variability, Physiological traits, Principal component analysis.

## INTRODUCTION

Blackgram is extensively grown pulse crop in India. It accounts for about 10% of total pulse production. It contains high amount of protein (25%) and shares major protein requirement in vegetarian diet. It is very sensitive to high temperature especially during reproductive stage coincides with temperature rise (Anitha *et al.*, 2015). Temperatures over 35°C during flowering causes significant reduction in the yield due to damage to reproductive organs. So, identification of blackgram genotypes that can perform better in the high temperature stress conditions has gained utmost importance in the current global warming scenarios.

Higher chlorophyll content is one of the key trait for selecting photosynthetically efficient genotypes under heat stress conditions (Chaudhary *et al.*, 2022). 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.

<sup>1</sup>Department of Crop Physiology, Acharya N.G. Ranga Agricultural University, Agricultural college, Bapatla-522 101 Andhra Pradesh, India.

<sup>2</sup>Department of Genetics and Plant Breeding, Acharya N.G. Ranga Agricultural University, Regional Agricultural Research Stations, Lam, Guntur-522 034, Andhra Pradesh, India.

<sup>3</sup>Department of Plant Physiology, Central Research Institute for Dryland Agriculture, Hyderabad-500 059, Telangana, India.

<sup>4</sup>Department of Biotechnology and Molecular Biology, Acharya N.G. Ranga Agricultural University, Regional Agricultural Research Stations, Lam, Guntur-522 034, Andhra Pradesh, India.

**Corresponding Author:** N. Pavithra, Department of Crop Physiology, Acharya N.G. Ranga Agricultural University, Agricultural college, Bapatla-522 101 Andhra Pradesh, India.  
Email: pavithranuthalapati1430@gmail.com

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Plants have adopted various physiological mechanisms to cope with unfavorable conditions. One such mechanism is transpirational cooling, which protects plants from heat stress. Transpirational cooling can be measured by canopy

temperature. Numerous reports stated that cool canopies were associated with better yield output as a result of higher transpiration and photosynthesis (Pinto *et al.*, 2015).

Genotypic variability exists among the genotypes in response to heat stress. Genetic variability can be assessed using principal component analysis (PCA). It also helps in the identification of desirable physiological traits governing heat stress tolerance in the thermotolerant genotypes. Information regarding the physiological responses of blackgram genotypes to heat stress during reproductive stage is rarely available. 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.

## 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 physiological traits under natural high temperature conditions during summer 2022 and 23 at College Farm, Agricultural College, Bapatla, Acharya N.G Ranga Agricultural University. The experimental site was geographically located at 15°54'N latitude and 80°47'E longitude and at an altitude of 5.49 m above mean sea level (MSL), which is about 8 km away from the Bay of Bengal in the Krishna Agro-Climatic zone of Andhra Pradesh, India. Observations such as chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, membrane injury index (MI), chlorophyll stability index (CSI), canopy temperature depression (CTD) and total biomass production (TB) were recorded at 50% flowering during both the years as it is more sensitive to high temperatures during flowering stage. The mean maximum temperature

was 35.5 and 37.2°C at flowering during summer, 2022 and 23, respectively.

### Physiological traits

#### Photosynthetic pigments

0.1 g of fresh leaf material was placed in a test tube and was heated in a water bath for 1 hr at 65°C and after heating, 10 mL of DMSO was added to it. Keep both treated and untreated samples for overnight. Chlorophyll extracted into DMSO solution was collected from test tubes and the absorbance of chlorophyll extract of heat treated samples was measured with a spectrophotometer at 652 nm and untreated samples at 652, 663, 645, 480 and 510 nm.

$$\text{Total chlorophyll (mg g}^{-1}\text{)} = \frac{D_{.652} \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll a} = 12.7 (A_{663}) - 2.69 (A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9 (A_{645}) - 4.68 (A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Carotenoid content (mg g}^{-1}\text{)} = 7.6 (D_{480}) - 1.49 (D_{510}) \times \frac{V}{1000 \times W}$$

Then, calculated the chlorophyll stability index of the leaf material using the following formula (Koleyoreas, 1958).

CSI (%) =

$$\frac{\text{Total chlorophyll content of the treated sample}}{\text{Total chlorophyll content of the untreated sample}} \times 100$$

#### Total biomass production

The total biomass was estimated from the five adjacent plants sampled from each treatment in two replications

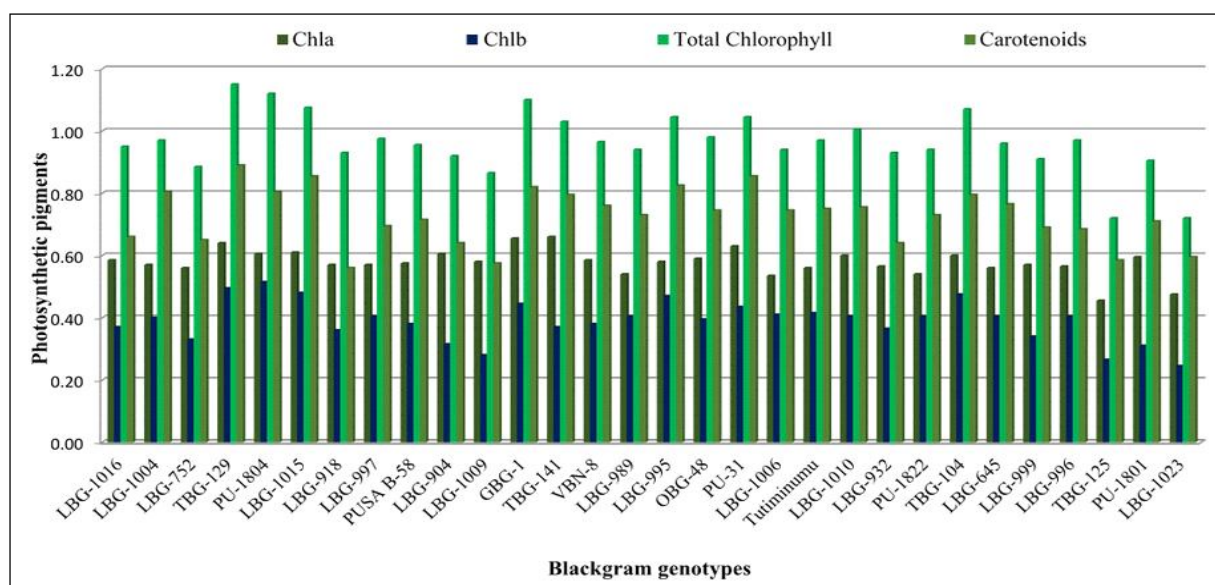


Fig 1: Effect of high temperature stress on photosynthetic pigments of blackgram genotypes (pooled data of two years).

and then separated into leaves, stems and pods. The plant parts were dried to a constant weight in hot-air-oven at 80°C for two days and the dry weights were recorded and expressed in g plant<sup>-1</sup>.

#### Membrane Injury Index

Leaf sample (0.1 g) was cut into pieces of uniform size and taken in test tubes (two sets four each) containing 10 mL of distilled water in each tube. One set of tubes were kept at 40 - 45°C for 30 minutes and another set, at 100°C for 10 -15 minutes in a water bath. After hot water bath incubation, samples were allowed to reach room temperature and electrical conductivity of water in both the sets of test tubes was recorded by using a conductivity meter. (Premchandra *et al.*, 1990).

$$\text{Membrane injury index (\%)} = \frac{\text{Conductivity at 45}^\circ\text{C}}{\text{Conductivity at 100}^\circ\text{C}} \times 100$$

#### Canopy temperature depression

Canopy temperature was monitored three times a day (7.00 am, 1.00 pm and 5.00 pm) by a hand hold Infrared thermometer (SMART SENSOR, AR802A, China). The gun focussed on the canopy target by holding gun pistol-grip at an angle of 45°C with a distance of 0.5 to 1 m above the canopy. Average of six readings from two plants was recorded in each plot. The air temperature was measured constantly by the instrument and the differences between canopy and air temperatures were recorded in differential mode.

Canopy temperature depression =

$$\text{Ambient temperature} - \text{Canopy temperature}$$

#### Yield and yield attributes

Yield parameters such as number of pods per plant (NPP), pod length (PL), no of seeds per pod (NSP) and seed yield per plant (SYP) 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 physiological and yield traits were subjected to correlation analysis in OPSTAT and PCA was performed using R software.

## RESULTS AND DISCUSSION

### Photosynthetic pigments

The data pertaining to photosynthetic pigments in the blackgram genotypes were recorded at flowering during summer, 2022 and 2023 and the pooled data were presented in the Fig 1. Pooled data of two seasons revealed that there was significant variation among all the genotypes with

respect to chlorophyll a content. Chlorophyll a content ranged between 0.46 to 0.66 mg g<sup>-1</sup>. Maximum chlorophyll a content was recorded in GBG-1 (0.66 mg g<sup>-1</sup>) followed by TBG-141 (0.66 mg g<sup>-1</sup>), TBG-129 (0.64 mg g<sup>-1</sup>), PU-31 (0.63 mg g<sup>-1</sup>), PU-1804, LBG-1015, LBG-904 (0.61 mg g<sup>-1</sup>), TBG-104, LBG-1010 and PU-1801 (0.60 mg g<sup>-1</sup>) whereas minimum chlorophyll a content was recorded in TBG-125 (0.46 mg g<sup>-1</sup>) followed by LBG-1023 (0.48 mg g<sup>-1</sup>). Chlorophyll b content ranged between 0.25 and 0.52 mg g<sup>-1</sup>. It was significantly higher in PU-1804 (0.52 mg g<sup>-1</sup>) followed by TBG-129 (0.50 mg g<sup>-1</sup>), LBG-1015 and TBG-104 (0.50 mg g<sup>-1</sup>) while, lower in LBG-1023 (0.25 mg g<sup>-1</sup>) followed by TBG-125 (0.27 mg g<sup>-1</sup>). This increase in the chlorophyll a and chlorophyll b content might be due to increase in the content of PS I and PS II subunits, which might have protected the chl a/b proteins from proteosomal degradation thereby maintaining high chl a and b content even under stress conditions (Shan *et al.*, 2018).

Heat stress significantly affected the total chlorophyll content with mean values ranging from 0.72 to 1.15 mg g<sup>-1</sup>. Significantly higher total chlorophyll content was recorded in TBG-129 (1.15 mg g<sup>-1</sup>) followed by PU-1804 (1.12 mg g<sup>-1</sup>), GBG-1 (1.10 mg g<sup>-1</sup>), LBG-1015 (1.08 mg g<sup>-1</sup>), TBG-104 (1.07 mg g<sup>-1</sup>), LBG-995 (1.05 mg g<sup>-1</sup>) and TBG-141 (1.03 mg g<sup>-1</sup>) while, lesser total chlorophyll content was recorded in TBG-125 and LBG-1023 (0.72 mg g<sup>-1</sup>). This reduction in photosynthetic pigments with increase in temperature might be due to oxidative damage caused by outburst of ROS and inhibition of chlorophyll biosynthesis. Similar findings of higher levels of chlorophyll content in the thermotolerant chickpea genotypes under heat stress conditions were previously reported by Devi *et al.* (2022). Our results also concur with the published reports of Jincy *et al.* (2022) in greengram and Chaudhary *et al.* (2022) in blackgram.

Carotenoid content varied significantly among the genotypes with mean values of 0.59 and 0.89 mg g<sup>-1</sup> f.wt. Higher carotenoid content was recorded in TBG-129 (0.89 mg g<sup>-1</sup>) followed by LBG-1015, PU-31 (0.86 mg g<sup>-1</sup>), LBG-995 (0.83 mg g<sup>-1</sup>), GBG-1 (0.82 mg g<sup>-1</sup>), PU-1804 and LBG-1004 (0.81 mg g<sup>-1</sup>) whereas, lesser carotenoid content was recorded in TBG-125 (0.59 mg g<sup>-1</sup>) followed by LBG-1023 (0.60 mg g<sup>-1</sup>), LBG-752 (0.65 mg g<sup>-1</sup>) and LBG-1016 (0.66 mg g<sup>-1</sup>). Carotenoids act as molecular antioxidants in cells by scavenging singlet oxygen (Knox and Dodge, 1985). They also 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.

### Total biomass production

Pooled data of two seasons revealed that total biomass varied significantly among the blackgram genotypes with mean values ranged from 1.98 and 3.54 g plant<sup>-1</sup>. Higher

biomass accumulation was recorded in TBG-129 (3.54 g plant<sup>-1</sup>) followed by TBG-104 (3.52 g plant<sup>-1</sup>), PU-1804 (3.40 g plant<sup>-1</sup>), GBG-1 (3.28 g plant<sup>-1</sup>), LBG-989 (3.24 g plant<sup>-1</sup>), TBG-141 (3.29 g plant<sup>-1</sup>) and PU-31 (3.18 g plant<sup>-1</sup>) while, the genotype TBG-125 (1.98 g plant<sup>-1</sup>) recorded lower biomass accumulation which was at par with LBG-645 (2.23 g plant<sup>-1</sup>), LBG-1023, PU-1822 (2.26 g plant<sup>-1</sup>), Tutiminumu (2.33 g plant<sup>-1</sup>) and LBG-1016 (2.35 g plant<sup>-1</sup>). Our results corroborate with the findings of Kumar *et al.* (2013) in chickpea who reported the similar decline in total biomass production in the heat sensitive genotypes.

**Chlorophyll stability index**

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

to CSI. The mean values of CSI ranged from 62.31 and 88.02% (Fig 2). Higher CSI was recorded in TBG-129 (88.02%) followed by TBG-141 (87.38%), PU-1804 (87.27%), TBG-104 (85.35%), LBG-1015 (85.29%), PU-31 (83.22%), LBG-1004 (81.05%), GBG-1 (80.26%) and LBG-995 (80.17%), whereas it was lower in LBG-1023 (62.31%) which was at par with TBG-125 (63.07%), LBG-996 (66.39%), LBG-999 (68.57%), LBG-997 (68.58%), OBG-48 (69.87%), Tutiminumu (70.09%) and LBG-1009 (70.31%). The decrease in CSI in the susceptible genotypes might be due to exposure of the crop to high temperatures that caused destruction of chlorophyll. CSI is one of the important traits that reflects the ability of plants to sustain photosynthesis under stress conditions (Sayed and Suzan, 1999). Similar finding were previously reported in green

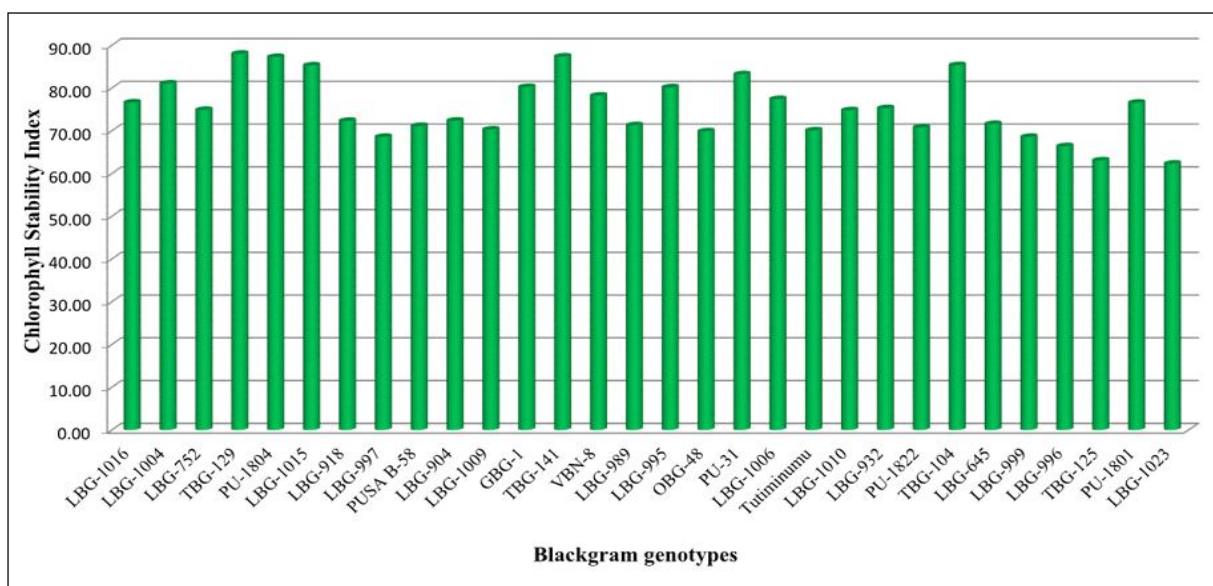


Fig 2: Effect of high temperature stress on CSI of blackgram genotypes (pooled data of two years).

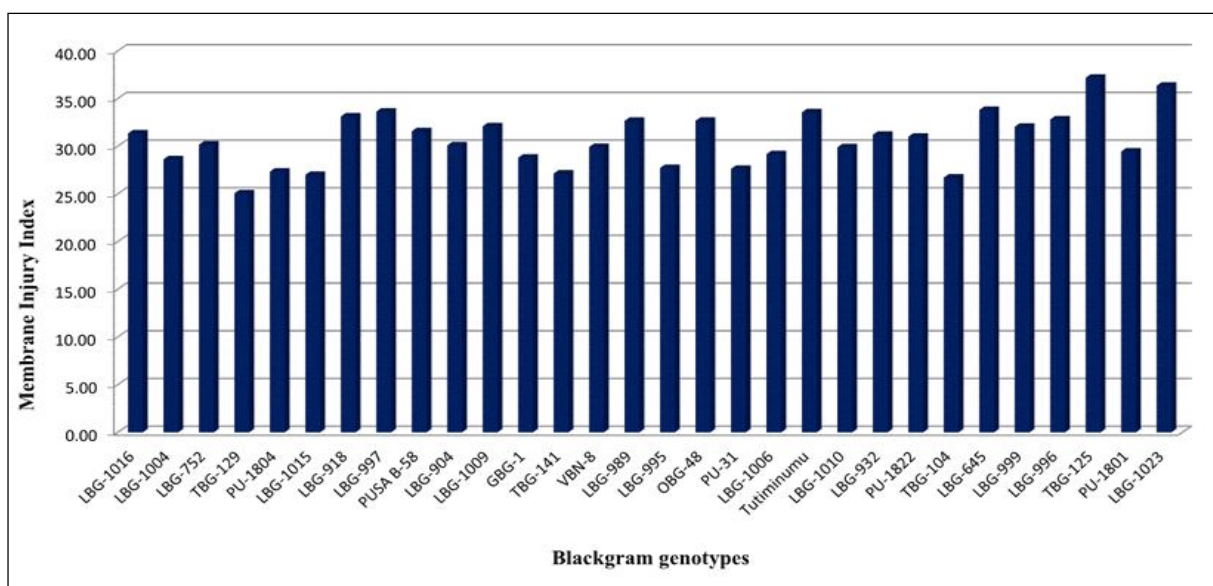


Fig 3: Effect of high temperature stress on MI of blackgram genotypes (pooled data of two years).



gram by Jincy *et al.* (2022) who stated the maximum CSI in heat and drought stress tolerant genotypes.

#### Membrane injury index

MII is one of the important physiological trait for selecting the genotypes to heat stress tolerance as the membranes are the primary sites of injury. Pooled data of two seasons revealed that there was significant variation among all the genotypes with respect to MII (Fig 3). The blackgram genotype TBG-129 (25.08%) recorded lower values of MII which was at par with TBG-104 (26.73%), LBG-1015 (27.01%), TBG-141 (27.15%), PU-1804 (27.35%), PU-31 (27.64%) and LBG-995 (27.73%) indicating their cell membrane stability and heat stress tolerance, whereas it was higher in TBG-125 (37.19%) which was at par with LBG-1023 (36.36%). Our reports concur with the published reports of Chaudhary *et al.* (2022) in blackgram and Almeselmani *et al.* (2009) in wheat which support our current findings.

#### Canopy temperature depression

CTD varied significantly among the genotypes with mean values of  $-0.24^{\circ}\text{C}$  to  $-2.04^{\circ}\text{C}$ . The blackgram genotype TBG-141 ( $-0.24^{\circ}\text{C}$ ) recorded higher CTD values which was at par with LBG-1004, TBG-129 ( $-0.59^{\circ}\text{C}$ ), PU-1804 and PU-31 ( $-0.67^{\circ}\text{C}$ ) indicating cooler canopies compared to other genotypes, whereas, lower CTD values were recorded in TBG-125, LBG-1023 ( $-2.34^{\circ}\text{C}$ ) and LBG-996 ( $-2.04^{\circ}\text{C}$ ). Transpirational cooling helps plants to conduct photosynthesis under heat stress conditions. Hence, it can be used as a measure for assessing heat stress tolerance. Devasirvatham *et al.* (2015) stated that the CTD, as a potential indirect selection criterion for yield under heat stress.

#### 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. The total NPP was higher in LBG-1015 (21.5) followed by PU-1804 (20.2), TBG-104 (19.8) and TBG-129 (19.9), whereas it was lower in TBG-125 (3.75) followed by LBG-996 (4.4), LBG-1023 (4.7), and LBG-999 (5.1) and PU-1822 (5.5) (Fig 4). The thermotolerant genotypes, TBG-129, LBG-1015, TBG-104 and PU-1804 recorded higher number of pods per plant which might be due to higher chlorophyll retention, CSI, CTD, lesser electrolyte leakage and higher antioxidant enzyme activity. 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) in blackgram and Omae *et al.* (2012) in common bean who reported higher number of pods in thermotolerant genotypes.

Maximum PL was recorded in TBG-104 (4.4 cm) followed by LBG-1015 (4.1 cm), PU-1804 (4.0 cm) and TBG-129 (3.9), whereas PL was minimum in Tutiminumu and TBG-125 (3.3 cm), which were at par with LBG-1023 (3.4 cm) and LBG-918 (3.5 cm). NSP were higher in PU-1804 (4.5) followed by LBG-1015 (4.3), TBG-129, GBG-1 and PU-31 (4.2) while, it was lower in TBG-125 (2.6) which was at par with Tutiminumu, LBG-932 (3.0), LBG-645, LBG-996, LBG-1023, LBG-918 and PUSA B-58 (3.1). Lesser number of seeds per pod in the susceptible genotypes might be due to sensitivity of pollen to high temperatures which in turn affects seed setting and pollination. The loss of viability of pollen or stigma under heat stress conditions may be the major reason for the reduction in the number of seeds

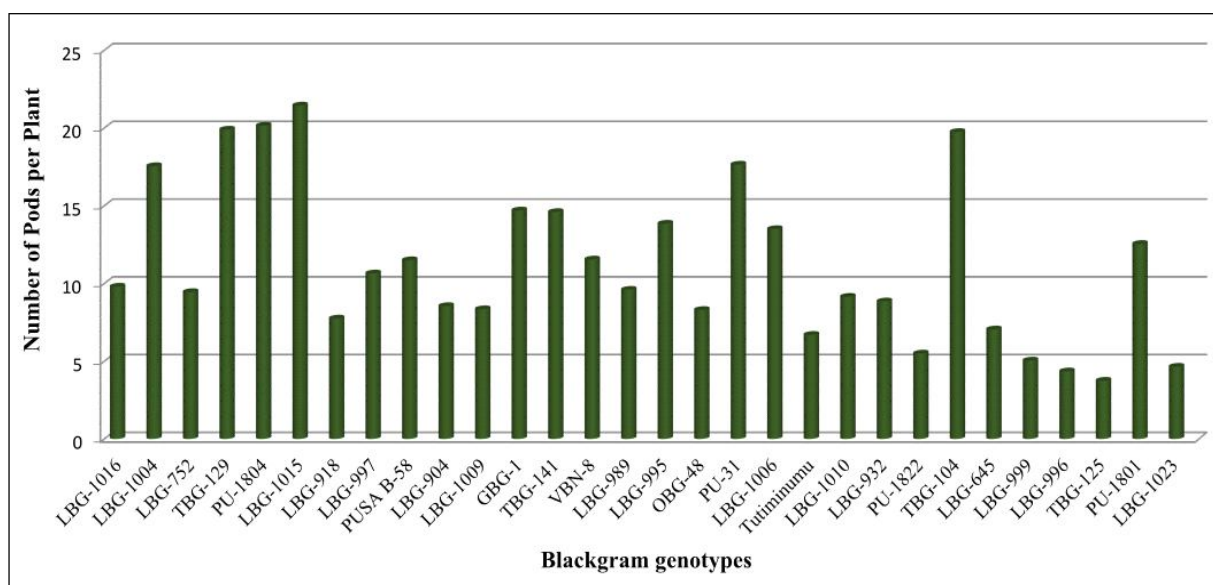


Fig 4: Effect of high temperature stress on NPP of blackgram genotypes (pooled data of two years).

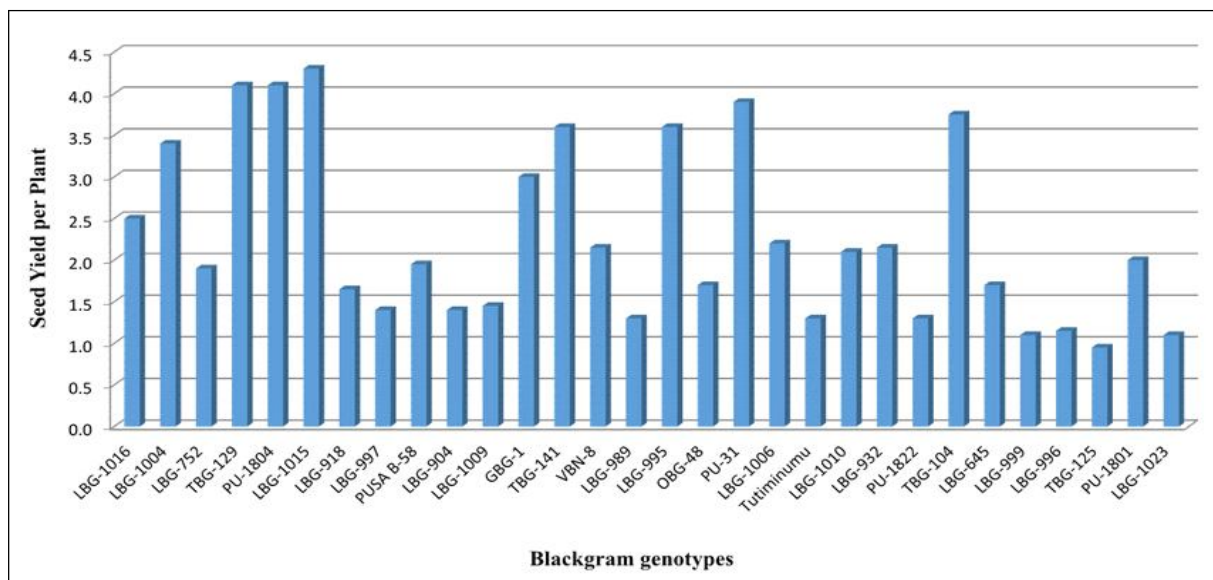
produced in legumes (Wang *et al.*, 2006). Our results concur with the published reports of Haritha (2020) who reported lower seed set in blackgram genotype TBG-125.

SYP was higher in LBG-1015 (4.3 g plant<sup>-1</sup>) followed by TBG-129 and PU-1804 (4.1 g plant<sup>-1</sup>) whereas, TBG-125 (1.0 g plant<sup>-1</sup>) recorded lower SYP which was at par with LBG-1023, LBG-999 (1.1 g plant<sup>-1</sup>), LBG-996 (1.2 g plant<sup>-1</sup>), Tutiminumu, PU-1822 and LBG-989 (1.3 g plant<sup>-1</sup>) (Fig 5). 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 seed yields. The prevailing high temperatures during summer, 2023 caused drastic reduction in seed yield which might be due to destruction of chlorophyll that in turn decreased the translocation of photosynthates from source to sink. Our results agree with the findings of Subrahmanyam and Rathore (1994) who reported that high temperature during reproductive stage in mustard significantly inhibited the

import of photosynthates by both upper and lower pods of terminal receme, and thereby reduced the sink strength.

**Correlation analysis**

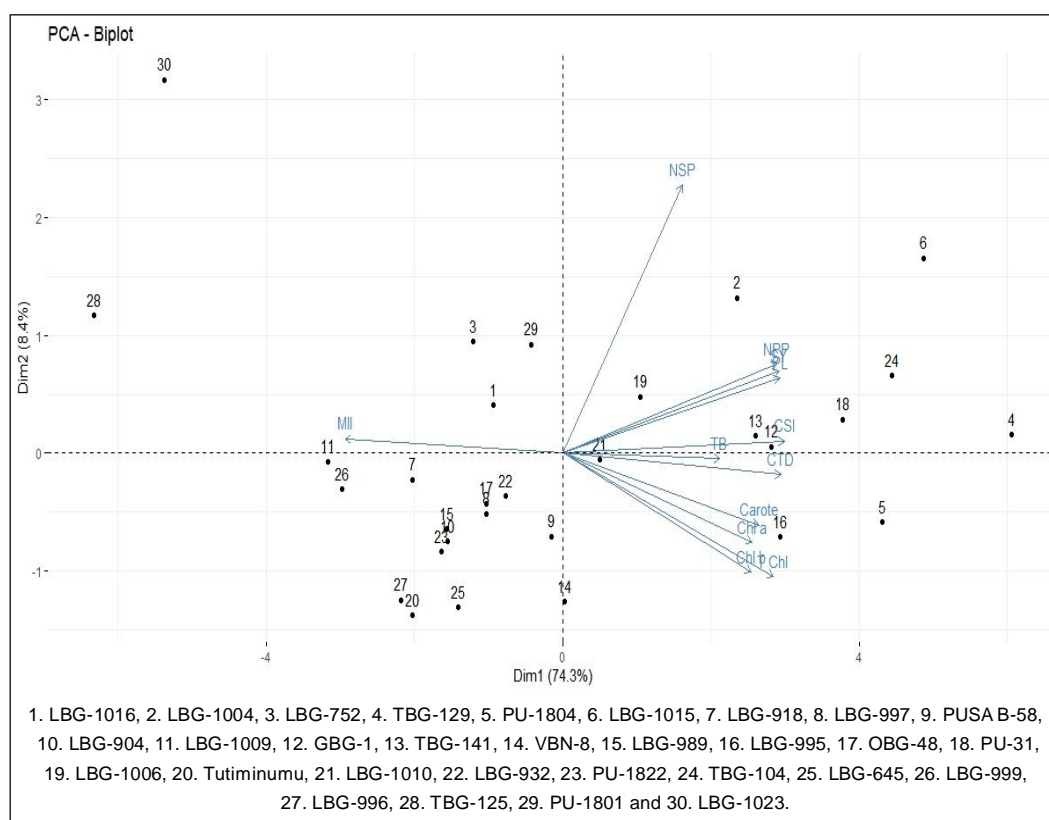
Correlation studies among various physiological traits and their association revealed the vital results under heat stress conditions (Table 1). Under heat stress conditions, seed yield per plant, pod length and number of pods per plant showed significant positive association with all the physiological traits except MII. MII showed negative correlation with chl a, chl b, total chlorophyll, carotenoids, CSI, TB, NPP, PL, NSP and SYP. SYP was found to be highly correlated with CSI and total chlorophyll indicating the ability of genotypes to sustain photosynthesis even under high temperature stress conditions. SYP and total chlorophyll was also found to be positively correlated with carotenoids indicating the protective role of carotenoids in quenching the singlet oxygen thereby protecting chlorophyll pigments from destruction with concomitant increase in photosynthetic rate resulting in higher yield under high temperature stress



**Fig 5:** Effect of high temperature stress on seed yield per plant of blackgram genotypes (pooled data of two years).

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

	Chl a	Chl b	Total Chl	Carotenoids	CSI	MII	TB	NPP	PL	NSP	SYP
Chl a	1	0.548**	0.828**	0.617**	0.754**	-0.781**	0.636**	0.640**	0.516**	0.621**	0.662**
Chl b		1	0.920**	0.836**	0.693**	-0.672**	0.581**	0.703**	0.636**	0.727**	0.710**
Total Chl			1	0.838**	0.810**	-0.810**	0.689**	0.762**	0.655**	0.767**	0.775**
Carotenoids				1	0.759**	-0.757**	0.541**	0.762**	0.621**	0.812**	0.778**
CSI					1	-0.942**	0.723**	0.921**	0.741**	0.834**	0.947**
MII						1	-0.751**	-0.869**	-0.692**	-0.811**	-0.875**
TB							1	0.749**	0.683**	0.646**	0.677**
NPP								1	0.839**	0.874**	0.940**
PL									1	0.808**	0.795**
NSP										1	0.850**
SYP											1



**Fig 6:** Physiological and yield traits (pooled data) are represented in principal component analysis (PCA) biplot viz., chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, membrane injury index (MI), chlorophyll stability index (CSI), canopy temperature depression (CTD), total biomass production (TB), number of pods per plant (NPP), number of seeds per pod (NSP), pod length (PL) and seed yield per plant (SYP) genotypes.

conditions. SYP was found to be highly correlated with number of pods per plant which might be the reason behind higher seed yield. Similar findings of electrolyte leakage showing negative association with physiological and yield traits were previously reported in blackgram by Chaudhary *et al.* (2022). Positive association of total chlorophyll with seed yield was previously reported by Devi *et al.* (2022) in chickpea.

### Principal component analysis

Principal component analysis was performed based on physiological and yield traits of blackgram genotypes grown under heat stress environments. PCA analysis revealed that first two principal components with eigen value more than 1 explained 83.04% of total variability. The first PC explains 74.30% and second PC explains 8.35% of total variation. Biplots of investigated traits in blackgram genotypes under heat stress conditions are depicted in (Fig 6). The biplots under heat stress conditions during both the years revealed that SY showed a strong positive correlation with physiological parameters such as chl a, chl b, total chlorophyll, carotenoids, CSI, MI, CTD and TB by possessing a small angle between the corresponding vectors of above traits. The seed yield also showed a

significant negative association with MI as there was a largest angle between the corresponding vectors of SYP and MI. In PCA of all 30 genotypes, TBG-129, LBG-1015, PU-1804, PU-31 and TBG-104 recorded higher chl a, chl b, total chlorophyll, carotenoid, CSI, CTD, NPP, PL, NSP and SYP 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 membrane damage and lower CSI. Moreover, these genotypes were placed distantly from other genotypes in the 2D plot. Our results are in accordance with the published reports of Chaudhary *et al.* (2022) in blackgram.

### CONCLUSION

Genetic variability in various physiological and yield traits was assessed over 2 years in 30 blackgram genotypes grown under heat stress conditions during reproductive stage. Significant reduction in the SYP was observed in TBG-125 and LBG-1023 genotypes indicating their susceptibility to high temperatures whereas TBG-129, PU-1804, LBG-1015, PU-31 and TBG-104 showed less reduction indicating their tolerance to high temperatures during both the years of the study. The reason behind the higher seed yield in these genotypes under heat stress

conditions might be due to underlying physiological mechanisms such as retention of chlorophyll by maintaining higher CSI, higher carotenoid content, lesser leakage of solutes and maintenance of cooler canopies by exhibiting higher CTD values. These physiological traits can be further used for identifying heat tolerant genotypes. All the physiological parameters such as chl a, chl b, total chlorophyll, carotenoids, CSI and TB showed a positive correlation with SYP under heat stress conditions during both the years indicating the role of physiological mechanisms in conferring heat stress tolerance. The results of PCA revealed that seed yield showed a positive association with all the physiological traits by possessing small angle between the corresponding vectors for the above traits. Moreover, the results of PCA also revealed that the tolerant genotypes TBG-129, LBG-1015, PU-1804, TBG-104 and PU-31 and the susceptible genotypes TBG-125 and LBG-1023 were placed distantly from other genotypes in the 2D plot. Hybridization between these diverse genotypes can be suggested.

Our results have paved the way by identifying the key physiological traits governing the heat stress tolerance which can be further used for screening heat tolerant genotypes. The tolerant genotypes identified in this study can be further assessed for reproductive and biochemical efficiency under heat stress conditions for confirming their heat tolerance. In addition to this, these genotypes should be tested across multiple locations for assessing their heat tolerance. Further, the work can be focused on identifying genes involved in the up-regulation of physiological mechanisms governing heat tolerance in thermotolerant genotypes.

#### Conflict of interest

All authors declared that there is no conflict of interest.

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