



RESEARCH ARTICLE

Physiological and biochemical dynamics of black gram (*Vigna mungo* L.) genotypes under high temperature stress at pod formation stage

N Pavithra^{1*}, K Jayalalitha¹, T Sujatha², N Harisatyanarayana³, N Jyothi Lakshmi⁴ & V Roja⁵

¹Department of Crop Physiology, Agricultural College, Bapatla, Acharya N.G Ranga Agricultural University, Bapatla 522 101, Andhra Pradesh, India

²Department of Crop Physiology, Acharya N.G Ranga Agricultural University, Guntur 522 034, Andhra Pradesh, India

³Department of Genetics and Plant Breeding, Acharya N.G Ranga Agricultural University, Guntur 522 034, Andhra Pradesh, India

⁴Department of Plant Physiology, ICAR-Central Research Institute for Dryland Agriculture, Saidabad, Hyderabad 500 059, Telangana, India

⁵Department of Biotechnology and Molecular Biology, Acharya N.G Ranga Agricultural University, Lam, Guntur 522 034, Andhra Pradesh, India

*Correspondence email - pavithranuthalapati1430@gmail.com

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Abstract

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 and biochemical processes at the cellular level. Keeping this in view, the present investigation was carried out for a better understanding of 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. Thirty blackgram genotypes were evaluated for high temperature stress tolerance during summer 2022 and 2023 at College Farm, Agricultural College, Bapatla, Acharya N.G Ranga Agricultural University. All the observations were recorded at the pod formation stage and analysed statistically. The results showed that the genotypes TBG-129, LBG-1015, PU-1804 and PU-31 were found to withstand high temperatures by maintaining higher chlorophyll stability index, cooler canopies, more proline accumulation and higher antioxidant defence activity, reflecting the lesser membrane leakage due to decrease in free radicals and MDA accumulation, thereby recording higher seed yield compared to other genotypes. The PCA results revealed considerable variability among the traits, accounting for 81.37 % of total variability. The blackgram genotypes TBG-129, LBG-1015, PU-1804 and PU-31 can be used as donors in the breeding programme for the development of thermotolerant genotypes.

Keywords: blackgram; high temperature stress; pod formation stage; principal component analysis

Introduction

Blackgram is a short-duration leguminous crop grown extensively in all parts of India. It has high nutritive value and consists of high content of protein, vitamins and minerals (1). India currently represents the largest producer of blackgram which accounts for more than 70 % of global production. In India, blackgram is cultivated in an area of 46.33 lakh ha with an annual production of 27.75 lakh t and productivity of 1059 kg ha⁻¹ (2). To meet the demand of a growing population, an improvement in pulse production is required. Productivity of pulses is mainly limited by abiotic stresses such as heat stress, drought, salinity and heavy metal stress, etc. In particular, heat stress-related events are threatening global food security by adversely impacting pulse production. Blackgram, being a thermosensitive crop, its yield is highly sensitive to high temperatures exceeding 35 °C (3). Among the various crop phenophases, reproductive or seed formation stages are reported to be extremely sensitive to even a few degrees rise in temperatures, leading marked reduction in yield potential. Heat stress hampers plant growth by disturbing the physiological

processes of plants. At the cellular level, heat stress causes oxidative damage by exaggerating the production of reactive oxygen species (ROS), leading to protein denaturation, lipid peroxidation and thereby leakage of solutes. Therefore, electrolyte leakage and the degree of lipid peroxidation serve as an indicator of oxidative damage and can be successfully employed for the assessment of thermotolerance in the blackgram genotypes.

Plants respond to such damage by up-regulating the various pathways involved in the production of osmoprotectants, antioxidants, phytohormones and secondary metabolites. Plants accumulate a variety of low molecular weight compounds to cope with stressful conditions. Plants also activate several enzymatic antioxidant defence pathways to quench ROS. SOD plays a role in the dismutation of O₂⁻ to H₂O₂ and molecular oxygen. Different types of catalases and peroxidases are involved in quenching H₂O₂ into water and oxygen molecules by oxidation, thereby protecting the cell from oxidative damage (4). Moreover, heat stress at pod formation also causes seed abortion by altering the transport of seed-derived auxins and other signalling molecules to the

pericarp, facilitating pedicle abscission, leading to substantial yield losses (5). Genetic variability exists among the blackgram genotypes for heat tolerance. Assessing genetic variability for various physiological, biochemical and yield traits in the available germplasm could be one of the promising approaches for the identification of heat-tolerant genotypes. Keeping this in view, the present investigation was carried out to evaluate the genotypic differences in the physiological and biochemical traits that affect the heat tolerance in the blackgram genotypes.

Materials and Methods

Experimental details and climatic condition

A total of a hundred blackgram genotypes were subjected to the temperature induction response technique (TIR) in which lethal temperature was standardised as 54 °C for 3 hr at which 90 % mortality of seedlings was observed. The optimum induction temperature was standardised as 36–46 °C at which 49.26 % reduction in growth over the control was observed. Based on % reduction in seedling growth over the control, all the blackgram genotypes were categorised. Among the hundred blackgram genotypes, 27 genotypes such as LBG-1016, LBG-1004, LBG-752, TBG-129, PU-1804, LBG-1015, LBG-918, LBG-997, PUSA B-58, LBG-904, LBG-1009, GBG-1, TBG-141, VBN-8, LBG-989, LBG-995, OBG-48, PU-31, LBG-1006, Tutimumu, LBG-1010, LBG-932, PU-1822, TBG-104, GBG-645, LBG-999 and LBG-996 recorded higher survival percentage and less % reduction in seedling growth over control indicating their tolerance to high temperature. Three blackgram genotypes, such as TBG-125, PU-1801 and LBG-1023, showed the extremely lowest survival percentage and the highest % reduction in growth, indicating higher susceptibility to high temperatures.

The 27 thermotolerant and 3 susceptible genotypes selected from TIR technique were further evaluated for their physiological, biochemical and yield traits in the field under high temperature stress conditions during summer, 2022 and 23 at Agricultural College Farm, Bapatla. A field experiment was conducted at College Farm, Agricultural College, Bapatla, Acharya N.G Ranga Agricultural University, during the summer of 2022 and 2023 to study the impact of high temperature stress on the physiological and biochemical responses of blackgram genotypes. The experiment was laid out in a Randomised Block Design (RBD) with two replications. 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. The present study was carried out with thirty blackgram genotypes, which were procured from AICRIP (pulses), Regional Agricultural Research Station (RARS), Lam, Guntur, Andhra Pradesh, India. The weather parameters during the cropping season are presented in the Fig. 1.

Observations such as membrane injury index (MI), chlorophyll stability index (CSI), canopy temperature depression (CTD), superoxide radical (SOR), hydrogen peroxide (HP), malondialdehyde (MDA), proline, superoxide dismutase (SOD) and catalase (CAT) were recorded at pod formation stage and yield attributes such as number of pods per plant (NPP), pod weight (PW) and seed yield per plant (SYP) were recorded at harvest. The mean maximum temperature during the first season (summer, 2022) at the pod formation stage ranged from 35.0 to 37.7 °C. During the second season (summer, 2023) at the pod formation stage, it was ranged from 37.7 to 39.7 °C.

Assessment of physiological and biochemical traits

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 was kept at 40–45 °C for 30 minutes and another set, at 100 °C for 10–15 min in a water bath. After hot water bath incubation, samples were allowed to reach room temperature and the electrical conductivity of water in both sets of test tubes was recorded by using a conductivity meter. (6).

$$\text{Membrane injury index (\%)} = \frac{\text{Conductivity at 45 } ^\circ\text{C}}{\text{Conductivity at 100 } ^\circ\text{C}} \times 100 \quad (\text{Eqn. 1})$$

Chlorophyll stability index

0.1 g of fresh leaf material was placed in a test tube and 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 overnight. Chlorophyll extracted into DMSO solution was collected from test tubes and the absorbance of the chlorophyll extract of heat-treated and untreated samples was measured with a spectrophotometer at 652 nm.

$$\text{Total chlorophyll (mg g}^{-1}\text{)} = \frac{D.652 \times 1000}{34.5} \times \frac{V}{1000 \times W} \quad (\text{Eqn. 2})$$

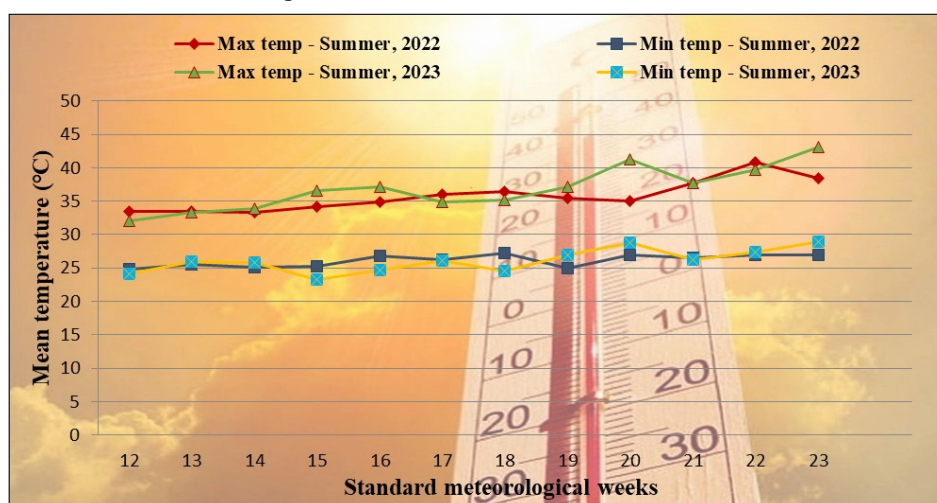


Fig. 1. Standard week wise mean temperatures during summer, 2022 and 23.

$$\text{CSI (\%)} = \frac{\text{Total chlorophyll content of the treated sample}}{\text{Total chlorophyll content of the untreated sample}} \times 100$$

(Eqn. 3.)

Then, the chlorophyll stability index of the leaf material is calculated using the following formula (7).

Canopy temperature depression (°C)

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 was focused on the canopy target by holding the gun pistol-grip at an angle of 45 °C with a distance of 0.5 to 1.0 m above the canopy. The 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.

$$\text{Canopy temperature depression} = \text{Air temperature} - \text{Canopy temperature}$$

(Eqn. 4)

Quantification of free radicals

Superoxide free radical was quantified by its capacity to reduce nitroblue tetrazolium chloride (NBT) (8). 0.1 g of leaf sample was homogenised in 2 mL of precooled phosphate buffer (0.2M, pH 7.2). The homogenate was centrifuged at 10000 rpm for 10 min at 4 °C. A 3 mL reaction mixture contained 100 µL supernatant in 0.75 mM NBT, 25 mM sodium carbonate, 0.1 mM EDTA and 13.3 mM L-Methionine. The reaction mixture was incubated at 30 °C in a waterbath for 10 min and absorbance was recorded at 540 nm.

Hydrogen peroxide was estimated by the formation of the titanium-hydroperoxide complex (9). Leaf samples were ground in 3 mL of cooled acetone in a chilled mortar and pestle. The homogenate was filtered through Whatman No. 1 filter paper, followed by the addition of 2 mL of titanium reagent and 2.5 mL of ammonium hydroxide solution to precipitate the titanium hydroperoxide complex. The reaction mixture was centrifuged at 10000 rpm for 10 min. The precipitate was dissolved in 5 mL of 2 M concentrated sulphuric acid and recentrifuged. The supernatant was read at 415 nm against a blank and H₂O₂ was expressed as µmol H₂O₂g⁻¹FW.

Lipid peroxidation

The amount of MDA derived from unsaturated fatty acid peroxidation of membrane lipids was measured (10). A 250 mg leaf sample was weighed and homogenised with 5 mL of 0.1% TCA. 1 mL supernatant was taken and 4 mL of 20 % TCA containing 0.5 % TBA was added. The mixture was heated at 95 °C for 30 min. The content was cooled in an ice bath and again centrifuged at 10000 rpm for 10 min. The absorbance was measured at 532 nm and the result was expressed in nmol g⁻¹.

Proline content

Leaf samples (500 mg) were homogenised in 3 % aqueous sulfosalicylic acid (10 mL) and filtered through Whatman No. 42 filter paper. Two millilitres of acid ninhydrin (1.25 g ninhydrin in 30mL of glacial acetic acid and 20 mL of 6 M phosphoric acid) and 2 mL of glacial acetic acid were heated for 1 hr at 100 °C. The reaction mixture was extracted with 4 mL of toluene and mixed vigorously for 15–20 sec and the absorbance of the toluene layer was measured spectrophotometrically at 520 nm using toluene as the blank (11).

Superoxide dismutase activity

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) (12). 0.2 g of leaf sample was homogenised in 10 mL of 0.5 M phosphate buffer containing 1 % NEDD. The homogenate was centrifuged at 4 °C for 30 min. at 10000 rpm. The 3 mL reaction mixture contained 1.5 mL phosphate buffer, 0.2 mL of methionine solution. 0.1 mL of riboflavin was added at the end and the tubes were shaken and placed 10 cm below a light source consisting of two 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 15 min. The reaction was stopped by switching off the light and the tubes were covered with a black cloth. The absorbance of the reaction mixture was read at 560 nm. A non-irradiated reaction mixture did not develop colour and served as a control. One unit of SOD activity was defined as the amount of enzyme required to 50 % inhibition of the rate of NBT reduction at 560 nm.

Catalase activity

Catalase (CAT) activity was determined spectrophotometrically by measuring the rate of H₂O₂ disappearance at 240 nm (13). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) and 10.5 mM H₂O₂. The reaction was run at 25 °C for 2 min, after adding the enzyme extract and the rate of decrease in absorbance at 240 nm was measured. Catalase activity was calculated by the decrease in H₂O₂ absorbance at 240nm and expressed in µg H₂O₂ min⁻¹g⁻¹.

Yield and yield attributes

Yield parameters such as number of pods per plant (NPP), pod weight (PW) and seed yield per plant (SYP) were recorded at harvest during both years.

Statistical analysis

The data were analysed statistically by following the Analysis of Variance (ANOVA) technique (14) for Randomised Block Design (RBD). The statistical hypothesis of equality of treatment means was tested by the F-test at 1 to 5 %, % level of significance. The data collected on physiological, biochemical and yield traits were subjected to correlation analysis in OPSTAT and PCA was performed using R software.

Results

Impact of high temperature stress on physiological responses of blackgram genotypes

Membrane injury index

Membrane injury index (MI) is a crucial physiological trait for selecting genotypes that are tolerant to heat stress, as membranes are the primary sites of injury. Pooled data from 2 seasons revealed significant variation in MI among all the genotypes (Fig. 2). The genotype TBG-129 (27.80 %) recorded a lower MI value which was at par with PU-31 (29.2 %), LBG-1015 (29.6 %), GGB-1 (29.8 %) and LBG-995 (30.0 %) indicating their cell membrane stability and heat stress tolerance while, it was higher in TBG-125 (40.4 %) followed by LBG-996 (39.8 %) and LBG-999 (39.2 %) indicating more membrane leakage and susceptibility to high temperatures.

Chlorophyll stability index

The data pertaining to chlorophyll stability index in the blackgram genotypes were recorded at the pod formation stage during summer, 2022 and 2023 and the pooled data were presented in

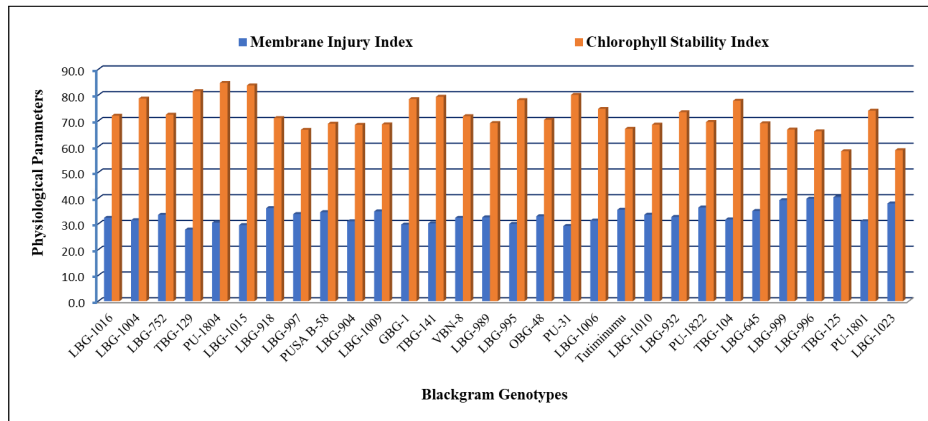


Fig. 2. Effect of high temperature stress on MII and CSI of blackgram genotypes during pod formation stage (pooled data of 2 years)

the Fig. 2. Pooled data of two seasons revealed that there was significant variation among all the genotypes with respect to the chlorophyll stability index. The genotype PU-1804 (84.8 %) recorded a higher CSI, which was at par with LBG-1015 (83.8 %), TBG-129 (81.6 %) and PU-31 (80.1 %); it was lower in TBG-125 (58.2 %), followed by LBG-1023 (58.6 %). The decrease in CSI in the susceptible genotypes might be due to exposure to high temperatures, which caused chlorophyll degradation.

Canopy temperature depression

Pooled data of two seasons revealed that there was significant variation among all the genotypes with respect to CTD. The genotype, PU-31 (-0.88 °C) recorded higher CTD values, which was at par with PU-1804 (-0.96 °C), LBG-1015 (-0.96 °C) and TBG-104 (-0.96 °C) while, while lower in TBG-125 (-2.51 °C) followed by LBG-1023 (-2.51 °C) and LBG-996 (-2.17 °C). Transpirational cooling helps plants maintain photosynthesis under heat stress conditions. Hence, CTD can be used as a measure for assessing heat stress tolerance.

Biochemical traits

Influence of high temperature stress on biochemical responses of blackgram genotypes

Free radical content

The data about superoxide radical content in the blackgram genotypes were recorded at the pod formation stage during summer, 2022 and 2023 and the pooled data were presented in the Fig. 3. Pooled data of two seasons revealed that there was significant variation among all the genotypes with respect to SOR and HP content. The genotype TBG-129 (1.39 change in OD min⁻¹ g⁻¹ FW) recorded lesser SOR content which was at par with TBG-141 (1.43 change in OD min⁻¹ g⁻¹ FW), LBG-1015 (1.46 change in OD min⁻¹ g⁻¹ FW) and LBG-1004 (1.47 change in OD min⁻¹ g⁻¹ FW) whereas, it was higher in LBG-1023 (2.06 change in OD min⁻¹ g⁻¹ FW) followed by TBG-125 (1.97 change in OD min⁻¹ g⁻¹ FW). This decrease in SOR content in thermotolerant genotypes was due to increased SOD activity, which might have scavenged the superoxide radicals.

The genotype TBG-129 (6.64 μ mol H₂O₂ g⁻¹ FW) recorded lesser HP radical content which was at par with PU-1804 (7.03 μ mol H₂O₂ g⁻¹ FW), TBG-141 (7.18 μ mol H₂O₂ g⁻¹ FW) and PU-31 (7.18 μ mol H₂O₂ g⁻¹ FW) while, higher in TBG-125 (10.74 μ mol H₂O₂ g⁻¹ FW) followed by LBG-1023 (9.96 μ mol H₂O₂ g⁻¹ FW). This increase in HP content in the susceptible genotypes indicates a decrease in the capacity of the hydrogen peroxide scavenging system.

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Lipid peroxidation

MDA content varied significantly among the genotypes during summer, 2022 and 2023 and the pooled data were presented in the Fig. 4. The genotype PU-31 (13.1 μ mol g⁻¹ FW) recorded lower MDA content which was at par with TBG-129 (13.4 μ mol g⁻¹ FW), PU-1804 (13.9 μ mol g⁻¹ FW) and LBG-1015 (14.5 μ mol g⁻¹ FW), whereas, it was higher in TBG-125 (19.4 μ mol g⁻¹ FW) followed by LBG-1023 (17.12 μ mol g⁻¹ FW). This reduction in MDA content of heat-tolerant genotypes was due to increased activity of antioxidant defence enzymes, which might have scavenged the ROS, thereby protecting cellular membranes from lipid peroxidation.

Proline

Significant genetic variability was observed among the genotypes with respect to proline content during summer, 2022 and 2023 and the pooled data were presented in the Fig. 4. Higher proline content was recorded in TBG-129 (1.96 μg g⁻¹ FW) which was at par with LBG-1015 (1.90 μg g⁻¹ FW), TBG-141 (1.87 μg g⁻¹ FW) and PU-

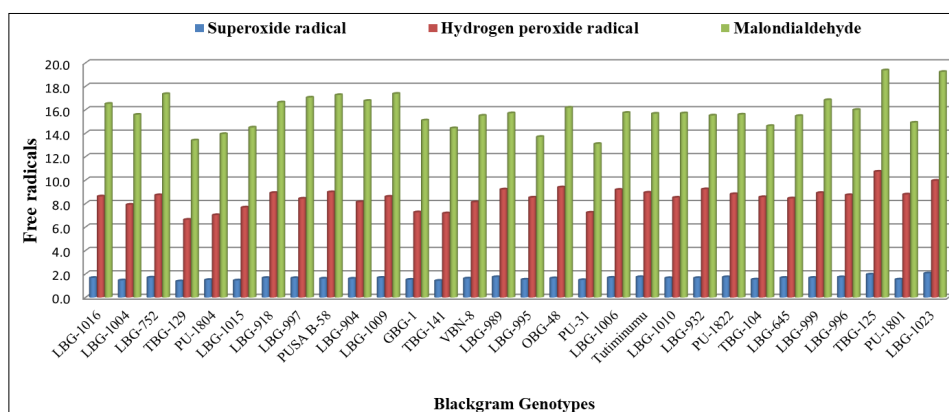


Fig. 3. Effect of high temperature stress on free radicals and lipid peroxidation of blackgram genotypes during pod formation stage (pooled data of two years).

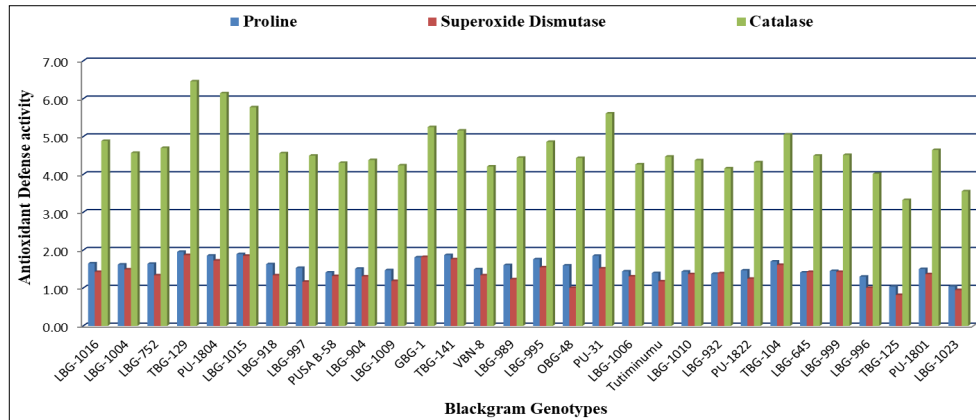


Fig. 4. Effect of high temperature stress on proline and antioxidant defence activity of blackgram genotypes during pod formation stage (pooled data of 2 years).

1804 (1.86 $\mu\text{g g}^{-1}$ FW) indicating their intrinsic tolerance to high temperatures while, it was lesser in LBG-1023 followed by TBG-125 (1.04 $\mu\text{g g}^{-1}$ FW) indicating the susceptibility of these genotypes to high temperatures during both the years.

Antioxidant enzyme activity

SOD activity varied significantly among the genotypes during summer, 2022 and 23 and the pooled data were presented in the Fig. 4. The genotype TBG-129 (1.87 U. g^{-1} FW min^{-1}) recorded higher SOD values which was at par with LBG-1015 (1.85 U. g^{-1} FW min^{-1}), GBG-1 (1.82 U. g^{-1} FW min^{-1}), TBG-141 (1.76 U. g^{-1} FW min^{-1}) and PU-1804 (1.73 U. g^{-1} FW min^{-1}) whereas, lower SOD activity was recorded in TBG-125 (0.82 U. g^{-1} FW min^{-1}) followed by LBG-1023 (0.95 U. g^{-1} FW min^{-1}) indicating more oxidative damage.

Significant genotypic variability was observed among the blackgram genotypes with respect to catalase activity during summer, 2022 and 23 and the pooled data were presented in the

Fig. 4. Higher catalase activity was recorded in TBG-129 (6.46 $\mu\text{g H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$) which was at par with PU-1804 (6.15 $\mu\text{g H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$) and LBG-1015 (5.78 $\mu\text{g H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$) while, lower in TBG-125 (3.33 $\mu\text{g H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$) followed by LBG-1023 (3.56 $\mu\text{g H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$) which might be due to heat stress induced inactivation of enzymes which resulted in accumulation of ROS leading to membrane damage.

Yield and yield attributes

Temperature stress at the pod formation stage affects the seed yield by decreasing the pod number per plant, which is the main yield contributing trait (Table 1). 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. PW ranged from 1.9 to 6.0 g plant^{-1} . Higher PW was recorded in TBG-129 (6.0 g plant^{-1}), which was at

Table 1. Yield and yield attributes of blackgram genotypes grown under high temperature stress conditions (pooled data).

Genotypes	Yield and yield attributes		
	No. of pods plant^{-1}	Pod weight (g plant^{-1})	Seed yield (g plant^{-1})
LBG-1016	9.8	4.2	2.5
LBG-1004	17.6	5.3	3.4
LBG-752	9.5	3.2	1.9
TBG-129	19.9	6.0	4.1
PU-1804	20.2	5.7	4.1
LBG-1015	21.5	5.8	4.3
LBG-918	7.8	3.2	1.7
LBG-997	10.7	2.4	1.4
PUSA B-58	11.5	3.9	2.1
LBG-904	8.6	3.0	1.4
LBG-1009	8.4	2.7	1.5
GBG-1	14.7	5.1	3.0
TBG-141	14.6	5.1	3.6
VBN-8	11.6	3.8	2.2
LBG-989	9.6	2.6	1.3
LBG-995	13.9	4.6	3.6
OBG-48	8.3	2.8	1.7
PU-31	17.7	5.6	3.9
LBG-1006	13.5	3.8	2.2
Tutimumumu	6.7	2.3	1.3
LBG-1010	9.2	4.0	2.1
LBG-932	8.9	3.2	2.2
PU-1822	5.5	2.5	1.3
TBG-104	19.8	5.7	3.8
LBG-645	7.1	3.2	1.7
LBG-999	5.1	1.9	1.1
LBG-996	4.4	1.9	1.2
TBG-125	3.8	2.0	1.0
PU-1801	12.6	3.5	2.0
LBG-1023	4.7	1.9	1.1
SEm \pm	0.7	0.3	0.2
CD (%)	2.0	0.7	0.5
CV (%)	8.9	9.6	10.7

par with PU-1804 (5.8 g plant⁻¹), PU-1804 and TBG-104 (5.7 g plant⁻¹), while it was lower in LBG-999, LBG-996, LBG-1023 (1.9g plant⁻¹), followed by TBG-125 (2.0 g plant⁻¹). SYP ranged from 1.0 to 4.3g 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 fertilisation, affecting seed filling and ultimately reducing the seed yield.

Correlation analysis

Seed yield per plant showed significant positive correlation with CSI, proline, SOD and CAT, whereas MII, SOR, HP and MDA showed significant negative correlation with SYP during summer, 2022 and 2023 (Table 2). Apart from this, superoxide radical and hydrogen peroxide 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, SOD and

CAT showed a negative association with free radicals, MII and MDA. The occurrence of a negative association of antioxidant defence enzymes with free radicals under heat stress conditions indicates the strong antioxidant defence activity, which might be the reason for heat tolerance at the cellular level. Moreover, all the antioxidant defence enzymes showed positive correlation with all the yield attributes such as NPP, PW and SYP during both years. This might be the reason behind the higher seed yield in thermotolerant blackgram genotypes.

Principal component analysis

Principal component analysis was performed based on physiological, biochemical and yield traits of blackgram genotypes grown under a heat stress environment. PCA analysis revealed that the first principal component with eigen value more than 1 explained 81.37 of the total variability. Biplot of investigated traits in blackgram genotypes under heat stress conditions are depicted in Fig. 5. The biplots under heat stress conditions during both years revealed that SYP showed a strong positive correlation with CSI, CTD, proline, SOD, CAT, NPP and PW by possessing a small angle

Table 2. Correlation between physiological, biochemical and yield traits of blackgram genotypes grown under high temperature stress conditions (pooled data)

	MII	CSI	SOR	HP	MDA	Proline	SOD	CAT	NPP	PW	SYP
MII	1										
CSI	-0.850**	1									
SOR	0.816**	-0.901**	1								
HP	0.732**	-0.811**	0.858**	1							
MDA	0.746**	-0.854**	0.824**	0.745**	1						
Proline	-0.818**	0.905**	-0.882**	-0.843**	-0.785**	1					
SOD	-0.758**	0.887**	-0.843**	-0.841**	-0.749**	0.851**	1				
CAT	-0.750**	0.875**	-0.818**	-0.865**	-0.770**	0.916**	0.858**	1			
NPP	-0.779**	0.896**	-0.937**	-0.830**	-0.817**	0.886**	0.866**	0.876**	1		
PW	-0.844**	0.911**	-0.827**	-0.742**	-0.723**	0.809**	0.811**	0.828**	0.833**	1	
SYP	-0.830**	0.910**	-0.834**	-0.773**	-0.739**	0.815**	0.866**	0.821**	0.855**	0.943**	1

MII - Membrane injury index, CSI - Chlorophyll stability index, CTD - Canopy temperature depression, SOR - Superoxide radical, HP - Hydrogen peroxide, MDA - Malondialdehyde, SOD - Superoxide dismutase, CAT - Catalase, NPP - Number of pods per plant, PW - Pod weight, SYP - Seed yield per plant.

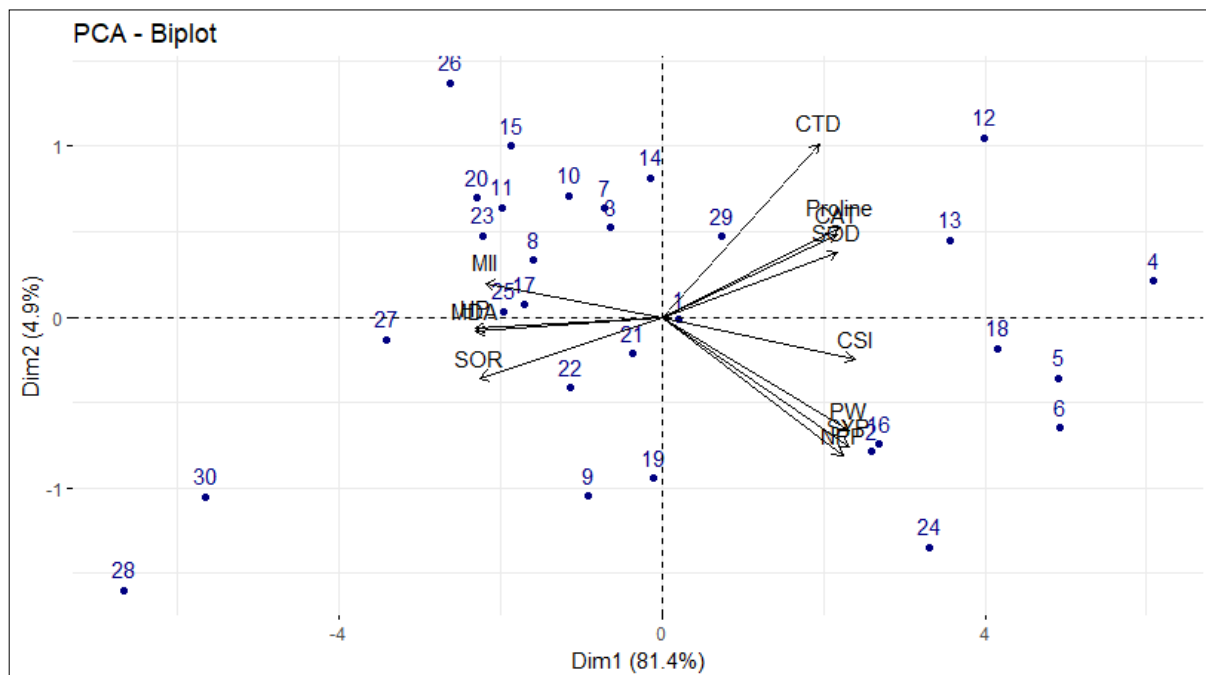


Fig. 5. Physiological, biochemical and yield traits (pooled data) are represented in PCA - Principal component analysis, MII - Membrane injury index, CSI - Chlorophyll stability index, CTD - Canopy temperature depression, SOR - Superoxide radical, HP - Hydrogen peroxide, MDA - Malondialdehyde, SOD - Superoxide dismutase, CAT - Catalase, NPP - Number of pods per plant, PW - Pod weight, SYP - Seed yield per plant. Genotypes are 1. LBG-1016, 2. LBG-1004, 3. LBG-752, 4. TBG-129, 5. PU-1804, 6. LBG-1015, 7. LBG-918, 8. LBG-997, 9. PUSA B-58, 10. LBG-904, 11. LBG-1009, 12. GBG-1, 13. TBG-141, 14. VBN-8, 15. LBG-989, 16. LBG-995, 17. OBG-48, 18. PU-31, 19. LBG-1006, 20. Tutiminumu, 21. LBG-1010, 22. LBG-932, 23. PU-1822, 24. TBG-104, 25. LBG-645, 26. LBG-999, 27. LBG-996, 28. TBG-125, 29. PU-1801 and 30. LBG-1023.

between the corresponding vectors of the above traits during both years. The seed yield showed a significant negative correlation with MII, SOR, HPR and MDA, as there was the largest angle between the corresponding vectors of the above traits during both years. In PCA of all 30 genotypes, TBG-129, LBG-1015, PU-1804 and PU-31 recorded higher CSI, CTD, proline, SOD, CAT, NPP, PW 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 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.

Discussion

Elevated temperatures influenced most of the physiological and biochemical traits of blackgram cultivars. To understand the realistic impact of high temperature stress on important physiological and biochemical traits of blackgram genotypes, various parameters such as MII, CSI, CTD, SOR, HP, MDA, Proline, SOD, CAT, NPP, PW and SYP were assessed at the pod formation stage. Three important basic physiological traits were used for identifying heat-tolerant blackgram genotypes, viz. MII, CSI and CTD. The degree of influence of high temperature stress on physiological traits varied with the type of blackgram cultivar. Membrane injury index is a measure of the extent of damage to cell membranes, which is often exacerbated by environmental stresses such as high temperatures. When the index is lower, it suggests that the cell membranes are more stable and less prone to damage under stress conditions. Genotypes TBG-129, LBG-1015, GBG-1, LBG-995 and PU-31 recorded lower MII values, indicating their cell membrane stability and heat stress tolerance. Research indicates similar results in the previous studies in blackgram (1, 15). Leaves exposed to excessive heat at certain times can cause changes in chlorophyll content (16, 17). Hence, the chlorophyll stability index under high-temperature stress is crucial in determining the resilience of plants. This index measures the stability and retention of chlorophyll in plant tissues when subjected to elevated temperatures. Higher chlorophyll stability indicates better photosynthetic efficiency and overall plant health under stress conditions, aiding in the selection and breeding of heat-tolerant crop varieties. In the current study, the genotypes PU-1804, LBG-1015, TBG-129 and PU-31 recorded higher CSI during both years. The decrease in CSI in the susceptible genotypes, viz., TBG-125 and LBG-1015, might be due to exposure of the crop to high temperatures that caused destruction of chlorophyll. Retention of chlorophyll under high temperature stress was reported in greengram (18). Moreover, research has shown that leaf chlorophyll content itself under stress conditions would possibly act as a proxy for leaf photosynthetic capacity (19).

Canopy temperature depression is a vital physiological trait that provides insights into a plants' ability to manage high-temperature stress. Plants that can maintain cooler canopies under high temperatures are usually more effective at transpiring water, which helps in cooling the plant tissues and maintaining physiological functions. It helps in identifying and developing crop varieties that are more resilient to heat, thereby enhancing agricultural productivity and stability in the face of climate change. In the present study, the blackgram genotypes, PU-1804, LBG-1015 and TBG-104 recorded higher CTD values during both years. Hence, CTD can be used as a measure for assessing heat stress tolerance.

CTD is a potential indirect selection criterion for yield under heat stress conditions (20). Biochemical traits used for identifying heat-tolerant blackgram genotypes were SOR, HP, MDA, Proline, SOD and CAT. The degree of influence of high temperature stress on biochemical traits also varied with the type of blackgram cultivar. Superoxide radicals (SOR) are a type of reactive oxygen species that are commonly produced in cells under stress conditions, including high temperature stress. These radicals are formed when oxygen molecules gain an extra electron, resulting in a highly reactive species. In the present study, it was noticed that the genotypes TBG-129, LBG-141, LBG-1015 and LBG-1004 recorded lower SOR content, indicating lesser oxidative damage and higher antioxidant defence activity, while higher SOR was recorded in TBG-125 and LBG-1023. Similar findings of lower levels of SOR content in heat-tolerant genotypes were previously reported in greengram (18). Monitoring and managing hydrogen peroxide levels are crucial for improving the stress tolerance of plants and other organisms. It was observed that the blackgram genotypes TBG-129, PU-1804, TBG-141 and PU-31 recorded lesser HP radical content. Similar findings of lower levels of HP content in heat-tolerant genotypes were previously reported in wheat and greengram (18, 21).

Malondialdehyde (MDA) is a byproduct of lipid peroxidation, which is a process where free radicals attack lipids containing carbon-carbon double bonds, particularly polyunsaturated fatty acids. MDA is commonly used as a biomarker to measure oxidative stress and lipid peroxidation levels in cells and tissues. Minimised lipid peroxidation under high temperature stress conditions with higher stable membranes, as shown by blackgram genotypes TBG-129, PU-1804, LBG-1015 and PU-31 in the current study, helps in overcoming the stress impacts adequately. Similar findings of lower levels of MDA in heat-tolerant genotypes were previously reported in greengram, chickpea and fieldpea (4, 18, 22). Proline is an amino acid that plays a crucial role in plants' response to various abiotic stresses, including high temperature stress. Under such conditions, proline accumulation serves multiple protective functions. In the current study, the genotypes TBG-129, LBG-1015, TBG-141 and PU-1804 recorded higher proline content, while lesser proline content was recorded in TBG-125 and LBG-1023. Similar findings of an increase in proline content in the heat-tolerant genotypes were previously reported in greengram and chickpea (18, 23). Proline protects the plant cell from ROS damage by osmotic adjustment, ROS detoxification, membrane and enzyme stabilisation (16).

Superoxide dismutase (SOD) plays a crucial role in responding to high-temperature stress in organisms. High temperatures can increase the production of reactive oxygen species (ROS) such as superoxide radicals, which can damage cellular components, including lipids, proteins and DNA. SOD helps mitigate this damage by converting superoxide radicals into less harmful molecules, thus protecting cells and tissues. The genotypes TBG-129, LBG-1015, PU-1804, GBG-1 and TBG-141 recorded higher SOD activity, indicating their better scavenging capacity and higher tolerance to heat stress. Lower SOD activity was recorded in TBG-125 and LBG-1023 during both years, indicating the susceptibility of these genotypes to high temperatures. Similar findings of an increase in SOD activity in the heat-tolerant genotypes were previously reported in wheat (21). Catalase is another key enzyme in the cellular defence against oxidative stress, particularly under high-temperature conditions. It works alongside SOD to neutralise ROS and protect cells from oxidative damage. The genotypes TBG-129, PU-1804 and LBG-1015 recorded higher catalase activity, while lower

catalase activity was recorded by TBG-125 and LBG-1023 during both years of the study. Lower catalase activity in susceptible genotypes might be due to heat stress induced inactivation of enzymes, which resulted in the accumulation of ROS, leading to membrane damage. Variations in heat stress-induced antioxidant enzyme activities between two wheat cultivars were previously reported in wheat (21).

At high temperatures, a significant impact on yield through a sharp reduction in the number of pods per plant was observed. Pod formation stage being highly sensitive to high temperature stress in blackgram, higher yield is possible by the genotypes having higher chlorophyll stability, cooler canopies and higher antioxidant defence enzyme activity (24). In the current study, the genotypes LBG-1015, TBG-129 and PU-1804 recorded higher NPP, PW and SYP, lesser was recorded in TBG-125, LBG-1023, LBG-999 and LBG-996, indicating their sensitivity to high temperatures. Reduction in seed yield of sensitive genotypes might be due to triggered flower abortion, pollen and ovule dysfunction, which resulted in failure of fertilisation, affecting seed filling and ultimately reducing the seed yield. Our results agree with the findings in mustard (25). The present study assessed the diverse responses of blackgram genotypes to high temperature stress and the complex relationships among traits using correlation analysis and PCA (4, 25). PCA analysis revealed that the first principal component with eigen value more than 1 explained 81.37 of the total variability. Our correlation studies showed that all the observed traits except MII, SOR, HP and MDA were positively linked with seed yield per plant. Our results were in accordance with the published reports on cotton (26). Thus, identified blackgram genotypes (TBG-129, LBG-1015 and PU-1804) with increased temperature stress tolerance could be a better option for use in crop improvement for developing heat-tolerant blackgram varieties.

Conclusion

Genotypic variability for various physiological, biochemical and yield traits was assessed over 2 years in 30 blackgram genotypes under high temperature stress conditions during the pod formation stage. The genotypes TBG-129, LBG-1015, PU-1804 and PU-31 were identified with tolerance to high temperature stress by possessing higher CSI, cooler canopies, higher proline and antioxidant defence activity compared to other genotypes, which could be utilised as a potential source of high temperature stress tolerance in blackgram. 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 defence enzyme activity during both years. Correlation analysis also revealed the presence of a significant positive association of CSI, proline, SOD and CAT with SYP during both years. 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. Hybridisation between these diverse genotypes can be suggested. Further work can be focused on identifying the genes involved in the upregulation of physiological and antioxidant defence pathways under high temperature stress

conditions. The genotypes should be evaluated across multilocations for confirming their tolerance.

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Authors' contributions

NP and KJ conducted the experiment, data generation and tabulation and manuscript writing. TS, NH, NJL and VR guidance in conducting experiments and manuscript editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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