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ABSTRACT

Background: In the present climate change scenario, the rising temperatures are imposing severe threat to blackgram production leading to marked reduction in yield potential. The reduction in the yield potential is mainly due to reproductive failures caused by heat stress. Keeping this in view, the present study was carried out to find out the reasons behind reproductive failures and identify the genotypes that yield better under heat stress.

Methods: Thirty blackgram genotypes selected from temperature induction response technique were evaluated for reproductive efficiency under natural high temperature conditions. Field experiment was conducted at Agricultural College Farm, Acharya N.G Ranga Agricultural University, Agricultural College, Bapatla. Dry matter partitioning, reproductive efficiency and yield traits were recorded at flowering and the data were analyzed statistically and pooled.

Result: Genetic variability was observed among the blackgram genotypes with respect to dry matter partitioning, reproductive efficiency and yield traits under high temperature stress conditions. Among the 30 genotypes tested for thermotolerance, the genotypes TBG-129, PU-1804 and LBG-1015 were found to withstand high temperature stress at reproductive stage by possessing higher dry matter, number of flowers per plant, pollen viability, pollen germination percentage, pollen load on stigma, stigma receptivity, flower to pod setting percentage and higher yield. Correlation analysis revealed a strong positive association of total dry matter and all the reproductive efficiency characteristics with seed yield under heat stress conditions. The PCA results revealed considerable variability among the traits accounting for 86.6% of total variability. The genotypes TBG-129, PU-1804 and LBG-1015 can be potentially used as donors in the breeding programmes for the development of heat tolerant genotypes.

Key words: Blackgram, Dry matter partitioning, High temperature stress, Reproductive efficiency.

INTRODUCTION

Blackgram is a short duration legume with high content of proteins, vitamins and minerals. In India, it is cultivated in uplands in *kharif* and also as a fallow crop after rice and grown in various agroecological conditions and cropping systems with diverse agricultural practices. Temperatures are predicted to rise in many parts of the world posing threat to crop productivity and food security. The rising temperatures are challenging the plants at various organizational levels with deleterious effects on crop growth. Blackgram being a thermosensitive crop, its yield is very sensitive to high temperature above 35°C (Anitha *et al.*, 2015). These rising temperatures are imposing challenges to blackgram at various phenological stages leading to drastic reduction in yield.

Reproductive growth is highly vulnerable to high temperatures. Exposure of crop even to short episodes of heat stress during reproductive phase causes severe reduction in the yield. Numerous reports have stated that even a few degree rise in temperature during reproductive organs development in plants results in marked reduction in the yield. Heat stress reduces the crop yield by targeting reproductive components during development that contribute to a reduction in harvest index (Hedhly *et al.*, ¹Department of Crop Physiology, Agricultural College, Acharya N.G Ranga Agricultural University, Bapatla-522 101, Andhra Pradesh, India. ²Department of Genetics and Plant Breeding, Acharya N.G Ranga Agricultural University, Regional Agricultural Research Station, Lam, Guntur-522 034, Andhra Pradesh, India.

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2003). High temperature stress disrupts male and female gametophytes, resulting in poor pollen viability, pollen

germination, loss of stigma receptivity and ovule function, fertilization arrest, limited embryogenesis, decreased ovule viability, increased ovule abortion leading to poor seed set (Kumar *et al.*, 2013). Moreover, heat stress also impairs the transport of sucrose to developing reproductive organs leading to flower abortion and poor seed set (Bhandari *et al.*, 2016).

Genotypes vary in their ability to withstand high temperature stress (Pavithra *et al.*, 2022). Principal component analysis (PCA) was used to assess genetic variability in blackgram genotypes. Information regarding genetic variability in terms of reproductive efficiency under heat stress is rarely available. In addition to this, lack of suitable varieties that can adapt to environmental extremes is the major problem affecting the productivity of blackgram. Therefore, it became important to understand genetic variability and identify the blackgram genotypes that can tolerate high temperatures.

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 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 weather parameters during cropping season were presented (Fig 1). Observations such as leaf dry matter (LDM), stem dry matter (SDM), reproductive parts dry matter (RDM), Total dry matter (TDM), number of flowers per plant (NFP), pollen viability (PV), pollen germination percentage (PGP), pollen load on stigma, stigma receptivity, flower to pod setting percentage (FPSP), number of flower clusters per plant (NPCP), number of pods per plant (NPP), pod weight (PW), seed yield per plant (SYP) and harvest index (HI) were recorded at reproductive stage as it is more sensitive to high temperature stress during both the years. The mean maximum temperature was 36.0 and 37.2°C at flowering during summer, 2022 and 23, respectively.

Dry matter production and its partitioning (g plant⁻¹)

The total dry matter accumulation and its partitioning were estimated from the five adjacent plants sampled from each treatment in two replications and then separated into leaves, stems and pods. The plant parts were dried to a constant weight in a hot-air-oven at 80°C for two days and the dry weights were recorded and expressed in g plant⁻¹.

No. of flowers/plant

The number of flowers plant¹ was counted for five plants selected and tagged for non destructive measurement continuously for 15 days. The mean value was calculated and expressed as number of flowers plant¹.

Pollen viability (%)

Around 100 pollen grains were tested per each genotype for the pollen viability with 0.5% acetocarmine per genotype in three replicates. Selection of viable pollen grains was made on the basis of size (fully expanded), shape (triangular or spherical) and concentration of stain taken by them. Pollen grains were collected from freshly opened flowers and were pooled and checked for their viability (Kaushal *et al.*, 2013).

Pollen germination (%)

Flower buds were randomly collected from each genotype during the morning hours. Pollen was dusted onto a petriplate with pollen germination medium (15 g sucrose, 0.03 g calcium nitrate, 0.01 g boric acid and 5 g agar in 100 mL distilled water). The contents were heated in a microwave oven and poured into petriplates. The plates were sealed and left at room temperature for incubation. Germinated pollen grains on a petriplate were counted after 40 hours of incubation under a light microscope (LEICA) to determine pollen tube germination. Partheeban and colleagues calculated the percentage of pollen germination in each field view by dividing the total number of pollen grains by the number of germinated pollen grains and expressing it as a percentage (Partheeban and Vijayaraghavan, 2017).

Stigma receptivity

Stigma receptivity refers to the stigma's ability to hold pollen grains and allow them to germinate in germination media. An esterase test was performed to detect stigma receptivity using α -napthyl acetate as the substrate in the azo-coupling reaction with fast blue B, as modified by Mattson *et al.* (1974). Stigmas were removed one day before flower opening and immersed in a phosphate buffer solution containing α -napthyl acetate and fast blue B for 15 minutes at 37°C. On a scale of 1 to 5, the reddish brown colour that developed on the surface of the stigma was rated.

Flower to pod setting percentage (%)

It denotes the reproductive efficiency of a genotype. Two days after the beginning of flowering, all of the flowers and buds were counted and recorded from 10 plants selected at random within each plot. The plants were tagged and numbered and at the physiological maturity the tagged plants were removed from the plot and the number of pods were counted. Number of flowers converted to pod was expressed in percentage and calculated as:

No. of flowers converted to pod (%) =

 $\frac{\text{No. of flowers converted into pods}}{\text{No. of flowers tagged}} \times 100$

Yield and yield attributes

Yield parameters such as number of pod clusters per plant (NPCP), number of pods per plant (NPP), pod weight (PW), 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, (1984) 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

Dry matter production and its partitioning

The data pertaining to dry matter production and partitioning 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 the all the genotypes with respect to dry matter production and partitioning. LDM ranged from 1.14 to 1.96 g plant⁻¹. Higher LDM was recorded in LBG-989 (1.96 g plant⁻¹) which was at par with LBG-932 (1.90 g plant⁻¹), TBG-129 (1.89 g plant⁻¹), TBG-141 (1.84 g plant⁻¹) and LBG-752, GBG-1, TBG-104 (1.83 g plant⁻¹) while, lower in TBG-125 (1.14 g plant⁻¹) followed by Tutiminumu (1.28 g plant⁻¹) and LBG-1023 (1.29 g plant⁻¹).

SDM ranged from 1.39 to 2.43 g plant⁻¹. Higher SDM was recorded in TBG-104 (2.43 g plant⁻¹) which was at par with TBG-129 (2.25 g plant⁻¹) and PU-1804 (2.21 g plant⁻¹) while, lower in TBG-125 (1.39 g plant⁻¹) followed by LBG-1016 (1.54 g plant⁻¹) and PU-1822 (1.56 g plant⁻¹).RDM ranged from 0.12 to 0.70 g plant⁻¹. Higher RDM was recorded in TBG-104 (0.70 g plant⁻¹) followed by LBG-995 (0.66 g plant⁻¹) and TBG-129 (0.63 g plant⁻¹) while, it was lower in TBG-125, LBG-932, PU-1822 and LBG-752 (0.12 g plant⁻¹) which was at par with LBG-1023 (0.14 g plant⁻¹). TDM ranged from 2.65 to 4.96 g plant⁻¹. Higher TDM accumulation was recorded in TBG-104 (4.96 g plant-1) which was at par with TBG-129 (4.77 g plant⁻¹) and PU-1804 (4.55 g plant⁻¹) while, it was lower in TBG-125 (2.65 g plant⁻¹) followed by PU-1822 (2.99 g plant⁻¹) and LBG-1023 (3.03 g plant⁻¹).

Reproductive efficiency traits

No. of flowers plant⁻¹

Pooled data of two seasons revealed that all the blackgram genotypes exhibited wider variation in reproductive efficiency traits. The genotype PU-1804 (87.3) recorded higher NFP which was at par with TBG-129 (83.3) while, it was lower in TBG-125 (56.8) followed by LBG-1023 (58.8). Our results agree with the published reports of Partheeban and Vijayaraghavan (2017) who reported more number of flowers in heat tolerant blackgram genotypes.

Pollen viability (%)

Pooled data of two seasons revealed that there was significant variation among the all the genotypes with respect to pollen viability with mean values ranging from 53.7 to 79.1%. PV was higher in TBG-129 (79.1%) which was at par with PU-1804 (76.6%) andLBG-1015 (76.3%), while, it was lower in TBG-125 (53.7%) followed by LBG-1023 (57.3%) (Fig 2 and 3). Similar findings of decrease in pollen viability in thermosensitive genotypes were previously reported by Haritha (2020) and Chaudhary *et al.* (2022) in blackgram.Heat stress decreases the accumulation of carbohydrates in pollen grains and stigmatic tissue by changing partitioning of the assimilates and the proportion between symplastic and apoplastic loading of the phloem (Taiz and Zeiger, 2006), which affects pollen viability (Kaushal *et al.*, 2013).

Pollen germination (%)

Heat stress significantly affected the pollen germination with mean values ranging from 47.4 to 70.9%. Higher PGP was recorded in PU-1804 (70.9%) which was at par with LBG-1015 (67.4%) and TBG-129 (67.0%) whereas, it was lower in TBG-125 (47.4%) followed by LBG-1023 (51.4%) (Fig 2 and 4). Similar findings of decrease in PGP in thermosensitive genotypes were previously reported by Chaudhary *et al.* (2022) in blackgram. Poor pollen germination could be the result of undernourished pollen during development due to stress, as reported in tomato (Pressman *et al.*, 2006).

Pollen load on stigma

Stereomicroscopic observation revealed that the genotypes TBG-129, LBG-1015 and PU-1804 maintained higher pollen load during summer, 2022 and 23 while, the genotypes TBG-125 and LBG-1023 showed less pollen load on stigma (Fig 5). Our results concur with the published reports of Ahmed *et al.* (1992) who reported that pollen load was inhibited severely at high temperature in the sensitive genotypes, which might be due to some restrictions in the dehiscence of anthers. Pollen adhesion to the stigma surface has been reported to decline with increases in temperature, as observed in sweet cherry by Hedhly *et al.* (2003).

Stigma receptivity (0-5 scale)

The genotypes TBG-129 (4.75), PU-1804 (4.5), LBG-1015 (4.5) and TBG-141 (4.5) showed higher stigma receptivity by absorbing more stain on stigma surface, whereas the genotypes TBG-125 (1.25) and LBG-1023 (1.25) showed lower stigma receptivity by absorbing less stain on stigma surface during first season (summer, 2022). The genotypes TBG-129 (4.5), PU-1804 (4.5) and LBG-1015 (4.25) showed higher stigma receptivity by absorbing more stain on stigma surface, whereas, the genotypes TBG-125 (1.25)

and LBG-1023 (1.25) showed lower stigma receptivity by absorbing less stain on stigma surface during second season (summer, 2023) (Fig 6). Similar findings of decrease in stigma receptivity with increase in temperature in both tolerant and susceptible genotypes of chickpea were previously reported by Kumar *et al.* (2013).

Flower to pod setting percentage (%)

Heat stress significantly affected the total chlorophyll content with mean values ranging from 68.5 to 45.4%. Higher FPSP was recorded in TBG-129 (68.5%) which was at par with LBG-1015 (66.3%) and PU-1804 (63.1%) while, lower was recorded in TBG-125 (45.4%) followed by LBG-1023 (49.1%). (Fig 2). High temperatures may cause drying up of stigma and ovary or it disturbs anthers viability due to which hybridization fails and thus flower shed occurs without initiating pod (Khattak *et al.*, 1998). It also causes pollination failure that affects flower abortion, leading to a decrease in the number of flowers converted into pods (Ahmed *et al.*, 1992). Similar findings of decrease in FPSP in susceptible genotypes under high temperature stress conditions were previously reported by Haritha *et al.* (2020) in blackgram and Khattak *et al.* (2009) in greengram.

Yield and yield attributes

Pooled data of two seasons revealed that there was significant variation among the all the genotypes with respect to yield and yield attributes. NPCP ranged from 2.2 to 6.9. The highest NPCP was recorded in LBG-1015 (6.9) which was at par with TBG-129 (6.8) and PU-1804 (6.4) whereas, lowest was recorded in LBG-1023 (2.2) followed by TBG-125 (2.4). Similar findings of higher NPCP in



Fig 1: Effect of high temperature stress on dry matter partitioning of blackgram genotypes (pooled data).



Fig 2: Effect of high temperature stress on pollen viability, pollen germination and flower to pod setting percentage of blackgram genotypes (pooled data).



Fig 3: Effect of high temperature stress on pollen viability of blackgram genotypes during summer, 2022 and 23.



Fig 4: Effect of high temperature stress on pollen germination percentage of blackgram genotypes during summer, 2022 and 23.



Fig 5: Effect of high temperature stress on pollen load on stigma of blackgram genotypes during summer, 2022 and 23.



Fig 6: Effect of high temperature stress on stigma receptivity of blackgram genotypes during summer, 2022 and 23.

thermotolerant blackgram genotypes were previously reported by Anitha *et al.* (2015).

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.

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 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 (1.9 g plant⁻¹) followed by TBG-125 (2.0 g plant⁻¹). Similar results of higher PW in thermotolerant blackgram genotypes were previously reported by Anitha *et al.* (2015).

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 (Boote *et al.*, 2005). Similar results were previously reported by Devasirvatham *et al.* (2015) in chickpea.

Correlation analysis

Correlation studies among the dry matter production, reproductive and yield traits of blackgram genotypes grown under high temperature stress at flowering revealed vital results (Table 1). RDM, TDM, PV, PGP and FPSP were positively associated with SYP. In addition to this, PV, PGP and FPSP were found to be positively correlated with NPP. This strong positive association of PV, PGP and FPSP with NPP might be the reason behind higher seed yield in heat tolerant genotypes. Similar findings of PV and PGP showing positive correlation with NPP and seed yield were previously reported by Chaudhary et al. (2022) in blackgram and Devi et al. (2022) in chickpea. NPCP, NPP and PW were also found to be positively associated with seed yield during both the years, respectively. This strong positive association of NPP, NPCP and PW with seed yield might be the reason behind higher yield under high temperature stress. Positive association of NPP, NPCP and PW with seed yield was previously reported by Devi et al. (2022) in chickpea.

Principal component analysis

Principal component analysis was performed based on dry matter production and partitioning, reproductive efficiency

Table 1	: Correlati	on between	dry matter p	artitioning,	reproductive	efficiency and	yield traits of	f blackgram g	enotypes grow	ın under high	temperature st	ress (Pooled o	lata).
	LDM	SDM	RDM	TDM	NFP	ЪЧ	PGP	FPSP	NPCP	ЧЧИ	PW	SYP	Ŧ
LDM	-	0.789**	0.376*	0.900**	0.550**	0.636**	0.560**	0.593**	0.486**	0.554**	0.436*	0.445*	0.448*
SDM		~	0.253NS	0.785**	0.342NS	0.365*	0.324NS	0.263NS	0.229NS	0.263NS	0.161NS	0.173NS	0.180NS
RDM			-	0.697**	0.749**	0.743**	0.815**	0.727**	0.833**	0.853**	0.803**	0.856**	0.842**
TDM				-	0.712**	0.761**	0.739**	0.710**	0.695**	0.740**	0.641**	0.670**	0.670**
NFP					-	0.939**	0.883**	0.899**	0.881**	0.820**	0.809**	0.816**	0.889**
P۷						£	0.950**	0.937**	0.869**	0.846**	0.838**	0.842**	0.906**
РСР							-	0.902**	0.911**	0.888**	0.895**	0.906**	0.918**
FPSP								-	0.900**	0.906**	0.911**	0.890**	0.927**
NPCP									-	0.948**	0.953**	0.966**	0.935**
NPP										-	0.944**	0.941**	0.895**
PW											-	0.973**	0.940**
SΥΡ												-	0.961**
Ŧ													1



Evaluation of Blackgram (Vigna mungo L.) Genotypes for Dry Matter Partitioning, Reproductive Efficiency and Yield under High...

Fig 7: Dry matter partitioning, reproductive efficiency and yield traits (pooled data) are represented in principal component analysis (PCA) biplot.

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 86.6% of total variability. Biplots of investigated traits in blackgram genotypes under heat stress conditions are depicted in Fig 7, respectively. The biplots under heat stress conditions during both the years revealed that SYP showed a strong positive correlation with RDM, NFP, PV, PGP, NPCP, NPP, PW and HI by possessing a small angle between the corresponding vectors of above traits. In PCA of all 30 genotypes, TBG-129, LBG-1015 and PU-1804 recorded higher RDM, PV, PGP FPSP, NPCP, NPP, PW 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 reproductive failure due to high temperature stress.

CONCLUSION

Rising temperatures in the present climate scenario posing a serious threat to blackgram production, particularly at the time of flowering. Thus, the genotypes TBG-129, LBG-1015 and PU-1804 identified with tolerance to high temperature stress by possessing higher reproductive capabilities in terms of higher pollen viability, pollen germination and stigma receptivity and thereby higher seed yield compared to other genotypes, could be utilized as potential source of high temperature stress tolerance in blackgram. Also correlation analysis revealed that all the RDM, TDM and all the reproductive efficiency traits showed positive association with seed yield. PCA also revealed that SYP showed a strong positive association with RDM, NFP, PV, PGP, NPCP, NPP, PW and HI by possessing a small angle between the corresponding vectors. In addition to this, physiological and biochemical efficiency characteristics should be analyzed in these genotypes to understand the physiological and biochemical basis for heat tolerance. The genotypes should be evaluated across multilocations for confirming their tolerance.

Conflict of interest

All authors declared that there is no conflict of interest.

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