RESEARCH ARTICLE Legume Research- An International Journal, Volume 47 Issue 3: 435-440 (March 2024)

Effect of High Minimum Temperature and Enriched Night CO_2 on Yield and Seed Quality of Black Gram (*Vigna mungo*) under Soil Plant Atmospheric Research (SPAR)

M. Guna[1], S.P. Ramanathan[1], V. Geethalakshmi[1], K. Chandrakumar[1], S. Kokilavani[1], M. Djanaguiraman[1] 10.18805/LR-4989

ABSTRACT

Background: Black gram is a leguminous plant species. This pulse crop has been grown on the Indian subcontinent for a long time. Black gram a drought-resistant crop that grown both in the summer and the winter, usually in rotation with rice but occasionally in mixed farming. The objective of this research was to (i) quantify the short-term effects of high minimum temperature and enriched night CO_2 (HMT and *en*CO_2) on yield contribution factors and (ii) quantify seed quality parameters employing biochemical analysis.

Methods: An investigation was carried out during summer 2021 and 2022 to evaluate the effect of high minimum temperature (ambient+3°C) and enriched night CO_2 (600 ppm) (HMT and *en*CO_2) on yield and seed quality parameters of black gram (*Vigna mungo*) under soil plant atmospheric research (SPAR) and ambient condition at Agro Climate Research Centre, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu with ten treatments which were replicated thrice.

Result: Black gram yield and quality parameters were significantly (p=0.05) reduced under HMT and *en*CO_2. Pooled data from two successive summer seasons revealed that stress from (i) 50 to 56 DAS (Days after sowing), the number of flowers dropped per plant significantly increased by 24% and pod setting percent decreased by 19.9% (ii) 43 to 49 DAS, grain yield and biomass/plant decreased by 25.4% and 16.2%, respectively. Seed quality parameters revealed that stress during 50 to 56 DAS, seed protein, total sugar content, polyphenols, calcium and iron decreased by 19.4%, 23.7%, 16.2%, 29.6% and 30.2%, respectively. The proline and phytic acid were statistically reduced by 53.9% and 59.1%, respectively stress during 50 to 56 DAS. Stress from 64 to 70 DAS, seed moisture content was significantly decreased by 32.1%. Overall, black gram yield and seed quality was negatively affected on two treatments: stress imposed from 43 to 49 DAS (T_7) and stress imposed from 50 to 56 DAS (T_8) (50% flowering to pod filling stage).

Key words: Black gram, Enriched night CO_2, High minimum temperature, Soil Plant Atmospheric Research, Seed quality.

INTRODUCTION

Pulses are major food crops that can help address future global food security and widely cultivated in the semi-arid tropics as a source for nutritional protein, feed and fodder. Black gram is a significant legume crop with high protein and mineral content (FAO, 2021). Variability and change in climatic parameters, such as temperatures, rainfall and changes in atmospheric carbon dioxide concentrations as a result of global warming, have a significant impact on the agricultural production system. Many research on crops reveals that the negative effects of climate change on crop yields have been more common than the positive effects at the global level. Carbon dioxide (CO_2) concentrations in the atmosphere have risen from 280 parts per million (ppm) before the industrial revolution to 418.7 parts per million (ppm) today. CO_2 is increasing at a rate of 3 ppm per every year and is anticipated to reach 750 parts per million by the end of the twenty-first century (WMO, 2021). The diurnal variation of global CO_2 concentration revealed that it is higher at night than during the day due to natural activities (Metya *et al.,* 2021). Rapid rises in minimum night temperatures compared to maximum day temperatures are expected in the future, causing significant crop productivity. Between 1970 and 2019, India's minimum temperature increased at a higher rate than its maximum temperature (Ray *et al.,* 2019). Major crops like rice, wheat, sorghum and legumes that are vulnerable to high minimum temperature stress (HMT) (Impa *et al.,* 2020). Physiological systems affected by HMT, include carbon balance, source-sink metabolite changes and reactive oxygen species. The effects of HMT on legume grain developmental dynamics are reviewed, with a focus on grain-filling length, post-flowering senescence, changes in grain starch, protein composition and starch metabolism enzymes (Garcia *et al.,* 2018). A minimum temperature of above 24°C was identified as the key threshold beyond which yields and quality began to decline

[1]Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

Corresponding Author: M. Guna, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India. Email: hawkgunams@gmail.com

How to cite this article: Guna, M., Ramanathan, S.P., Geethalakshmi, V., Chandrakumar, K., Kokilavani, S. and Djanaguiraman, M. (2024). Effect of High Minimum Temperature and Enriched Night CO_2 on Yield and Seed Quality of Black Gram (*Vigna mungo*) under Soil Plant Atmospheric Research (SPAR). Legume Research. 47(3): 435-440. doi: 10.18805/LR-4989.

Submitted: 23-06-2022 **Accepted:** 23-08-2022 **Online:** 02-09-2022

consistently in wheat (Garcia *et al*., 2016). However, under HMT and *en*CO_2 in black gram, are still not clearly understood and need attention. In this contest, the role of high minimum temperatures on black gram needs to be understood. Specifically, we aim to study the short episodes of HMT and *en*CO_2 on yield and seed quality parameters in black gram.

MATERIALS AND METHODS

To evaluate the impact of HMT and *en*CO_2 on yield and seed quality characteristics of variety of black gram CO6, a pot culture experiment was done at SPAR (Soil Plant Atmospheric Research) unit, Agro Climate Research Centre, Tamil Nadu Agricultural University, Coimbatore (11.013251° - N, 76.939725° - E) during the summer season (March to May) 2021 and 2022. The two years (2021 and 2022), daily weather data with respect to maximum and minimum temperature (°C), average relative humidity (%) and rainfall (mm) prevailed during the summer season from March to May 2022 were collected from SPAR automatic data logger and Agro Climate Research Centre, Tamil Nadu Agricultural University, Coimbatore (Fig 1). Three replications were used in the CRD (Completely Randomized Design) experiment. A high yielding black gram culture CO6 (COBG 653) is a cross derivative of DU 2 × VB 20 and matures in 65-70 days. This culture recorded an average yield of 877 Kg/ha and found to be susceptible to yellow mosaic disease. The SPAR system contains a plexiglass chamber 2 × 1.5 metres in cross section and 2.5 metres height where crop is being grown. It also contains an air conditioner, as well as other required devices like a humidifier and dehumidifier, installed on a sturdy steel frame made of plexiglass with a 6 mm thickness. The software, named EMCON (environment control), was used to stimulate the required and accurate levels of temperature and CO_2 inside the SPAR unit. The CO_2 gas was supplied to the unit to maintain CO_2 concentration at 600 ppm and an air conditioner was used to maintain the temperature levels at ambient +3°C during the night time (1800 to 0600 hrs IST), also plants were kept under ambient condition during the day time. The pot culture was maintained with well-watered condition and the pot culture was treated with RDF (Recommended dose of fetilizer) (25 kg N + 50 kg P_2O_5 + 25kg K_2O + 40 kg S)/ ha and appropriate measurements were taken for pest and disease management during the crop season (TNAU crop production guide 2021).

Stress: Ambient+3°C and 600 ppm CO_2 (HMT and *en*CO_2) during the night time. Experiment is designed with 10 treatments *viz.,* T_1: Control, T_2: stress imposed from 7 to 14 DAS (Day After Sowing), T_3: stress imposed from 15 to 21 DAS, T_4: stress imposed from 22 to 28 DAS, T_5: stress imposed from 29 to 35 DAS, T_6: stress imposed from 36 to 42 DAS, T_7: stress imposed from 43 to 49 DAS, T_8: stress imposed from 50 to 56 DAS, T_9: stress imposed from 57 to 63 DAS, T_{10}: stress imposed from 64 to 70 DAS.

The yield attributes *viz.,* number of flowers dropped/ plant, Pod setting percent, Grain yield/plant and biomass/ plant were recorded after the harvesting of the crop. The seed quality parameters *viz.,* total sugars, protein, polyphenols, seed moisture, proline, phytic acid, calcium and iron were measured by standard methods. The powdered black gram grains (100 mg) were kept overnight in 25 ml of 0.1 N NaOH to extract total proteins and total proteins were quantified using the supernatant after centrifugation at 3000 rpm (5000xg) (Lowry *et al*., 1951). 80% ethanol was employed to extract free sugars from black gram powder, followed by 70% ethanol (Kaur *et al*., 2000). Total soluble sugars were determined from the pooled extract (Dubois *et al.,* 1956). By refluxing dried seed powder with 80% aqueous methanol, the phenolic components were extracted. After filtering, total phenols were estimated using

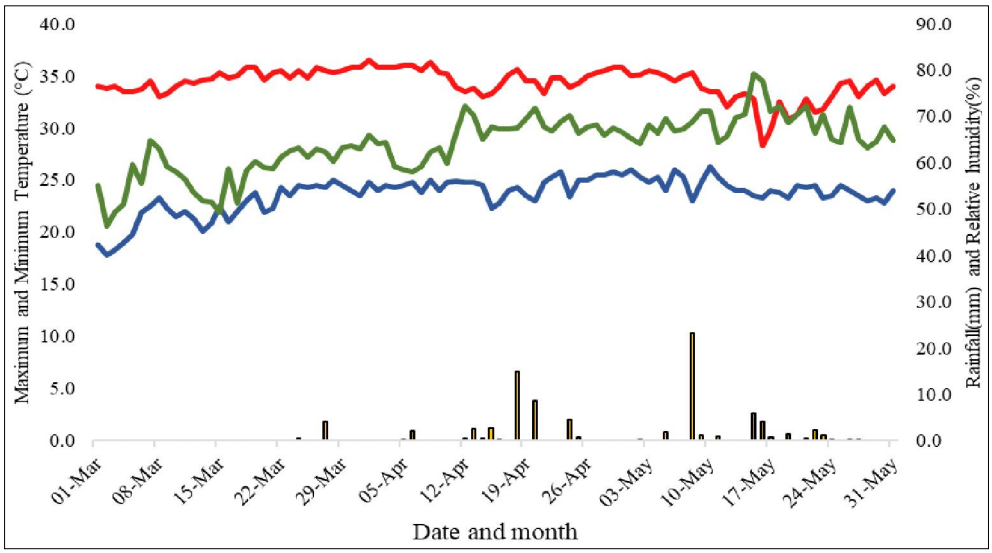

Fig 1: Two years (2021 and 2022), average daily weather prevailed during March to May.

the refluxed sample (Swain and Hills, 1959). The AOAC method (2000) has been used to determine the seed moisture content of the samples. Proline was measured using the acid ninhydrin procedure and the absorbance was obtained spectrophotometrically at 520 nm after homogenization in 10 mL of 3% (w/v) sulfosalicylic acid Bates *et al.* (1973). The phytic acid was extracted from the powdered seeds with 1.2% HCl and precipitated with 0.4% ferric chloride (Zemel and Shelef, 1982). The Rouser *et al.* (1974) technique, which involved treating the sample with strong HCl and perchloric acid, has been used to determine the sample's organic phosphorus level. In a muffle furnace, total ash was determined by burning and weighing a sample and the mineral content was calculated after it was dissolved in water. Using Wong's Method, iron was identified colorimetrically (Raghuramulu *et al.,* 2003). Calcium oxalate was precipitated, diluted in hot dilute H_2SO_4 and titrated against a potassium permanganate standard (Helrich, 1990).

SPSS 16.0 was used to perform statistical analysis on the data (SPSS Inc., Chicago, IL). For all data, the mean and standard deviation were determined and as suggested by Gomez and Gomez (1984), significant differences between mean values were evaluated using the Least Significant Difference (LSD) at a 5% probability level.

RESULTS AND DISCUSSION

Yield and yield attributes

Number of flowers dropped per plant ranged from 32.5 to 40.3 (Table 1). The lowest flower drop per plant (32.5) was recorded in black gram plants grown under ambient condition (T_1), which was statistically on par with stress imposed from 7 to 14 DAS (T_2) (33.9). This differed statistically from stress imposed during 50 to 56 DAS (T_8) (40.3) which was on par with stress imposed from 43 to 49 DAS (T_7) (40). This present study corroborates with Prasad *et al.,* (2008) and Bahuguna *et al.* (2017), they reported that the peak flowering stage of the rice was most sensitive to high night temperature and reduced the overall number of flowers per plant.

The data analysis shows that the HMT and en CO_2 had significant (p = 0.05) influence on the pod setting per cent of black gram (Table 1). The highest pod setting per cent of black gram was recorded under ambient condition (T_1) (57.1%) which was lower under stress imposed from 50 to 56 DAS (T_8) (47.7%). This current study was in conformity with findings of Shi *et al.,* (2017), which reported that the 8% reduction was measured at grain filling percent in rice crop under high minimum temperature.

From the Table 1, the grain yield per plant was highest under stress imposed from 7 to 14 DAS (T_2) (13.8 g/plant) followed by ambient condition (T_1) and stress imposed from 15 to 21 DAS (T_3) (13.4 g/plant). There was a significant reduction in the grain yield with stress imposed from 43 to 49 DAS (T_7) and stress imposed from 57 to 63 DAS (T_9) (10 g/plant) which on par with stress imposed from 50 to 56 DAS (T_8) (10.2 g/plant). The current findings are consistent with the results of Hein *et al.,* (2019), who reported that field-based heat tents experiment shows stress imposed from 50 percent flowering to maturity on wheat will reduce grain yield by 7 to 14%.

When compared to stress imposed from 7 to 14 DAS (17.6 g/plant), HMT and *en*CO_2 significantly reduced the total biomass (14.4 g/plant) when stress imposed from 43 to 49 DAS (T_7) followed by stress imposed from 50 to 56 DAS (T_8) (14.7 g/plant) and stress imposed from 36 to 42 DAS (T_6) (14.8 g/plant) (Table 1). This result correlated with the findings of Impa *et al.* (2020), who reported that increasing the high night temperature during anthesis and maturity stage would reduce the above ground biomass by 2 to 46% in wheat.

Table 1: Effect of HMT and *en*CO_2 on number of flowers dropped, pod setting percentage (%), yield (g/plant), total biomass (g/plant) of black gram (two years data pooled).

Treatment	Number of flower dropped/plant		Pod setting percentage (%)		Yield (g/plant)		Total biomass (g/plant)	
	Pooled data	% increase	Pooled data	% reduction	Pooled data	% reduction	Pooled data	% reduction
T_1	32.5		57.1		13.4		17.2	
T_2	33.9	4.3	56.7	-4.9	13.8	2.8	17.6	2.1
T_3	35.0	7.7	56.6	-5.0	13.4	0.3	17.5	1.5
T_4	35.2	8.3	59.6	0.1	11.7	-13.1	16.7	-3.0
T_5	35.9	10.5	56.4	-5.3	11.2	-16.1	17.0	-1.2
T_6	38.6	18.8	52.8	-11.4	10.3	-23.5	14.8	-14.2
T_7	40.0	23.1	50.6	-15.1	10.0	-25.4	14.4	-16.2
T_8	40.3	24.0	47.7	-19.9	10.2	-24.0	14.7	-14.3
T_9	36.9	13.5	51.8	-13.2	10.0	-25.2	16.5	-4.2
T_{10}	36.7	12.8	53.6	-10.0	12.1	-9.9	16.7	-3.1
Mean	36.5	13.7	54.3	-9.4	11.4	-14.9	16.3	-5.8
SEd	0.67		1.22		0.31		0.34	
Sem	0.21		0.39		0.10		0.11	
CD (p = 0.05)	1.39		2.56		0.64		0.72	

Nutritional and anti-nutritional compositions

The black gram plants that were grown in the ambient condition (T_1) recorded high seed protein content (21.6%) on par with stress imposed from 7 to 14 DAS (T_2) (21.2%) and was significantly (p=0.05) different from stress imposed from 50 to 56 DAS (T_8) (17.4%). This was statistically on par with stress imposed from 43 to 49 DAS (T_7) (17.7%) and stress imposed from 57 to 63 DAS (T_8) (18.1%) (Table 2). Also, according to Impa *et al.,* (2019), the seed protein of wheat crop was sensitive to high night temperature and illustrated 21% reduction of protein content in wheat.

Total sugar content (492.3 mg/g) was higher in black gram seed grown under stress imposed from 7 to 14 DAS (T_2), which was statistically on par with ambient condition (T_1) (491.3 mg/g). A significant difference was observed stress during 50 to 56 DAS (T_8) (375 mg/g), which was on par with stress imposed from 36 to 42 DAS (T_6) (377.8 mg/g) and stress imposed from 43 to 49 DAS (T_7) (378.2 mg/g) (Table 2). According to Gogoi *et al.* (2018) total sugar content of groundnut seed was reduced by 24.5% under heat stress.

The total polyphenols content (7.4 mg/g) was significantly higher in black gram seeds grown under ambient condition (T_1) which was statistically on par with stress imposed from 15 to 21 DAS (T_3) (7.3 mg/g) and stress imposed from 7 to 14 DAS (T_2) (7.2 mg/g) and lower polyphenol was recorded in stress imposed from 50 to 56 DAS (T_8) (6.2 mg/g) (Table 2). Total polyphenols help to overcome various stresses like heat and cold stress (Kaushal *et al.,* 2016). Singh *et al.,* 2015, reported that the flowering to pod development stage of legume crops would be affected by the high temperature stress.

The level of seed moisture as influenced by the HMT and *en*CO_2 are given in Table 2. The range varies from 8 to 11.9% among the treatments. The stress imposed from 64 to 70 DAS (T_{10}) had the lowest moisture content (8%), followed by stress imposed from 57 to 63 DAS (T_9) and stress imposed from 50 to 56 DAS (T_8) (8.4%) whereas the highest seed moisture content (11.9%) was recorded under stress imposed from 7 to 14 DAS (T_2). Based on the research findings of Sehgal *et al.,* 2018**,** the heat stress on pulse crops reduced the seed moisture content under the maturity stage than the other stages. In the present study, lower seed moisture content was observed during the maturity stage of the crop.

Significantly (p = 0.05) lower proline content (39.9 mg/g) was recorded under ambient condition (T_1) which was statistically on par with stress imposed from 15 to 21 DAS (T_3) (40.5 mg/g). The highest proline content (61.4 mg/g) was found in stress imposed from 50 to 56 DAS (T_8) followed by stress imposed from 43 to 49 DAS (T_7) (59 mg/g) (Table 3). Similar to present studies Liu *et al.,* 2019 found that increased proline content by 40% in bean seeds.

The phytic acid content was found significantly lower (8.3 mg/g) under stress imposed from 15 to 21 DAS (T_3) followed by stress imposed from 7 to 14 DAS (T_2) (8.6 mg/g) and ambient condition (T_1) (8.8 mg/g). Higher phytic content was recorded in stress imposed from 50 to 56 DAS (T_8) (14 mg/g) which was on par with stress imposed from 43 to 49 DAS (T_7) (12.8 mg/g) (Table 3). Temperature stress on pulse crops during reproductive stages increases phytic acid, which leads to malnutrition, according to Gillman *et al.* (2021). The essential minerals like calcium, iron and zinc were known to be absorbed by phytic acid. As a result, increasing the high minimum temperature in the black gram seeds will increase the phytic acid content. Choukri *et al.,* 2022 findings reveal that 14% phytic acid content had increased during the reproductive stage in lentils.

Significantly (p = 0.05) higher calcium content (239 mg/100 g) was recorded under stress imposed from 7 to 14 DAS (T_2) which was on par with ambient condition (T_1) (238 mg/100 g). Comparatively, lower calcium content (168 mg/100 g) was found when stress imposed from 50 to 56 DAS (T_8)

Table 2: Effect of HMT and *en*CO_2 on seed protein (%), total sugars (mg/g), polyphenols (mg/g) and seed moisture (%) of black gram (two years pooled data).

Treatment	Seed protein (%)		Total sugars (mg/g)		Polyphenols (mg/g)		Seed moisture (%)	
	Pooled data	% reduction	Pooled data	% reduction	Pooled data	% reduction	Pooled data	% reduction
T_1	21.6		491.3		7.4		11.7	
T_2	21.2	-2.1	492.9	0.3	7.2	-3.4	11.9	1.3
T_3	19.4	-10.4	478.7	-2.6	7.3	-2.0	11.0	-6.4
T_4	18.9	-12.5	472.0	-3.9	6.9	-7.4	11.1	-5.1
T_5	19.1	-11.6	461.7	-6.0	6.8	-8.1	10.7	-8.5
T_6	18.9	-12.7	377.8	-23.1	6.4	-13.5	10.6	-9.8
T_7	17.7	-18.3	378.2	-23.0	6.4	-14.2	10.0	-14.5
T_8	17.4	-19.4	375.0	-23.7	6.2	-16.2	8.4	-28.2
T_9	18.1	-16.4	383.7	-21.9	6.9	-6.8	8.4	-28.6
T_{10}	19.1	-11.6	459.4	-6.5	7.3	-1.4	8.0	-32.1
Mean	19.1	-12.8	437.1	-12.3	6.9	-8.1	10.2	-14.7
SEd	0.31		8.58		0.12		0.19	
Sem	0.10		2.72		0.04		0.06	
CD (p = 0.05)	0.66		17.90		0.25		0.41	

Table 3: Effect of HMT and *en*CO_2 on proline (mg/g), phytic acid (mg/g), calcium (mg/100 g) and iron (mg/100 g) of black gram (two years pooled data).

Treatment	Proline (mg/g)		Phytic acid (mg/g)		Calcium (mg/100 g)		Iron (mg/100 g)	
	Pooled data	% increase	Pooled data	% increase	Pooled data	% reduction	Pooled data	% reduction
T_1	39.9		8.8		238		33.1	
T_2	41.1	2.9	8.6	-2.3	239	0.4	34.3	3.6
T_3	40.5	1.5	8.3	-5.7	222	-6.7	34.0	2.7
T_4	41.7	4.4	8.9	1.1	221	-7.4	29.3	-11.5
T_5	46.4	16.3	9.7	10.2	219	-8.2	29.5	-10.9
T_6	52.6	31.8	10.4	17.6	180	-24.6	27.8	-16.0
T_7	59.0	47.7	12.8	45.5	175	-26.5	24.2	-27.0
T_8	61.4	53.9	14.0	59.1	168	-29.6	23.1	-30.2
T_9	55.8	39.8	11.1	25.6	196	-17.6	28.0	-15.6
T_{10}	42.7	7.0	10.0	13.6	208	-12.8	32.2	-2.7
Mean	48.1	22.8	10.3	18.3	206.3	-14.8	29.5	-12.0
SEd	0.88		0.28		4.07		0.54	
Sem	0.28		0.09		1.29		0.17	
CD (p = 0.05)	1.85		0.58		8.50		1.13	

followed by stress imposed from 43 to 49 DAS (T_7) (175 mg/100 g) (Table 3). The present study corroborates the results of Farooq *et al.,* (2018), also found that decreased calcium content in legume crops under the high night temperature on the pod filling stage.

Higher iron content of black gram (34.3 mg/100 g) was recorded in stress imposed from 7 to 14 DAS (T_2) which was on par with stress imposed from 15 to 21 DAS (T_3) (34 mg/100 g) and ambient condition (T_1) (33.1 mg/100 g) and statistically differed with stress imposed from 50 to 56 DAS (T_8) (23.1 mg/100 g) followed by stress imposed from 43 to 49 DAS (T_7) (24.1 mg/100 g) due to HMT and *en*CO_2 (Table 3). The current investigation supports the findings of Sarkar *et al.* (2021), who assessed decreased iron content in legume crops under temperature and CO_2 stress during the pod filling stage.

CONCLUSION

Increase in minimum temperature could have greater global effect due to its persistence over long periods during crop cycle, possibly affecting a wide range of growth and development stages. According to the findings of this study, HMT and *en*CO_2 have an adverse effect on black gram production and seed quality. However, there was only a minimal effect observed on black gram from emergence to vegetative phase (stress from 7 to 28 days after sowing). As a result, high minimum temperature and enriched night CO_2 will have a negative impact on food security in the future.

Conflict of interest

All authors declared that there is no conflict of interest.

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