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Research Paper

Effect of high night temperature and CO_2 on yield and seed quality of summer greengram (*Vigna radiata*) under soil plant atmospheric research (SPAR)

M. GUNA[1], SP. RAMANATHAN[1], V. GEETHALAKSHMI[2], K. CHANDRAKUMAR[3], S. KOKILAVANI[1], and M. DJANAGUIRAMAN[4]

[1]*Agro Climate Research Centre, Tamil Nadu Agricultural University, Coimbatore- 641003, India*
[2]*Tamil Nadu Agricultural University, Coimbatore- 641003, India*
[3]*Department of Renewable Energy Engineering, Tamil Nadu Agricultural University, Coimbatore- 641003, India*
[4]*Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore- 641003, India*
**Corresponding author email: hawkgunams@gmail.com*

ABSTRACT

An experiment was designed in Coimbatore, Tamil Nadu during two successive summer seasons, 2021 and 2022, to investigate the effects of high night temperature (HNT) (ambient minimum temperature+3°C) and CO_2 (600ppm at night) on the yield and seed quality parameters of greengram under ten treatments in SPAR (Soil Plant Atmospheric Research) and ambient conditions. The objective of this research was to (i) quantify the short-term effects of HNT and CO_2 on yield contribution factors and (ii) quantify seed quality parameters employing biochemical analysis. Greengram yield and quality parameters were significantly reduced under HNT and CO_2 when compared to ambient conditions. Pooled data from two successive summer seasons revealed that when stress was imposed from (i) 43 to 49 DAS (Day After Sowing), the number of flowers dropped per plant significantly increased by 41.3% (ii) 29 to 35 DAS, pod setting percentage decreased by 18.5% (iii) 36 to 42 DAS, grain yield and biomass/plant decreased by 26.9 % and 29.3% respectively. In aspects of seed quality parameters, data revealed that under stress (i) Seed protein decreased by 20.5% from 50 to 56 DAS. (ii) Total sugars, polyphenols, calcium and iron decreased by 17.7, 19.9, 19.9 and 37.3%, respectively from 43 to 49 DAS. (iii) Seed moisture was reduced by 19.7% during 57 to 63 DAS (iv) The levels of proline and phytic acid increased by 64.3 and 33.8%, respectively, from 50 to 56 DAS and 43 to 49 DAS. Overall, greengram yield was adversely affected on three treatments from 35 to 56 DAS (flower initiation stage to pod filling stage) and seed quality parameters such as protein, total sugars, polyphenols, seed moisture, proline, phytic acid, calcium and iron were reduced under HNT and CO_2 from 50% flowering to pod development stage (42 to 56 DAS).

Keywords: Soil plant atmospheric research, greengram, high night temperature and CO_2, seed quality, proline, phytic acid

Legumes are the world's third most widely cultivated crop, next to cereals and oilseeds. Greengram is an important legume crop that is a good source of protein and minerals. Climate change would boost global temperatures and have a substantial detrimental impact on food production (FAO. 2020). Rising temperatures, erotic precipitation, and increased CO_2 concentrations are all possible outcomes of climate change scenarios. Carbon dioxide (CO_2), methane (CH_4), water vapour (H_2O), nitrous oxide (N_2O), and other greenhouse gases (GHGs) produced by natural and anthropogenic activities would contribute to global warming. CO_2 levels surged from 275 ppm to 417.2 ppm, CH_4 and N_2O concentrations increased from 715 ppb to 1889 ppb and 227 ppb to 333.2 ppb, respectively between 1970 and 2020 (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. Since the pre-industrial era, the global average temperature has risen by about 1.2°C. In the latter half of the twentieth century, the worldwide average minimum temperature grew twice as much as the maximum temperature (Rao *et al.,* 2010). The negative impact of increasing the minimum temperature on major cereals like rice (Mohammed and Tarpley, 2011), wheat (Garcia *et al.,* 2018), sunflower (Sreenivas *et al.,* 2021) and soybean (Cheng-Zhi *et al.,* 2021) and millets like sorghum (Prasad and Djanaguriraman, 2011) are well documented. Shorter periods of increasing maximum temperature may affect crop yield differently depending on the geographical location (Kumari *et al.,* 2019). However, increasing the minimum temperature would negatively impact the crop yield and increase the likelihood of prolonged warming (Hein *et al.,* 2019). Also, the

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prolonged warming in minimum temperature would negatively alter the growth and development, yield and seed quality of the particular crop (Bahuguna *et al.,* 2017). Research findings from various field and controlled experiments revealed that C_3 crops are negatively impacted by higher night temperatures than the day temperature (Impa *et al.,* 2020). Crops exposed to high night temperature shortened the grain filling stage and also early grain development, which affected the yield attributes (Shi *et al.,* 2017). A control chamber study conducted in 2018 shows that the minimum temperature is more than 23°C, which reduces the yield and yield attributes by 14.6% in wheat (Lu *et al.*, 2019). Beyond 29°C, the grain weight was reduced by 9-10% but there was no variation in rice seed quantity under open-top chamber (OTC) (Garcia *et al.,* 2016; Morita *et al.,* 2005). However, HNT and CO_2 in greengram, are still not clearly understood and need attention. In this context, the role of high night temperatures on greengram needs to be understood. Specifically, we aim to study the short episodes of HNT and CO_2 on yield and seed quality parameters in greengram.

MATERIALS AND METHODS

Study area

A pot culture experiment was conducted during the summer season (1st March to 8th May) 2021 and 2022 at SPAR unit, Agro Climate Research Centre, Tamil Nadu Agricultural University, Coimbatore (11.013251° - N, 76.939725° - E) to determine the effect of HNT and CO_2 on yield and seed quality parameters of greengram. The SPAR system has a plexi glass chamber made up of plexi /acrylic material having 6 mm thickness, mounted on a strong metallic frame containing air conditioner and other necessary gadgets like humidifier and dehumidifier. Dimension of the plexi glass: 2 x 1.5 m in cross section with 2.5 m height. The SPAR unit was used to raise the ruling greengram CO8 variety under HNT and CO_2. Automatic temperature, relative humidity and elevated CO_2 was developed by adapting software by EMCON (environment control) to automatically maintain the desired 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 conditions during the day time.

Treatment Details

The experiment was laid out in CRD (Completely Randomized Design) with three replications. The stress *viz.,* ambient night minimum temperature +3°C and elevated night CO_2 (600 ppm) (HNT and CO_2). There were 10 treatments, *viz.,*

T_1: Control,

T_2: stress imposed during 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.

According to the TNAU crop production guide, the pot culture was treated with RDF (Recommended dose of fertilizer) (25kg N + 50kg P_2O_5 + 25kg K_2O + 40kg S)/ ha and adequate measurements were taken for pest and disease control during the crop season.

Yield attributes, nutritional and anti-nutritional compositions

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 required chemicals were procured from Sigma Chemicals Company, USA and analytical grade. To extract total proteins, the powdered mung bean grains (100 mg) were stored overnight in 25 ml of 0.1 N NaOH and the total proteins were estimated using the supernatant after centrifugation at (5000xg). To extract free sugars from greengram powder, 80% ethanol was used followed by 70 % ethanol. From the pooled extract, total soluble sugars were estimated (Dubois *et al.* 1956). The phenolic components were extracted by refluxing the seed powder with 80 percent aqueous methanol. Total phenols were estimated using the refluxed sample after filtration (Swain and Hills, 1959). The seed moisture content of samples was estimated by AOAC (2000) method. Proline was estimated using the method of Bates *et al*. (1973). After homogenization in 10 mL of 3 percent (w/v) sulfosalicylic acid, proline was measured using the acid ninhydrin technique and the absorbance was determined spectrophotometrically at 520 nm. 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 organic phosphorus content of the sample was determined using the Rouser, Fleisher, and Yamamoto method (1974), which involved treating the sample with strong HCl and perchloric acid. Total ash was assessed by burning and weighing the sample in a muffle furnace. This was dissolved in water and used to calculate mineral content. Iron was detected colourimetrically as ferric iron using Wong's Method (Raghuramulu *et al.,* 2003). Calcium oxalate was precipitated, diluted in hot dilute H_2SO_4 and titrated against a potassium permanganate standard (Helrich, 1990).

Data analysis

The data were statistically analyzed using statistical software SPSS 16.0 (SPSS Inc., Chicago, IL). Mean and standard deviation for all values were calculated and the significant differences between mean values were evaluated using the Least Significant Difference (LSD) at a 5 per cent probability level as suggested by Gomez and Gomez (1984).

Table 1: Effect of high night temperature and CO_2 concentration on number of flower dropped, pod setting percentage (%), grain yield (g/plant) and biomass (g/plant) of greengram (two years pooled data)

Treatment	Number of flower dropped/plant		Pod setting percentage (%)		Grain yield (g/plant)		Biomass (g/plant)	
	Pooled data	% increase	Pooled data	% reduction	Pooled data	% reduction	Pooled data	% reduction
T_1	21.8		55.3		16.0		15.7	
T_2	24.8	13.8	52.2	5.7	15.3	4.7	14.6	6.9
T_3	26.0	19.3	50.2	9.3	14.4	10.0	13.7	12.5
T_4	26.7	22.2	49.8	10.0	12.9	19.4	13.0	17.5
T_5	28.2	29.1	45.1	18.5	12.7	20.6	13.4	15.0
T_6	29.6	35.8	45.3	18.2	11.7	26.9	11.1	29.3
T_7	30.8	41.3	45.6	17.6	12.1	24.7	12.5	20.7
T_8	28.6	31.2	46.6	15.8	12.1	24.3	13.0	17.2
T_9	26.5	21.6	49.7	10.2	13.3	16.8	13.8	12.4
T_{10}	26.3	20.4	52.5	5.1	13.9	13.4	15.1	3.8
Mean	26.9	26.1	49.2	12.3	13.1	17.9	13.6	13.6
SEd	0.69		1.16		0.31		0.28	
CD(p=0.05)	1.43		2.43		0.65		0.60	

RESULTS AND DISCUSSION

Yield attributes

The data analysis shows that the HNT and CO_2 had a significant (p = 0.05) influence on the yield parameters (Table. 1). The lowest number of flower dropped/plant of greengram (21.8) was recorded under ambient condition (T_1) which was higher under stress during 43 to 49 DAS (T_7) (30.8) followed by 36 to 42 DAS (T6) and 50 to 56 DAS (T_8). This current study conformed with the findings of Prasad *et al.,* (2008)**,** which reported that the panicle initiation stage to the flowering stage of the rice was most sensitive to HNT stress and reduced the overall number of flowers per plant.

Pod setting percent ranged from 45.1 to 55.3 % (Table. 1). The highest pod setting percent (55.3%) was recorded in greengram plants grown under ambient conditions (T_1), which was statistically on par with the stress imposed from 7 to 14 DAS (T_2) (52.2%) and stress imposed from 64 to 70 DAS (T_{10}) (52.5%). This differed statistically from stress imposed during 29 to 35 DAS (T_5) (45.1%) which was on par with the stress imposed from 36 to 42 DAS (T_6) (45.3%) and stress imposed during 43 to 49 DAS (T_7) (45.6%). The present study corroborates with (Impa *et al.,* 2020), they reported that the grain filling was accelerated at an early stage in rice and also when the crops were exposed to HNT, the grain filling percentage was reduced.

When compared to ambient condition (T_1) (16 g/plant), HNT and CO_2 significantly reduced the grain yield (11.7g/plant) when stress was imposed from 36 to 42 DAS (T_6) followed by stress imposed from 43 to 49 DAS (T_7) and 50 to 56 DAS (T_8) (12.1g/plant) (Table.1). This result correlated with the findings of Garica *et al.,* 2016**,** who reported that increasing the minimum temperature during the grain filling stage would reduce the grain yield by 7 to 9 percent in barley and wheat.

As depicted in Table.1, the biomass per plant (15.7 g/plant) was highest under ambient conditions (T_1) followed by stress from 64 to 70 DAS (T_{10}) (15.1 g/plant) and 7 to 14 DAS (T_2) (14.6 g/plant). There was a significant reduction in the plant when stress was imposed from 36 to 42 DAS (T_6) (11.1 g/plant) which was on par with Stress imposed from 43 to 49 DAS (T_7) (12.5 g/plant). The current findings are consistent with the findings of Impa *et al.,* 2020, who reported that controlled environment research beyond the minimum temperature threshold will reduce total dry matter and shoot biomass.

Nutritional and anti-nutritional compositions

Data on seed protein as influenced by the HNT and CO_2 are given in Table.2. The range varied from 23.1 to 29 percent among the treatments. The stress imposed during 50 to 56 DAS (T_8) had the lowest protein content (23.1%), followed by stress imposed during 43 to 49 DAS (T_7) and57 to 63 DAS (T_9) whereas the highest protein content (29%) was recorded under the ambient condition (T_1). Based on the research findings of Van and Esawe, 2014**,** the heat stress on pulse crops reduced the protein content and also 19.6 percent reduction in groundnut (Gogoi *et al.,* 2018).

The greengram plants that were grown in the ambient condition (T_1) recorded high total sugar content (505.5 mg/g) and were significantly (P=0.05) different from the stress imposed during 43 to 49 DAS (T_7) (416 mg/g). This was statistically on par with the stress imposed from 36 to 42 DAS (T_6) (420 mg/g) and 29 to 35 DAS (T_5) (429.7 mg/g) (Table.2). Also, according to Gogoi *et al.,* 2018, the total sugar content of legumes was sensitive to high temperature and illustrated a 24.5 percent reduction of total sugar content in peanut

Total polyphenol content (8.8 mg/g) was higher in plants grown under ambient conditions (T_1), which was statistically on par with the stress imposed from 7 to 14 DAS (T_2) (8.4 mg/g),

Table 2: Effect of high night temperature and CO_2 concentration on protein (%), total sugars (mg/g), calcium (mg/100g) and iron (mg/100g) of greengram (two years pooled data)

Treatment	Protein (%)		Total Sugars (mg/g)		Calcium (mg/100g)		Iron (mg/100g)	
	Pooled data	% reduction	Pooled data	% reduction	Pooled data	% reduction	Pooled data	% reduction
T_1	29.0		505.5		151		20.1	
T_2	28.7	1.2	499.3	1.2	140	7.3	19.5	3.0
T_3	28.2	2.9	462.5	8.5	139	7.9	18.7	7.0
T_4	27.9	4.0	441.6	12.6	140	7.3	18.4	8.5
T_5	26.3	9.3	429.7	15.0	141	6.6	15.4	23.6
T_6	27.1	6.6	420.0	16.9	127	15.9	14.4	28.6
T_7	24.4	15.9	416.0	17.7	121	19.9	12.6	37.3
T_8	23.1	20.5	432.9	14.4	126	16.9	13.0	35.6
T_9	25.6	11.7	431.2	14.7	131	13.2	14.1	30.1
T_{10}	28.9	0.5	477.5	5.5	137	9.3	18.1	10.0
Mean	26.9	8.1	451.6	11.8	135.3	11.6	16.4	20.4
SEd	0.68		10.98		2.59		0.33	
CD(p=0.05)	1.42		22.91		5.40		0.70	

Table 3: Effect of high night temperature and CO_2 concentration on polyphenols (mg/g), seed moisture (%), proline content (mg/g) and phytic acid (mg/g) of greengram (two years pooled data)

Treatment	Polyphenols (mg/g)		Seed moisture (%)		Proline content (mg/g)		Phytic acid (mg/g)	
	Pooled data	% reduction	Pooled data	% reduction	Pooled data	% increase	Pooled data	% increase
T_1	8.8		9.9		23.4		6.5	
T_2	8.4	5.1	9.8	1.5	23.3	0.4	6.7	2.3
T_3	8.3	6.3	9.0	9.1	23.8	1.5	6.5	0.0
T_4	8.0	9.7	9.3	6.6	25.9	10.7	6.7	2.3
T_5	7.7	13.1	9.2	7.1	29.8	27.4	7.0	6.9
T_6	7.1	19.6	9.1	8.1	32.4	38.5	8.3	26.9
T_7	7.1	19.9	9.4	5.1	37.4	59.6	8.7	33.8
T_8	7.5	15.3	8.3	16.2	38.5	64.3	8.6	32.3
T_9	8.0	9.7	8.0	19.7	33.3	42.1	8.1	23.8
T_{10}	8.3	5.7	8.1	18.7	26.8	14.5	7.2	10.8
Mean	7.9	11.6	9.0	10.2	29.4	28.7	7.4	15.5
SEd	0.16		0.17		0.64		0.19	
CD(p=0.05)	0.34		0.35		1.34		0.40	

15 to 21 DAS (T_3) (8.3 mg/g) and 64 to 70 DAS (T_{10}) (8.3 mg/g). A significant difference was observed during 43 to 49 DAS (T_7) and 36 to 42 DAS (T_6) (7.1 mg/g), which was on par with stress imposed from50 to 56 DAS (T_8) (7.5 mg/g) (Table. 3). Singh *et al.,* (2015), reported that the flowering to the pod development stage of legume crops would be affected by the high-temperature stress. Total phenols help to overcome various stress like heat and cold stress (Dunja *et al.,* 2021).

The seed moisture content (9.9%) was significantly (P=0.05) higher in greengram plants grown under ambient condition which was statistically on par with stress imposed from 7 to 14 DAS (T_2) (9.8%) and lower seed moisture was recorded in stress imposed from 57 to 63 DAS (T_9) (8%) and 64 to 70 DAS (T_{10}) (8.1%) (Table. 3). Reduction in seed moisture content could be observed during the maturity stage than other stages of the legume's crop (Sehgal *et al.,* 2018). In the present study, lower seed moisture content was observed during the maturity stage of the crop.

Table 3 shows that significantly (p=0.05) lower proline content (23.3 mg/g) was recorded under Stress from7 to 14 DAS (T_2), which was statistically on par with ambient condition (T_1)

(23.4 mg/g) and under Stress from15 to 21 DAS (T_3) (23.8 mg/g). The highest proline content (38.5 mg/g) was found under stress from 50 to 56 DAS (T_8) (38.5 mg/g) followed by 43 to 49 DAS (T_7) (37.4 mg/g). Similar to present study, Sugenith Arteaga *et al.,* (2020) found 48 % increased proline content in bean seeds under high temperature stress condition.

Significantly (p=0.05) lower phytic acid content (6.5 mg/g) was recorded in ambient condition (T_1) and under stress from 15 to 21 DAS (T_3) which was statistically on par with Stress imposed from 7 to 14 DAS (T_2) and 22 to 28 DAS (T_4) (6.7 mg/g). Comparatively, higher phytic acid content (8.7 mg/g) was found when stress imposed from 43 to 49 DAS (T_7) which was on par with 50 to 56 DAS (T_8) (8.6 mg/g) (Table. 3). Choukri *et al.,* (2022), reported that temperature stress on pulse crop during reproductive stage would increase an anti-nutrient, (phytic acid) which leads to malnutrition. The phytic acid was known to absorb the essential nutrients like calcium, iron and zinc. So, increasing the high minimum temperature will increase the phytic acid content in the greengram seeds. Nakandalage and Seneweera, (2018) revealed that 15.9 per cent of phytic acid content had increased during the reproductive stage.

As shown in the Table.2 calcium content was found significantly higher (151mg/100g) under ambient conditions which was lower in stress imposed from 43 to 49 DAS (T_7) (121 mg/100g) which was on par with stress imposed from 50 to 56 DAS (T_8) (126 mg/100g) and 36 to 42 DAS (T_6) (127 mg/100g). The present investigation revealed that the greengram grown under ambient conditions had significantly higher calcium content as compared with the Stress imposed from 43 to 49 DAS (T_7). Vadivel and Janardhanan, (2001) discovered that the calcium content of pulse seeds decreased as phytic acid levels increased from pod development to pod filling, which is consistent with the current finding.

Higher iron content of greengram (20.1 mg/100g) was recorded under T_1 (ambient condition) which differed statistically when stress was imposed from 43 to 49 DAS (T_7) (12.6 mg/100g), on par with 50 to 56 DAS (T_8) (13 mg/100g). In comparison to the maximum iron content recorded under ambient conditions (T_1), the lowest iron content was observed when the plants were stressed from 43 to 49 DAS (T_7) due to HNT and CO_2 (Table.2). The current investigation supports the findings of Lazarte *et al.,* 2015, who assessed deceased iron content in legume crops under temperature and CO_2 stress during the pod filling stage.

CONCLUSION

Climate is the dominant factor of agricultural productivity, and has a profound influence on global food production. Agriculture sectors are extremely vulnerable to climate change, such as increased CO_2 levels, precipitation, and temperature changes, which can cause heat or cold stress, restricting production and ultimately jeopardising global food security. According to the findings of this study, HNT and CO_2 have a negative impact on greengram production and seed quality. However, there was only a minor effect on greengram production and seed quality from emergence to vegetative stage (7 to 28 days after sowing). As a result, high night temperatures and CO_2 levels will have an adverse effect on food security in the future.

Conflict of Interest Statement: The author (s) declares (s) that there is no conflict of interest.

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