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Impact of Heat Stress on Physiological traits of Greengram

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Abiotic stress seriously affect the productivity of legumes worldwide. Among legumes, greengram is very popular and extensively grown in India, however, the production is affected by hostile environments, especially heat stress due to climate change. The global air temperature is predicted to rise by 0.2ºC per decade, which will lead to (1.8–4.0ºC) higher temperature than the current level by 2100. In the above view, an experiment was conducted in CO 8 green gram by elevating the temperature up to 2°C and 4°C from ambient level to study the effect of elevated temperature on morpho-physiological, biochemical and yield attributes of greengram during different growth stages. The results revealed that when CO 8 greengram exposed to elevated temperature of 4°C from the ambient, showed higher variation in its growth, development and yield attributes when compared with 2ºC raise. The impact was lesser when plants subjected to an elevated temperature of 2°C from ambient when compared to 4°C raise. This study helps us to know the response of green gram to different elevated temperature at various crop growth stages.Thus, the present investigation opened new vistas in the field of developmental physiology for identifying the heat tolerant genotypes in greengram.

Key words: Leaf area, Total dry matter, Electrolyte leakage, DPPH assay, Yield traits, Greengram

Pulses are very important in our diet because of its rich protein content and ability to fix atmospheric nitrogen in soils. Among pulses, greengram is very popular and its production is affected by hostile environments, especially elevated temperature due to climate change. The global air temperature is predicted to rise by 0.2ºC per decade, which will lead to (1.8–4.0ºC) higher temperature than the current level by 2100 (IPCC 2007). Elevated temperature both as a seasonal phenomenon and as a part of climate change, is currently the leading threat to world for the production of crop yields (Darkwab *et al*., 2016). Suitable climate for cultivation of green gram should be warm, humid and within cardinal temperature range of 25°C to 35°C, with moderate rainfall.

The flowering time in green gram is adversely affected by conditions comprising high mean temperature and long day photoperiod. Among the various yield limiting factors, environmental stresses particularly water and temperature play a major role. Pulses are very sensitive to drought, water logging and high temperature.Temperature stress has devastating effects on plant growth and metabolism, as these processes have optimum temperature limits in every plant species. Therefore, it was found that the mechanism of heat stress in plants is important to investigate for future generations (Mahla *et al*., 2012). With this background the current study is undertaken to know the impacts and effects caused by elevated temperature stress in greengram for designing the future perspectives.

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Material and Methods

The pot culture experiment was conducted during 2017 at the open top chambers located in the Department of Crop Physiology, TNAU, Coimbatore. The greengram variety CO 8 was taken for the experiment. The treatments were ambient temperature that exists under open field condition (T_1), Elevated temperature of 2°C from the ambient temperature (T_2), Elevated temperature of 4°C from the ambient temperature (T_3). The treatments were imposed during Vegetative (S_1), Flowering (S_2) and Pod development stages (S_3). The physiological parameters and yield traits were recorded under elevated temperature stress. The experiment was laid out in Factorial Completely Randomized Design (FCRD) with four replications.

Leaf area per plant was measured using a Leaf area meter (LICOR, Model LI 3000) and expressed as cm^2 $plant^{-1}$. Total dry matter production (TDMP) was estimated by randomly pulling out four plants with intact root system at the end of each stage in each treatment and weighed after drying the plants at 80ºC for 48 hour and expressed in mg $plant^{-1}$. Electrolyte leakage was measured by the method described by Bajji *et al.* (2002) and expressed in per cent. The antioxidant activity to scavenge the stable free radical DPPH was assayed by the method of Brand-William *et al.* (1995). Different concentration of extracts (20-100 µg/ml) was mixed with 1 ml DPPH solution (0.2 mM/ml in methanol) and incubated at 20°C for 40 minutes in dark condition. After 40 minutes of incubation, the discolourisation of the

purple colour was measured at 518nm. The radical scavenging activity was calculated as follows:

$$\text{Percentage DPPH radical scavenging} = \frac{Ac - At}{Ac} \times 100$$

Where,

Ac = Absorbance of the control;

At = Absorbance of the test sample and expressed as per cent.

Number of nodules was observed through electron microscope (LEICA model). The number of pods produced in each plant was taken randomly from four plants in each replication from each treatment at the harvest stage. The total pod weight was weighed and expressed in gram plant^{-1}. The seed yield was expressed in gram plant^{-1}. The data arrived and collected on various parameters from the pot culture experiment were analyzed statistically in FCRD (Factorial Completely Randomized Design) as per the procedure suggested by Gomez and Gomez (1984).

Results and Discussion

Leaf area and total dry matter production

Leaf area is a photosynthetic surface area of plants. In our study, leaf area (cm^2 plant^{-1}) showed that it was decreased in all the stages *viz.,* vegetative stage (S_1), flowering stage (S_1) and pod development stage (S_3) under elevated temperature stress (Table 1). Higher leaf area was observed in T_1 (97.29 at S_1, 134.56 at S_2, 148.91 at S_3) when compared with T2 (86.67 at S_1, 121.40 at S_2, 132.51 at S_3) and T_3 (74.93 at S_1, 104.66 at S_2, 116.15 at S_3). Extension of cell wall is decreased by stress and high pH which results in structural changes in the plant cell wall, finally reducing the rate of growth of the stressed plants compared to unstressed plants (Burssens *et al.,* 2000; Pakorn *et al.*, 2009). Total dry matter production (TDMP) is an index of biological output from the net photosynthesis.

Table 1. Effect of elevated temperature on leaf area, total dry matter production (TDMP) and number of nodules

Treatments	Leaf Area (cm^2 plant^{-1})				TDMP (g plant^{-1})				No. of nodules (plant $^{-1}$)		
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	Mean
T_1	97.29	134.56	148.91	126.91	0.90	1.43	6.10	2.80	8.50	19.00	13.75
T_2	86.67	121.40	132.51	113.52	0.77	1.31	5.30	2.46	5.00	12.50	8.75
T_3	74.93	104.66	116.15	98.57	0.62	0.90	3.99	1.83	1.75	4.50	3.12
Mean	86.29	120.20	132.52	113.00	0.76	1.21	5.12	2.36	5.08	12.00	8.54
		T	S	T×S		T	S	T×S	T	S	T×S
SEd		2.70	2.70	4.69		0.07	0.07	0.13	0.59	0.48	0.84
CD (P≤0.05)		5.55**	5.55**	9.62(NS)		0.16**	0.16**	0.27**	1.25**	1.02**	1.77*

*, ** and NS denote significance level at P<0.05, P<0.01 and non-significance, respectively.

TDMP significantly differed among the treatments *viz.,* T_1 (ambient condition), T_2 (2ºC raise from ambient), T_3 (4ºC raise from ambient) and also at different stages (S_1, S_2 and S_3). Among all the stages, TDMP (g plant^{-1}) was significantly higher in T_1 (0.90 at S_1, 1.43 at S_2, 6.10 at S_3) followed by T_2 (0.77 at S_1, 1.31 at S_2, 5.30 at S_3) and the lowest TDMP was found in T_3 (0.62 at S_1, 0.90 at S_2, 3.99 at S_3). In the aspect of number of nodules, it was found to be peak at flowering stage (S_2). The number of nodules per plant recorded in T_1 (8.5 at S_1, 19.0 at S_2) was the highest among the treatments. In T_2 (5.0 at S_1, 12.5 at S_2) and T_3 (1.75 at S_1, 4.50 at S_3) there was a significant reduction in the nodule numbers per plant (Table 1.). Present study can be supported with Kurdali (1996) who reported that biological nitrogen fixation (BNF) in legumes is very sensitive to elevated temperature and almost ceases beyond 35°C. Basu *et al.* (2016) also stated that nodulation and nitrogen fixation terminates at high temperature.

Table 2. Effect of elevated temperature on yield traits

Treatments	Total Pod weight (g plant^{-1})				Seed yield (g plant^{-1})			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
T_1		4.24				3.67		
T_2	3.82	2.83	3.24	3.29	3.50	3.00	2.89	3.11
T_3	3.27	2.11	2.31	2.56	3.11	2.02	2.47	2.53
Mean	3.77	3.05	3.26	3.36	3.41	2.89	3.00	3.10
		T	S	T×S		T	S	T×S
SED		0.18	0.18	0.32		0.12	0.12	0.20
CD (P≤0.05)		0.38**	0.38*	0.66(NS)		0.24**	0.24*	0.42(NS)

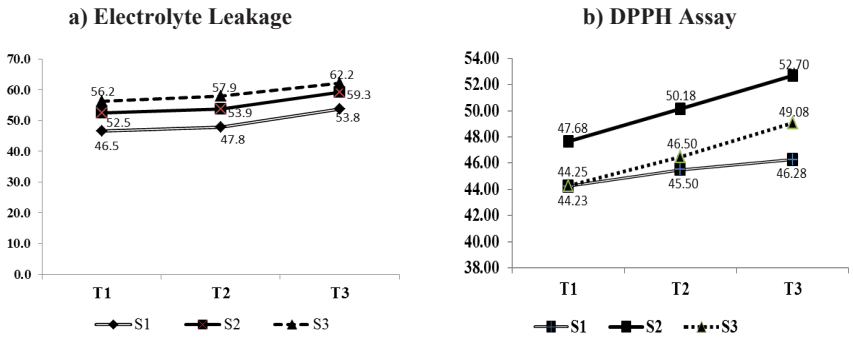
*, ** and NS denote significance level at P<0.05, P<0.01 and non-significant respectively

Electrolyte leakage and DPPH assay

Electrolyte leakage, a measure of electrolyte diffusion, resulting from elevated temperature induced cell membrane leakage, has been used to screen and evaluate different wheat genotypes for thermal

tolerance (Blum and Ebercon, 1981; Saadalla *et al.,* 1990) and other plants including wheat (Blum *et al.,* 2001), cotton (Ashraf *et al.,* 1994). In our data on electrolyte leakage was found to be maximum in T_3 (53.8 at S_1, 59.3 at S_2, 62.2 at S_3) followed by T_2 (47.8 at S_1, 53.9 at S_2, 57.9 at S_3) (Fig.1). Minimum leakage was observed in T_1 (46.5 at S_1, 52.5 at S_2, 56.2 at S_3) when compared with other treatments. Plants exposed to 4º C elevated temperatures from ambient recorded the maximum electrolyte leakage. The results on DPPH radical scavenging activity showed higher value under elevated temperature and the data was given in Fig.1. It was observed that in T_3 plants (46.28 at S_1, 52.70 at S_2, and 49.08 at S_3) the DPPH assay exhibited maximum scavenging activity. In T_2 (45.50 at S_1, 50.18 at S_2, 46.50 at S_3) there was a decline in scavenging activity was observed than T_3.

Among the treatments, T_1 (44.23 at S_1, 47.68 at S_2, 44.25 at S_3) had minimum scavenging activity than other treatments. The DPPH scavenging activity was high in 4ºC elevated temperature proving the



T_1: Ambient temperature that exists under open field condition S_1: Vegetative stage
T_2: Elevated temperature of 2ºC from the ambient temperature S_2: Flowering stage
T_3: Elevated temperature of 4ºC from the ambient temperature S_3: Pod development stage

Fig.1 Effect of elevated temperature on electrolyte leakage and DPPH Assay

presence and increase in total anti-oxidants. Present research revealed that the plants grown under 4ºC elevated temperature (T_3) showed a higher activity of DPPH radical scavenging activity. Thus the plants with higher accumulation of antioxidant enzymes to detoxify reactive oxygen species and mitigate oxidative stress-induced damage under elevated temperature (Shah *et al.,* 2001).

Yield traits

Among the treatments, T_1 (4.24) recorded maximum pod weight showing its better performance under ambient temperature condition (Table 2). Number of pods was decreased when temperature was elevated (T_2 and T_3) from the ambient condition. Irrespective of the stages, plants imposed with elevated temperature have recorded the lowest number of pod weight with a mean value of 2.83 (T_2) and 2.11 (T_3). The data on seed yield depicted in Table 3 showed similar trend like total pod weight. Plants under ambient temperature (T_1) showed highest seed yield per plant (3.67). It was observed that T_3 had greater impact on seed yield (Table 2.). In T_2, the per cent reduction over control was 18 at S_2, and 21 at S_3 respectively but in T_3 plants (44% at S_2, 32.6% at S_3), the reduction was higher. Our results confirmed by the earlier findings of the Wang *et al*. (2006), Krishnamurthy *et al*. (2011) and Kumar *et al*. (2013).

In green gram, the leaf area was drastically reduced when the temperature was elevated to 4°C from the ambient condition. The reduction in the TDMP was visualized in almost all the stressed plants. The per cent reduction was more in flowering stage (37.1%) of plants kept under 4°C elevated temperature condition. The present study also indicates that electrolyte leakage was high at flowering stage (59.3%) followed by pod development stage (62.2%). In case of number of nodules per plant, the per cent reduction was very high in plants exposed to 4ºC (T_3) elevated temperature at vegetative (79.4%) and flowering stage (76.3%) over control. Elevated temperature mainly affected the pod and seed yield. Seed yield per plant was reduced up to 45% over control during flowering stage when the temperature increased 4°C from the ambient. From the present study, it was concluded that, when greengram crop undergoes to an elevated temperature of 4°C from the ambient, showed significant changes in its physiology and yield attributes.

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