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Physiological response of greengram under heat stress

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Abstract
Greengram is a major grain legume used for consumption from ancient days for its higher protein content. Due to climate change, high temperature stress seriously affects the productivity of legumes and nutritional balance worldwide. Elevated temperature stress during sensitive crop growth stages affects biochemistry of the crops by production of reactive oxygen species which leads to membrane degradation and less physiological activity that affects the yield in greengram. With this background, the experiment was carried out in CO 8 variety to study the biochemical and yield traits of greengram in the temperature controlled growth chambers. Stress was given by raising the temperature up to 2ºC and 4ºC from the ambient at different growth stages. Results showed, that an increased production of malondialdehyde and H_2O_2 under elevated temperature than ambient and reduced the photosynthetic rate was also recorded. Antioxidant enzymes *viz*., Superoxide dismutase and catalase activity were increased under elevated temperature so as to scavenge the free radicals. Yield traits like pod length and weight were drastically reduced in stressed plants. From the results, it was concluded that temperature stress on CO 8 greengram during flowering stage was more critical which caused poor fertility and ultimately resulted in low yield.

Keywords: H_2O_2, malondialdehyde content, photosynthetic rate, antioxidants and yield

Introduction
Pulses are popularly known as Poor man's meat and rich man's vegetable and contribute to nutritional security globally. Food and Agriculture Organization (FAO) declared 2016 as the International Year of Pulses recognizing protein deficiency as a global concern. Greengram is considered as extensively grown pulse crop of India. The percent share in total production of greengram is 9.72 during the year 2015-2016 (Directorate of Economics and Statistics, GOI). The productivity of greengram in India is very low and far below when compared to other greengram growing countries. This is mainly due to various abiotic stress factors. Among the various environmental stresses water and temperature play a major role. Pulses are very sensitive to drought, water logging and high temperature. Many countries could experience unprecedented heat stress due to global climate change (Gaur *et al.*, 2015) [10]. High temperature is implicated as a major limiting factor for yield decline in greengram (Zinn *et al*., 2010) [25]. The elevated temperature stress causes oxidative damage to leaves due to production of Reactive Oxygen Species (ROS). ROS are produced continuously as byproducts of different metabolic pathways which are located in different cellular compartments such as chloroplast, mitochondria and peroxisomes (Navrot *et al.*, 2007) [18]. The increased ROS, decreased cell membrane permeability in chickpea (Deshmukh *et al*., 2002) [7]. ROS by causing oxidative damage impairs photosynthetic efficiency, which also affects nitrogen fixing ability of mung bean rhizobia by restricting formation and spread of root hair. The detoxification of these ROS is very important and plants have evolved complex strategies to deal with them (Asthir *et al.*, 2012) [2]. The scavenging enzymes are called as antioxidant enzymes. They remove free radicals and prevent the membranes and DNA from oxidative damage and make the plant to survive under stress. There are various evidences indicating increased activity of antioxidant enzymes under heat stress which is crop specific (Babu *et al.*, 2007) [3]. The major enzymatic antioxidants are superoxide dismutase (SOD) and catalase (CAT)) are reported to increase under various environmental stress (Munne-Bosch and Alegre, 2000) [17]. Heat stress during reproductive stage is becoming a serious constraint to grain legumes productivity as their

cultivation is expanding to warmer environment (Gaur *et al*., 2015) [10]. Global climate change is making elevated temperature a critical factor for plant growth and productivity. In this light of view, the study aims to understand the ROS production, antioxidants, photosynthetic rate and yield of CO 8 variety for studying the physiological and biochemical changes in greengram under elevated temperature stress.

Materials 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 latest variety in greengram 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 biochemical parameters and yield traits were recorded under elevated temperature stress. The experiment was laid out in Factorial Completely Randomized Design (FCRD) with four replications.

The lipid peroxidation level was determined by quantifying the malondialdehyde equivalents using 2-Thiobarbituric acid (TBA), as described by Hodges *et al*., (1999) [12] and expressed in µmol g^{-1} of fresh weight. H_2O_2 accumulation in leaves was visually detected by staining with 3,3-diaminobenzidine (DAB) using the method as described by Thordal Christensen *et al*. (1997) [20]. Leaves were immediately removed from plants, submerged in DAB solution (1 mg ml^{-1} at pH 3.8) and incubated under light for six hours at 25° C for reaction and examined under light microscope by using LEICA 6SD. Photosynthetic gas exchange was measured from non-detached young and fully expanded leaves using a portable photosynthetic system (PPS) (ADC Bio-Scientific Ltd.) and expressed in µmol CO_2 m^{-2} s^{-1}. Catalase activity was measured by the method described by Aebi (1974) [1] and expressed in µg H_2O_2 $min^{-1}g^{-1}$. Superoxide dismutase activity was determined by using nitro blue tetrazolium (NBT) salt as described by Champ and Fridovich (1971) [4] and expressed as enzyme units mg^{-1} protein. The important components contributing to the yield potential of the crop were recorded at harvest. For pod length ten pods per plant were randomly taken from four selected plants at maturity and it was measured in cm. For pod weight 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 per plant.

Results and Discussion

In this experiment, malondialdehyde (MDA) content was measured to determine the lipid peroxidation level because MDA is a byproduct of lipid peroxidation.

The content of MDA and hydrogen peroxide (H_2O_2) has been considered as an indicator of oxidative injury (Mandhania *et al*., 2006; Moller *et al*., 2007) [15, 16]. Results showed that T_3 have more MDA than T_1 and T_2. Among the treatments, T_1 (0.48 at S_1, 0.93 at S_2, 0.63 at S_3) was observed to have lowest value. The treatment T_3, (1.85 at S_1, 2.06 at S_2, 1.77 at S_3) recorded highest MDA content than other treatments (Fig.1.). We can visuvalize (Plate.1.) the production of H_2O_2 concentrations remained almost constant in leaves under ambient temperature condition in all the stages of plant growth. However, under elevated temperature, substantial increase in H_2O_2 accumulation was observed. More accumulation of H_2O_2 observed in T_2 and T_3 at S_1, S_2 and S_3 than in T_1 plants where ROS accumulation strikingly lesser when compared with others. Among the treatments, more H_2O_2 accumulation was observed in T_3. Heat stress impairs mitochondrial functions thereby resulting in the induction of oxidative damage that manifests in lipid peroxidation, detected by malondialdehyde (MDA) content (Larkindale and Knight 2002; Vacca *et al*., 2004) [14, 21].

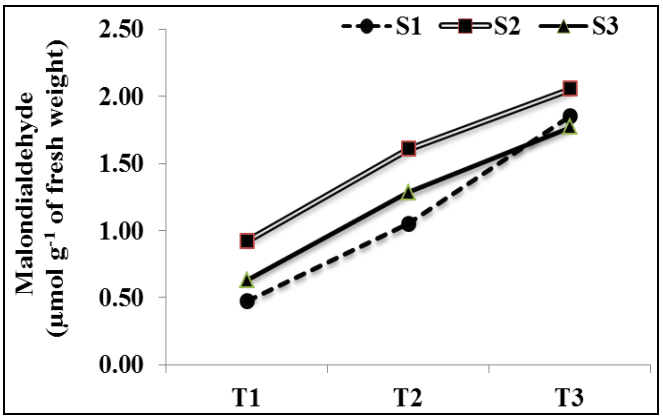


Fig 1: Effect of elevated temperature on malondialdehyde content

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

The *in situ* detection of H_2O_2 in greengram leaves at vegetative stage

The *in situ* detection of H_2O_2 in greengram leaves at flowering stage

The *in situ* detection of H_2O_2 in greengram leaves at pod development stage

Plate 1: Effect of elevated temperature in H_2O_2 production

Photosynthetic rate was recorded during vegetative (S_1) and flowering stages (S_2) alone. In control (T_1) the photosynthetic rate found to be 16.8 at S_1 and 23.5 at S_2.

The photosynthetic rate was reduced under elevated

temperature in T_2 (13.5 at S_1, 21.8 at S_2) and in T_3 (11.3 at S_1, 17.0 at S_2). Among the treatments, plants grown in ambient temperature condition (T_1) performed better in all the stages when compared to elevated temperature condition (T_2 and T_3) (Fig.2.). The reduction of whole leaf photosynthesis by high temperature might be caused by disruption of the functional integrity of the photosynthetic apparatus associated with the production of Reactive Oxygen Species (Camejo *et al.*, 2005; Guo *et al.*, 2006) [5, 11] that cause damage to the thylakoid membrane. The results of the present study is similar to the findings of Djanaguiraman *et al.* (2011) [9] who found that high temperature stress during flowering in soybean plants decreased the photosynthetic rate by 20.5 per cent compared with those grown at ambient temperature. The results of the present study lead to the conclusion that decrease in photosynthetic rates are more pronounced when temperature stress was applied during flowering stage (S_2).

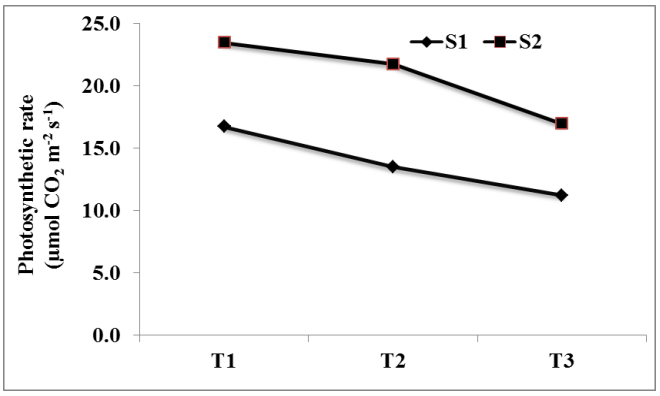
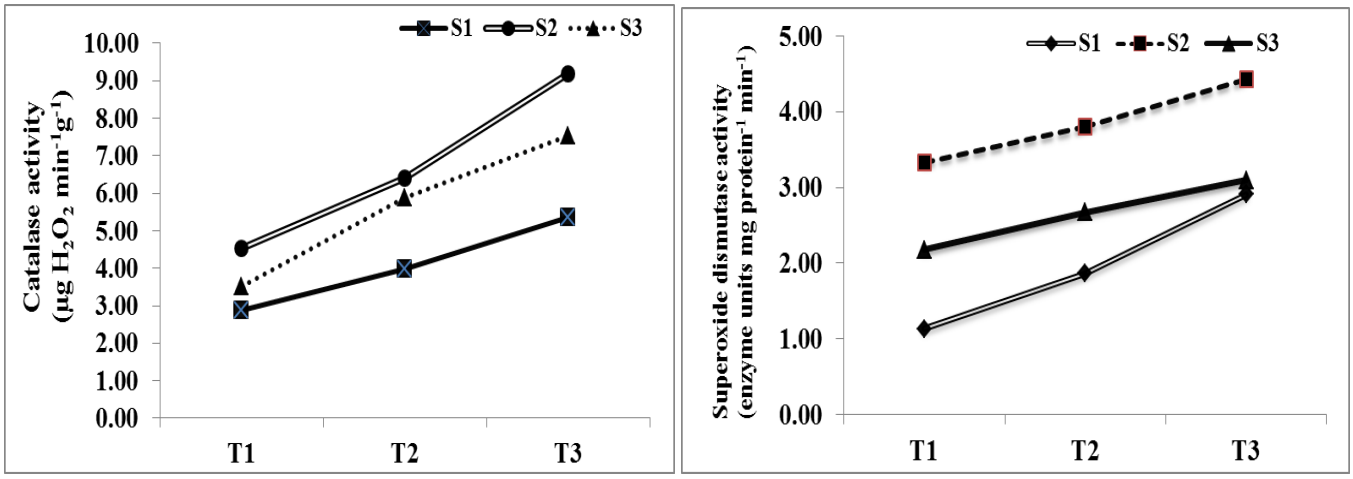


Fig 2: Effect of elevated temperature on Photosynthetic rate

Plants use antioxidant enzymes to detoxify reactive oxygen species and mitigate oxidative stress-induced damage under elevated temperature (Shah *et al.*, 2001) [19]. Many studies demonstrated that high temperature injury was caused by the excessive production of reactive oxygen radicals, the low activities of antioxidant enzymes, and the membrane damage in plants (Zhang *et al.*, 2006; Zhu *et al.*, 2005) [11, 23, 24]. Antioxidants activity shows the plants condition and stress level. Activities of different antioxidant enzymes are temperature sensitive and activation occurs at different temperature ranges but the activities of these enzymes increase with increasing temperature (Chakraborty and Pradhan, 2011) [6]. Similar finding was observed in the present investigation, Catalase and Superoxide dismutase activity (SOD) was increased under temperature stress condition (Fig.3a and 3b). The CAT activity of T_3 (5.36 at S_1, 9.18 at S_1, 7.53 at S_1) had lower activity than T_2 and T_1. Whereas T_1 (2.89 at S_1, 4.54 at S_2, 3.51 at S_3) showed its better performance than other treatments. The data on CAT activity in T_2 (3.99 at S_1, 6.41 at S_2, 5.89 at S_3) was higher than T_3. SOD also showed similar trend that under elevated temperature, the enzyme SOD activity was high in T_3 (2.91 at S_1, 4.43 at S_2, 3.10 at S_3) whereas T_1 recorded (1.13 at S_1, 3.33 at S_2, 2.18 at S_3) the lowest SOD activity. The per cent increase in SOD activity was 39.2 at vegetative stage (S_1), 12.4 at flowering stage (S_2) and 18.4 at pod development stage (S_3). When the temperature was increased to 4ºC, the SOD activity still enhanced to 61.2% increase at vegetative stage, 24.8% increase at flowering stage and 29.7% increase at pod development stage. This explains that the plants are trying to survive under elevated temperature to maintain its growth and development process.



a. Catalase activity

b. Superoxide dismutase activity

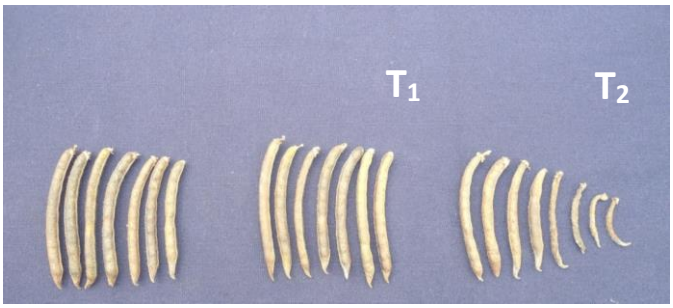
Fig 3: Effect of elevated temperature on

The brief exposure of high temperature stress (32-35ºC) in chickpea reduced pod set and hence grain yield in the controlled environments (Devasirvatham *et al.* 2010) [8]. In results, T_1 (6.46) showed higher pod length than other treatments (Table.1.). In both the treatments, T_2 (6.12 at S_1, 5.53 at S_2, 5.39 at S_3) and T_3 (5.72 at S_1, 4.14 at S_2, 4.59 at S_3) the pod length were reduced than T_1. Especially the plants where elevated temperature (T_3) imposed during pod development stage (S_3) have recorded the lowest pod length. Total Pod weight showed similar trends that T_1 (4.24) recorded maximum pod weight showing its better performance under ambient temperature condition. 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). Elevated temperature imposed at vegetative stage did not show much reduction in the yield components. But the impact was greater when plants exposed to temperature stress at flowering stage and pod development stage (Plare.2). Similar results observed by Kumar *et al.* (2013) [13] in chickpea. Our results confirmed by the earlier findings of the Wang *et al.* (2006) [22] and Kumar *et al.* (2013) [13].

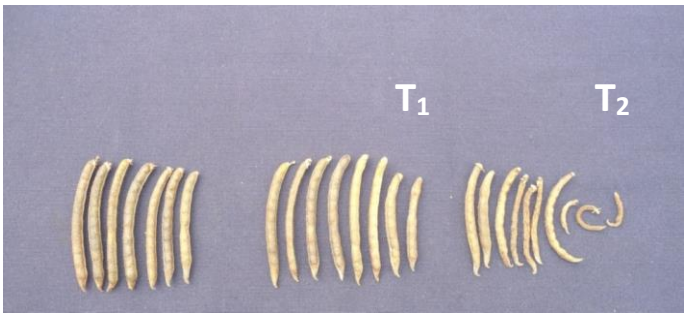
Table 1: Effect of elevated temperature on Yield traits

Treatments	Total Pod weight (g plant^{-1}) Crop growth stages				Hundred seed weight (g) Crop growth stages			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
T_1	4.24				3.75			
T_2	3.82	2.83	3.24	3.29	3.59	3.45	3.43	3.48
T_3	3.27	2.11	2.31	2.56	3.26	2.93	3.20	3.12
Mean	3.77	3.05	3.26	3.36	3.53	3.37	3.45	3.45
		T	S	T×S	T	S	T×S	T
SED		0.18	0.18	0.32	0.06	0.06	0.11	0.06
CD ($P \leq 0.05$)		0.38^{**}	0.38^{*}	$0.66^{(NS)}$	0.13^{**}	$0.13^{(NS)}$	$0.22^{(NS)}$	0.13^{**}

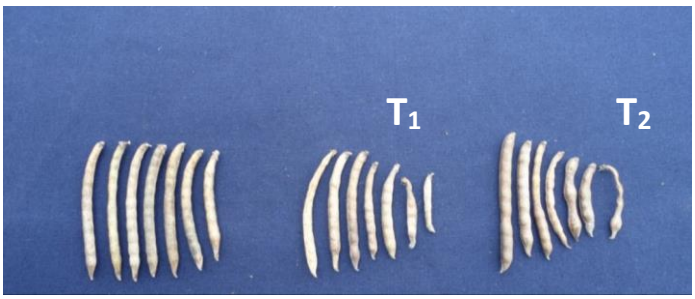
*, ** and NS denote significance level at $P<0.05$, $P<0.01$ and non-significant respectively



Stress imposed at vegetative stage



Stress imposed at flowering stage



Stress imposed at pod development stage

Plate 2: Effect of elevated temperature on yield (pods) during different crop growth stages

Conclusion

The results of this study showed that high temperature caused negative effect on growth which could be due to the generation of high levels of ROS. Antioxidants produced scavenged the ROS and made the plants to survive under elevated temperature stress at an extent. From the present study, it was concluded that, when greengram crop undergoes to an elevated temperature of than 2°C and 4°C from the ambient, showed significant changes in its physiology, biochemical and yield attributes.

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