



Effect of Heat Stress on Photosystem II, Antioxidant Activity and Micronutrient Concentration in Contrasting Cultivars of Greengram (*Vigna radiata* (L.) Wilczek)

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Authors' contributions

This work was carried out in collaboration among all authors. Author MA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author CV managed the analyses of the study. Authors KSKS and TNS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Heat stress around flowering has negative effect on greengram (*Vigna radiata* (L.) Wilczek) grain yield. The pot culture experiments were conducted to study the response of antioxidant system, photosystem II and micronutrient concentration to above-optimum temperature at flowering stage in two cultivars of greengram (*Vigna radiata* (L.) Wilczek) cv. SAMRAT (heat tolerant) and VBN-2 (heat susceptible). The plants were grown under natural light and atmospheric conditions (33/22°C day/night) up to the first appearance of flower. Then, cohort of plants were: (a) exposed to natural environment (33/22°C day/night) for 13 days and were (b) exposed to controlled environment 33/22 to 45/30°C (day/night) for 13 days by gradually increasing 1°C per day in day/night temperature and then pots were shifted to natural environment for 5 days for recovery. The activity of antioxidant enzyme (SOD, CAT and APX) were increased significantly as the temperatures increased upto

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38/26°C in both cultivars and showed less reduction appropriately at 45/30°C in SAMRAT compared to VBN-2. It is possible that better tolerant to heat stress of SAMRAT was related to its ability to maintain higher levels of activity of antioxidant enzymes in all the temperature regimes. Quantum yield (F_v/F_m) showed significant reduction at 41/28°C in VBN-2 while at 45/30°C in SAMRAT indicating relative tolerance to heat stress. Micronutrient composition was not affected at 45/30°C except Cu content in leaves and Mn and Fe content in shoots. Upon heat stress treatment SAMRAT showed relatively less reduction of micronutrients like Cu, Mn and Fe content in leaves compared to VBN-2. The tolerant cultivar SAMRAT can serve as parents for breeding for heat stress tolerant variety.

Keywords: *Vigna radiata*; heat stress; antioxidants; photosystem-II; micronutrients.

1. INTRODUCTION

Heat stress is one of the primary stresses limiting the performance of plants over the globe [1]. The rise in temperatures is drastically affecting the growth and production potential of crops, especially for those being grown in the tropical conditions. The above-optimum temperatures can induce chlorosis, sunburns on vegetative tissues, senescence and abscission of leaves, inhibition in the growth of roots and shoots, and finally substantial reduction in seed yield in various plant species [2,3]. Heat stress induces the rapid production and accumulation of reactive oxygen species (ROS) and cause loss in crop productivity worldwide [4].

Greengram (*Vigna radiata* (L.) Wilczek) is an important vegetable crop of India and rich in proteins, vitamins, and minerals [5]. In northern parts of India, it is sown during spring/summer seasons and experiences heat stress (35-45°C) as it grows during its various developmental stages leading to reduction in potential yield. Our preliminary observations on greengram plants experiencing temperatures exceeding 40°C indicate damage as chlorosis, reduced vegetative and reproductive growth, abscission of buds, flowers and pods suggesting its sensitivity to heat stress [6].

One of the ways to overcome the negative effects of heat stress may involve use of some antioxidant enzymes having proven protective functions against the stresses. The major enzymatic antioxidants are Superoxide dismutase (SOD), Catalase (CAT) and Ascorbate peroxidase (APX) are reported to increase under various environmental stress [7]. In enzymatic antioxidant system, SOD converts free O_2^- radicals to H_2O_2 and O_2 [8]. The CAT and APX scavenge the accumulated H_2O_2 to nontoxic levels or form water and oxygen [9].

Chlorophyll fluorescence, an indication of the fate of excitation energy in the photosynthetic apparatus, has been used as an early, in vivo, indication of many types of plant stress [10] including temperature stress [11]. One of the first responses of a plant to environmental stress is an increase in nonradioactive energy dissipation, which is reflected by the amount of chlorophyll fluorescence [12].

Rising temperature may enhance organic matter mineralization and mineral weathering rates thus alleviating, at least temporarily, some nutrient limitation. Micronutrient content decreased in shoots of chickpea crops under elevated temperature [13]. The increasing in plant growth in elevated CO_2 with increasing temperature can only be achieved if some combination of increased nutrient absorption and improved nutrient use efficiency is attained pulses. Understanding the response of greengram to heat stress at photosystem II (chlorophyll fluorescence), antioxidant enzyme and micronutrients level will accumulate, making the genetic manipulation easier which could lead to more research on mechanism of heat stress tolerance to plants in future. The objective of this study was to assess the heat stress tolerance by comparing efficiency of photosystem II, activity of antioxidant enzymes and micronutrient concentration associated with heat tolerance in two contrasting cultivars of greengram in heat tolerance.

2. MATERIALS AND METHODS

2.1 Experimental Materials

The two greengram cultivars SAMRAT (heat tolerant) and VBN-2 (heat susceptible) were grown in a controlled environment with seven replications. Seeds of each cultivar were sown in pots (5 L volume) containing a mixture of sandy loam soil and vermi-compost (3:1) and two plants

were maintained in each pot. The plants were grown under natural light and atmospheric conditions (33/22°C day/night) up to the first appearance of flower. Then, cohort of plants were: (a) exposed to natural environment (33/22°C day/night) for 13 days maintained as control and were (b) exposed to controlled environment 33/22 to 45/30°C (day/night) for 13 days by gradually increasing 1°C per day in day/night temperature and then pots were shifted to natural environment for 5 days for recovery.

2.2 Antioxidant Enzymes

Leaf samples were taken at various temperature regimes (33/22°C, 38/26°C and 45/30°C) for observations of antioxidant enzyme viz., Superoxide dismutase (SOD), Ascorbate peroxidase (APX) and Catalase (CAT) activity. The SOD activity was estimated by using nitro-blue tetrazolium dye, as described by [14]. The APX enzyme activity was estimated using the method as described by [15]. The CAT enzyme activity was computed by calculating the amount of H₂O₂ decomposed using the method as developed by [16].

2.3 Efficiency of Photosystem II

The chlorophyll fluorescence readings were taken during different temperature regimes (33/22°C, 35/24°C, 38/26°C, 41/28°C and 45/30°C) by using an OS-30p portable chlorophyll fluorometer (Opti-Sciences Hudson, NH, USA). The maximum quantum efficiency of photosystem II (F_v/F_m) was calculated by adopting the procedure of [17].

2.4 Micronutrient Analysis

Micronutrients were estimated using the method described by [18].

2.5 Statistical Analysis

The data obtained in this study were analyzed in CRD design by using AGRIS statistical software.

3. RESULTS

3.1 Antioxidant Enzymes

The effect of heat stress on activities of catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) were highly significant. The activity of SOD was increased significantly as the temperatures increased to

38/26°C, but decreased at 45/30°C in both cultivars. The activity of SOD was increased by 17.4 and 31.7% at 38/26°C and it was decreased by 14.3 and 34.6% at 45/30°C over control (33/22°C) in SAMRAT and VBN-2 respectively. But the cultivar SAMRAT showed less reduction in SOD activity than VBN-2 at 45/30°C. The SOD activity was significantly higher in SAMRAT than VBN-2 in all the temperature regimes.

The significant increase in CAT activity was observed by 48.6 and 46.1% in SAMRAT and VBN-2 respectively at 45/30°C, compared with the control. The CAT activity was significantly higher in SAMRAT than VBN-2 in all the temperature regimes. On the other hand, APX activity followed the same increasing trend at 38/26°C and decreased at 45/30°C in both cultivars. The activity of APX was increased by 83.1 and 40.1% in SAMRAT and VBN-2 at 45/30°C respectively over control.

3.2 Chlorophyll Fluorescence (F_v/F_m)

The effect of heat stress on efficiency of photosystem II (F_v/F_m) showed that, cultivar VBN-2 was not affected significantly when the temperature increased from 33/22 to 38/26°C. However, F_v/F_m decreased significantly when the temperature increased at 41/28°C in VBN-2. The changes in F_v/F_m increased by 6.1 and 12.5% when temperature increased from 33/22°C to 41/28°C, whereas decreased by 13.9 and 24.5% at 45/30°C in SAMRAT and VBN-2 respectively. In addition, the temperature increased from 38/26°C to 41/28°C did not affect F_v/F_m significantly in SAMRAT. Though the cultivar SAMRAT showed significant decrease in F_v/F_m at 45/30°C, indicating relative tolerance compared to VBN-2.

3.3 Micronutrients

The effect of temperature and the interaction between cultivar and temperature were not significantly differed on Zn concentration in both leaves and shoots under heat stress. Copper (Cu) concentration was significantly affected by heat stress in leaves but not in shoots. Copper content decreased in the leaves of SAMRAT and VBN-2 by 5.7 and 18.4% while, in the shoots by 19.7 and 4.8%, respectively at 45/30°C. Copper content in leaves and shoot decreased when plants exposed to 38/26°C in both cultivars except in the shoots of VBN-2. Manganese (Mn) content increased by 16.8 and 54.6% in shoots, while decreased in leaves by 26.6 and 26.9% in

Table 1. Details of temperature regime and parameters observation under controlled environments

Days	0	1	2	3	4	5&6	7	8	9	10	11	12&13
Temp regime (day & night °C)	33/22	34/23	35/24	36/25	37/25	38/26	39/27	40/27	41/28	42/29	43/29	45/30
Chlorophyll fluorescence observation	*	-	*	-	-	*	-	-	*	-	-	*
Antioxidant enzyme analysis	*	-	-	-	-	*	-	-	-	-	-	*
Micronutrient analysis	*	-	-	-	-	*	-	-	-	-	-	*

*Note: The symbol * indicates the temperature regime for observation*

SAMRAT and VBN-2, respectively at 45/30°C but no significant changes observed at 38/26°C in both cultivars except in the stem of VBN-2. Iron content (Fe) content decreased by 34.9 and 45.8% in shoots of SAMRAT and VBN-2, respectively at 45/30°C, but no significant differences observed in leaves. Also no significant changes in Fe content was observed at 38/26°C in both cultivars except in the stem of SAMRAT.

Table 2. Effect of heat stress on the superoxide dismutase activity in greengram cultivars at flowering stage

Cultivars	Superoxide dismutase (unit g ⁻¹ fresh weight min ⁻¹)			
	33/22°C	38/26°C	45/30°C	Mean
SAMRAT	13.2±0.36	15.5±0.40 (17.4%)	11.3 ±0.31 (-14.3%)	13.3
VBN(Gg) 2	10.4±0.50	13.7±0.40 (31.7%)	6.8±0.56 (-34.6%)	10.3
Mean	11.8	14.6	9.1	
CD (p≤0.05)				
Cultivar (C)	0.702*			
Temperature (T)	0.860*			
C × T	1.21**			

*, ** and n.s. denote significance level at p≤0.05, p≤ 0.01, and non-significant, respectively. Values in parentheses indicate percent change over 33/22°C (control)

Table 3. Effect of heat stress on the catalase activity in greengram cultivars at flowering stage

Cultivars	Catalase (μmol g ⁻¹ fresh weight min ⁻¹)			
	33/22°C	38/26°C	45/30°C	Mean
SAMRAT	0.286±0.01	0.396±0.01 (38.4%)	0.425±0.02 (48.6%)	0.369
VBN(Gg) 2	0.247±0.01	0.382±0.12 (54.6%)	0.361±0.01 (46.1%)	0.330
Mean	0.267	0.389	0.393	
CD (p≤0.05)				
Cultivar (C)	0.020*			
Temperature (T)	0.024 ^{n.s}			
C × T	0.035*			

* and n.s. denote significance level at p≤0.05 and non-significant, respectively. Values in parentheses indicate percent change over 33/22°C (control)

Table 4. Effect of heat stress on the ascorbate peroxidase activity in greengram cultivars at flowering stage

Cultivars	Ascorbate peroxidase (μmol g ⁻¹ fresh weight min ⁻¹)			
	33/22°C	38/26°C	45/30°C	Mean
SAMRAT	2.31±0.13	4.72±0.15 (104.3%)	4.23±0.07 (83.1%)	3.75
VBN(Gg) 2	1.87±0.15	3.85±0.13 (105.8%)	2.62±0.09 (40.1%)	2.78
Mean	2.09	4.29	3.43	
CD (p≤0.05)				
Cultivar (C)	0.210*			
Temperature (T)	0.258 ^{n.s}			
C × T	0.365**			

*, ** and n.s. denote significance level at p≤0.05, p≤ 0.01, and non-significant, respectively. Values in parentheses indicate percent change over 33/22°C (control)

Table 5. Effect of heat stress on quantum yield of PSII of the greengram cultivars at flowering stage

Cultivars	Quantum yield PSII (F_v/F_m)					Mean
	33/22°C	35/24°C	38/26°C	41/28°C	45/30°C	
SAMRAT	0.783±0.012	0.772±0.007	0.748±0.003	0.735±0.011	0.683±0.014	0.744
VBN(Gg) 2	0.764±0.007	0.753±0.022	0.713±0.006	0.688±0.011	0.576±0.007	0.699
Mean	0.773	0.763	0.731	0.711	0.630	
CD ($p \leq 0.05$)						
Cultivars (C)	0.030**					
Temperature (T)	0.048 ^{n.s.}					
C × T	0.068 ^{n.s.}					

** and n.s. denote significance level at $p \leq 0.01$ and non-significant, respectively

Table 6. Effect of heat stress on mineral elements concentration in leaves of greengram cultivars at flowering stage

Cultivars	Temperature	Micronutrients (ppm)			
		Zn	Cu	Mn	Fe
SAMRAT	33/22°C	62.5±1.96	29.6±1.06	232.9±7.35	737.1±4.54
	38/26°C	58.9±2.11	24.7±0.82	213.2±5.44	742.7±4.68
	45/30°C	48.6±2.05	31.3±1.26	170.9±4.22	709.3±9.57
VBN(Gg) 2	33/22°C	53.4±2.04	22.2±1.23	181.6±3.32	734.5±8.52
	38/26°C	49.4±1.81	22.5±0.64	166.7±2.88	726.3±9.75
	45/30°C	41.2±2.16	26.3±0.81	132.7±3.18	657.2±6.97
Mean		52.33	26.16	183.1	717.8
CD ($p \leq 0.05$)					
Cultivar (C)		3.36*	1.65*	19.59*	30.5 ^{n.s.}
Temperature (T)		4.11 ^{n.s.}	2.02*	24.0 ^{n.s.}	37.3 ^{n.s.}
C × T		5.82 ^{n.s.}	2.86 ^{n.s.}	33.9 ^{n.s.}	52.8 ^{n.s.}

* and n.s. denote significance level at $p \leq 0.05$ and non-significant, respectively

Table 7. Effect of heat stress on mineral elements concentration in shoots of greengram cultivars at flowering stage

Cultivars	Temperature	Micronutrients (ppm)			
		Zn	Cu	Mn	Fe
SAMRAT	33/22°C	30.7±1.18	29.8±1.24	49.2±1.48	309.1±10.67
	38/26°C	31.3±1.60	31.4±1.18	51.7±2.24	317.3±10.36
	45/30°C	36.2±1.65	35.7±2.11	57.5±2.24	201.3±8.30
VBN(Gg) 2	33/22°C	45.4±1.91	26.8±0.99	32.6±2.33	328.2±8.73
	38/26°C	38.9±1.81	23.3±0.86	23.8±1.70	339.2±5.35
	45/30°C	39.2±1.97	28.1±0.84	50.4±1.45	177.6±7.70
Mean		36.95	29.23	44.2	278.7
CD ($p \leq 0.05$)					
Cultivar (C)		2.83 ^{n.s.}	2.22*	3.2 ^{n.s.}	14.41**
Temperature (T)		3.47 ^{n.s.}	2.72 ^{n.s.}	3.95*	17.65*
C × T		4.91 ^{n.s.}	3.85 ^{n.s.}	5.58**	24.96*

*, ** and n.s. denote significance level at $p \leq 0.05$, $p \leq 0.01$, and non-significant, respectively

4. DISCUSSION

Cells produce enzymatic antioxidants to defend against oxidative stress in plants. SOD removes peroxides while converting them to H_2O_2 , which is quickly acted upon by catalase to convert into

water and oxygen. The enzymes APX is a components of the ascorbate–glutathione cycle and are implicated in the removal of H_2O_2 . CAT and APX play a role in the protection of the plants from the damages of upward accumulation of H_2O_2 . In the present study, plants exposed to

38/26°C showed an appreciable increase in these antioxidant enzymes (*SOD*, *CAT* and *APX*) in both heat tolerant and sensitive cultivars. It could be due to, increase in *SOD* activity as a result of heat stress as reported by [19]; such increase could be due to the accumulation of *Cu/Zn-SOD* mRNA [20]. The increase in *SOD* activity at heat stress could be mainly due to the isoform pattern of *SOD*. However, transcript level of *APX* gene is increased in heat, drought and combined stress treated tobacco plants [21].

On the other hand, in the present study, it was found that these antioxidant enzymes were significantly decreased at 45/30°C. It appears that at extreme temperatures, the expression of antioxidants are inactivated or inhibited and it was in concordance with previous reports about the decline in the transcript level of *SOD*, *CAT* and *APX* related genes in the heat stress treated chick pea [22], cotton [23] and tomato [24]. Though these enzymes were significantly decreased in both cultivars at 45/30°C, comparatively the tolerant cultivar SAMRAT differed from the sensitive cultivar VBN-2 with respect to a less reduction in the activity of *SO*, *CAT* and *APX* appropriately at 45/30°C. Maintenance of antioxidant enzyme activity in the tolerant cultivar SAMRAT reflects the efficient detoxification of H_2O_2 compared with the sensitive cultivar VBN-2 which possibly reduced heat-stress induced damage in the former. Tolerant genotypes are able to maintain *SOD*, *CAT* and *APX* at higher levels, which might be significant in the differential heat sensitivity of the contrasting cultivars. These findings are similar to that of heat-stressed wheat in which the tolerant genotypes possessed greater activity levels of all the antioxidants such as *SOD*, *APX*, *CAT* [25] and [26].

Under heat stress conditions, particularly at reproductive stage, the level gene expression and the upstream regulatory regions of the genes that regulate the antioxidant enzymes such as *SOD*, *CAT* and *APX* needs to be studied in detail to better understand the mechanism of heat tolerance in greengram. Molecular markers associated with the genes encoding antioxidant enzymes and the genes playing role in heat tolerance would be another step towards developing better performing greengram plants under elevated temperatures.

In the present study, increased day temperature above 38/26°C caused significant decrease in quantum yield of PSII in susceptible cultivar

VBN-2, while it was significantly not affected by this temperature in tolerant cultivar SAMRAT, indicating relative tolerance to this level of heat stress. But in tolerant cultivar the quantum yield starting to declined above the temperature 41/28°C and showed significant reduction at 45/30°C. The investigated contrasting cultivars SAMRAT and VBN-2 were starting to differ significantly by F_v/F_m at 41/28°C treatment. Heat stress affected the chlorophyll fluorescence only at 41/28°C. So, it could be used as a screening temperature in breeding for heat-tolerance of greengram. This is in line with the findings of [27] obtained from garden bean and [28] obtained from maize and sunflower plant.

Heat stress alter the concentration for various macro and micro nutrients. In present study heat stress significantly affected nutrient content in both the cultivars in particular temperature regimes. In this study, at 45/30°C temperature Copper (Cu) content significantly decreased in the leaves of both the cultivars SAMRAT and VBN-2. At the same temperature Manganese (Mn) content significantly increased in the shoot dry matter. Zinc (Zn) content was not affected by this heat stress in either cultivar of leaves and shoot. However, iron (Fe) content was significantly decreased in shoot of both cultivars at 45/30°C. Reduction in mineral like Fe in chickpea under low temperature but higher levels of iron and other minerals have been reported under elevated temperature which is similar to the present finding [29]. Heat stress may affect the accumulation of elements in above ground biomass differently depending on sensitivity of their uptake process to temperature and the changes may be species and element specific [30]. Micronutrients like Zn, Mn, Cu play a major role on the growth, development and activity of *SOD* isoforms in seedlings of narrow leaved *Lupines angustifolius* [31]. Zn, Mn, Cu deficiencies are recognized as the most common and widespread micronutrient deficiency in Rice-wheat cropping system belt of peninsular India where greengram cultivation more practiced. Micronutrient deficiency changes the activities of *SOD* and *APX* in tobacco seedlings [32]. Upon heat stress treatment SAMRAT showed relatively less reduction of micronutrients like Cu, Mn and Fe content in leaves as against VBN-2. So, this nutrient variations which could have made changes in the activities of *SOD*, *APX* which could also be considered an important step in understanding mechanism of heat stress tolerance as SAMRAT showed positive response under heat stress environment.

5. CONCLUSION

Activities of antioxidant enzymes (SOD, CAT and APX) were affected under heat stress in both tolerant and susceptible cultivars. The activity and levels of antioxidant enzyme were increased significantly as the temperatures increased to 38/26°C, but decreased at 45/30°C in both contrasting cultivars. It is possible that better to heat stress of SAMRAT was related to its ability to maintain higher levels of activity of antioxidant enzymes in all the temperature regimes. Quantum yield showed significant reduction at 41/28°C in VBN-2 while at 45/30°C in SAMRAT indicating relative tolerance to this level of this heat stress. Micronutrient composition in contrasting greengram cultivars were not affected at 45/30°C temperature except Cu content in leaves and also Mn and Fe content in shoots. Upon heat stress treatment SAMRAT showed relatively less reduction of micronutrients like Cu, Mn and Fe content in leaves than VBN-2. The level of gene expression that regulate the antioxidant enzymes such as SOD, CAT and APX needs to be studied in more detail to better understand the mechanism of heat tolerance in greengram and also investigate the role of micronutrients in antioxidant enzyme activity under heat stress.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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