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Effect of heat stress on lipid peroxidation and antioxidant enzymes in mung bean (*Vigna radiata L*) seedlings

Simeen Mansoor[1]* and Farzana Nasir Naqvi[2]

Department of Genetics, University of Karachi, Karachi-75270, Pakistan.

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The aim of this research was to estimate the effects of heat stress on lipid peroxidation and antioxidant enzymes in seedlings of four mung bean genotypes (NM 19-19, NM 20-21, NM 121-123 and NCM 89). Sterilized seeds were grown in petri dishes at optimum temperature (30°C) for 24 h, exposed to 50°C for 2 h (lethal temperature) as well as pretreated to 40°C for 1 h prior to 50°C for 2 h. Changes in malondialdehyde (MDA) content which is an indicator of lipid peroxidation and the activity of guaiacol peroxidase (POD), ascorbate peroxidase (APX), superoxide (SOD) and catalase (CAT) were measured. The results showed that the growth in lethal temperature was extremely poor which improved when pretreatment of 40°C was applied before 50°C. The content of MDA in seedlings treated with lethal temperature was highest at any harvest, which reduced when seedlings were pretreated with 40°C prior to lethal stress. Variable response of antioxidant enzymes activity amongst different genotypes was observed. POD and SOD activities increased under stress in all genotypes except in NM 20-21, APX activity also increased under heat stress in all genotypes. However, the activity of CAT showed reduction under stress for NM 19-19 and NM 121-123 but increase in NM 20-21 and NCM 89. Low MDA content and increased antioxidant enzymes under heat stress were observed in NM 19-19, indicating that it was most thermotolerant genotype. However, high MDA content with decreased activity of antioxidant enzymes were detected in NM 20-21 which could be suggested a least thermotolerant genotype

Key words: Antioxidant enzymes, lethal temperature, mung bean, malandialdehyde, lipid peroxidation.

INTRODUCTION

Low crop yield, reduction in viability, and low plant quality are caused by abiotic stresses including temperature extremes (Anderson and Sonali, 2004). Among these, heat stress is an alarming factor due to global warming (Mahla et al., 2012). Heat stress causes oxidative stress which is marked by the generation of reactive oxygen species (ROS). These species include singlet oxygen, superoxide anion, peroxide hydrogen and hydroxyl radical; all of them are said to damage the cell membrane (Kumar et al., 2012). Lipid peroxidation, protein denaturation and DNA mutation are caused when the above mentioned species of oxygen react with lipids, proteins and nucleic acid (Breusegem et al., 2001). The chloroplastic, mitochondrial, and plasma membrane linked electron transport systems of higher plants create ROS even under optimum conditions (Alscher et al., 2002). Fortunately, Plants have learnt to form and remove ROS through defensive enzymatic and non-enzymatic scavenging systems for the balancing of oxygen under unstressed circumstances (Alscher et al., 2002; Liu and Bingru, 2000).

Heat stress causes increased membrane damage due

*Corresponding author. E-mail: simeenm@uok.edu.pk.

to lipid peroxidation (Amirjani, 2012). Lipid peroxidation is a natural metabolic process under normal aerobic conditions and it is one of the most investigated consequences of ROS action on membrane structure and function (Blokhina et al., 2003). Lipid peroxidation is a commonly utilized stress indicator of membrane damage (Taulavuori et al., 2001). Heat stress impairs mitochondrial functions thereby resulting in the induction of oxidative damage that manifests in lipid peroxidation, detected by malondialdehyde (MDA) content (Davidson and Schiestl, 2001; Larkindale and Knight, 2002; Vacca et al., 2004).

The dismutation of superoxide anion to O_2 and H_2O_2 is important in the protection of cell, which is catalyzed by superoxide dismutase (Kumar et al., 2012). Different types of catalases and peroxidases present in the plant enzymatically regulate the relatively stable levels of H_2O_2 to water and oxygen molecule. The reactive oxygen species-scavenging enzymes for example ascorbic peroxidase, catalase, guaiacol peroxidase and superoxide dismutase are enhanced by heat stress (Chaitanya et al., 2002).

Normally, these oxidative enzymes control oxidative damage by processing ROS; however, cellular damage occurs when ROS production exceeds the antioxidant defense capacity (Almeselmani et al., 2006). Studies have shown that tolerance to high temperature in crop plants is linked to the increase in antioxidant enzymes activity (Zhau et al., 1995). Due to global warming, plants and their yield is negatively affected. Therefore, it was found that the mechanism of heat stress in plants is important to investigate for future generations (Mahla et al., 2012).

MATERIALS AND METHODS

Seeds of four mung bean genotypes (NM 19-19, NM 20-21, NM 121-123 and NCM 89) were obtained from National Agricultural Research Center (NARC), Islamabad, Pakistan. Seeds were treated according to the method of Chen et al. (1986) with minor modifications. Seeds were imbibed in distilled water (d/w) for 5 h and sterilized with 1% sodium hypochlorite solution. They were allowed to germinate for 24 h in d/w at 30°C (optimum temperature).

Seedlings were transferred in the Petri dishes lined with two layers of filter papers soaked with 6 ml of incubating buffer (0.001M sodium phosphate buffer pH 6.0 and 1% sucrose solution). Temperature treatment A (control) was 30°C, treatment B (pretreatment) was 40°C for 1 h, and then lethal temperature of 50°C for 2 h was given, where as treatment C (lethal) was 50°C for 2 h. Seedlings were then transferred back to 30°C in the Petri dishes lined with filter paper soaked with d/w. Seedlings were harvested at 0, 24, 48, and 72 h after treatment and used for the estimation of lipid peroxidation and antioxidant enzymes.

Estimation of lipid peroxidation

The level of lipid peroxidation was measured by estimating MDA content according to the method of Heath and Packer, (1968). Seedlings (0.2 g) were homogenized in 1.0 ml of 5% trichloroacetic acid (TCA) solution in cold pestle and mortar. Homogenate was centrifuged at 12 000 rpm for 15 min at room temperature. The supernatant was collected for the estimation of MDA. Reaction mixture contained 1.0 ml of aliquot and 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA solution (freshly prepared), heated at 96°C for 30 min. The reaction was stopped by placing tubes quickly in ice chilled water following centrifugation at 2000 rpm for 10 min. Absorbance was taken at 532 and nonspecific absorbance was taken at 600 nm. Lipid peroxidation was calculated by subtracting the absorption value at 600 nm from 532 nm. The concentration of MDA was calculated by means of extinction coefficient of 155 mM^{-1} cm^{-1} and the results were expressed as µmol/mg protein.

Extraction and estimation of antioxidant enzymes

Antioxidant enzymes were extracted by the method of Jiang and Bingru, (2001). Seedlings (2.0 g) were homogenized in 4 ml extraction buffer (0.05 M phosphate buffer, pH 7.0 containing ascorbic acid and polyvinylpyrrolidone). The homogenate was centrifuged at 12 000 rpm for 30 min at 4°C. Supernatant was saved for the estimation of proteins using the method of Lowry et al. (1951) and antioxidant enzymes.

Guaicol peroxidase (POD) activity was estimated by the method of Evers et al. (1994) by taking 50 µL of enzyme, 3.75 ml sodium phosphate buffer (pH 5.6), 100 µL H_2O_2, 100 µL Guaiacol. Test tubes were left at room temperature for 8 min. Volume was raised to 5 mL of d/w. The oxidation of guaiacol into tetraguaiacol was estimated by measuring the absorbance at 470 nm against the reagent blank, using extinction coefficient of 26.6 mM^{-1} cm^{-1}.

Ascorbate peroxidase (APX) activity was measured according to Nakano and Asada, (1981) with minor modifications. For assay of the enzyme activity, the rate of hydrogen peroxide-dependent oxidation of ascorbic acid was determined in a reaction mixture that contained 0.25 ml of 0.05 M phosphate buffer pH 7.0, 0.25 ml of 0.0001 M ethylenediaminetetraacetic acid (EDTA), 0.25 ml of 0.05 M ascorbate and 150 µL supernatant. The reaction was initiated by addition of 0.25 ml of 0.0001 M H_2O_2 and the oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290 nm for 1 min. The APX specific activity was recorded as µmol ascorbate oxi./mg protein/2 min with the extinction coefficient of 2.8 mM^{-1} cm^{-1}.

Superoxide dismutase (SOD) activity was assayed by using photochemical nitroblue tetrazolium (NBT) by the method of Sairam et al. (2002). The reaction mixture was composed of 100 µL of Na_2CO_3, 200 µL methionine, 100 µL NBT, 100 µL EDTA, 1.5 ml potassium buffer (pH 7.5), 1.0 ml d/w and 150 µL enzyme. The reaction mixture was incubated in the dark box (24×12 inches) for 5 to 8 min. Then 100 µL Riboflavin was added and samples were incubated in the box attached with two fluorescent bulbs of 20 Watt each for 30 min. A complete reaction mixture without enzyme, which gave maximal colour, served as control. Reaction was stopped by switching -off the light and putting the tubes in the dark by covering the box with black cloth for further 10 min. A non-irradiated complete reaction mixture served as a blank. Absorbance was recorded at 560 nm. One unit of SOD activity was defined as the amount of enzyme which reduced the absorbance reading to 50% in comparison with tubes lacking enzyme.

Specific activity was calculated on O.D as follows:

Sample O.D = Blank O.D-Sample O.D/ Blank O.D÷2
Sp/Activity = Sample O.D/µg protein in 150 µL extract × 1000
Therefore, Specific activity was calculated as unit/ mg protein /30 min.

Catalase (CAT) activity was assayed by monitoring the decrease in absorbance due to hydrogen peroxide at 240 nm as described by Aebi, (1984). The reaction mixture contained 2.5 ml of 0.05 M Potassium phosphate buffer pH 7.0, 250 µL d/w, 50 µL enzyme and 0.2 ml of 0.125 M H_2O_2. Catalase activity was estimated at 240 nm by the decrease in the absorbance for 1 min which reflects the decomposition of H_2O_2 by catalase. Specific activity was calculated

Figure 1. Growth performance of mung bean seedling, harvested 72 h after temperature treatment. **A,** 30°C; **B,** 30°C+40°C+50°C; **C,** 30°C+ 50°C.

using extinction coefficient of 40 mM^{-1} cm^{-1}

Statistical analysis

Experiment was conducted in factorial as CRD with three replications, considering temperature as factor A and genotype as factor B. Analysis of variance was performed by a computer program SPSS version 13. DMR was performed by Steel and Torrie (1980) at $P< 0.05$ level of significance.

RESULTS

Figure 1 shows that the growth was extremely retarded during lethal stress (50°C for 2 h; treatment C), whereas considerable improvement was seen when exposed to mild temperature of 40°C for 1 h before lethal temperature (treatment B). By looking at the growth of 72 h old mung bean seedlings, the length was greatest at control (A) and extremely poor at lethal (C). However, when pretreatment of 40°C was given prior to lethal temperature, seedling length was improved as compared to lethal temperature. These seedlings along with control were used for determining membrane damage by estimating MDA content and activity of antioxidant enzymes. They showed variable response.

Effect of heat stress on lipid peroxidation

The effect of temperature on the growth of mung bean seedlings was studied by estimating lipid peroxidation and antioxidant enzymes. Lipid peroxidation as indicated by MDA content was measured in seedlings of all four genotypes of mung bean at various harvests. Table 1 shows that mean MDA content was lowest in control samples at all harvests and genotypes and it was significantly highest in samples treated with lethal shock (C). It was further noticed that MDA level decreased significantly when a pretreatment of 40°C for 1 h was given prior to lethal shock but it was not less than that in control. As MDA content reflects the degree of membrane lipid peroxidation, pretreatment of 40°C probably makes the cell membrane ready for the next exposure to lethal stress (50°C). Furthermore, it was also noticed that the level of MDA increased as the growth proceeded for all genotypes and treatments including control. Genotypic

Table 1. Mean comparison of MDA content (µmol/mg protein) in seedlings of four mung bean (*Vigna radiata L*) genotypes, harvested 0, 24, 48 and 72 h after temperature treatments.

Harvest (h)	Treatment	Genotype			
		NM 19-19	NM20-21	NM121-123	NCM 89
0	30°C(A)	22.6±1.5[b]	39.9±2.5[c]	35.0±0.5[c]	47.7±1.4[c]
	30°C+40°C+50°C (B)	25.6±0.3[b]	66.3±3.4[b]	51.0±2.0[b]	64.2±2.2[b]
	30°C+50°C (C)	43.1±1.6[a]	80.0 ±1.7[a]	77.09 ±0.1[a]	80.1±1.8[a]
24	30°C (A)	47.3±1.4[c]	54.8±2.5[c]	51.3±2.4[c]	52.6±1.2[b]
	30°C+40°C+50°C (B)	59.5±1.0[b]	76.4± 0.2[b]	71.3 ±2.0[b]	71.3 ± 3.4[a]
	30°C+50°C (C)	71.0±1.2[a]	94.6±1.4[a]	92.9±1.6[a]	78.6±0.8[a]
48	30°C (A)	63.0±2.4[b]	67.1±1.5[c]	64.8 ±0.8[c]	66.1±1.8[c]
	30°C+40°C+50°C (B)	78.9±1.9[a]	90.3 ±1.4[b]	85.1±1.4[b]	86.8±0.9[b]
	30°C+50°C (C)	83.7±1.5[a]	98.6±0.3[a]	96.1±1.7[a]	96.1±0.5[a]
72	30°C (A)	77.5 ±0.7[c]	85.9 ±2.9[c]	78.5 ±1.7[c]	83.7±0.9[c]
	30°C+40°C+50°C (B)	92.7±1.5[b]	106.0±1.2[b]	96.3±1.5[b]	101.4±0.4[b]
	30°C+50°C (C)	101.9 ±1.7[a]	130.1±1.7[a]	108.3±0.9[a]	121.0 ±2.0[a]

variations revealed highest level of MDA in MN 20-21 and lowest in NM 19-19 at any harvest and treatment.

Effect of heat stress on antioxidant enzymes

Mung bean seedlings of various genotypes subjected to heat stress respond differently for antioxidant enzymes activity.

POD activity

Table 2 shows the mean comparisons of POD among treatments of all harvests and genotypes. It was observed that when seedlings were exposed to lethal temperature (C), highest activity of POD was seen, which reduced when pretreatment of 40°C was given before lethal temperature (B) at 0, 24 and 48 h except for NM 20-21. However, at 72 h, POD activity was reduced in treatment B which was further decreased at treatment C of all genotypes except in NM 121-123.

APX activity

From the experimental results it was clear that the activity of APX was significantly high in treatment B (pretreatment), further induced significantly in treatment C (lethal) as compared to A (control) for all genotypes, except in genotype NM 20-21at 72 (Table 3). It was also observed that highest promotion in APX was seen in NM 19-19 under heat stress among all genotypes.

SOD activity

As SOD is an important antioxidant enzyme to keep the balance of scavenging POD and CAT enzyme as well as H_2O_2. It was also responsible for the low levels of ROS. Table 4 represents mean comparison of the activity of superoxide dismutase enzyme at all harvest and genotypes of mung bean. This shows that when the temperature stress was lethal (50°C), SOD activity was highest. However, it did not significantly reduce during pretreatment (B) for all genotypes and harvests. It indicates that increase stress will result in an increased SOD activity; whereas, there was decline in SOD activity during pretreatment (B), which reduced further in lethal treatment (C).

CAT activity

Genotypic variation with respect to CAT activity was prominent. Table 5 shows that the CAT activity in MN 19-19 and NM 121-123 at 0 and 48 h was reduced in pretreated samples (B) and further reduced in lethal treatment (C). At 24 h of NM 19-19 there was reduction in CAT activity at treatment B but slight increase in treatment C, that was still less than in control (A). However, CAT activity revealed an increasing pattern in NM 20-21 and NCM 89. Variable response of antioxidant enzymes for different genotypes was observed. However, activity was highest in NM 19-19 and lowest in NM 20-21 for any of the antioxidant enzymes or harvests. By looking at the performance of mung bean genotypes under heat stress, the hierarchy for the heat tolerant genotype would be NM19-19 > NM 121-123 > NCM 89 > NM 20-21.

Table 2. Mean comparison of POD activity (µmol tetraguaiacol/mg protein /8 min) in seedlings of four mung bean *(Vigna radiate L)* genotypes, harvested 0, 24, 48 and 72 h after temperature treatments.

Harvest (h)	Treatment	Genotype			
		NM 19-19	NM20-21	NM121-123	NCM 89
0	30°C (A)	1.90±0.055^{b}	0.58±0.02^{b}	0.95±0.026^{c}	0.87±0.014^{c}
	30°C+40°C+50°C (B)	1.88±0.068^{b}	0.733±0.020^{b}	1.34±0.072^{b}	1.32±0.089^{b}
	30°C+50°C (C)	3.84±0.123^{a}	2.44±0.254^{a}	1.75±0.082^{a}	1.82±0.040^{a}
24	30°C (A)	9.2±0.14^{c}	7.33±0.345^{a}	7.33±0.345^{a}	7.86±1.10^{b}
	30°C+40°C+50°C (B)	18.10±1.20^{b}	6.71±0.420^{a}	6.71±0.420^{a}	9.46±0.53^{a}
	30°C+50°C (C)	22.13±1.20^{a}	4.60±0.23^{b}	4.60±0.23^{b}	10.76±1.08^{a}
48	30°C (A)	14.18± 1.4^{c}	9.6±1.301^{a}	8.82±0.43^{b}	8.46±0.6^{c}
	30°C+40°C+50°C (B)	19.51±0.70^{b}	8.3±0.66^{a}	14.7±1.04^{a}	11.46±0.72^{b}
	30°C+50°C (C)	24.0±1.156^{a}	6.6±0.352^{a}	17.0±0.577^{a}	13.3±0.43^{a}
72	30°C (A)	27.16±1.23^{a}	11.9±0.75^{a}	18.25±0.406^{b}	14.9± 1.17^{a}
	30°C+40°C+50°C (B)	25.4±0.84^{a}	9.3±0.47^{b}	18.25±0.406^{b}	11.33±0.88^{b}
	30°C+50°C (C)	20.1±1.15^{b}	8.0±0.52^{c}	43.47±0.746^{a}	9.6± 0.7^{b}

Table 3. Mean comparison of APX activity (µmol ascorbate oxi. /mg protein/2min) in seedlings of four mung bean (*Vigna radiata L*) genotypes, harvested 0, 24, 48 and 72 h after temperature treatments.

Harvest (h)	Treatment	Genotype			
		NM 19-19	NM20-21	NM121-123	NCM 89
0	30°C (A)	0.22± 0.014^{c}	0.19±0.005^{b}	0.55±0.029^{b}	0.21±0.02^{a}
	30°C+40°C+50°C (B)	0.28±0.020^{b}	0.22±0.017^{ab}	0.70±0.005^{a}	0.24±0.011^{a}
	30°C+50°C (C)	0.62±0.032^{a}	0.27± 0.014^{a}	0.71±0.023^{a}	0.28±0.037^{a}
24	30°C (A)	0.61±0.048^{b}	0.26±0.005^{b}	0.51±0.044^{c}	0.27±0.008^{c}
	30°C+40°C+50°C (B)	0.92±0.062^{b}	0.29±0.017^{a}	0.63±0.018^{b}	0.30±0.005^{b}
	30°C+50°C (C)	1.55 ±0.294^{a}	0.31±0.00 5^{a}	0.82±0.037^{a}	0.33±0.005^{a}
48	30°C (A)	1.31±0.105^{c}	0.30±0.014^{b}	0.43±0.018^{b}	0 .33±0.015^{b}
	30°C+40°C+50°C (B)	4.52±0.289^{b}	0.32 ±0.018^{b}	0.76±0.030^{a}	0.49± 0.006^{a}
	30°C+50°C (C)	5.28±0.148^{a}	0.52 ± 0.014^{a}	0.80±0.011^{a}	0.53±0.027^{a}
72	30°C (A)	0.83±0.065^{c}	0.83±0.065^{a}	0.39±0.006^{c}	0.25±0.029^{b}
	30°C+40°C+50°C (B)	2.64±0.320^{b}	0.44±0.02^{b}	0.56±0.016^{b}	0.36±0.030^{b}
	30°C+50°C (C)	4.47±0.290 ^{a}	0.30±0.057^{b}	0.75±0.029^{a}	0.79±0.115^{a}

DISCUSSION

The results of this study show that lethal temperature caused negative effect on growth which could be due to the generation of high levels of ROS and this effect was suppressed by pretreatment of 40°C. Plants generate ROS during growth, but the generation of ROS significantly increases when the plant is under stressful conditions (Zhang et al. 2006), which cause severe oxidative damage to different cell organelles and biomolecules (Amirjani, 2012). This phenomenon was revealed when the seedling length of mung bean decreased with increased MDA content under lethal temperature treatments and improved in pretreatment of 40°C, with decreased MDA content. Results of this study indicate that lipid peroxidation resulted in membrane damage, which occurred due to highest levels of MDA content in lethal temperature stressed samples, while a considerable decrease was seen in pretreated samples. High level of MDA was reported under different stresses by many researchers. Esfandari et al. (2007) reported increased MDA during salt stress in wheat seedling. Similarly, Nagi

Table 4. Mean comparison of SOD activity (units/mg protein) in seedlings of four mung bean (*Vigna radiata L*) genotypes, harvested 0, 24, 48 and 72 h after temperature treatments.

Harvest (h)	Treatment	Genotype			
		NM 19-19	NM20-21	NM121-123	NCM 89
0	30°C (A)	1.2 ± 0.057[a]	0.89 ±0.020[a]	0.81±0.005[b]	1.01±0.013[a]
	30°C+40°C+50°C (B)	1.26±0.088[a]	0.86 ±0.030[a]	0.93±0.033[b]	1.01 ±0.092[a]
	30°C+50°C(C)	1.40±0.029[a]	0.76±0.030[b]	1.26 ±0.145[a]	1.1±0.057[a]
24	30°C (A)	1.25±0.144[a]	0.50±0.057[a]	1.1 ± 0.10[a]	1.03 ±0.088[a]
	30°C+40°C+50°C (B)	1.35± 0.028[a]	0.27±0.043[b]	1.28±0.060[a]	1.06 ± 0.030[a]
	30°C+50°C (C)	1.46± 0.045[a]	0.21 ± 0.005[b]	1.38±0.109[a]	1.10 ±0.058[a]
48	30°C (A)	1.06±0.068[a]	0.426±0.014[a]	0.92±0.033[a]	0.84±0.023[a]
	30°C+40°C+50°C (B)	1.23±0.185[a]	0.25±0.017[b]	0.95±0.077[a]	0.85±0.028[a]
	30°C+50°C (C)	1.25±0.144[a]	0.20±0.006[b]	1.17±0.088[a]	0.87±0.026[a]
72	30°C (A)	1.06±0.068[a]	0.14±0.026[b]	0.48±0.018[c]	0.67±0.039[b]
	30°C+40°C+50°C (B)	1.23±0.185[a]	0.23±0.017[b]	0.80±0.014[b]	0.79±0.020[a]
	30°C+50°C (C)	1.25±0.144[a]	0.35±0.034[a]	0.94±0.037[a]	0.81±0.008[a]

Table 5. Mean comparison of CAT activity (µmol H_2O_2/mg protein/2 min) in seedlings of four mung bean *(Vigna radiata L)* genotypes, harvested 0, 24, 48 and 72 h after temperature treatments.

Harvest (h)	Treatment	Genotype			
		NM 19-19	NM20-21	NM121-123	NCM 89
0	30°C (A)	0.30±0.011[a]	0.09± 0.005[b]	0.20±0.003[a]	0.08±0.003[b]
	30°C+40°C+50°C (B)	0.20±0.014[b]	0.19± 0.008[a]	0.13±0.014[b]	0.12±0.003[a]
	30°C+50°C (C)	0.15±0.002[c]	0.22±0.011[a]	0.12±0.017[b]	0.13±0.014[a]
24	30°C (A)	0.28±0.011[a]	0.09±0.008[b]	0.26±0.015[a]	0.10±0.003[c]
	30°C+40°C+50°C (B)	0.22± 0.011[b]	0.14±0.018[a]	0.17±0.008[b]	0.11±0.003[b]
	30°C+50°C (C)	0.24±0.023[ab]	0.16±0.008[a]	0.10±0.008[c]	0.14±0.005[a]
48	30°C (A)	0.27±0.014[a]	0.11 ± 0.005[a]	0.23±0.011[a]	0.12 ± 0.005[b]
	30°C+40°C+50°C (B)	0.25±0.029[a]	0.15± 0.022[a]	0.15±0.021[b]	0.13±0.008[ab]
	30°C+50°C (C)	0.17±0.008[b]	0.15± 0.021[a]	0.11±0.005[c]	0.15±0.008[a]
72	30°C (A)	0.10±0.008[c]	0.09±0.014[b]	0.10±0.005[a]	0.15±0.028[a]
	30°C+40°C+50°C (B)	0.20±0.006[b]	0.16±0.005[ab]	0.09±0.005[a]	0.18±0.018[a]
	30°C+50°C (C)	0.29±0.003[a]	0.18±0.008[a]	0.05±0.005[b]	0.24±0.034[a]

and Devaraj (2011) found high levels of MDA in temperature and salt stressed horse gram. MDA inactivates proteins and enzymes of cellular membranes, which are important products of lipid peroxidation. Hence, antioxidant enzymes prevent cell damage by reducing and regulating levels of ROS (Ge et al. 2005).

Antioxidant enzymes like POD, APX, SOD and CAT play significant role in the detoxification of ROS. When mung bean seedlings of different genotypes were subjected to lethal temperature and pretreatment of 40°C before lethal temperature, respond differently in terms of antioxidant enzymes. POD and CAT are the major enzymes involved in the metabolism of H_2O_2 produced by SOD in all aerobic organisms. During temperature stress, most of the antioxidant enzymes were elevated for all genotypes. However, the response of NM 20-21 was different amongst all genotypes.

Present study showed the highest enhancement of POD activity in lethal treatment which declined in most of the pretreated samples but remained higher as compared

to control except in NM 20-21. Same results were observed by Mahla et al. (2012) in wheat seedlings. The response of NM 20-21 was different from rest of the mung bean genotypes, where it showed reduction in POD activity during pretreatment with more reduction in lethal treatment. Similarly, He, (2010) detected a decline in POD activity in two cultivar of Kentucky Blue grasses under heat stress, suggested that heat induced decline could be due to the reason that POD is sensitive to heat stress and not related to heat tolerance. It could be suggested that NM 20-21 was heat sensitive genotype.

Results from current study shows that the APX activity was increased in all genotypes. However, the higher values were found in NM 19-19, which may be considered as heat tolerant genotype. Increased APX activity under heat stress was also detected in Kentucky Blue grass by He and Huang (2007). On the other hand the values of APX activity were least in NM 20-21, which could reflect that it was heat sensitive genotype. The higher APX activity could be due to the enhanced H_2O_2 scavanging system in chloroplast (He, 2010).

SOD activity was highest in lethal treatment 'C' which reduced in pretreated samples 'B' but still higher than that in control for all genotypes except for NM 20-21. He (2010) reported decreased SOD activity in heat sensitive Kentucky blue grass cultivar 'Brilliant' during 28 d of heat stress. Increased SOD activity is responsible to control the formation of superoxide (Ostrovskaya et al. 2009). Therefore, we may suggest that NM 20-21 was heat sensitive genotype as it showed decreased SOD activity hence gave less protection against superoxide

The activity of CAT reduced in NM 19-19 and NM 121-123 where as it increased in NM 20-21 and NCM 89. Kele and Oncel, (2002), reported increase CAT acivity in *T. aestivum* but decrease activity in *T.durum* genotypes during heat stress. However, Kumar et al. (2012) observed increased CAT activity in heat stressed wheat plants. Our results suggest that increase in CAT activity under heat stress could be due to the increase in H_2O_2 production leading heat injury. There are several reports of decreased activities of key antioxidant enzymes (SOD and CAT) and increased lipid peroxidation, following heat shock. The antioxidant defense mechanism thus impaired by heat shock and lead to increased antioxidant concentrations (Foyer et al. 1997). Zhao et al. (2010) reported that decreased activities of POD, SOD and CAT were closely related to MDA accumulation, and our findings were similar for genotype NM 20-21 where MDA level was high with low levels of POD and SOD. However, the activity of CAT rose non-significantly in most of the harvests under stress. On the other hand Zhao et al. (2010) found that when the antioxidant enzyme activities were high, MDA content as well as relative membrane lipid peroxidation was low. NM 19-19 showed lowest level of MDA and highest levels of antioxidant enzymes when compared with all genotypes. On the other hand, high MDA and low levels of antioxidant enzymes were seen for NM 20-21.

Conclusion

Plants are continuously exposed to change in temperature during the day or by sudden episodes of high temperatures due to global warming. Mung bean is one of the most important legume and cheapest source of protein in Asian countries including Pakistan. Therefore, it is necessary to screen those genotypes which are most or least thermotolerant, so that one can grow more thermotolerant genotype to get better yield. It is reported that heat stress is responsible to retard the growth of mung bean seedlings which was due to the increased level of MDA and low level of antioxidant enzymes. By looking at the performance of all four genotypes with respect to MDA and antioxidant enzymes activity, our results suggest NM19-19 as heat tolerant and NM 20-21 as heat sensitive genotype.

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