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# Role of Heat Shock and Salicylic Acid in Antioxidant Homeostasis in Mungbean (*Vigna radiata* L.) Plant Subjected to Heat Stress

Amal A.H. Saleh, Dina Z. Abdel-Kader and Amr M. El Elish
Department of Botany, Faculty of Science, Suez Canal University, Ismailia, Egypt

**Abstract:** The objective of the present study was to examine the role of heat shock and salicylic acid in antioxidant homeostasis in mungbean (*Vigna radiata* L.) plant. Fifteen-day mungbean seedlings were divided into 6 groups. The first one kept in open air then harvested after 25 and 32 day post germination (negative control).The second one were exposed to 50°C for 3 h (heat stress) and then harvested after 10 days (positive control). The 3rd and 4th groups were exposed to 2 different heat shock temperature (40 and 45°C) for 1, 1.5 and 2 h. The fifth and sixth groups were sprayed with two different Salicylic Acid (SA) concentrations (0.5 or 1 mM). The groups from third to sixth were exposed to heat stress (50°C) for 3 h either directly or after week. The seedlings were harvested 10 days post heat stress. The results showed that, high temperature stress induced lipid peroxidation, electrolyte leakage and increased hydrogen peroxidase level. High temperature stress decreased catalase, peroxidase, ascorbic peroxidase activities and glutathione content. It was also found that high temperature stress induced a significant increase in endogenous SA concentration and superoxide dismutase activity. These deteriorative symptoms in the mung bean seedlings were ameliorated by heat shock treatments or SA application by decreasing lipid peroxidation and increasing catalase, peroxidase, ascorbic peroxidase, superoxide dismutase activities and glutathione content. Based on these results, it was presumed that the stress protection caused by heat shock treatments or SA application contributes to some extent to the enhanced activity of the free-radical scavenging systems.

**Key words:** High temperature stress, heat shock, SA, $H_2O_2$, antioxidant enzymes, mungbean (*Vigna radiata* L.)

## INTRODUCTION

One of the major environmental factors affecting plant growth and productivity is high temperature (Havaux, 1993). Field grown plants are often subjected to fluctuating temperature that has a profound effect on the plant metabolism. Many of the changes that appear during acclimation to heat stress are reversible, but if the stress is too great, irreversible changes can occur and these can lead to plant death. Electrolyte leakage is an effective means of measuring cell membrane thermostability and has been used as an indicator of direct heat injury (Saelim and Zwiazek, 2000).

Heat stress induces or enhances the active oxygen species-scavenging enzymes like superoxide dismutase, catalase, peroxidase (PRX) and several antioxidants (Chaitanya *et al*., 2002). PRX enzyme has been related to the appearance of physiological injuries caused in plants by thermal stress and its activity was enhanced by high temperature stress (Chaitanya *et al*., 2002; Mazorra *et al*., 2002).

It has been estimated that 1% of the oxygen consumed by plants is diverted into active oxygen (Asada and Takahashi, 1987). Consequently, plant cells have developed an array of nonenzymatic and enzymatic mechanisms for scavenging this toxic component. Nieto-Sotelo (1989) found an increase of

**Corresponding Author:** Dina Z. Abdel-Kader, Department of Botany, Faculty of Science, Suez Canal University, 41522, Ismailia, Egypt Tel: 0020643219455

GSH content and a decrease of cysteine content in maize subjected to heat stress. In maize GSH increased only in the tolerant genotype after cultivation at 40°C for 3 days (Kocsy *et al*., 1998a). Moreover, heat stress increased the amount of GSH in wheat (Kocsy *et al*., 1998b).

Induction of protein synthesis or altered protein function may be one of the several mechanisms of adaptation to high temperature (Teeri, 1980). Plants respond to Heat Stress (HS) by changing their metabolic pathways. Under HS, synthesis of most proteins is repressed and some proteins, which are called Heat Shock Proteins (HSPs), start to be synthesised (Vierling, 1991). Lin *et al*. (1984) reported that soybean seedlings exposed to 40°C for 2 h produced HSPs and tolerate temperature of 45°C, but plants transferred directly from 28 to 45°C did not produce HSPs. Moreover, Chen *et al*. (1982) mentioned that tomato plants grown in temperature regimes below 30°C their leaf tissues were killed in about 15 min at 50°C, while tomato plants increased significant tolerance when exposed to temperatures above 30°C for 24 h.

Salicylic acid has been defined as a new potential plant hormone (Raskin, 1992a) and found to play an important role in disease resistance (Raskin, 1992b) and abiotic stress tolerance. SA could also regulate physiological adaptation to some environmental stresses including oxidative damage (Borsani *et al*., 2001), cold injury (Janda *et al*., 1999) and ozone excess (Rao *et al*., 1996). There are data about SA induction of heat shock proteins synthesis in tobacco plants in suspension cell culture at osmotic stress (Mikolajczyk *et al*., 2000).

Thus, the objective of this study was to examine the relation between heat shock and salicylic acid treatments in controlling antioxidant homeostasis in mungbean (*Vigna radiata* L.) plant under heat stress conditions. It was aimed also to investigate whether the effect of heat stress or SA will continue even after week of application or not.

## MATERIALS AND METHODS

### Plant Material and Plantation

Mung bean (*Vigna radiata* L.) seeds were obtained from the Agricultural Research Center (ARC), Giza, Egypt. Seeds were surface sterilized with 2.5% sodium hypochlorite for 15 min then washed thoroughly with distilled water. It was found that the highest germination percentage for mung bean (*Vigna radiata* L.) was achieved at 30°C. Based on these results, the heat shock temperature was determined. The seeds were grown in plastic pots (20 cm in height and 15 cm in diameter) equally filled with a pre-sieved sandy loam soil. All pots were watered up to saturation and irrigated regularly every two days until heat shock and salicylic acid treatments. Seedlings were exposed to (14: 10) light: dark periods allover the experimental period. Mung bean was cultivated in open-air temperature range between 25 and 38°C. Fifteen days post germination; the planted seedlings were treated as follow:

### Negative Control I and II

Seedlings was kept in control condition (38°C: 25°C day/night temperature and 14: 10 light: dark periods) all over the experimental period and were harvested 25 and 32 days post germination (negative control I and II) respectively.

### Positive Control

Fifteen days old seedlings were subjected to heat stress at 50°C for 3 h.

### Heat Shock Treatment (Two Groups)

Mung bean seedlings group (15 days old) were divided to 6 sub-groups. Three groups were subjected to heat shock at 40°C and the others were subjected to heat shock at 45°C for 1, 1.5 and

2 h. The six sub-groups were allowed to recover at 35°C for 3 h. Each sub group was divided into two sub- sub groups one of them was directly subjected to heat stress (50°C for 3 h) and the other was subjected to the same conditions of heat stress after one week.

**Salicylic Acid Treatment (Two Groups)**

Fifteen days old seedling of mung bean plants were divided into two subgroups, one group was sprayed twice (two days interval) with 0.5 mM Salicylic acid and the other group was sprayed twice (two days interval) with 1 mM Salicylic acid. Each subgroup was subjected to heat stress as those in the heat shock treatment. The plants of all groups were harvested 10 days post heat stress treatments (25 and 32 days post germination).

**Assay of $H_2O_2$**

$H_2O_2$ concentration in the incubation medium of treated leaf explants was measured by the FOX[2] method (Jiang *et al*., 1990; Wolff, 1994) based on the peroxide-mediated oxidation of $Fe^{2+}$ with xylenol orange.

**Determination of Lipid Peroxidation Level (LP)**

Estimation of lipid peroxidation was assayed spectrophotometrically using thiobarbituric acid-malondialdehyde (TBA-MDA) assay. Lipid peroxides were extracted from 0.5 g fresh leaves with 5 mL 5% (w/v) metaphosphoric acid and 100 μL 2% (w/v in ethanol) butyle hydroxytoluene butanol (Minotti and Aust, 1987). The extract was centrifuged at 6,000 g for 20 min. An aliquot of the supernatant was reacted with thiobarbituric acid at low pH and 95°C and cooled to room temperature. The resulting thiobarbituric acid-malondialdehyde adduct was extracted with 1-butanol

**Membrane Leakage**

One leaflet of upper foliage mung bean leaves was transferred into de-ionized water. Leaves were centrifuged for 80 min at 300 rpm. Electric conductivity of the solution was determined using conductivity meter (ECOSCAN Handheld Series, Eutech Instruments EC/pH meter). Electric conductivity micro-siemens (μS) represent the total ion leakage from mung bean leaves (Vahala, 2003).

**Determination of Catalase Activity**

Catalase activity was assayed in a method following Aebi (1983). Activity was determined by following the decomposition of $H_2O_2$ at 240 nm.

**Determination of Peroxidase Activity**

Peroxidase activity was determined by following the dehydrogenation of guaiacol at 436 nm (Malik and Singh, 1980).

**Ascorbate Peroxidase (ASPX) Activity**

ASPX activity was determined using the method of Nakano and Asada (1987). The assay mixture contained 90 mM potassium phosphate buffer (pH = 7.0), 0.1 mM EDTA, 0.65 mM ascorbate and 1.0 mM $H_2O_2$. Activity was determined by following the $H_2O_2$ dependent decomposition of ascorbate at 290 nm.

**Superoxide Dismutase Activity (SOD)**

SOD was measured by the photochemical method as described by Giannopolitis and Ries (1977). Assays were carried out under illumination. One unit SOD activity is defined as the amount of enzyme required to cause 50% inhibition of the rate of p-nitro blue tetrazolium chloride reaction at 560 nm.

**Determination of Glutathione (GSH) Content**

Glutathione content was determined spectrophotometrically following the method described by Griffith (1980).

**Estimation of Carotenoid Content**

Carotenoid were estimated in the fresh mung bean leaves according to the procedure of Lichtenthaler (1987).

**Statistical Analyses**

Data are mean of three replicas each consists of ten seedlings. Differences with each treatment were statistically analyzed using one way ANOVA. Differences in the mean values were regarded as significant and highly significant at the levels of $p<0.05$* and $p<0.01$**, respectively. Pearson Rank Correlation test was used between variables. Statistical analyses were performed using SPSS (Version 9.00).

## RESULTS

**$H_2O_2$ Level**

The data showed that heat stress-after week treatment induced a significant increase in $H_2O_2$ content when compared to negative control. While heat shock pretreatment at 40°C/60 min and 45°C/90 and 120 min significantly reduced $H_2O_2$ contents. Application of 0.5 mM SA in both heat stress treatments (Directly and after week) showed a significant increase in $H_2O_2$ content (Table 1).

**Lipid Peroxidation (LP) Level**

Pretreatment of heat stressed seedlings witheat shock or SA significantly reduced lipid peroxidation hen compared to non-treated heat stressed seedlings. The decrease was more pronounced at 40°C/90 min heat shock in both directly or after week heat stress treatments, while the decrease was dose dependent in SA treatments (Table 2).

Table 1: $H_2O_2$ level expressed as μmole $g^{-1}$ fresh wt. in the leaf extract of Mung bean seedlings subjected to two heat shock temperatures (40 and 45°C) for 60, 90 and 120 min or sprayed with 0.5 and 1 mM salicylic acid. The seedlings then subjected directly or after week to heat stress at 50°C for 3 h

| | | Heat stress (50°C) | | | |
|---|---|---|---|---|---|
| | | Directly | | After week | |
| Treatments | Time (min) | Mean±SE | LSD | Mean±SE | LSD |
| Negative control | | 1.23±0.02 | - | 1.26±0.02 | - |
| Positive control | | 1.33±0.01* | - | 1.59±0.01* | - |
| Heat shock (40°C) | 60 | 1.25±0.01 | 0.00 | 1.22±0.01[a] | 0.06 |
| | 90 | 1.26±0.01 | 0.56 | 1.54±0.01* | 0.00 |
| | 120 | 1.37±0.10 | 0.11 | 1.66±0.00* | 0.00 |
| Oneway ANOVA | F- ratio | 5453 | | 51324 | |
| | p-value | 0.000** | | 0.000** | |
| Heat shock (45°C) | 60 | 1.24±0.02 | 0.02 | 1.74±0.00[a]* | 0.00 |
| | 90 | 1.26±0.05 | 0.56 | 1.37±0.01[a] | 0.00 |
| | 120 | 1.25±0.00 | 0.01 | 1.35±0.01[a] | 0.01 |
| Oneway ANOVA | F- ratio | 18 | | 47149 | |
| | p-value | 0.009* | | 0.000** | |
| SA 0.5 mM | | 1.46±0.00[a] | 0.00 | 2.02±0.01[a]** | 0.00 |
| SA 1.0 mM | | 1.35±0.00* | 0.00 | 1.53±0.00* | 0.00 |
| Oneway ANOVA | F- ratio | 20020 | | 215419 | |
| | p-value | 0.000** | | 0.000** | |

*, **: Significant and highly significant at $p<0.05$ and 0.01 respectively comparing with negative control. [a]: Significant at $p<0.01$ comparing with positive control. Plants were harvested 10 days post heat stress. Data represented are mean of 3 replica±SE

**Membrane Leakage**

Both heat stress treatments (Directly and after week) induced a significant increase in membrane leakage percentage when compared to negative control. Increasing heat shock time intervals increased membrane leakage at both heat stress treatments. Heat shock pretreatment at 40 and 45°C for 1 h and application of 1 mM SA induced a significant decrease in membrane leakage % at both heat stress treatments (Table 3).

Table 2: Lipid peroxidation as malondialdehye level (MDA) expressed as μmole $g^{-1}$ fresh wt. in the leaf extract of Mung bean seedlings subjected to two heat shock temperatures (40 and 45°C) for 60, 90 and 120 min or sprayed with 0.5 and 1 mM salicylic acid. The seedlings then subjected directly or after week to heat stress at 50°C for 3 h

| | | Heat stress (50°C) | | | |
|---|---|---|---|---|---|
| | | Directly | | After week | |
| Treatments | Time (min) | Mean±SE | LSD | Mean±SE | LSD |
| Negative control | | 10.42±0.00 | - | 12.21±0.00 | - |
| Positive control | | 13.85±0.30* | - | 13.88±0.30 | - |
| Heat shock (40°C) | 60 | 9.39±0.40[a] | 0.21 | 9.49±0.00[a]* | 0.00 |
| | 90 | 8.27±0.00[a] | 0.00 | 8.69±0.40[a]* | 0.00 |
| | 120 | 13.24±0.40* | 0.00 | 8.94±0.10[a]* | 0.00 |
| Oneway ANOVA | F-ratio | 5878.556 | | 3411.889 | |
| | p-value | 0.000** | | 0.000** | |
| Heat shock (45°C) | 60 | 10.03±0.30[a] | 0.22 | 11.38±0.00[a] | 0.00 |
| | 90 | 10.06±0.32[a] | 0.32 | 9.36±0.00[a]* | 0.00 |
| | 120 | 10.13±0.00[a] | 0.93 | 12.28±0.20 | 0.12 |
| Oneway ANOVA | F-ratio | 20 | | 11793.333 | |
| | p-value | 0.007* | | 0.000** | |
| SA 0.5 mM | | 11.54±0.00[a] | 0.00 | 12.12±0.00[a] | 0.08 |
| SA 1.0 mM | | 10.26±0.00[a] | 0.01 | 10.03±0.03[a] | 0.00 |
| Oneway ANOVA | F-ratio | 1425.000 | | 2214.500 | |
| | p-value | 0.000** | | 0.000** | |

*, **: Significant and highly significant at $p<0.05$ and 0.01 respectively comparing with negative control. [a]: Significant at $p<0.01$ comparing with positive control. Plants were harvested 10 days post heat stress. Data represented are mean of 3 replica±SE

Table 3: Membrane leakage expressed as (%) fresh wt. in the leaf extract of Mung bean seedlings subjected to two heat shock temperatures (40 and 45°C) for 60, 90 and 120 min or sprayed with 0.5 and 1 mM salicylic acid. The seedlings then subjected directly or after week to heat stress at 50°C for 3 h

| | | Heat stress (50°C) | | | |
|---|---|---|---|---|---|
| | | Directly | | After week | |
| Treatments | Time (min) | Mean±SE | LSD | Mean±SE | LSD |
| Negative control | | 73.70±0.02 | - | 74.84±2.34 | - |
| Positive control | | 85.41±0.68* | - | 84.50±0.00* | - |
| Heat shock (40°C) | 60 | 85.13±0.48 | 0.00 | 83.33±0.57[a]* | 0.53 |
| | 90 | 94.76±0.72[a] | 0.00 | 86.10±0.46[a]* | 0.46 |
| | 120 | 93.23±2.12[a]* | 0.00 | 89.91±0.60[a]* | 0.05 |
| Oneway ANOVA | F-ratio | 71.041 | | 4.716 | |
| | p-value | 0.001** | | 0.084 | |
| Heat shock (45°C) | 60 | 78.06±4.34[a] | 0.01 | 66.09±0.98[a]* | 0.01 |
| | 90 | 87.97±1.14* | 0.25 | 78.61±5.39[a] | 0.23 |
| | 120 | 97.06±0.98** | 0.00 | 81.84±1.84[a]* | 0.53 |
| Oneway ANOVA | F-ratio | 20.740 | | 6.978 | |
| | p-value | 0.007* | | 0.045 | |
| SA 0.5 mM | | 88.89±0.00* | 0.00 | 82.92±0.42* | 0.53 |
| SA 1.0 mM | | 72.81±0.00[a] | 0.07 | 79.69±2.27[a] | 0.15 |
| Oneway ANOVA | F-ratio | 1527 | | 2 | |
| | p-value | 0.000** | | 0.296 | |

*,**: Significant and highly significant at $p<0.05$ and 0.01 respectively comparing with negative control. [a]Significant at $p<0.01$ comparing with positive control. Plants were harvested 10 days post heat stress. Data represented are mean of 3 replica±SE

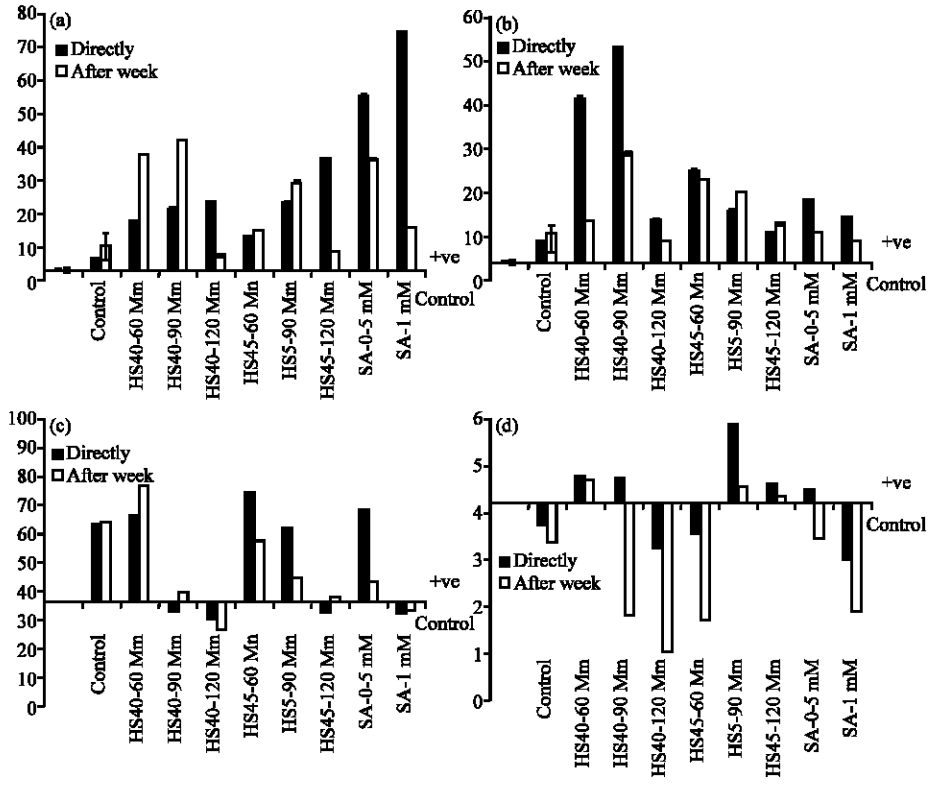


Fig. 1: Activities of (a) Catalase activity, (b) Peroxidase activity, (c) Ascorbic peroxidase and (d) Superoxide dismutase activity expressed as unit $g^{-1}$ fresh wt. in the leaf extract of Mung bean seedlings subjected to two heat shock temperatures (40 and 45°C) for 60, 90 and 120 min or sprayed with 0.5 and 1 mM salicylic acid. The seedlings then subjected directly or after week to heat stress at 50°C for 3 h. Plants were harvested 10 days post heat stress. Data represented are mean of 3 replica±SE

**Antioxidant Enzymes**

Heat stress significantly decreased CAT, peroxidase and ASPX activities in mung bean seedling while it induced a significant increase in SOD activity when compared to negative control (Fig. 1a-d). Heat shock pretreatment and SA application enhanced CAT and peroxidase activity at both heat stress treatments (direct or after week). The effect was decreased at 120 min. heat stress duration. The decrease in SOD activity was observed at after-week heat stress in seedlings subjected to heat shock pretreatment 40 and 90°C for 120 min and 45°C for 60 min and in both SA concentrations.

**Glutathione (GSH) Content**

Heat stress induced a significant decline in glutathione content when compared to negative control. Subjecting mung bean seedlings to the direct heat shock at 45°C for 90 min and after week heat shock at 40°C for 60 min achieved the highest glutathione content and their increases were 97.2 and 99%, respectively. The glutathione level was significantly increased after spraying with SA. Both SA concentrations (0.5 and 1 mM) in both heat stress treatments (Directly and after week) improved the injurious effect of lethal high temperature stress by increasing glutathione content when compared to positive control level (Fig. 2).

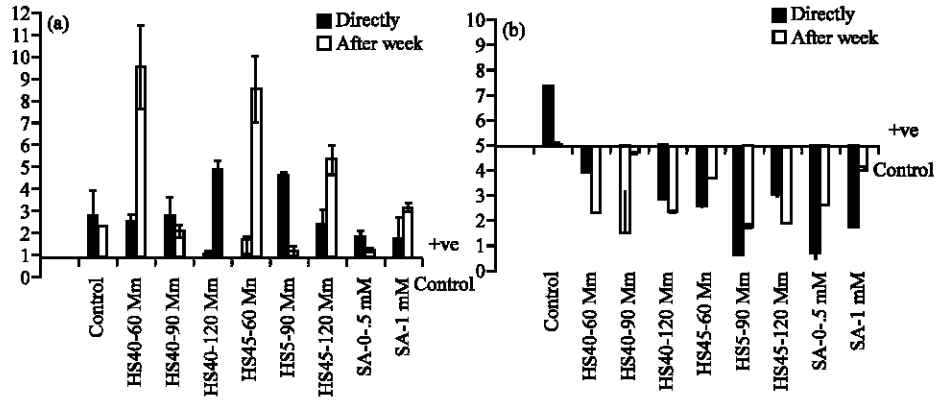


Fig. 2: (a) Glutathione content (μg $g^{-1}$ fresh wt.) and (b) carotenoid contents (μg $g^{-1}$ dry wt.) expressed as μg $g^{-1}$ fresh wt. in the leaf extract of Mung bean seedlings subjected to two heat shock temperatures (40 and 45°C) for 60, 90 and 120 min or sprayed with 0.5 and 1 m salicylic acid. The plants then subjected directly or after week to heat stress at 50°C for 3 h. Plants were harvested 10 days post heat stress. Data represented are mean of 3 replica±SE

**Carotenoid Content**

The data in Fig. 2b showed that heat stress significantly reduced carotenoid content when compared to negative control. Moreover, heat shock treatments (40 and 45°C) for different time intervals (60, 90 and 120 min) and SA application at both concentrations (0.5 and 1 mM) significantly reduced carotenoid content when compared to both negative and positive control.

## DISCUSSION

Abiotic stresses, including temperature extremes, are among the primary causes of reduced crop yields. High temperature injury can result in considerable pre-harvest and post-harvest crop losses. One mechanism of injury involves the generation and reactions of Activated Oxygen Species (AOS) (Liu and Huang, 2000). AOS can also react with pigments, membranes, enzymes and nucleic acids, thereby modifying their functions (Elstner, 1982).

The results in Table 1 revealed that heat stress in mungbean induced a significant increase in hydrogen peroxide ($H_2O_2$) content which could be attributed to a decrease in catalase activity. As catalase has a rapid turnover, conditions inhibiting catalase synthesis will lower the steady-state level of this enzyme (Streb and Feierabend, 1996; Scandalios *et al*., 1997). There are several reports of decreased activities of key antioxidant enzymes (SOD and CAT) following heat shock; the antioxidant defenses may thus be impaired by heat shock and lead to increased oxidant concentrations (Willekens *et al*., 1995; Foyer *et al*., 1997; Polle, 1997). Moreover, application of SA in mung bean plants showed a significant increase in $H_2O_2$ content (Table 1). The increase in $H_2O_2$ following heat shock in the dark could be explained by the model of Doke *et al*. (1994) in which abiotic stresses are accompanied by an oxidative burst (Doke, 1997), similar to that involved in signalling during plant-pathogen interactions (Levine *et al*., 1994; Baker and Orlandi, 1995). It is tempting to associate this $H_2O_2$ increase with an oxidative burst similar to that observed during other forms of abiotic stress including chilling (Prasad *et al*., 1994) and during incompatible pathogen interactions (Tenhaken *et al*., 1995).

As a result of heat stress, lipid peroxidation and membrane leakage was increased in mung bean seedlings (Table 2, 3). These results are in agreement with those obtained by Larkindale and Huang (2004). They found changes in membrane lipid compositions and saturation levels in three cultivars

of creeping bentgrass (*Agrostis stolonifera*). Their results also showed significant increases in Thiobarbituric acid reactive substances (TBARS); a marker of oxidative damage increases in membrane leakage of leaves during 28 days of heat stress (35°C). Growth at high temperature usually results into a decrease in the polyunsaturated fatty acid content; such a decrease is associated with reduced membrane fluidity (Santarius and Weiss, 1988). One of the primary symptoms of stress injury (Foyer *et al*., 1997). Larkindale and Huang (2004) reported that there might be some connection between the degree of saturation of leaf membrane lipids prior to heat stress and the ability of that plant to limit heat-induced damages during the stress period. They also suggested that lipid composition or saturation level of roots could be an important factor in controlling plant tolerance to heat stress.

The decrease in lipid peroxidation after heat shock treatments or SA treatments (Table 2, 3) may be attributed to the increase in zeaxanthin, as a result of carotenoid conversion which provide direct protection against lipid peroxidation (Horton, 2002). The decrease in carotenoid content observed in this study (Fig. 2b) confirmed this suggestion. Carotenoid play a protective role against oxidative damage by quenching of chlorophyll triplets, which otherwise could give rise to highly reactive singlet oxygen species and thus limiting membrane damage (Cuttriss and Pogson, 2004; Pogson *et al*., 2006).

Loss of enzyme activities at elevated temperatures may be due to conformational changes, production of inhibitors, diminished rates of enzyme synthesis and/or elevated enzyme degradation. Acute heat stress injury probably involves changes in protein structure that impair enzymatic function. Alternatively, enzyme thermostability may have been moderated by protective mechanisms, such as molecular chaperones (Nagao *et al*., 1990). Moreover, heat stress may enhance inactivation of catalase by preventing synthesis of new enzyme (Hertwig *et al*., 1992; Feierabend and Dehne, 1996), resulting in a decline in CAT activity.

Under heat or other stress, the level of oxygen radicals is increased, leading to denaturation and inactivation of cytosolic enzymes. APX plays an important role under stressed conditions. But, on the other hand, APX is also a subject to thermal damage (Zhong *et al*., 2000). Panchuk *et al*. (2002) stated that the total cellular activity of APX appeared to be more sensitive to heat stress in plant a compared with Glutathione Reductase (GR) and SOD.

A significant increase in the peroxidase (PRX) activity indicates the formation of large amounts $H_2O_2$ in leaves as a result of heat shock or SA pretreatments. In addition, the greater increase in PRX activity and lower electrolyte leakage may indicate an involvement of PRX in cell membrane integrity. In other words, there is a possible association between PRX activity and recovery of cell membrane damage due to high temperature.

It is well documented that heat stress induces oxidative injury and alters the activities of antioxidant enzymes including SOD, CAT, APX and GR in many plant species (Jagtap and Bhargava, 1995; Gong *et al*., 1997). The results of this work showed that only direct 0.5 mM SA application significantly increased SOD activity. He *et al*. (2005) suggested that SA-induced heat tolerance could be related to higher $O^{-}_{2}$ and $H_2O_2$ scavenge potential due to higher SOD and CAT activities under heat stress. The results in Kentucky bluegrass agree with the reports in *Arabidopsis* and creeping bentgrass that SA is involved in protection against heat stress-induced oxidative damage (Larkindale and Knight, 2002; Larkindale and Huang, 2004).

GSH proved to be involved during high-temperature acclimation, as observed by Nieto-Sotelo and Ho (1986) in heat shock in maize roots. Accumulation of GSH during stress has been reported during HS of maize roots (Nieto-Sotelo and Ho, 1986) and during chilling stress in zucchini (Wang, 1995), maize (Kocsy *et al*., 1996) and Arabidopsis (O'Kane *et al*., 1996). The increases in GSH and glutathione disulphide (GSSG) occurred during the period of induced thermoprotection, when catalase activity declined (Dat *et al*., 1998). A decreased redox state of the glutathione pool was also observed following a temperature shift of sorghum from 37 to 27°C (Badiani *et al*., 1997), growing seedlings at supraoptimal temperatures (Paolacci *et al*., 1997) and other abiotic stresses (Fadzilla *et al*., 1997;

Karpinski *et al*., 1997). Such changes in the redox state of the glutathione pool may be involved in acclimatory stress signalling (Foyer *et al*., 1997; May *et al*., 1998). High GR activity maintains the pool of glutathione in the reduced state, allowing GSH to be used by dehydroascorbate reductase (DHAR) to reduce dehydroascorbate (DHA) to the reduced form of ascorbate (AA) (Noctor *et al*., 1998). Observations suggest that SA could be involved in heat acclimation and that its action may be linked to oxidative stress. There is also evidence that SA can alter the antioxidant capacity in plants (Chen *et al*., 1997; Fodor *et al*., 1997; Rao *et al*., 1997).

The decrease in carotenoid, observed in the present study, may be attributed to the accumulation of anhydroeschsoltzxanthin, a chromoplast carotenoid in *Buxus sempervirens* leaves (Elstner *et al*., 1988). One explanation is that the extended chromophores of rhodoxanthin and anhydroeschsoltzxanthin provide better photodynamic screens for protection from the oxidative stresses generated under these conditions than do normal leaf carotenoid (Hirayama *et al*., 1994; Edge *et al*., 1997).

In conclusion, it was found, heat stress significantly increased oxidative stress damage in mungbean plant by increasing $H_2O_2$ level, lipid peroxidation and membrane leakage. In addition, we showed that protection from heat stress injury could be induced in mungbean plants by heat shock and SA treatments. This protection could be attributed to the enhancement of antioxidative enzyme activities including catalase, peroxidase, ascorbate peroxidase and superoxide dismutase. It also could be attributed to the increase in carotenoid and GSH contents.

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