INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–230/MFA/2012/14–5–705–712
http://www.fspublishers.org

Full Length Article

Possible Antioxidative Role of Endogenous Vitamins Biosynthesis in Heat Stressed Maize (*Zea mays*)

SAQIB MAHMOOD[1], ABDUL WAHID[1]†, RIZWAN RASHEED, IQBAL HUSSAIN AND SHAHZAD M.A. BASRA‡
Department of Botany, Faculty of Science and Technology, Government College University, Faisalabad-38000, Pakistan
†*Department of Botany, University of Agricultural, Faisalabad-38040, Pakistan*
‡*Department of Crop Physiology, University of Agriculture, Faisalabad-38040, Pakistan*
[1]Corresponding author's e-mail: drsaqibm@yahoo.com; drawahid2001@yahoo.com

ABSTRACT

Maize (*Zea mays* L.) shows reduced crop stand under stressful conditions; sensitivity to abiotic stresses is the main reason for this. Short term exposure to abiotic stresses may help more precisely understand the stress tolerance mechanisms. High temperature (heat stress) is more crucial amongst the abiotic factors as it aggravates the effects of other abiotic stresses. This study was conducted on six-day old plants heat-tolerant (Sultan) and heat-sensitive (S-2002) maize varieties to explore the biochemical changes taking place at three harvests at two-day intervals. The determinations were made for oxidative damages, antioxidants and reducing power assay in shoot and root of tested cultivars. Results from H_2O_2 and MDA data revealed that heat stress produce oxidative stress on both the varieties, although lowly in the tolerant one. Although heat stress altered their production in both varieties, enhanced or steady state levels of niacin (Nia), ascorbic acid and riboflavin (Rib) as well as enhanced ability of reducing powers led to a greater alleviation of oxidative damage in the tolerant variety. In conclusion, heat tolerance in maize was associated with better metabolites adjustments in the shoot than root. This is the first report on the possible contributions of Nia and Rib as antioxidants in plants subjected heat stress.

Key Words: Oxidative stress; Niacin; Riboflavin; Reducing powers; ROS scavenging

INTRODUCTION

Gradually increasing global temperature is a leading constraint responsible for altered crop performance and reduced profitable production, where it alters morphology, biochemistry and physiology of plants (Wahid *et al*., 2007). Major consequence of environmental perturbations is the overproduction of activated oxygen species (AOS) e.g., H_2O_2, OH^-, O_2^- and $O_2^{\cdot}$ etc. Since 2.7 billion years of aerobic life origin; these partially reduced forms of oxygen have been unwanted companions of molecular oxygen. They execute dual impact on life; their balanced production contributes towards the regulation of growth, development and defense, whereas their overproduction leads to lipid peroxidation (Van Breusegem *et al*., 2008), denatures proteins and increase unsaturation of fatty acids with ultimate loss of cell contents (Savchenko *et al*., 2002). Moreover, they negatively affect the activity of antioxidant enzymes (Liu & Huang, 2000), the levels of metabolites (Wahid *et al*., 2007) and self-control of cells (Taiz & Zeiger, 2010), and may shorten life cycle of stressed plants (Anderson-Teixeira *et al*., 2012). Hence, a balanced level of AOS in cells is needed to be firmly synchronized.

A range of surviving strategies has been evolved in plants to sense and combat AOS. Reducing power is a vital feature in the cell's knack to counter the drastic effects of unrestrained oxidation. A pool of reductants and antioxidants perks-up the reducing powers in the cells. Cellular redox system powers plant growth and defense in crop species (Foyer *et al*., 2009). Antioxidants assist in normalizing cellular redox homeostasis and avert oxidation of molecules such as proteins and nucleic acids. Reducing power develops in concomitance with the antioxidant property. In fact antioxidant activity and reducing power are linked as reductones to donate a hydrogen atom that culminates in the termination of free radical chain reaction (Duh *et al*., 1999). Therefore, reducing capacity of a compound may be a noteworthy gauge of its antioxidant potential (Patil *et al*., 2009).

Antioxidants mediated thermotolerance is a selection factor as well as a driving force for improved resistance and adaption to stress (Jochum *et al*., 2007). Plant scientists are working for years to emphasize plant responses to ameliorate stressful conditions. So far, there are anatomical, physiological, biochemical and molecular studies with information highlighting this aspect; nonetheless understanding of vitamins as antioxidants in plants is still scarcely understood as compared to bacteria, yeast and mammals (Smirnoff *et al*., 2004). Vitamins detoxify AOS to encounter biotic and abiotic challenges (Giovannoni, 2007).

To cite this paper: Mahmood, S., A. Wahid, R. Rasheed, I. Hussain and S.M.A. Basra, 2012. Possible antioxidative role of endogenous vitamins biosynthesis in heat stressed maize (*Zea mays*). *Int. J. Agric. Biol*., 14: 705-712

Amongst plant vitamins ascorbic acid (AsA) is the most studied with a comprehended quantification for its nutritive value. However, as cellular redox state sensor still it is not as much reviewed in plants as in animals and bacteria (Smirnoff, 2000; Netto, 2001). Under adverse ecological conditions, it mitigates the stress effects by acting as an antioxidant (Smirnoff *et al*., 2004; Iriti & Faoro, 2007; Zhang *et al*., 2007). Optimum levels of AsA attenuate its oxidative impairment (Sairam *et al*., 2000), where it reacts with ROS and stabilize them in the form of monodehydroascorbate radical, which douse free radicals by self dismutation (Halliwell & Gutteridge, 1999). Moreover, AsA promotes biosynthesis of α-tocopherol (Asada, 1994) and confers resistance in plants by conferring tolerance against stresses such as ozone and sulfur dioxide (Luwe *et al*., 1993) and heat stress (Almeselmani *et al*., 2006; Farooq *et al*., 2011).

Recent evidences suggest that vitamins other than ascorbate, also play a noteworthy role as antioxidants in plant cells (Havaux *et al*., 2009; Tunc-Ozdemir *et al*., 2009). The evidence has been obtained for vitamin B6 (Titiz, 2006; Havaux *et al*., 2009), which although provides low Trolox-equivalent antioxidant capacity (a measure of defense against free radicals) and that are effectual quenchers/scavenger of singlet oxygen but not good trappers of free radicals (Triantaphylide & Havaux, 2009). Among vitamin B compounds, riboflavin (B2; Rib) and niacin (B3; Nia) have been well reported for their crucial role in animal defensive system, where Rib is of vital importance in respiratory mechanics, promoting the release of energy from blood. Furthermore, it facilitates resistance against some skin and eye disorder (Oram, 1983). In a similar fashion, evidence shows that Nia is an antioxidant against some human diseases (Tazer, 1986). However, the role(s) of Rib and Nia in plant physiological functions are obscure yet.

Changes in ambient temperature are more important to plant growth and development than other stressors, since a rise or drop by a single degree may substantially changes the physiology of living organisms. High temperature hampers the plant growth and final yield. The effects of heat stress are more pronounced at initial growth stages. Although a C_4 plant, maize (*Zea mays* L.) shows a range of changes in growth and physiological phenomena when exposed to high temperature. This study was conducted to determine the role of endogenous vitamins biosynthesis in metabolic changes and important adjustments responsible for heat tolerance in selected maize varieties.

MATERIALS AND METHODS

General experimental details: Laboratory experiments were conducted to compare changes in oxidative damage and the possible role of vitamin (AsA, Rib & Nia) and reducing powers assay (RPA) using a heat tolerant (Sultan) and heat sensitive (S-2002) varieties of maize (*Zea mays* L.) at seedling stage in a time course manner. The varieties were selected during screening experiments (Mahmood, 2010). Seeds were surface sterilized with 0.1% $HgCl_2$ for 3 minutes and washed several times with sterilized distilled water before sowing in pots containing 2 kg of washed river sand. Six days old seedlings were divided into two sets; one set placed in an illuminated growth chamber (FLI, Eyelatron, Rikkakai, Japan) 27/22°C±2 (control) whereas second set was stressed at 42/37°C±2 (light/darkness period 14/10 h). The plants were supplemented with half strength Hoagland and Arnon (1949) nutrient solution whenever required. The experiments were laid out in a completely randomized design (CRD) with three replications and repeated to confirm the results. Seedlings were harvested at 2, 4 and 6 day after heat treatment. For biochemical determinations, the shoots were cut from its attachment with the root, while the roots were washed blotted dried for 10 seconds and then used for measurements.

Oxidative damage parameters: The H_2O_2 concentration was determined as described by Velikova *et al*. (2000). Fresh shoot and root samples (0.1 g) were homogenized in an ice bath with 1 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000×*g* for 15 min. Supernatant (0.5 mL) was added to 0.5 mL 10 m*M* potassium phosphate buffer (pH 7.0) and 1 mL 1 *M* potassium iodide, vortexed and absorbance read at 390 nm using water as blank. The H_2O_2 concentration was determined from standard curve prepared by using 35% H_2O_2.

For determination of malondialdehyde (MDA) concentration, with the method of Heath and Packer (1968), plant tissue (0.1 g) was homogenized in 1 mL of 5% TCA, centrifuged at 12,000×g for 15 min and mixed 1 mL supernatant an equal volume of thiobarbituric acid [0.5% in 20% (w/v) TCA]. The mixture was heated for 30 min at 95°C, cooled and centrifuged at 7500×g for 5 min to clarify the solution. The absorbance of the mixture was recorded at 532 nm and 600 nm, while using 5% TCA as blank. Non-specific turbidity was corrected by subtracting the absorbance at 600 nm from that taken at 532 nm. MDA contents were calculated using its absorption coefficient of 155000 nmol/mol as: MDA (nmol/mL) = $[(A_{532}\text{-}A_{600})/155000]10^6$

The relative membrane permeability (RMP) was determined in terms of ion leakage from the control and stressed seedlings following the protocol of Yang *et al*. (1996). Fresh shoots and root samples (0.5 g) were taken, put in 10 mL distilled water, vortexed for 5 sec and measured for electrical conductivity at 0 h (EC_0). The test tubes containing samples in distilled water and covered with aluminum foil were placed in refrigerator at 4°C for 24 h and measured for EC_1. Then these test tubes were autoclaved, filtered and filtrate measured for EC_2 (of dead tissues). The relative membrane permeability (RMP) was determined by applying the following formula:

$$RMP\ (\%) = [EC_1\text{-}EC_0 / (EC_2\text{-}EC_0)] \times 100$$

Determination of vitamins and reducing powers assay (RPA): For AsA determination following AOAC (1990), fresh sample (0.5 g) was extracted in 100 mL d.H_2O. A 25 mL of 20% glacial acetic acid was added to 10 mL of the sample extract and titrated against standardized 2, 6-dichloroindophenol (0.05 g/100 mL) solution. The Rib and Nia were analyzed with the method of Okwu and Josiah (2006), using fat free sample (defatted as described by AOAC, 1996). For Rib determination, 0.5 g fat free sample was extracted with 10 mL of 50% ethanol (v/v), shaken for 1 h, filtered. Extract (0.1 mL) was pipette in an Eppendorf tube followed by addition of 0.1 mL of 5% potassium permanganate solution and 0.1 mL of 30% H_2O_2, and allowed to stand in a hot water bath (at 50°C) for 30 min. Then 200 µL of 40% sodium sulfate was added up to 0.5 mL mark, vortexed and absorbance taken at 510 nm on a spectrophotometer (U-2000, Hitachi, Japan). For Nia estimation, 0.5 g defatted sample was treated with 5 mL of 1 N sulfuric acid, and shaken for 30 min, followed by addition of three drops of ammonia solution and filtered. Filtrate (1 mL) was pipetted into Eppendorf tube and 0.5 mL potassium cyanide solution followed by 0.5 mL of 0.02 N H_2SO_4 were added. Final absorbance of the reaction mixture was taken at 470 nm, and amount in the unknown samples were determined from standard curve constructed from the Rib and Nia solutions.

For RPA determination, samples were prepared following the protocol of Sofowora (1993). The samples were air-dried at room temperature and blended to a mesh size of 1 mm. Sample (5 g) was soaked in 20 mL of 98% methanol for 48 h, filtered and concentrated to dryness using rotary evaporator, removed and refrigerated for analysis. The RPA of maize roots and shoots extracts was quantified by the method of Perumal and Becker (2003) with modification. Methanolic extract was re-dissolved in 80% methanol and 1 mL of this extract was mixed with phosphate buffer (5.0 mL of 2.0 M, pH 6.6) and potassium ferricyanide (5.0 mL of 1.0%). The mixtures were incubated at 50°C for 20 min. A portion (5.0 mL) of TCA (10%) was added and the mixture was centrifuged at 3000×*g* for 10 min. The upper layer of the solution (5.0 mL) was mixed with distilled water (5.0 mL) and ferric chloride (1.0 mL of 0.1%), and absorbance of the pink color complex was measured at 700 nm. Increased absorbance of the mixture indicates increased reducing power.

Statistical analysis: The data were statistically analyzed to find the significance of variance sources using MSTAT-C computer software. The means were compared to denote differences amongst them using Duncan's new multiple range (DMR) test at $P < 0.05$.

RESULTS

Oxidative stress and RMP: There was significant ($P<0.01$) difference in the varieties, heat treatments and harvests with significant ($P<0.01$) interactions of these factors for shoot and root H_2O_2. Under control condition, there was no difference in the varieties at all harvests for H_2O_2 content of both parts (Fig. 1). However, under heat stress although shoot H_2O_2 contents increased in both the varieties, Sultan manifested a much lower H_2O_2 content than S-2002 (Fig. 1a). However, for root H_2O_2, Sultan displayed a decreased root H_2O_2 at all harvests, while S-2002 indicated a steady state level (Fig. 1b). Of the two parts, shoot indicated a much greater H_2O_2 accumulation than root.

For shoot MDA, there was significant ($P<0.01$) difference in the varieties and harvests but a non-significant one ($P>0.05$) in the heat treatments. Varieties × harvests and heat treatments × harvests interactions were significant ($P<0.01$). Under control condition, Sultan showed a steady state level of MDA in shoot, while S-2002 indicated a substantial increase at day-4 and then a small decrease at day-6. However, under heat stress although shoot MDA increased in both the varieties, Sultan manifested a much lower shoot MDA at day-2, increased at day-4 and attained a steady state level at day-6, while it increased linearly in S-2002 (Fig. 2a). For root MDA, there was significant ($P<0.01$) difference in the varieties but a non-significant ($P>0.05$) difference in the heat stress treatments and harvests, although interactions of these factors were significant ($P<0.01$). Under control condition, Sultan showed decreased root MDA at day-2 and 4 and an increased at day-6, while S-2002 showed a steady state increase in this attribute at all harvests (Fig. 2b). Of the two parts, shoot indicated a relatively higher level of MDA than root.

Shoot RMP showed significant ($P<0.01$) difference in the varieties, heat stress and harvests, while the varieties × heat treatments and heat treatments × harvests interactions were significant ($P<0.01$). Control plants of both the varieties showed similar shoot RMP at all harvests. However, under heat stress, although increased in both the varieties, Sultan showed a lower shoot RMP than S-2002 (Fig. 3a). For root RMP, there was significant ($P<0.01$) difference in the varieties, heat stress treatments and harvests, with significant ($P<0.01$) interactions of these factors. With no difference under control, Sultan showed a little decrease in root RMP at day-4 and then an increase at day-6, while S-2002 showed increased root RMP up to day-4 and then attained a steady level at day-6 under heat stress (Fig. 3b). In both parts, shoot indicated a much higher RMP than root.

Endogenous vitamins biosynthesis and RPA: For shoot AsA, data indicated significant ($P<0.01$) difference in the varieties, heat treatments and harvests with significant ($P<0.01$) interactions of all these factors, except a non-significant ($P>0.05$) varieties × harvests interaction. Under control condition, Sultan displayed a similar shoot AsA at all harvests while S-2002 indicated a consistent increase. Under heat stress, on the contrary, both the varieties indicated increased shoot AsA but Sultan indicated a substantial increase, particularly at day-6, while S-2002

Fig. 1: Time course accumulation of H_2O_2 in the shoot (a) and root (b) of differentially heat tolerant maize varieties under heat stress at three harvests (2, 4 & 6 days). The figures with alphabets on columns indicate significant (P<0.05) interaction of varieties, harvests and stress treatments

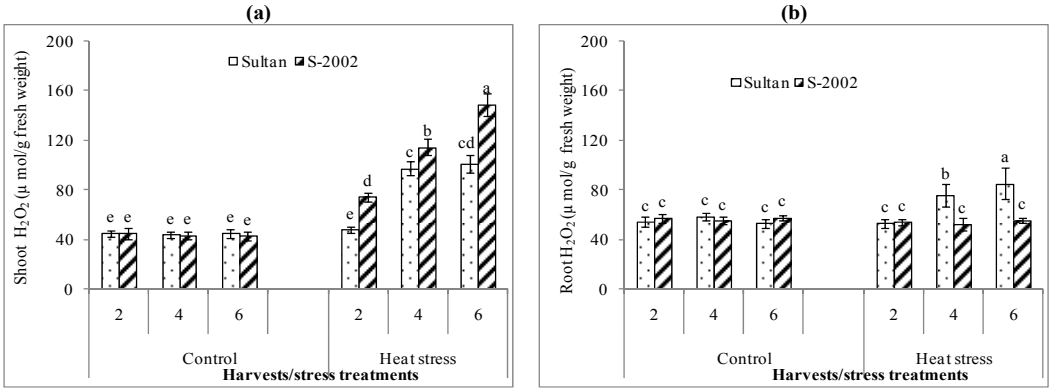


Fig. 2: Time course accumulation of malondialdehyde (MDA) in shoot (a) and root (b) of differentially heat tolerant maize varieties under heat stress at three harvests (2, 4 & 6 days). The figures with alphabets on columns indicate significant (P<0.05) interaction of varieties, harvests and stress treatments

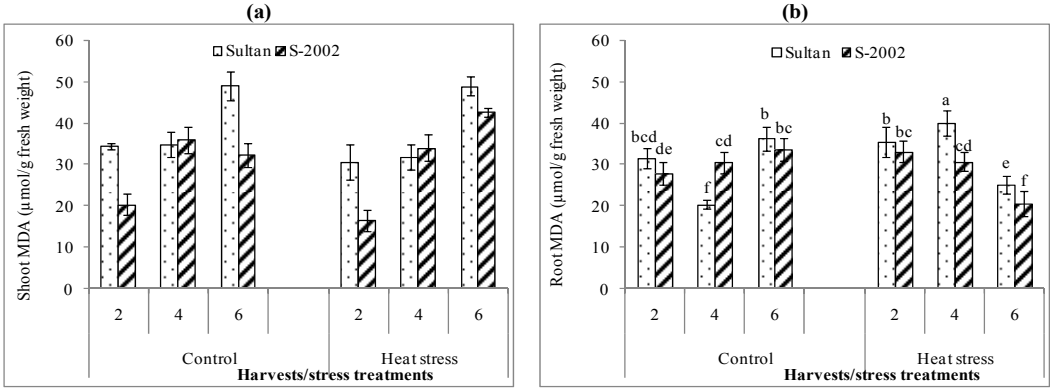
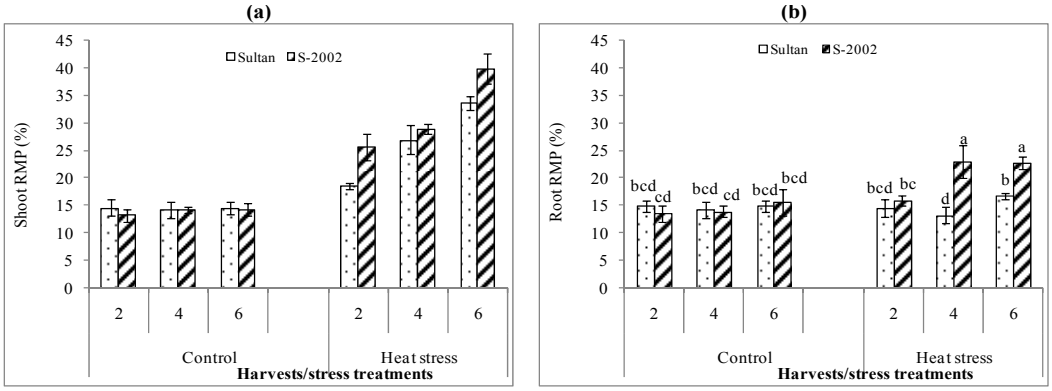


Fig. 3: Time course changes in relative membrane permeability (RMP) of shoot (a) and root (b) of differentially heat tolerant maize varieties under heat stress at three harvests (2, 4 & 6 days). The figures with alphabets on columns indicate significant (P<0.05) interaction of varieties, harvests and stress treatments



indicated a lesser increase (Fig. 4a). For root AsA, results revealed significant (P<0.01) difference in the varieties heat stress treatments and harvests were observed, while among the possible interactions, temperature × harvests and varieties × heat stress treatments × harvests interactions were significant (P<0.01). Under control condition, Sultan indicated a steady state level while S-2002 indicated increased root AsA. However, under heat stress Sultan showed a consistent increase, while S-2002 indicated increased AsA contents at day-4 but a decreased one at day-6 (Fig. 4b). Of the two parts shoot displayed relatively higher AsA level than root.

Fig. 4: Time course changes in the ascorbic acid concentration of shoot (a) and root (b) of differentially heat tolerant maize varieties under heat stress at three harvests (2, 4 & 6 days). The figures with alphabets on columns indicate significant (P<0.05) interaction of varieties, harvests and stress treatments

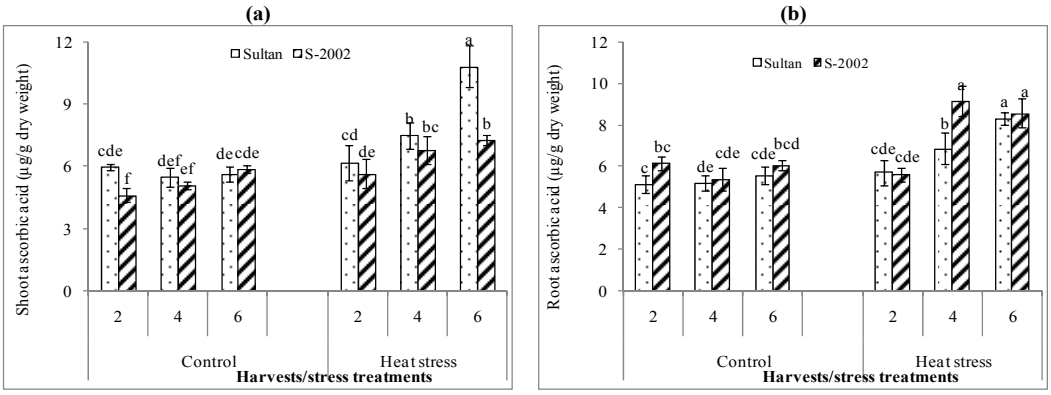
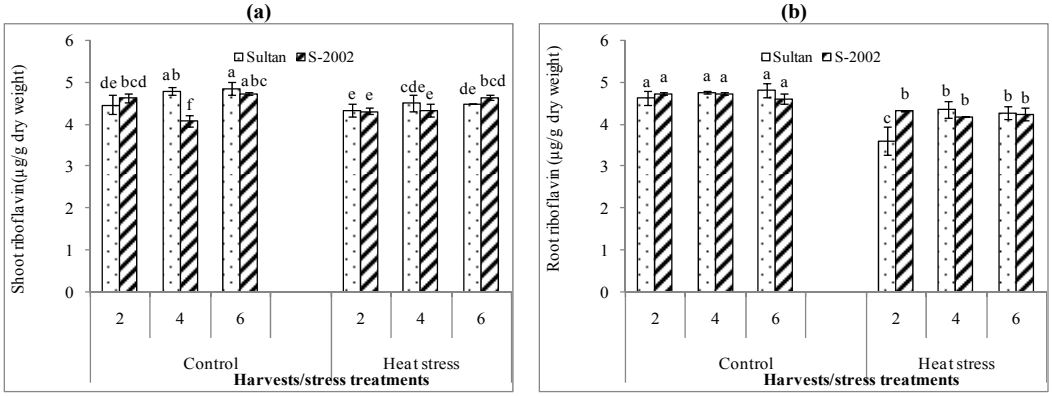


Fig. 5: Time course changes in the riboflavin concentration of shoot (a) and root (b) of differentially heat tolerant maize varieties under heat stress at three harvests (2, 4 & 6 days). The figures with alphabets on columns indicate significant (P<0.05) interaction of varieties, harvests and stress treatments



Shoot Rib indicated significant difference in the varieties (P<0.05), heat stress and harvests with significant (P<0.01) interactions of these factors except a non-significant (P>0.05) heat stress treatments × harvests interaction. Control plants of Sultan displayed a consistently increased shoot Rib, while plants of S-2002 showed a decreased at day-4 and then an increased at day-6. However, under heat stress Sultan displayed a fairly steady state level while S-2002 an increased shoot-Rib concentration (Fig. 5a). For root-Rib, there was non-significant (P>0.05) difference in the varieties but significant difference in heat stress treatments and harvests (P<0.01), while significant (P<0.01) interactions of all these factors was evident except a non-significant (P>0.05) temperature × harvests interaction. At 27°C, both the varieties indicated no difference in the root Rib, while at 42°C, Sultan showed an increased while S-2002 a steady state level of Rib at all harvests (Fig. 5b).

For shoot Nia, there was significant (P<0.01) difference in the varieties and harvests but not the heat stress treatments (P>0.05), while there was no interaction (P>0.05) of all these factors for shoot Nia. At 27°C, Nia decreased in both the varieties at day-4 and day-6, although this reduction was greater in Sultan. Under heat stress too, both the varieties displayed a reduction in shoot Nia concentration, but S-2002 exhibited a greater reduction (Fig. 6a). For root Nia, although there was non-significant (P>0.05) difference in the varieties, heat stress and harvests had significant (P<0.01) difference, with significant (P<0.01) interactions of these factors. Under control condition, Sultan indicated a steady state level while S-2002 indicated consistent decline in the root Nia. Under heat stress, Sultan showed a steady state level of root Nia while S-2002 displayed a linear decline at all harvest (Fig. 6b). Of the two parts, shoot indicated substantially higher Nia than root.

For shoot RPA there was significant (P<0.01) difference in the varieties, heat treatments and harvests with significant (P<0.01) interactions of all these factors. Under control condition, Sultan displayed a similar shoot RPA at all harvests while S-2002 indicated a similar shoot RPA at day-2 and day-4 but a decreased one on day-6. However, under heat stress both the varieties indicated increased shoot RPA but Sultan indicated a substantially higher increase than S-2002 (Fig. 7a). For root-RPA data

Fig. 6: Time course changes in the niacin concentration of shoot (a) and root (b) of differentially heat tolerant maize varieties under heat stress at three harvests (2, 4 & 6 days). The figures with alphabets on columns indicate significant (P<0.05) interaction of varieties, harvests and stress treatments

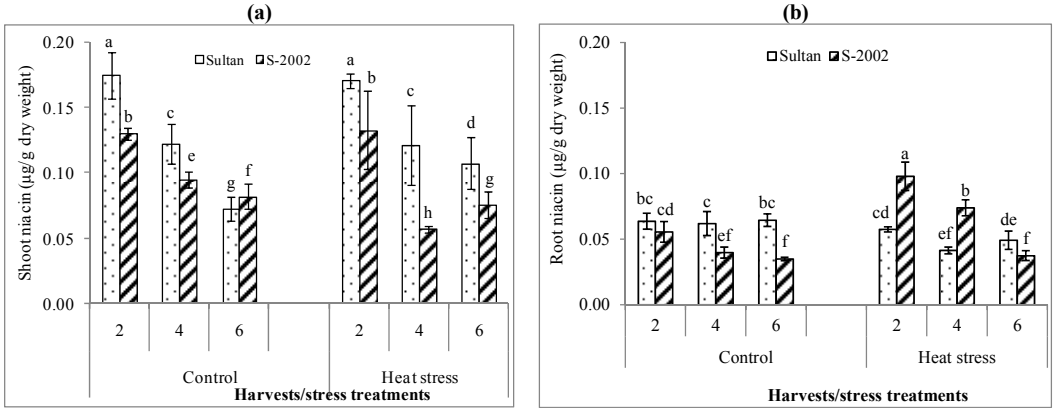
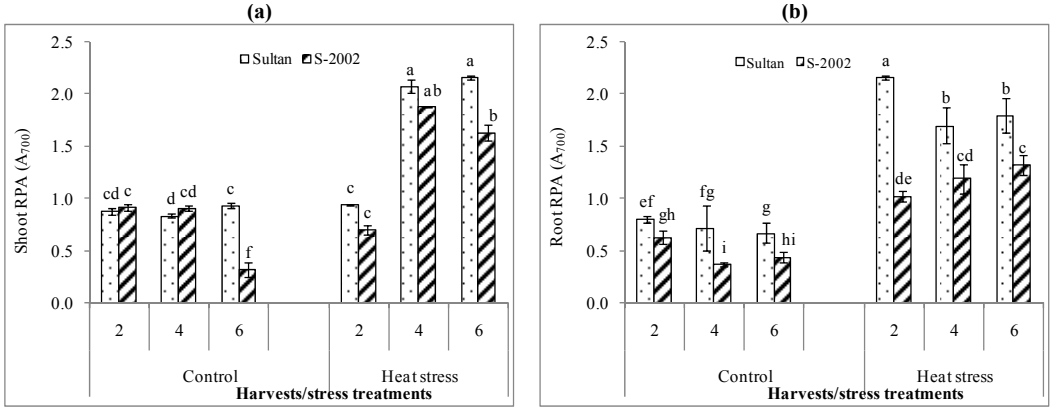


Fig. 7: Time course changes in the reducing powers ability (RPA) of shoot (a) and root (b) of differentially heat tolerant maize varieties under heat stress at three harvests (2, 4 & 6 days). The figures with alphabets on columns indicate significant (P<0.05) interaction of varieties, harvests and stress treatments



exhibited significant (P<0.01) difference in the varieties heat stress treatments and harvests, while the possible interactions of these factors were significant (P<0.01) except a non-significant temperature × harvests interaction. Under control condition, Sultan indicated a steady state level while S-2002 indicated a reduced and then an increased root RPA. However, under heat stress Sultan manifested much greater increase in root-RPA at day-2, which decreased at day-4 and then did not change at day-6. However, S-2002 displayed an increased root RPA at all harvest, but its level was much lesser than that noted in Sultan (Fig. 7b).

DISCUSSION

Heat stress leads to the generation of AOS such as H_2O_2, singlet oxygen, hydroxyl ions etc. (Skopelitisa *et al*., 2006; Foyer *et al*., 2009). H_2O_2, being longer lived amongst the ROS, is more damaging to the membrane of organelles where they are generated (Jones, 2000; Adachi *et al*., 2009). The effects are evident on the production of MDA after the peroxidation of lipid bilayer and enhanced ion-leakage under heat stress (Liu & Huang, 2000; Wahid *et al*., 2007). In the present case the determinations made for the H_2O_2 accumulation in the shoot and root of differentially heat tolerant maize varieties indicated no remarkable differences under normal temperature but a substantial increase under heat stress in the shoot but not in the root, and the effects were time and varieties dependent, tolerant variety being less affected (Fig. 1). Likewise, heat stressed plants showed a greater MDA accumulation in the shoot while roots indicated the MDA almost similar to the control plants (Fig. 2). An increased production of both H_2O_2 and MDA leads to enhanced ion-leakage from the leaf surface (Wahid & Shabbir, 2005; Wahid *et al*., 2008). The relative membrane permeability measured from the leaves indicated no significant changes in the control samples but greatly increased in the heat stressed shoot but lowly in the roots

(Fig. 3). A parallel set of changes in the H_2O_2, MDA and RMP specifically in the shoot suggested that shoot being directly exposed to the hot environment is highly impinged upon by the heat stress.

When exposed to hot environments, the tolerant plants try to evade or cope with the oxidative damage with the measurable metabolic adjustments, which include both enzymatic and non-enzymatic ROS scavengers (Liu & Huang, 2000; Zobayed *et al*., 2005; Skopelitisa *et al*., 2006; Wang *et al*., 2012). Among the non-enzymatic antioxidants attention has been mainly given to glutathione (Mittler, 2002), phenolics including salicylates (Llusia *et al*., 2005; Wahid *et al.*, 2007), soluble phenolics and anthocyanins (Wahid, 2007), while roles of vitamins including Nia, AsA and Rib are not explored in plant systems. This study showed that Nia and AsA concentration in the shoot of tolerant variety (Sultan) were higher than S-2002 while Rib contents were steady (Fig. 4-5). These metabolites appeared to help maize to withstand high temperature environment. Changes in the root, however, were not consistence with the heat tolerance in both the varieties.

Reducing powers ability has been regarded as measure of the scavenging of AOS (Bukhari *et al*., 2008). Heat tolerant variety indicated an increased RPA than the sensitive variety both in the shoot and root (Fig. 4). It is plausible that the increased RPA in the tolerant variety is due to increased concentrations of these vitamins, which scavenged the ROS and enabled the tolerant maize to withstand high temperature environment.

In nutshell, being directly exposed, shoot compared to root was more prone to the oxidative damage caused by heat stress. Data suggest that tolerant maize coped with the oxidative damage by reduced production of H_2O_2 and MDA and reduced RMP while greater concentrations of Nia, AsA and Rib, and higher RPA. Thus the endogenous synthesis of vitamins as metabolites is an adaptive mechanism in maize for heat tolerance.

Acknowledgement: Data reported here are part of Ph.D. dissertation of first author. Critical reading of the paper by Dr. M. Farooq is gratefully acknowledged.

REFERENCES

Adachi, M., Y. Liu, K. Fujii, S.K. Calderwood, A. Nakai, I. Kohzoh and S. Yasuhisa, 2009. Oxidative stress impairs the heat stress response and delays unfolded protein recovery. *PLoS ONE*, 4: e7719

Almeselmani, M., P.S. Deshmukh, R.K. Sairam, S.R. Kushwaha and T.P. Singh, 2006. Protective role of antioxidant enzymes under high temperature stress. *Plant Sci.*, 71: 382–388

Anderson-Teixeira, K.J., P.K. Snyder, T.E. Twine, S.V. Cuadra, M.H. Costa and E.H. DeLucia, 2012. Climate-regulation services of natural and agricultural ecoregions of the Americas. *Nat. Climate Change*, published online

AOAC, 1996. *Official Methods of Analysis of AOAC International*. 16th edition, Vol. II. AOAC International, Gaithersburg, Maryland, USA

AOAC, 1990. Official method of analysis. *In:* Adrich, R.C. (ed.), *Association of Official Analytical Chemists: Food Composition, Additives Natural Contaminant*. Association of Official Analytical Chemist Inc., USA

Asada, K., 1994. Mechanisms for scavenging reactive molecules generated in chloroplasts under light stress. *In*: Baker, N.R., J.R. Bowyer (eds.), *Photoinhibition of Photosynthesis*, pp: 129−142. From Molecular Mechanisms to the Field. Bios Scientific Publishers, Oxford, UK

Bukhari, S.B., M.I. Bhanger and S. Memon, 2008. Antioxidative activity of extracts from Fenugreek seeds (*Trigonella foenum-graecum*). *Pakistan J. Anal. Environ. Chem*., 9: 78−83

Duh, P.D., Y.Y. Tu and G.C. Yen, 1999. Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat). *LWT-Food Sci. Technol*., 32: 269–277

Farooq, M., H. Bramley, J.A. Palta and K.H.M. Siddique, 2011. Heat stress in wheat during reproductive and grain-filling phases. *Crit. Rev. Plant Sci.*, 30: 491−507

Foyer, C.H., A. Bloom, G. Queval and G. Noctor, 2009. Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. *Annu. Rev. Plant Biol.*, 60: 455−484

Giovannoni, J.J., 2007. Completing a pathway to plant vitamin C synthesis. *Proc. Natl. Acad. Sci. USA*, 104: 9109–9110

Halliwell, B. and J.M.C. Gutteridge, 1999 *Free Radicals in Biology and Medicine,* 3rd edition. Clarendon Press, Oxford, UK

Havaux, M., B. Ksas, A. Szewczyk, D. Rumeau, F. Franck, S. Caffarri and C. Triantaphylidès, 2009. Vitamin B6 deficient plants display increased sensitivity to high light and photo-oxidative stress. *BMC Plant Biol.*, 9: 130

Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts. *Arch. Biochem. Biophys.*, 125: 189–198

Hoagland, D.R. and D.I. Arnon, 1950. *The Water-culture Method for Growing Plants Without Soil*. California Agricultural Experiment Station Circular 347

Iriti, M. and F. Faoro, 2007. Review of innate and specific immunity in plants and animals. *Mycopathologia*, 164: 57–64

Jochum, G.M., K.W. Mudge and R.B. Thomas, 2007. Elevated temperatures increase leaf senescence and root secondary metabolite concentrations in the understory herb *Panax quinquefolius* (Araliaceae). *American J. Bot.*, 94: 819–826

Jones, A., 2000. Does the plant mitochondrion integrate cellular stress and regulate programmed cell death? *Trends Plant Sci.*, 5: 225–230

Liu, X. and B. Huang, 2000. Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. *Crop Sci.*, 40: 503–510

Llusia, J., J. Peñuelas and S. Munné-Bosch, 2005. Sustained accumulation of methyl salicylate alters antioxidant protection and reduces tolerance of holm oak to heat stress. *Physiol. Plant.*, 124: 353–361

Luwe, M.W.F., U. Takahama and U. Heber, 1993. Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia oleracea*) leaves. *Plant Physiol.*, 101: 969–976

Mahmood, S., 2010. Time course changes in metabolite accumulation and their implications for heat stress tolerance in maize (*Zea mays* L.) seedlings. *Ph.D. thesis*. Department of Botany, University of Agriculture, Faisalabad, Pakistan

Mittler, R., 2002. Oxidative stress, antioxidant and stress tolerance. *Trends Plant Sci.*, 7: 405–410

Netto, L.E.S., 2001. Oxidative stress response in sugarcane. *Genet. Mol. Biol.*, 24: 1–4

Okwu, D.E. and C. Josiah, 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. *African J. Biotechnol.*, 5: 357–361

Oram, R.F., 1983. *Biology: Living Systems.* A. Bell and Howell Company, Columbus, Ohio, USA

Patil, A.P., V.V. Patil and V.R. Patil, 2009. *In-vitro* free radicals scavenging activity of *Madhuca indica* Gmel. *Pharmacologyonline,* 2: 1344–1352

Perumal, S. and K. Becker, 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J. Agric. Food Chem.*, 51: 2144–2155

Sairam, R.K., G.C. Srivastava and D.C. Saxena, 2000. Increased antioxidant activity under elevated temperature: a mechanism of heat tolerance in wheat genotypes. *Biol. Plant.*, 43: 245–251

Savchenko, G.E., E.A. Klyuchareva, L.M. Abrabchik and E.V. Serdyuchenko, 2002. Effect of periodic heat shock on the membrane system of etioplasts. *Russian J. Plant Physiol.*, 49: 349–359

Skopelitisa, D.S., N.V. Paranychianakisa, K.A. Paschalidisa, E.D. Pliakonisa, I.D. Delisa, D.I. Yakoumakisa, A. Kouvarakisb, A.K. Papadakisa, E.G. Stephanoub and K.A. Roubelakis-Angelakisa, 2006. Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. *Plant Cell*, 18: 2767–2781

Smirnoff, N., 2000. Ascorbic acid: metabolism and functions of a multifaceted molecule. *Curr. Opin. Plant Biol.*, 3: 229–235

Smirnoff, N., J.A. Running and S. Gaztek, 2004. Ascorbate biosynthesis: a diversity of pathways. *In*: Asard, H., J.M. May and N. Smirnoff (eds.), *Vitamin C: Functions and Biochemistry in Animals and Plants*, pp: 7–29. BIOS Scientific Publishers, London

Sofowora, A., 1993. *Phytochemical Screening of Medicinal plants and traditional medicine in Africa*, 2nd edition, pp: 150–156. Spectrum Books Limited, Nigeria

Taiz, L. and E. Zeiger, 2010. *Plant Physiology*, 5th edition. Sinauer Associates Inc. Publishers, Massachusetts, USA

Tazer, C., 1986. *Biology and Human Progress,* 7th edition, p: 544. Prentice Hall Inc. Englewood Cliffs New Jersey, USA

Titiz, O., 2006. PDX1 is essential for vitamin B6 biosynthesis, development and stress tolerance in Arabidopsis. *Plant J.*, 48: 933–946

Triantaphylide, C. and M. Havaux, 2009. Singlet oxygen in plants: production, detoxification and signaling. *Trends Plant Sci.*, 14: 219–228

Tunc-Ozdemir, M., G. Miller, L. Song, J. Kim, A. Sodek, S. Koussevitzky, A.N. Misra, R. Mittler and D. Shintani, 2009. Thiamin confers enhanced tolerance to oxidative stress in *Arabidopsis*. *Plant Physiol.*, 151: 421–432

Van Breusegem, F., J. Bailey-Serres and R. Mittler, 2008. Unraveling the tapestry of networks involving reactive oxygen species in plants. *Plant Physiol.*, 147: 978–988

Velikova, V., I. Yordanov and A. Edreva, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Sci.*, 151: 59–66

Wahid, A., 2007. Physiological implications of metabolites biosynthesis in net assimilation and heat stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *J. Plant Res.*, 120: 219–228

Wahid, A. and A. Shabbir, 2005. Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with glycinebetaine. *Plant Growth Regul.*, 46: 133–141

Wahid, A, S. Gelani, M. Ashraf and M.R. Foolad, 2007. Heat tolerance in plants: an overview. *Environ. Exp. Bot.*, 61: 199–223

Wahid, A., S. Sehar, M. Perveen, S. Gelani, S.M.A. Basra and M. Farooq, 2008. Seed pretreatment with hydrogen peroxide improves heat tolerance in maize at germination and seedling growth stages. *Seed Sci. Technol.*, 36: 633–645

Wang, W.Y., H.X. Yin, J. Xu, X.J. Liu, Q. Mi, J.H. Du, L.L. Ma and H.K. Zhou, 2012. Effects of glycine pretreatment on the growth and oxidative damage in heat-stressed *Festuca sinensis* Keng seedlings. *J. Lanzhou. Univ. (Nat. Sci.)*, 48: 75–78

Yang, G., D. Rhodes and R.J. Joly, 1996. Effect of high temperature on membrane stability and chlorophyll fluorescence in glycinebetaine-deficient and glycinebetaine containing maize lines. *Australian J. Plant Physiol.*, 23: 437–443

Zhang, L., Z. Wang, Y. Xia, G. Kai, W. Chen and K. Tang, 2007. Metabolic engineering of plant L-ascorbic acid biosynthesis: recent trends and applications. *Crit. Rev. Biotechnol.*, 27: 173–182

Zobayed, S.M.A., F. Afreen and T. Kozai, 2005. Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. *Plant Physiol. Biochem.*, 43: 977–984

(Received 15 May 2012; Accepted 17 July 2012)