Journal of Environmental Biology **July 2009,** 30(4) 557-562 (2009)

Involvement of calcium and calmodulin in oxidative and temperature stress of *Amaranthus lividus* L. during early germination

Soumen Bhattacharjee

Post Graduate Department of Botany, Hooghly Mohsin College, Chinsurah - 712 101, India

(Received: August 24, 2007; Revised received: April 24, 2008; Accepted: July 28, 2008)

Abstract: *Both heat and chilling caused reduction in membrane protein thiol level and increased accumulation of thiobarbituric acid reactive substances in 72 hr old germinating tissues (indicators of oxidative stress) and reduced germination and early growth performances. Calcium chelator EGTA [Ethylene glycol-bis(2-aminoethylether)-N,N,N′·N, tetra acetic acid] calcium channel blocker $LaCl_3$ (Lanthanum chloride) and calmodulin inhibitor TFP (trifluroperazine) aggravated these effects of heat and chilling and added calcium reversed them. Imposition of heat and chilling stress during early germination also causes accumulation of reactive oxygen species (ROS) like O_2^- and H_2O_2. Calcium treatment significantly reduced the accumulation of both the toxic ROS, while EGTA, $LaCl_3$ and TFP treatment enhanced the accumulation. Activities of antioxidative enzymes catalase (CAT), ascorbate peroxidase (APOX) and glutathione reductase (GR) and total thiol content decreased significantly under both heat and chilling stress in germinating Amaranthus seedlings. Seedlings raised with Ca^{2+} treatment under heat and chilling stress exhibit higher activities of CAT, GR and APOX and total thiol level than the untreated plants. EGTA, $LaCl_3$ and TFP treatment, on the other hand significantly reduce the activities of all anti-oxidative enzymes and total thiol level. The work clearly supports the view that Ca^{2+}-signalling pathway plays significant role in limiting heat and chilling induced oxidative stress by up-regulating antioxidative defense during recovery phase of post- germination event in Amaranthus lividus.*

Key words: *Heat, Chilling, Germination, Calcium, Calmodulin, Oxidative stress*
PDF of full length paper is available online

Introduction

In nature, plants are subjected to changes in temperature both during seasonal and over the course of individual days. Temperature stress (both heat and chilling) is a major limiting factor limiting growth and development involving many physiological and biochemical changes including oxidative stress (Bewley and Black, 1982; Jiang and Huang, 2001; Bhattacharjee and Mukherjee, 1997, 2006). Both high and low temperature influences germination and subsequent seedlings growth (Bewley and Black,1982; Alka and Khanna-Chopra,1995; Bhattacharjee and Mukherjee,1997, 2003/ 2004). In fact, imbibitions and early germination event continue even at sub and supra-optimum temperature but embryo growth in most of the cases inhibited. Such damage can be ascribed to metabolic dysfunction pertaining to loss of membrane integrity in juvenile tissues, which are largely being instigated by secondary oxidative stress (Alka and Khanna-Chopra, 1995; Bhattacharjee and Mukherjee, 2003/2004).

Several studies showed that Ca^{2+} is involved in regulation of plant responses to various environmental stresses including heat and chilling (Bhattacharjee, 2008; Knight and Knight, 1993; Gong *et al*., 1998; Jiang and Huang, 2001; Nayyar, 2003). Increased cytosolic Ca^{2+} content under heat stress may alleviate heat injury and enable plant cells to better survive (Knight and Knight, 1993; Wang and Li, 1999).The link between cold treatment and calcium influx has been observed for some time. Increased influx of radiolabelled calcium ($^{45}Ca^{2+}$) into root was observed in winter wheat treated at 2°C at high calcium concentration (Eralandson and Jensen,1989) and similar observation has been noticed in maize roots (Rincon and Hanson, 1986).Cold induced cytosolic calcium elevation have been measured in tobacco (Knight *et al.,*1993) and *Arabidopsis* (Knight *et al*.,1996).However, excessive Ca^{2+} released into cytosol and maintaining high Ca^{2+} conc. might be cytotoxic (Wang and Li, 1999; Jiang and Huang, 2001).

Limited contradictory results are available concerning the effects of exogenous Ca^{2+} on heat and chilling tolerance. It is found that treatment of *Zea mays* and *Amaranthus* seeds with 15 and 20 mM Ca^{2+} solution enhanced intrinsic heat and chilling tolerance of seedlings (Gong *et al*., 1997; Bhattacharjee, 2008). In contrast, it has been reported that heat induced growth retardation could not be alleviated by external Ca^{2+} treatment in excised coleoptiles of wheat (Onwueme and Laude, 1972).

Therefore, the role of Ca^{2+} in regulation of tolerance to high and low temperature is still unclear. Some suggests that it may be involved in signal transdaction involving new gene expression (Trofimova *et al*., 1999) under oxidative and temperature stress. Others reported that Ca^{2+} control guard cell turgur and stomatal aperture (Mansfield *et al*., 1990; Webb *et al*., 1996) and helps in turgur maintenance (Hare *et al*., 1998).

The objective of the present study is to investigate the involvement of Ca^{2+} and calmodulin in the protection of membrane and survival during early germination in a tropical leaf crop, *Amaranthus lividus* L. and also to examine their effects on reactive oxygen species metabolism and subsequent oxidative injury of the juvenile germinating tissues.

Materials and Methods

Seeds of the tropical leaf crop *Amaranthus lividus* L., selected as experimental materials were supplied by local harvest. The surface sterilized seeds were pretreated separately for 20 hr in darkness with the following solutions -

i) Glass distilled water ii) 20 mM $CaCl_2$
iii) 1 mM $LaCl_3$ iv) 2 mM EGTA
v) 200 mM trifluroperazine

Seeds were then air dried in room temperature and finally sown in petri plates on moist filter paper (30 seeds per plate) and subsequently kept separately at 40 ± 2°C , 8 ± 2°C and at room temperature (25 ± 2°C) for 24 hr. Finally they were allowed to grow at 25 ± 2°C with 12 hr photoperiod (270 E m^{-2} s^{-1}) and 78 ± 2% RH.

Survival assay, growth performance, extent of oxidative membrane damage (in terms of thiobarbituric acid reactive substances and membrane protein thiol level), efficiency of antioxidative defense systems (in terms of the activities of ascorbate peroxidase, catalase, glutathione reductase, superoxide dismutase and total thiol content) and accumulation of reactive oxygen species (O_2^- and H_2O_2) were also performed after 72 hr of treatment.

For studying survival assay and growth performance, relative germination performance (RGP) and relative growth index (RGI) were calculated (Bhattacharjee and Mukherjee, 2003,2004). Relative germination performance (RGP) was calculated as

To estimate membrane lipid peroxidation test for thiobarbituric acid reactive substances (TBARS) was performed using the procedure of Heath and Packer (1968).

For the determination of membrane protein thiol content, the membrane was prepared according to Singh (1997) with some necessary modifications. 1 gram of plant tissue was homogenized in 10 cm^3 ice cold buffer (0.05 M Tris-HCl, pH 7.0). The homogenate was centrifuged at 10,000 g at 4°C for 30 minutes and the pellet was discarded. The membranes were then sedimented at 1,00,000 g at 4°C for 3 hr and the pellet containing the membrane fractions was suspended in ice cold buffer (0.05 M Tris-HCl, pH 7.0). The membrane fractions were stored under ice. The membrane associated protein bound thiol group were assayed after protein precipitation with TCA (10% mass/volume) and quantified with DTNB following the procedures of Ellman (1959) and Dekok and Kuiper (1986).

H_2O_2 was extracted and estimated following the procedure of MacNevin and Uron (1953) using titanic sulfate. For the determination of superoxide, the method of Chaitanya and Naithani (1994) was followed with some necessary modifications. 500 mg of tissues was homogenized in cold with 5 cm^3 of 0.2 M sodium phosphate buffer, pH 7.2, with addition of diethyldithiolcarbomate (10^{-3} M) to inhibit SOD activity. The homogenates was immediately centrifuged at 2000 g at 4°C for 1 min. In the supernatant, superoxide anion was measured by its capacity to reduce nitrobluetetrazolium (2.5 X 10^{-4} M). The absorbance of the end product was measured at 540 wave length. Formation of superoxide was expressed as ΔA_{540} g^{-1} (dry matter) min^{-1}.

For the extraction and estimation of catalase and superoxide dismutase the methods of Snell and Snell (1971) and Gianopolitis and Ries (1977) were followed.

Ascorbate peroxidase (APOX) activity was determined according to Nakano and Asada (1981) using homogenates previously supplemented with 0.5 mM ascorbic acid and 0.1 mM EDTA. Parallel experiments in presence of p-chloromercuribenzoate (50mM) were performed to rule out any interference from guaiacol peroxidases. Glutathione reductase (GR) activity was measured according to Schaedle and Bassham (1977). The reaction mixture contained 50 mM Tris-HCl (pH 7.6), 0.15 mM NADPH, 0.5 mM oxidized glutathione (GSSG), 3 mM $MgCl_2$ and 100 ml homogenate (7 mg protein ml^{-1}). NADPH oxidation was followed at 340 nm. In all cases enzyme activity was expressed according to Fick and Qualset (1975) as enzyme unit g^{-1} (dry matter) min^{-1}.

For the estimation of total thiol content the process of Tietze (1969) was followed. Total –SH content was assayed in acid soluble extract (in 3% w/v TCA solution) followed by a brief centrifugation. The supernatant was then diluted 10-fold in 100 mM phosphate buffer (pH 7.5). Thiol contents was determined measuring absorbance at 412 nm in presence of 0.5 mM 5, 5′-dithiobis 2-nitrobenzoic acid (DTNB), 0.5 U ml^{-1} glutathione reductase and 0.2 mM NADPH. For statistical analysis standerd error (SE) of three independent replicates for each parameters were estimated.

Results and Discussion

When germinating *Amaranthus lividus* seeds were treated separately at 40°C and 8°C for 24 hr and then referred to normal growth condition, they exhibit less survival (measured in terms of germination performance after 72 hr of treatment) as well as reduced early growth performances (measured in terms of relative growth index). The relative germination performance and relative growth index for heat stress raised *Amaranthus* have been reduced to 54.7% and 56.1% respectively as compared to the cent percent values of untreated control (Table 1).Similarly the relative germination performance and relative growth index for chilling stressed *Amaranthus* have been reduced to 50.3% and 51.1% respectively(Table 1). In order to ascertain whether the damage caused by adverse environmental temperature was due to the oxidative stress occurring during the recovery phase in germinating seedlings, the extent of oxidative damage suffered by the heat and

Table - 1: Effect of $CaCl_2$, $LaCl_3$, EGTA and Trifluroperazine on thiobarbituric acid reactive substances (μmole g^{-1}dry matter) and membrane protein thiol level (nmole g^{-1}dry meter)], survival (relative germination performances) and early growth performances (relative growth index) in response to heat (40°C for 24 hr) and chilling stress (8°C for 24 hr) in *Amaranthus lividus* L. values are mean of three replicates (± SE)

Treatments	Thiobarbituric acid reactive substances		Membrane protein thiol level		Survival RGP (%)	Growth performancs RGI (%)
	Root	Shoot	Root	Shoot		
Untreated	68.0(.11)	44.6(.09)	114.0(.28)	158.0(.30)	100.0	100.0
Untreated and heat stress	88.1(.14)	71.6(.12)	58.5(.11)	85.8(.21)	54.7(.11)	56.1(.19)
Ca^{2+} pretreated and heat stress	72.6(.12)	53.5(.10)	83.1(.20)	110.5(.26)	71.5(.17)	72.0(.10)
$LaCl_3$ pretreated and heat stress	136.5(.11)	122.5(.13)	48.8(.09)	72.6(.17)	44.4(.08)	44.0(.05)
EGTA pretreated and heat stress	101.7(.19)	92.1(.21)	52.7(.12)	83.2(.17)	46.4(.04)	48.2(.04)
TFP pretreated and heat stress	98.4(.08)	79.9(.10)	48.6(.08)	78.3(.11)	48.0(.03)	46.2(.04)
Untreated and chilling stress	93.4(.14)	85.2(.12)	60.4(.09)	100.4(.22)	50.3(.08)	51.1(.06)
Ca^{2+} pretreated and chilling stress	85.3(.11)	81.7(.21)	70.8(.15)	109.5(.23)	59.7(.11)	57.7(.12)
$LaCl_3$ pretreated and chilling stress	98.9(.17)	92.7(.22)	55.8(.16)	97.8(.22)	44.4(.05)	46.2(.05)
EGTA pretreated and chilling stress	98.2(.14)	92.1(.23)	59.8(.11)	97.2(.29)	44.9(.04)	46.1(.11)
TFP pretreated and chilling stress	99.1(.21)	92.3(.21)	58.2(.11)	96.1(.21)	45.1(.08)	47.1(.08)

RGI :Relative growth index, RGP : Relative germination performance

Table - 2: Effect of calcium $CaCl_2$, $LaCl_3$, EGTA and Trifluroperazine pretreatment on heat (40°C for 24 hr) and Chilling(8°C for 24 hr) induced changes in activities of ascorbate peroxidase, catalase, superoxide dismutase and glutathione reductase (Ug^{-1}dry matter min^{-1}) and total thiol content (μ mol g^{-1} dry matter) in *Amaranthus lividus* L. seedlings. Values are mean of three replicates (± SE)

Treatment	APOX		CAT		SOD		GR		Total thiols	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Untreated	9.80 (.04)	8.30 (.03)	4.14 (.01)	3.28 (.01)	4.80 (.02)	4.0 (.02)	0.17 (.001)	0.11 (.007)	1.07 (.007)	0.85 (.002)
Untreated and heat stress	8.06 (.30)	7.00 (.01)	3.30 (.02)	2.60 (.05)	3.70 (.02)	3.64 (.02)	0.12 (.001)	0.07 (.002)	0.75 (.002)	0.48 (.001)
Ca^{2+} pretreated and heat stress	8.5 (.07)	7.90 (.04)	3.79 (.01)	2.98 (.02)	3.71 (.01)	3.60 (.03)	0.16 (.002)	0.09 (.001)	0.91 (.003)	0.60 (.08)
$LaCl_3$ pretreated and heat stress	8.0 (.03)	6.75 (.02)	3.20 (.01)	2.41 (.01)	3.50 (.01)	3.19 (.02)	0.10 (.002)	0.05 (.001)	0.63 (.001)	0.35 (.004)
EGTA pretreated and heat stress	8.0 (.02)	6.80 (.01)	3.10 (.01)	2.50 (.02)	3.40 (.03)	3.10 (.02)	0.10 (.002)	0.06 (.002)	0.70 (.001)	0.40 (.003)
TFP pretreated and heat stress	7.90 (.02)	6.77 (.01)	3.15 (.02)	2.54 (.01)	3.50 (.03)	3.20 (.03)	0.09 (.002)	0.06 (.002)	0.72 (.004)	0.38 (.001)
Untreated and chilling stress	8.5 (.02)	8 (.02)	3.05 (01)	2.41 (.01)	3.91 (.01)	3.8 (.007)	0.14 (.003)	0.09 (.001)	0.80 (.003)	0.47 (.001)
Ca^{2+}pretreated and chilling stress	9.9 (.04)	8.18 (.01)	3.28 (.03)	2.64 (.01)	3.71 (.02)	3.4 (.008)	0.16 (.002)	0.11 (.003)	0.98 (.004)	0.67 (008)
$LaCl_3$ pretreated and chilling stress	8.1 (.03)	7.6 (.01)	3.01 (.01)	2.20 (.01)	2.81 (.01)	2.80 (.01)	0.13 (.001)	0.07 (.003)	0.72 (.002)	0.40 (.004)
EGTA pretreated and chilling stress	8 (.01)	7.5 (.01)	2.91 (.01)	2.22 (.01)	2.98 (.01)	2.98 (.02)	0.12 (.002)	0.08 (.002)	0.77 (.005)	0.46 (.006)
TFP pretreated and chilling stress	7.7 (.03)	7.3 (.02)	2.71 (.01)	2.12 (.01)	2.95 (.03)	2.95 (.01)	0.10 (.001)	0.06 (.003)	0.72 (.009)	0.40 (.004)

APOX: Ascorbate peroxidase,CAT: Catalase, GR: Glutathione Reductase, SOD: Superoxide dismutase

chilling raised seedlings were assessed. It was tested by measuring the accumulation of TBARS (which reflect lipid peroxidation measured in terms of accumulation of malondialdehyde). As shown in Table 1, both 40 and 8°C treatment caused significant increase in Thiobarbituric acid reactive substance (TBARS) in germinating *Amaranthus* seedlings. As TBARS assay measures oxidative damage to membrane, so to corroborate it further membrane protein thiol level (MPTL) was estimated for heat and chilling stress raised *Amaranthus* seedlings and compared with untreated control. It clearly exhibit significant decline in MPTL in both the heat and chilling

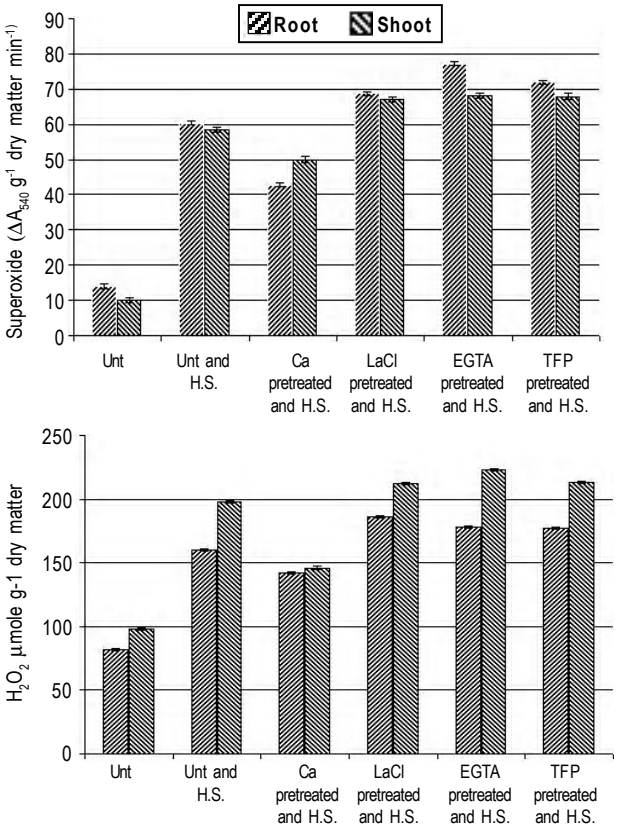

Fig. 1: Effect of $CaCl_2$, $LaCl_2$, EGTA and Trifluroperazine (TFP) on the accumulation Hydrogen peroxide and superoxide in response to heat stress (40ºC for 24 hr) in *Amaranthus lividus* L. Values are mean of three replicates (± SE), Unt = Untreated, HS = Heat stress

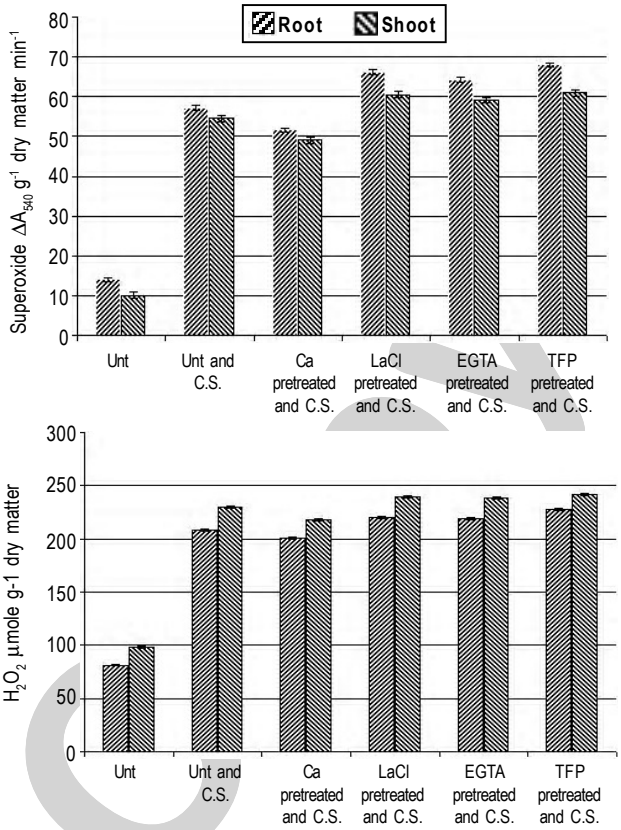

Fig. 2: Effect of $CaCl_2$, $LaCl_2$, EGTA and Trifluroperazine (TFP) on the accumulation Hydrogen peroxide and superoxide in response to chilling stress (8ºC for 24 hr) in *Amaranthus lividus* L. Values are mean of three replicates (± SE), Unt = Untreated, CS = Chilling stress

raised *Amaranthus* seedlings as compared to untreated control (Table 1).

To test the hypothesis whether Ca^{2+} play any role in mitigating oxidative stress and improving survival and growth performances Ca^{2+} (20 mM $CaCl_2$) pretreatment has been done during early imbibitional phase of germination and subsequently exposed to elevated and low temperature (40 and 8ºC for 24 hr). Ca^{2+} pretreated *Amaranthus* clearly shows reversal in oxidative damage as well as improve survival and early growth performances under adverse environmental temperature. Table 1 shows that there is significant reduction in the accumulation of TBARS and restoration of MPTL in calcium treated heat and chilling stressed *Amaranthus* seedlings, implying the involvement of Ca^{2+} in mitigating oxidative stress.

Effect of calcium channel blocker ($LaCl_3$), calcium chelator (EGTA) and inhibitors of calmodulin (trifluroperazine) pretreatment on subsequently heat and chilling stressed *Amaranthus* was also assessed in terms of germination and growth performances as well as oxidative membrane injury. All the inhibitors tested, increased heat and chilling induced accumulation of TBARS, with concomitant reduction in MPTL (Table 1). Their effects on survival and early growth performances were also negative (Table 1). La^{3+} had the greatest effect on increasing the oxidative membrane damage with a corresponding reduction in the survival level and early growth performances (measures in terms of relative germination performance and relative growth index).

Figure 1, 2 shows the effect of Ca^{2+}, EGTA, La^{3+} and TFP pretreatment on subsequently heat and chilling stress induced accumulation of two important ROS, superoxide and hydrogen peroxide. Imbibitional heat and chilling stress causes significant increase in the level of O_2^- and H_2O_2 as compared to untreated control. Ca^{2+} pretreatment prior to heat and chilling reduced the accumulation of both the ROS (O_2^- and H_2O_2), whereas all the inhibitor treatment enhances the accumulation of ROS.

Both heat and chilling stress during early phase of germination induces oxidative injury and significantly reduces the activities of antioxidative enzymes (during early phase of recovery) including CAT, APOX, SOD, GR as well as total thiol level (Table-2). External Ca^{2+} treatment helped to maintain higher APOX, CAT and GR activities and total thiol level. The external Ca^{2+} treatment, however, did not affect SOD activity under heat stress. Activities of

all these antioxidant enzymes and total thiol level decreased significantly when treated with Ca^{2+} channel blocker, chelator or calmodulin inhibitor, hinting the role of Ca^{2+} and calmodulin in the maintenance of the antioxidative defense during recovery phase of oxidative stress.

Data shows that a secondary oxidative stress and related oxidative membrane damage to the juvenile tissue occurs in *Amaranthus lividus* during early germination after imposing adverse temperature stress and the levels of the damage continues 3 days post exposure. This concurs with the work described by Gong *et al.,* 1998, Larkindale and Knight, 2002; Bhattacharjee and Mukherjee, 1997, 2003/2004, 2006; Bhattacharjee, 2005.

The levels of oxidative damage measured generally correlated with ultimate survival (germination) and early growth performances of *Amaranthus.* Although the two variables, *i.e.*, survival and oxidative damage are not completely linked, but in all experiments survival declined with higher levels of TBARS and reduced MPTL. This suggests that survival after heat and chilling stress requires ability to tolerate or repair oxidative damage (Bhatacharjee, 2008). It also requires ability to tolerate or minimize other kinds of adverse temperature induced damage (Woodstock *et al.,* 1983; Bhattacharjee 2001; Gong *et al.,* 1998; Larkindale and Knight, 2002; Bhattacharjee, 2005; Bhattacharjee and Mukherjee, 2006).

The development of thermo-tolerance (in terms of increased survival *i.e.*, germination and improved early growth performances) in Ca^{2+} pretreated and subsequently heat and chilling stress raised *Amaranthus* seedlings reinforced the idea that Ca^{2+} might be required in some signaling pathway in *Amaranthus* leading to improved survival. This suggests that a flux of Ca^{2+} ion is required to switch on some mechanism by which plants prevent or repair oxidative damage caused by high and low temperature. Thus a calcium flux is required for the plant to germinate under adverse environmental temperature.

Ca^{2+} inhibitor (Ca^{2+} channel blocker, Ca^{2+} chelator) and calmodulin inhibitor pretreatment before imposition of heat and chilling stress further substantiate the fact that a flux of Ca^{2+} is required to switch on some mechanism to mitigate oxidative damage of the germinating tissue. The significantly reduced accumulation of $O_2^{\cdot-}$ and H_2O_2 along with elevated activities of antioxidative enzymes (APOX, GR and CAT) and water soluble total thiol level in Ca^{2+} pretreated and heat and chilling stress raised *Amaranthus* seedlings further corroborate the involvement of Ca^{2+} in mitigating the oxidative stress. This Ca^{2+}-dependent pathway is presumed to act through calmodulin, as calmodulin inhibitor (trifluroperazine) significantly increased the level of oxidative stress after heat and chilling. A role for calmodulin is supported by the fact that higher levels of calmodulin have been obtained in thermo-tolerant maize cells than in those that are sensitive to heat (Gong *et al.,* 1997). Higher calmodulin levels have also been linked lower levels of heat induced membrane damage in maize (Gong *et al.,* 1997a) and in *Arabidopsis* (Larkindale and Knight, 2002). Price *et al.* (1996) have reported links between Ca^{2+} signalling and oxidative stress in tobacco plant where oxidative stress induces Ca^{2+} transients. Temperature stress induced Ca^{2+} peak also found to be involved the prevention of oxidative damage during recovery from heating (Price *et al.,* 1996).

The work therefore supports the hypothesis that Ca^{2+}-signaling offers significant role in limiting adverse temperature induced oxidative damage during the recovery phase of post germination event in *Amaranthus lividus,* which might have potential application in the field of Agriculture for the management of oxidative stress and the yield potential of the plant.

Acknowledgments

The author gratefully acknowledges University. Grants Commission, Govt. of India, for providing financial assistance in the form of Research Project, Sanction No. : FPS/007/04-05W (ERO).The author also acknowledges Prof. A.K. Mukherjee, Dean and Head of the Department of Biotechnology, Bengal College of Engineering and Technology, Durgapur for his valuable suggestions.

References

Alka, S. and R. Khanna-Chopra**:** Influence of temperature in germination and seedling growth and its relationship with amylase activity and respiration in wheat varieties differing in temperature tolerance. *Indian J. Exp. Biol.*, **33**, 775-779 (1995).

Bewley, J.D.and M. Black: In Physiology and Biochemistry of Seeds in Relation to Germination - Springer-Verlag, Berlin (1982).

Bhattacharjee, S. and A.K. Mukherjee: Heat and salinity induced oxidative stress and changes in protein profile in *Amaranthus lividus* L. *Indian J. Plant Physiol.*, **11**, 41-47 (2006).

Bhattacharjee, S. and A.K. Mukherjee: Role of free radicals in membrane deterioration in three rice cultivars under NaCl salinity at early germination stage. *Indian J. Exp. Biol.*., **35**, 1365-1369 (1997).

Bhattacharjee, S.: Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transdaction in plants. *Curr. Sci.*, **89**, 01-10 (2005).

Bhattacharjee, S.and A.K. Mukherjee: Implication of reactive oxygen species in heat shock induced germination and early growth impairment in *Amaranthus lividus* L. *Biol. Plant*, **47**, 571-522 (2003/2004).

Bhattacharjee, S.:Calcium-dependent signaling pathway in the heat-induced oxidative injury in *Amaranthus lividus*. *Biol. Plant,* **52**, 137-140 (2008).

Chaitanya, K.S.K. and S.C. Naithani: Role of superoxide, lipid peroxidation and superoxide dismutase in membrane perturbation during loss of viability of *Scorea robusta* Gaertn. F. *New Phytol.,* **126**, 623-627 (1994).

Dekok, L.J. and P.J.C. Kuiper: Effect of short term incubation with sulfate, chloride and selenate on glutathione content of spinach leaf discs. *Physiol. Plant*, **68**, 472-482 (1986).

Ellman, G.: Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*, **82**, 72-77 (1959).

Erlandson, A.G.I. and H. Jensen: Influence of low temperature on regulation of rubidium and calcium influx in roots of winter wheat. *Physiol. Plant*, **75**, 114-120 (1989).

Fick, N.G. and C.D. Qualset: Genetic control of endosperm amylase activity and gibberellin response in standard height and short stature wheat. *Proc. Nat. Acad. Sci.* USA., **72**, 892-895 (1975).

Gianopolities, C.N.and S.K. Ries: Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol.*, **59**, 309-314(1977).

Gong, M., A. Vander Luit, M.R. Knight, and A.J. Trewavas: Heat shock induced changes in intracellular Ca^{2+} level in tobacco seedlings in relation to thermotolerance. *Plant Physiol.*, **116**, 429-437(1998).

Gong, M., S. Chen, S.N. Song, Y.Q. and Z.G. Li: Effect of calcium and calmodulin on Involvement intrinsic heat tolerance in relation to antioxidant systems in maize seedlings. *Aust. J. Plant Physiol.*, **24**, 371-379 (1997a).

Gong, M., X.J. Li, X. Dai, M. Tian and G. Li: Involvement of calcium and calmodulin in the acquisition of thermotolerance in maize seedlings. *J. Plant Physiol*., **150**, 615-621 (1997).

Hare, P.D., W.A. Cress and S.J. Van: Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ*., **21**, 535-553 (1998).

Heath, R.L. and L. Packer: Photo-oxidation in isolated chloroplasts: kinetics and stoichiometry of fatty acid oxidation. *Arch. Biochem. Biophys*., **125**, 189-198 (1968).

Jiang, M. and J. Zhang: Effect of ABA on active oxygen species, antioxidative defense system and oxidative damage in leaves of maize seedlings. *Plant Cell Physiol*., **42**, 1265-1273 (2001).

Knight, H., A.J. Trewavas and M.R. Knight: Cold calcium signaling in *Arabidopsis* involved in two cellular pools and a change in calcium signature after acclimation. *Plant Cell.,* **8**, 489-503 (1996).

Knight, H. and M.R. Knight: Calcium signaling in plants responding to stress. *In*: Plant Responds to environmental stress (*Eds.*: M.F. Smallward, C.M. Calvert and D.J. Bowles). Bios Sc. Pub., Oxford, UK (1993).

Knight, M.R., N.D. Read, A.K. Campbell and A.J. Trewavas: Imaging calcium dynamics in living plants using semisynthetic recombinant aequorins. *J. Cell Biol.,* **121**, 83-90 (1993).

Larkindale, J. and M.R. Knight: Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, ABA, ethylene and salicylic acid. *Plant Physiol.*, **128**, 682-695 (2002).

MacNevin, W.M. and P.F. Uron: Spectrum of hydrogen peroxide from organic hydroperoxides. *Anal. Chem*., **2**, 1760-1761 (1953).

Mansfield, T.A., A.M. Hetherington and C.J. Atkinson: Some current aspects of stomatal physiology. *Annu. Rev. Plant Physiol. Plant Molecular Biol.,* **41**, 55-75 (1990).

Nakano, Y. and K. Asada: Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.*, **22**, 867-880 (1981).

Nayyar, H.: Calcium as environmental sensor in plant. *Curr. Sci*., **84**, 893-902 (2003).

Onwueme, I.C. and H.M. Laude: Heat-induced growth retardation and attempts at its prevention in barley and wheat coleoptiles. *J. Agric. Sci.,* **79**, 331-333 (1972).

Price, A.H., A. Taylor, S.J. Ripley, A. Griffiths, A.J. Trewavas and M.R. Knight: Oxidative signals in tobacco increase cytosolic calcium. *Plant Cell*., **6**, 1301-1310 (1996).

Rincon, M. and J.B. Hanson: Controls of calcium ion fluxes in injured and shocked corn (*Zea mays*) root cells: Importance of proton pumping and cell membrane potential. *Physiol. Plant,* **67**, 576-583 (1986).

Schaedle, M. and J.A. Bassham: Chloroplast glutathione reductase. *Plant Physiol.*, **59**, 1011-1112 (1977).

Singh, V.P.: Interaction of temperature and microsomal peroxidase in aflatoxin degradation by *Aspergillus flavus* 102566. *Curr. Sci*., **72**, 529-532 (1997).

Snell, F.D. and C.T. Snell: In Colorimetric methods of analysis - Van Nostard Reinford Co., New York (1971).

Tietze, F.: Enzymatic method for quantitative determination of nanogram amounts of total and oxidised glutathione : Application to mammalian blood and other tissues. *Anal. Biochem*., **27**, 502-522 (1969).

Trofimova, M.S., I.M. Andreev and V.V. Kuznetsov: Calcium is involved in regulation of the synthesis of HSPs in suspension-cultured sugar beet cells under hyperthermia. *Physiol. Planta*, **105**, 67-73 (1999).

Wang, J.B. and R.Q. Li: Changes of Ca^{2+} distribution in mesophyll cells of pepper under heat stress. *Acta. Horticul. Sinica*., **26**, 57-58 (1999).

Webb, A.A.R., M.R. McAinsh, J.E. Taylor and A.M. Hetherington: Calcium ions as intercellular second messengers in higher plants. *Adv. Bot. Res.,* **22**, 45-96 (1996).

Woodstock, L.M., S. Mason, K. Faul and L. Bass: Use of freeze drying and acetone impregnation with natural and synthetic antioxidants to improve storability of onion, pepper and parsley seeds. *J. Am. Soc. Hort. Sci.*, **108**, 692-696 (1983).