Indian Journal of Experimental Biology
Vol. 40, November 2002, pp. 1260-1268

Protection against photooxidative damage provided by enzymatic and non-enzymatic antioxidant system in sorghum seedlings

Sangeeta Sankhalkar & Prabhat Kumar Sharma*

Department of Botany, Goa University, Goa 403 206, India

Received 30 January 2002; revised 30 July 2002

Effect of photoinhibition of sorghum leaves and isolated chloroplasts on chlorophyll fluorescence, peroxidation of thylakoid lipids and activity of antioxidant enzymes were studied. Photoinhibition of intact leaves and isolated chloroplasts decreased Fv/Fm ratio and qP, while qN increased. Photoinhibitory damage was more at 5°C than at 30° or 50°C. Peroxidation of thylakoid lipids was 5 times greater when photoinhibited at 50°C compared to control. Photoinhibition of chloroplasts under low oxygen condition or when supplemented with anti-oxidants (β-carotene, ascorbate and GSH) resulted in significantly less damage to photosynthesis (Fv/Fm ratio) and peroxidation level. Photoinhibition also resulted in many fold increase in the activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) and decrease in catalase. Data presented here suggest that photoinhibition resulted in production of oxygen radicals and photoinhibition of chloroplasts in the presence of low oxygen level or when supplemented with antioxidants decreased the damage to Fv/Fm ratio and peroxidation level to a great extent since former prevented the formation of oxygen radicals and later could scavenge the oxygen radicals thus the protection. Increase activity of SOD and APX may also be to metabolise the oxygen radicals produced during photoinhibition treatment, thereby, protecting the seedlings against photooxidative damage.

Photoinhibition occurs when photosynthetic organisms are exposed to light level in excess to what can be dissipated by their normal photochemical processes resulting in decreased photosynthetic capacity[1]. Environmental stress factors such as temperature, draught, salinity etc. often amplify the photoinhibition. Inhibition of photochemical processes of photosynthesis proceed from an initial damaging event which affects components of PS II system such as D1 protein, P680, QA etc. It is thought that photoinhibition of photosynthesis was due to interaction of molecular oxygen with plastoquinone resulting in oxygen radicals which in turn cause damage to lipids and protein in the membrane. It has been shown that exposure of thylakoid to photoinhibitory illumination is accompanied by singlet oxygen production[2-4], which attack P680 itself, forming a triplet which subsequently interact with oxygen to form superoxide radicals which then attack the closest molecule. In other case oxygen directly alters P680 itself by absorbing second quanta and oxidising P680. Other oxygen radicals such as superoxide ($O_2^{\cdot-}$), hydroxy radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) are formed by reducing the oxygen under conditions where reductant generated in the photochemical reactions are not utilised by biochemical reaction[5]. These oxygen radicals generated in the photosynthetic reaction cause substantial damage to molecules such as lipids in thylakoid and other cell membranes, bleaching of pigments and oxidation of proteins[6-9].

Plants have developed various defence mechanisms in the chloroplasts to scavenge the toxic oxygen species or prevent their formation, thereby, preventing greater damage. This includes among other mechanism, an increase in the activity of enzymatic and non enzymatic antioxidant systems which metabolise oxygen radicals thus provide protection against oxidative damage. Only in cases where the anti-oxidant defence system becomes overcharged these oxygen species might have a significant damaging impact.

In this work, we have attempted to study activities of antioxidtive enzymes such as superoxide dismutase, ascorbate peroxidase, catalase and fluorescence characteristics in sorghum leaves after photoinhibition at 5°, 30° and 50°C as well. We have also examined effect of photoinhibition on fluorescence in isolated chloroplasts as well as effect of β-carotene, ascorbate and glutathione after photoinhibition treatment.

Material and Methods

Plant material—Seeds of *Sorghum bicolor* L. cultivar MSH-51 were obtained from Mahyco Hybrid Seeds Company, Jalna, Maharastra, India and were stored in a dessicator at room temperature.

*Correspondent author—E-mail: pksharma@unigoa.ernet.in; Fax: +91-832-451184.
Phone: +91-832-452345-48 Ext. 354

Growth conditions—Prior to sowing the seeds were soaked in tap water for 1 hr and were surface sterilised with $HgCl_2$ (0.1%) for 2 min. The seeds were grown densely in plastic pots (diam 10 cm) containing vermiculite and routinely bottom irrigated with half strength Hoagland solution. The plants were grown in a controlled environmental growth chamber with 14 hr photoperiod provided with incandescent bulbs and fluorescent tubes having a non photoinhibitory level of light of 250 µmol m^{-2} s^{-1} PAR. The day/night temperature was maintained at 30°±2°C and relative humidity at 60%. Seedlings of 8 day old were used for the experiments.

Isolation of chloroplasts—Leaves (25 g) of 8 day old sorghum seedlings were cut into pieces (50 mm) and homogenised in a kitchen blender (Braun) for 10 sec at full speed in 200 ml isolation medium containing sorbitol (0.33 *M*), 30 m*M* of TES buffer (*p*H 7.2 with KOH), EDTA (1 m*M*), $MgCl_2$ (1 m*M*), $MnCl_2$ (1 m*M*), dithiothreitol (5 m*M*; DTT) and 0.5% BSA (bovine serum albumin). The homogenate was filtered through 2 and 8 layers of muslin, respectively. The filtrate was again filtered through a nylon cloth (75 µ*M*) in order to remove the bundle sheath. The filtrate was centrifuged (1000 *g*) for 8 min in a swinging bucket rotor. the supernatant was discarded and the pellet was washed twice and resuspended in a suspension medium containing sorbitol (0.33 *M*), 50 m*M* of tricine buffer (*p*H 7.8 with KOH), $MgCl_2$ (4 m*M*), EDTA (2 m*M*), $MnCl_2$ (2 m*M*), NaCl (10 m*M*), DTT (5 m*M*) and 0.5% BSA. Chlorophyll concentration was measured according to Arnon[10]. Per cent intactness of isolated chloroplasts was 60% of A type, determined according to Leegood and Walker[11] by assaying the rate of light dependent oxygen evolution after addition of NH_4Cl with osmotically shocked chloroplasts (A) and unshocked chloroplasts (B) in assay medium containing sorbitol (0.33 *M*), 50 m*M* of HEPES buffer (*p*H 7.6 with KOH), $MgCl_2$ (1 m*M*), $MnCl_2$ (1 m*M*) EDTA (2 m*M*) and chloroplasts equivalent to 100 µg ml^{-1}. The percentage of intactness of the chloroplasts preparation was calculated as % intactness = A-B/A × 100.

Photoinhibition treatment—Intact leaves and isolated chloroplasts were exposed to irradiance of 3600 µmol m^{-2} s^{-1} at 5°, 30° and 50°C temperature for a duration 0-6 hr in case of leaves and for 0-60 min in case of isolated chloroplasts. Six halogen lamps of 15V and 250 W were used to provide the required light intensity. The leaves were kept on top of a metallic box with water circulating facility to maintain the desired temperature during the treatment. Photoinhibition of isolated chloroplasts was done in a metal cuvette used for measuring chlorophyll fluorescence under *in vitro* conditions attached with PAM chlorophyll fluorometer (Walz, Germany). Isolated chloroplasts (type A; 1 ml) of 1 mg chlorophyll ml^{-1} concentration were placed in the cuvette and irradiated from top to high light (3600 µmol m^{-2} s^{-1}) using Schott KL 1500 electronic light source. Chloroplasts were kept constantly stirred during the photoinhibition treatment.

Effect of non-enzymatic antioxidant—β-carotene, ascorbic acid and glutathione in final concentration of 0.5 m*M* was added to the chloroplasts prior to the photoinhibition. The photoinhibition treatment was given according to the method as described for photoinhibition of isolated chloroplasts.

Photoinhibition under anaerobic conditions—Chloroplasts were also photoinhibited under anaerobic conditions (low oxygen conditions) and normal aerobic conditions. The anaerobic condition during the photoinhibition treatment was created by flushing the chloroplasts with nitrogen throughout the treatment through the small pore provided in the cuvette for adding reagents or taking out sample without disturbing the fluorescence measurements.

Fluorescence measurements of intact leaves and isolated chloroplasts—Leaves after photoinhibition treatment were dark adapted for 30 min prior to measurements at room temperature. The leaf was enclosed in the leaf chamber provided for the measurement of chlorophyll fluorescence from intact leaf. Similarly, isolated chloroplasts were dark adapted for 5 min prior to measurement, and chlorophyll fluorescence of isolated chloroplast was measured in a cuvette (Walz, Germany). The dark adapted leaf or isolated chloroplasts, as the case may be, were exposed to a modulated light with an intensity of 4 µmol m^{-2} s^{-1} to measure initial fluorescence (Fo). This was followed by an exposure to a saturating pulse of white light of 4000 µmol m^{-2} s^{-1} to provide the maximum fluorescence (Fm). After measurement of Fm the leaf was allowed to reach a steady state fluorescence (Fs) while exposed to actinic light intensity of 800 µmol m^{-2} s^{-1}. Another short burst of saturating light at Fs state was given to measure Fm′. After reaching to the steady state again, leaf was exposed to far-red radiation to measure Fo′. The coefficient of variable fluorescence (Fv=Fm-Fo), photochemical quenching

(qP=Fm′-FS/Fm′-Fo′) and non-photochemical quenching (qN=Fm-Fm′/Fm-Fo′) were calculated according to Schreiber[12].

Peroxidation of thylakoid lipids—Peroxidation of thylakoid lipids was assayed according to Halliwell and Gutteridge[7] by 2-thiobarbituric acid-malondialdehyde (TBA-MDA) adduct formation. Malondialdehyde formation was followed by adding equal amount of 0.5% TBA in 20% tri-chloro acetic acid to an aliquot of the incubation buffer containing 50 m*M* tris (*p*H 8.0), 175 m*M*, NaCl and chloroplasts equivalent to 50 µg of chlorophyll. The solution was incubated at 95°C for 25 min and centrifuged at 250 *g* for 2 min to pellet out the protein precipitated by TCA. The clear supernatant was used for determination of lipid peroxidation. Absorbance at 600 nm was subtracted from absorbance at 530 nm in order to correct for the non specific turbidity. Amount of MDA formation was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Assay of antioxidant enzymes

Extraction—Leaves (0.5 g) were homogenised in chilled extraction buffer containing 50 m*M* potassium phosphate buffer (*p*H 7.5), EDTA (1 m*M*), triton X-100 (0.1%) and 1% (w/v) soluble polyvinyl pyrophosphate. The homogenate was filtered and centrifuged at 12000 *g* for 15 min at 4°C. Protein content of the supernatant was measured according to Lowry *et al*[13]. Supernatant equivalent to 50 µg was used for the estimation of enzyme activity.

Assay of superoxide oxidoreductase (EC 1.15.1.1)—Activity of superoxide dismutase (SOD) was assayed according to Badiani *et al*[14]. The assay medium contained 50 m*M* potassium phosphate (*p*H 7.8), EDTA (di sodium; 0.1 m*M*), cytochrome c (10 m*M*), xanthine (0.1 m*M*) and the leaf homogenate equivalent to 50 µg of protein. The reaction was followed spectrophotometrically as reduction of cytochrome c at 550 nm. One unit of SOD activity was defined as the amount of enzymes required to inhibit the rate of cytochrome c reduction by 50%.

Assay of hydrogen peroxide oxidoreductase (EC 1.11.1.7) peroxidase—Peroxidase activity was monitored according to Afilthile *et al*[15]. Activity was assayed in a reaction mixture containing guaicol (3.4 m*M*), H_2O_2 (0.9 m*M*) and 50 m*M* potassium phosphate (*p*H 6.0) and leaf extract equivalent of 50 µg of protein. The mixture was incubated for 5 min at 25°C and the reaction was stopped by adding 0.5 ml of 5% (v/v) sulphuric acid. Enzyme activity was calculated using an extinction coefficient of 25.5 mM^{-1} cm^{-1} at 470 nm for tetra-guaicol.

Assay of hydrogen peroxide: Hydrogen peroxide oxidoreductase (EC 1.11.1.6) catalase—Activity of catalase was determined spectrophotometrically by following the decomposition of hydrogen peroxide at 240 nm at 25°C in a reaction mixture containing potassium phosphate (50 m*M*), H_2O_2 (11 m*M*) and the leaf extract equivalent of 50 µg of protein, according to Mishra *et al*[16].

Results and Discussion

Room temperature chlorophyll fluorescence measurements (Fv/Fm ratio, photochemical quenching; qP and non-photochemical quenching; qN) of sorghum leaves photoinhibited at 3600 µmol m^{-2} s^{-1} at 5°, 30° and 50°C are shown in Fig. 1 A-C. It was observed that with the increase in the duration of photoinhibitory treatment of intact leaves, Fv/Fm ratio decreased linearly. Decrease in Fv/Fm ratio was greater in leaves photoinhibited at 5°C than at 30° or 50°C (Fig. 1A). Photochemical quenching (qP) also declined linearly when leaves were photoinhibited at 5°C, however, leaves photoinhibited at 30° and 50°C showed an initial increase in qP followed by a decrease (Fig. 1B). Non-photochemical quenching (qN), however, increased due to photoinhibition. Increase in qN was greater when photoinhibited at 30°C than at 5° or 50°C (Fig. 1C). Photoinhibition of isolated chloroplasts (up to 60 min) also resulted in linear decrease in Fv/Fm ratio (Fig. 1 D) and qP (Fig. 1 E) and increase in qN (Fig. 1F) at all the three temperatures. Photoinhibition at 5°C resulted in the maximum decrease in Fv/Fm ratio and qP and increase in qN (Fig. 1D- F).

Results indicated that photoinhibition caused damage to Fv/Fm ratio, suggested that efficiency of transfer of excitation energy from LHC to reaction centre was declined, more so in leaves photoinhibited at 5° and 50° than at 30°C. Results showed decrease in qP, an indicator of utilisation of harvested light by photochemical reaction. Results also showed increase in qN, indicative of dissipation of excessive energy in the pigment bed, thereby, preventing over excitation of reaction centre under high light condition. Chilling and high temperature exacerbated the effect. Photoinhibition of isolated chloroplasts also indicated similar pattern except that the extent of damage was greater in short duration. However, damage under *in vivo* and *in vitro* conditions cannot be compared with each other.

Photoinhibition of sorghum leaves increased peroxidation of thylakoid lipids (Fig. 2A) as indicated by MDA formation. Photoinhibition treatment at 5°C for 6 hr caused an increase of 265% in peroxidation level of thylakoid lipids compared to 128% observed at 30°C and 533% at 50°C for the same duration with respect to control (Fig. 2A). Photoinhibition of isolated chloroplasts showed similar results as seen for intact leaves except for greater level of peroxidation under *in vitro* conditions (Fig. 2B). Increase in MDA formation as a result of photooxidation of thylakoid lipids in our study indicated that photoinhibition caused oxidative damage to seedlings probably by producing oxygen radicals. These radicals if not quenched by the various processes such as carotenoids and enzymatic and non-enzymatic processes cause peroxidation of thylakoid lipids. Lipid peroxidation has also been linked to effects such as increased ion permeability, loss of fluidity, cross linking of amino lipids and polypeptides, inactivation of membrane enzymes and receptors resulting in a decrease in photosynthesis[6,17].

Earlier results indicate damage to photosynthesis which involves at least in part, peroxidation of thylakoid lipids, suggesting the involvement of lipids in the functioning of chloroplasts[18]. Thylakoid lipids in addition to fulfilling a structural role in membrane organisation, also play a functional role in the activity

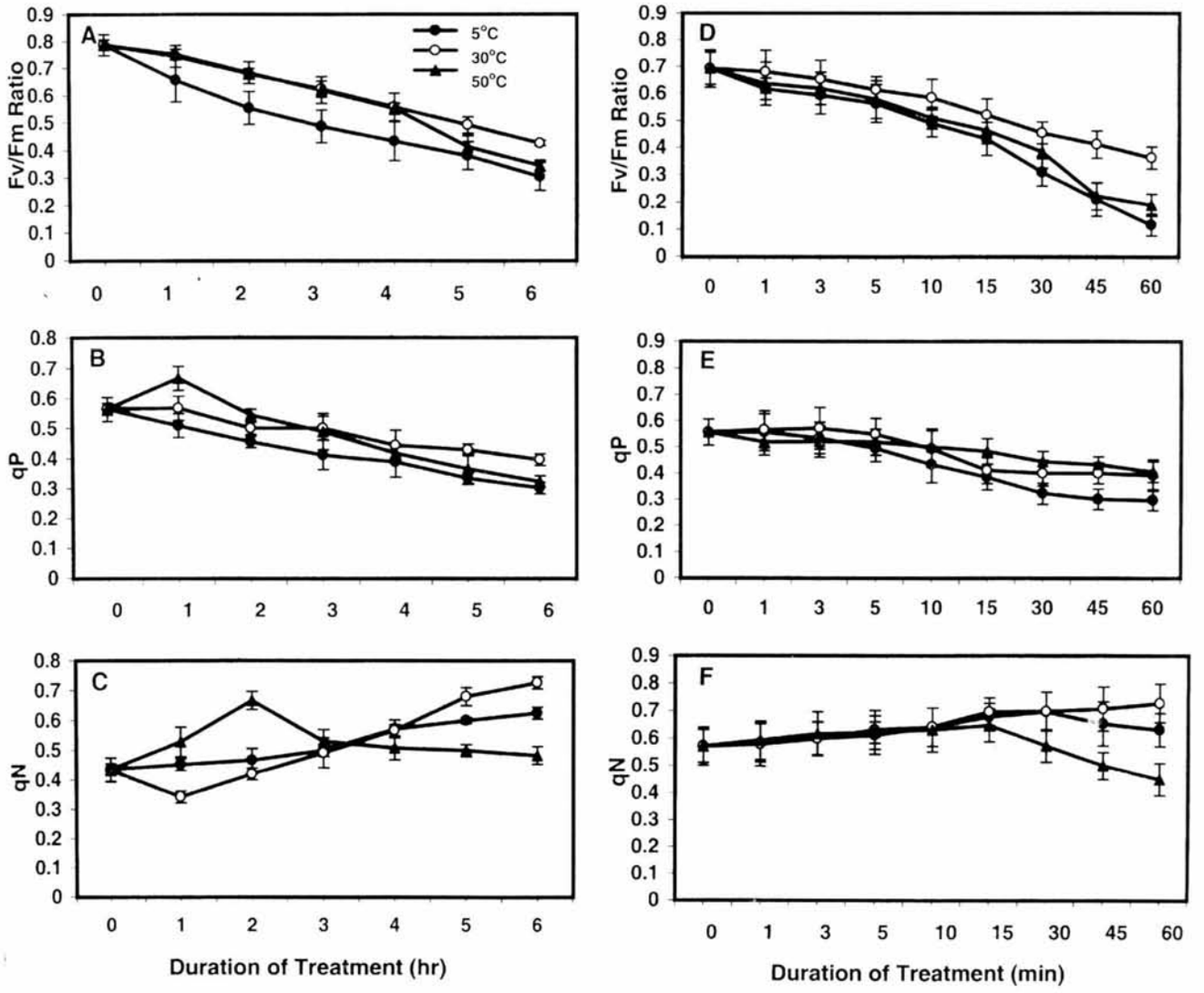

Fig. 1—Effect of photoinhibition of intact leaves (A, B & C) and isolated chloroplasts (D,E & F) at 5°C (---•---), 30°C (---o---) and 50°C (---▲---) temperature up to 6 hr for intact leaves and up to 60 min for isolated chloroplasts, on Fv/Fm ratio (A & D), photochemical quenching (B & E) and non-photochemical quenching (C & F). The photoinhibition treatment was given at 3600 μmole m^{-2} sec^{-1}. The points represent 5 separate set of experiments. Vertical bars show standard deviation.

of several integral membrane protein complex[19]. The composition of membrane lipids and the degree of their unsaturation appear to be important for protection against stress and photoinhibition may alter this balance. It has been shown that resistant plants to photoinhibitory damage show higher unsaturation level of chloroplastic and mitochondrial membranes[20]. A 4 fold increase in ratio of unsaturated to saturated fatty acids in cold tolerant cultivars of burmudagrass has been reported under photoinhibition conditions[21].

Data with photoinhibition of isolated chloroplasts flushed with N_2 gas showed that Fv/Fm ratio and peroxidation of thylakoid did not result in much damage compared to that observed in control (chloroplasts photoinhibited under normal aerobic condition; Table 1). These results indicated that oxygen radicals were involved in the photoinhibition of photosynthesis as photoinhibition under anaerobic conditions resulted in less damage to photosynthesis and peroxidation of thylakoid lipids. This may be due to the generation of low level of oxygen radicals under the experimental conditions, thereby, less damage[18].

Isolated chloroplasts were also supplemented with β-carotene, ascorbic acid and glutathione during photoinhibition in order to see if addition of these antioxidant provided any protection against photoinhibition of photosynthesis under *in vitro* conditions (Fig. 2 C-E). This was carried out as under *in vitro* condition inherent protection as well as cellular recovery processes were either not functioning or functioning at minimal level. The results were compared with the chloroplasts photoinhibited without adding antioxidants (Fig. 2B) to find out the relative extent of protection to photosynthesis provided by addition of the antioxidants. It was seen that addition of antioxidants during the photoinhibition decreased peroxidation of thylakoid

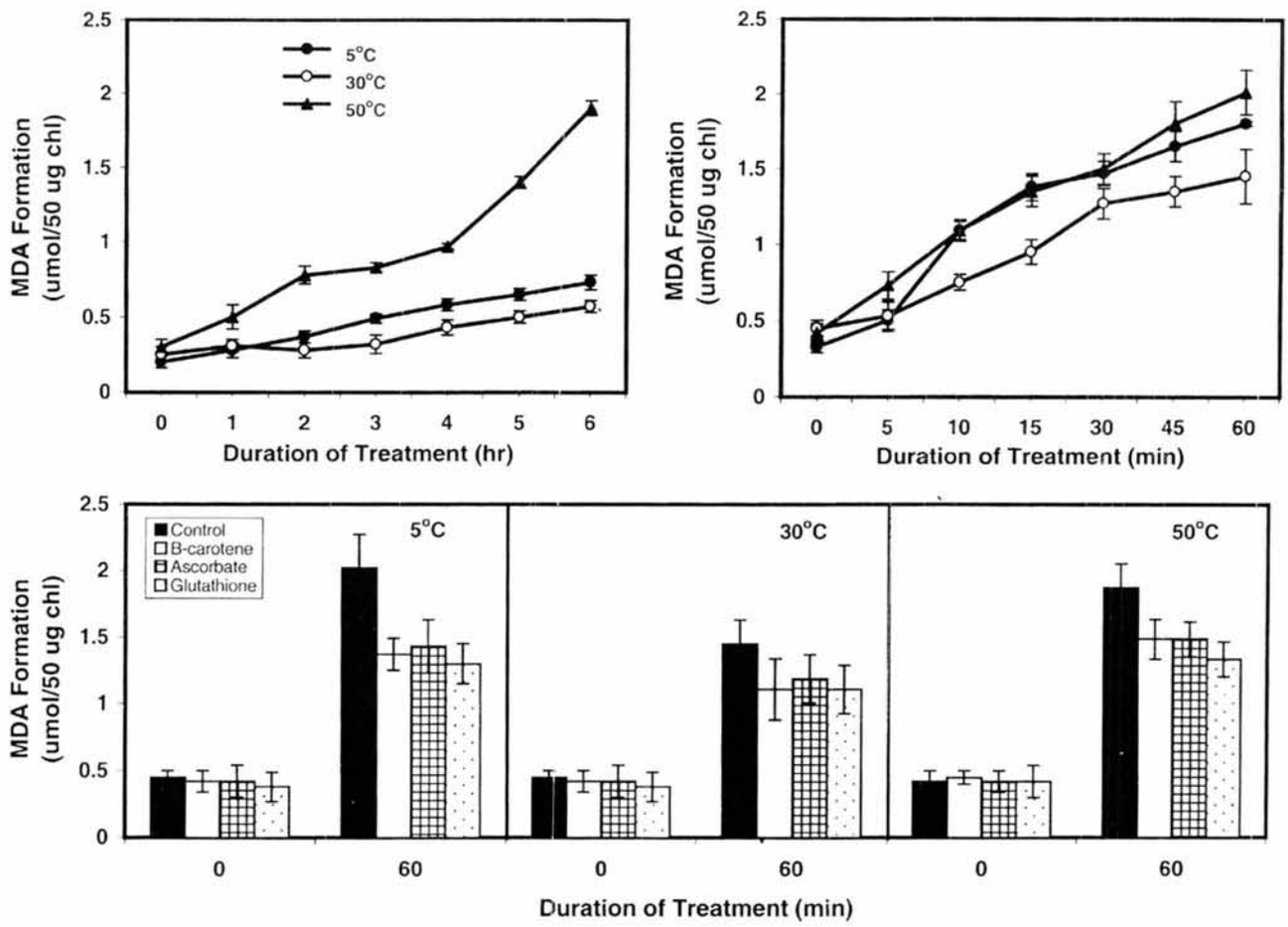

Fig. 2—Effect of photoinhibition on MDA formation of intact leaves (A), isolated chloroplasts (B) and isolated chloroplasts supplemented with antioxidants β-carotene, ascorbate and glutathione (C) prior to the treatment on peroxidation of thylakoid lipids (expressed as formation of μmol of MDA per 50 μg chlorophyll). The photoinhibition treatment was given at 3600 μmole m^{-2} sec^{-1} PAR at 5°C (---•---), 30°C (---o---) and 50°C (---▲---) temperature up to 6 hr to intact leaves and up to 60 min to isolated chloroplasts. The points represent 5 separate set of experiments. Vertical bars show standard deviation.

lipids, indicating less damage, compared to control (chloroplast photoinhibited without addition of antioxidants). Results show that photoinhibition of isolated chloroplasts supplemented with β-carotene, ascorbic acid and glutathione resulted in 23, 28 and 27% less peroxidation of thylakoid lipids respectively as compared to control (Fig. 2C-E) indicating protection against photodamage by non-enzymatic antioxidant.

Chlorophyll fluorescence was also measured in isolated chloroplasts supplemented with antioxidants (β-carotene, ascorbate and GSH) after photoinhibition. Results showed that damage to Fv/Fm ratio was less than control at 5° and 50°C when chloroplasts were supplemented with antioxidants during the photoinhibition treatment (Fig. 3A-C).

Role of β-carotene in radical trapping antioxidant is of much current interest and many aspects of this are still not understood[22]. Protective role of β-carotene has also been reported by others[4,23,24]. β-carotene can inhibit photo-oxidised oxidation[23] and is therefore, an efficient quencher of singlet oxygen[24]. Protection to photodamage provided by addition of ascorbate in this study may be due to ascorbate being a excellent scav-

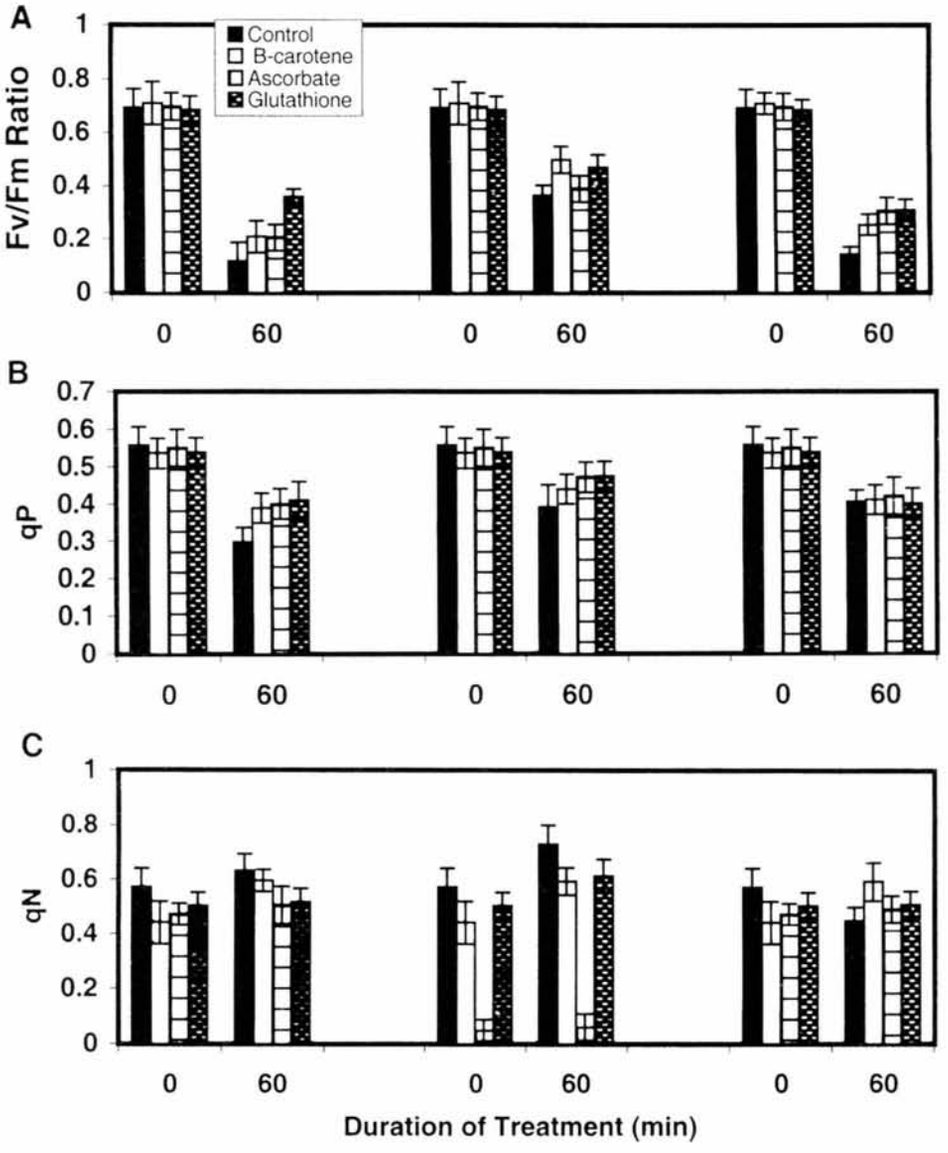

Fig. 3—Effect of photoinhibition of chloroplasts, isolated from normal leaves and supplemented with antioxidants β-carotene, ascorbate and glutathione at 5°, 30° and 50°C on chlorophyll fluorescence parameters, Fv/Fm ratio (A), qP (B) and qN (C). The photoinhibition treatment was given at 3600 μmole m^{-2} sec^{-1} PAR at 5°, 30° & 50°C. The points represent 5 set of experiments. Vertical bars show standard deviation.

enger of free radicals and act as reductant[5]. Ascorbate can also facilitate the activity of ascorbate peroxidase, an enzyme which catalyzes H_2O_2 and thereby prevent oxidative damage[5] and may also facilitate de-epoxidation of violaxanthin (V) to zeaxanthin (Z), since the same ascorbate pool (chloroplastic) is used for APX activity and de-epoxidation of V to Z under these conditions[25]. It has been reported that high concentration of ascorbate in sun acclimated tissue may be necessary for de-toxification of singlet oxygen[22]. Glutathione may also provide similar kind of protection against photodamage, as has been seen in this study. Glutathione is a substrate which is reduced by NADPH dependent glutathione reductase. Reduced glutathione in turn is used to maintain a pool of reduced ascorbate. Glutathione and ascorbate are major reductant of plant cells and offer protection against adverse effects of several active oxygen species[26,27]. Glutathione facilitates rapid regeneration of reduced ascorbate pool, thereby, better scavenging of oxygen radicals through activity of APX. Glutathione may also have effect on rapid de-epoxidation of V to Z by maintaining ascorbate pool essential for the activity of membrane bound violaxanthin de-epoxidase.

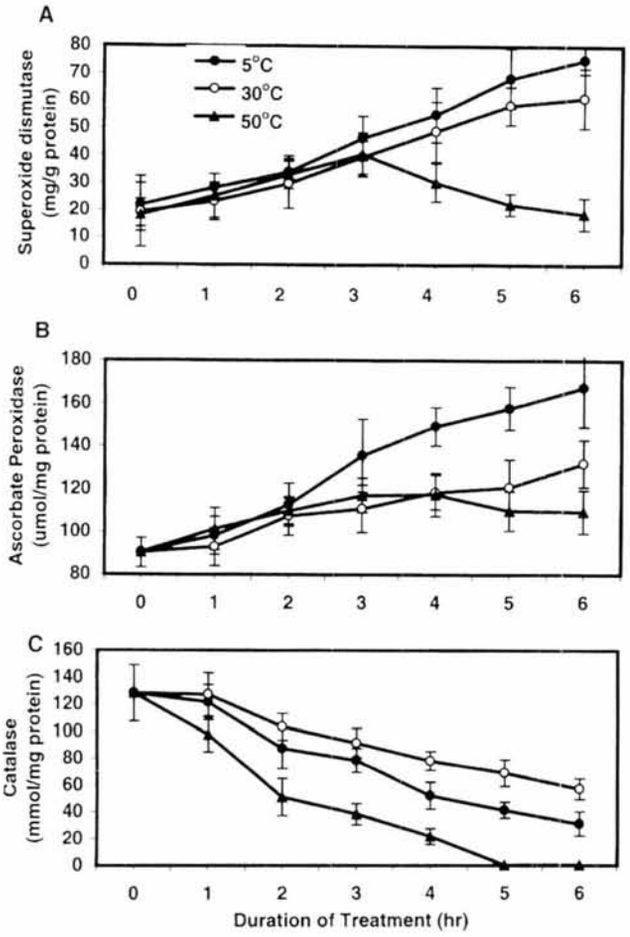

Fig. 4—Effect of photoinhibition of intact leaves at 3600 μmole m^{-2} s^{-1} PAR at 5°C (---•---), 30°C (---o---) and 50°C (---▲---) temperature up to 6 hr on activities of superoxide dismutase (A), ascorbate peroxidase (B) and catalase (C). The points represent 5 set of experiments. Vertical bars show standard deviation.

There are reports of induction of endogenous antioxidants in improving photoprotection[28,29]. Mortenssen *et al.*[30] have studied the mechanism and rate of free radical scavenging by carotenoids and reported that rate constant for radical scavenging are in the order of 10^7-10^9 m^{-2} sec^{-1}. They have also reported that mechanism and rate of scavenging is strongly dependent on the nature of oxidising radical species, but less dependent on structure of antioxidant. Mortensen and Skibsted[31] have established the hierarchy of antioxidant studied as α-tocopherol, lycopene, β-carotene, Z-λ-tocopherol and lutein.

Effect of photoinhibition treatment of intact leaves on various antioxidant enzymes was also studied (Fig. 4A-C). Photoinhibition treatment for 6 hr at 5°C resulted in an increase of 144% in superoxide dismutase as compared to 216 and 124% increase observed when

Table 1—Effect of photoinhibition treatment of isolated chloroplast at 3600 μmole m^{-2} sec^{-1} PAR at 30°C on chlorophyll fluorescence and peroxidation of thyalkoid lipids under aerobic and anaerobic (low oxygen condition).

[Values are mean ± SD of 4 replications]

Duration of treatment (min)	Chlorophyll fluorescence (Fv/Fm ratio)		Peroxidation of thylakoid lipids (μmole/50μg chl)	
	A	B	A	B
0	0.692 ± 0.07	0.710 ± 0.05	0.45 ± 0.04	0.45 ± 0.05
5	0.612 ± 0.05	0.685 ± 0.06	0.53 ± 0.09	0.48 ± 0.08
15	0.519 ± 0.06	0.648 ± 0.08	0.95 ± 0.08	0.56 ± 0.09
30	0.453 ± 0.04	0.623 ± 0.05	1.27 ± 0.1	0.64 ± 0.08
60	0.362 ± 0.04	0.608 ± 0.06	1.45 ± 0.18	0.88 ± 0.1

A—aerobic; B—anaerobic condition

photoinhibited at 30° and 50°C respectively, with respect to control (Fig. 4A). Activity of ascorbate peroxidase also showed similar trends. A 6 hr photoinhibition treatment at 5°C resulted in an increase of 124% in APX as compared to control (Fig. 4B). Unlike the activity of SOD and APX, catalase activity declined due to photoinhibition treatment at all the three temperatures (Fig. 4C).

Increase in the activity of antioxidant enzymes was mainly influenced by temperature (Fig. 4A-C). Photoinhibition at chilling temperature caused increase in APX and SOD. Apparently, the activity of these antioxidant enzymes increased in response to metabolise the increase level of oxygen radicals and free radicals generated due to treatment.

Biehler and Fock[32] have reported a role of Mehler-Peroxidase reaction in oxygen consumption in drought stressed wheat. Over-expression of glutathione reductase (5-8 times) and SOD (3-4 times) due to photoinhibition has been reported [33]. Some reports suggest that over production of SOD in the chloroplasts or in cytosol resulted in no protection against photoinhibition[34-35]. However, others have shown that higher production of SOD in light stressed plants provided protection against photodamage[36,37]. Jansen *et al.*[36] have reported that photoinhibition causes overexpression of several other antioxidant enzymes. Our results also showed similar results of elevated level of SOD and APX under photoinhibitory conditions. Wherever treatment resulted in higher activity of SOD and APX such as when leaves photoinhibited at 30°C the damage to photosynthesis (Fv/Fm ratio) and photooxidation of lipids was less indicating relationship between the two i.e. higher induction of SOD and APX and less photodamage. In most of the cases reported, even a moderate increase in chloroplastic SOD activity has led to improved tolerance to oxidative stress[38,39]. Arisi *et al.*[39] have also reported that in some cases enhancement of SOD activity brought about increase in the oxidative stress tolerance which afforded better tolerance against photodamage to PS II. Catalase is an enzyme which is limited in effectiveness to metabolise H_2O_2 to H_2O by its relatively poor affinity for H_2O_2 and its subcellular location in peroxisomes and also susceptible to photoinactivation and degradation [40-41], which could explain the decrease in activity of catalase under our experimental conditions. Also catalase being more functional during photorespiration reaction to metabolise H_2O_2 was not required to function at higher level as sorghum being C4 plants will not show a high photorespiratory rate.

Present results showed that sorghum seedlings illuminated with high light experienced inhibition of photosynthesis seen as decline in Fv/Fm ratio and qP. Damage was enhanced when photoinhibition was given at chilling or high temperature. The results also indicated oxidative damage to plants during photoinhibition treatment, which was indicated by higher peroxidation of thylakoid lipids under aerobic condition than seen under anaerobic conditions. The work showed that non-enzymatic antioxidant such as β-carotene, ascorbate and glutathione provided protection against photodamage, probably by scavenging oxygen radicals generated during photoinhibition. Activity of SOD and APX increased during the treatment which may be useful to metabolise the oxygen radicals and provide protection against photodamage.

Acknowledgement

The research grant from the DAE, India under the scheme of DAE young scientist research award (1994) to the PKS is highly acknowledged.

References

1 Sharma P K & Hall D O, Effect of high irradiance stress on primary photochemistry and light regulated enzyme of photosynthetic carbon metabolism, *J Plant Physiol*, 139 (1992) 719.

2 Hideg E, Spetea C & Vass I, Singlet oxygen and free radical production during acceptor and donar side induced photoinhibition studies with spin trapping EPR spectroscopy, *Biochim Biophys Acta*, 1186 (1994) 143.

3 Macpherson A N, Telfer A, Barber J & Truschott T G, Direct detection of singlet oxygen from isolated PS II reaction centres, *Biochim Biophys Acta*, 1143 (1993) 301.

4 Telfer A, Dhami S, Boshop S M, Phillips D & Barber J, β-carotene quenching singlet oxygen formed by isolated photosystem II reaction centres, *Biochemistry*, 33 (1994) 14469.

5 Krause G H, Photoinhibition induced by low temperature. in *Photoinhibition of photosynthesis from molecular mechanism to the field*, edited by N R Baker and J R Boyer (BIOS Sci, Oxford) 1994, 331.

6 Girotti A W, Photodynamic lipid peroxidation in biological systems, *Photochem Photobiol*, 51 (1990) 497.

7 Halliwell B & Gutteridge J M, *Free radicals in biology and medicine*, edited by B Halliwell and J M Gutteridge (Clarendon press, Oxford) 1989, 188.

8 Youngman R J, Oxygen activation is the hydroxyl radical always biologically relevant? *Trends Biochem Biol Sci*, 9 (1984) 581.

9 Hodgson R A J & Raison J K, Superoxide production by thylakoids during chilling and its implication in the succeptibility of plants to chilling-induced photoinhibition, *Planta*, 183 (1991) 222.

10 Arnon I, Copper enzyme in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*, *Plant Physiol*, 24 (1949) 1.

11 Leegood R C & Walker D A, Chloroplasts and protoplasts, in *Techniques in bioproductivity and photosynthesis*, edited by J

Coombs, D O Hall, S P Long & J M O Scurlock (Pergamon Press, Oxford) 1985, 118.
12 Schreiber U, Schliwa U & Bilger W, Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type modulation fluorometer, *Photosyn Res*, 10 (1986) 51.
13 Lowry O H, Rosebrough N J, Farr A L & Randall R L, Protein measurement with folin phenol reagent, *J Biol Chem*, 193 (1951) 262.
14 Badiani M, Paolacci A R, D'Annibale A & Giovannozzi Sermanni G, Antioxidants and photosynthesis in the leaves of *Triticum durum* L. Seedlings acclimated to low non-chilling temperature, *J Plant Physiol*, 142 (1993) 18.
15 Afitlhile M M, Rauchel M D & Cowan K A, Changes in carotenoid composition in senescing leaves of *Hordeum vulgare*, *J Plant Physiol*, 142 (1993) 43.
16 Mishra R K, Chauhan N P & Singhal G S, The role of secondary acceptor QB of the photosystem II in photoinhibition of isolated wheat chloroplasts, *J Plant Physiol*, 138 (1993) 602.
17 Kappus H, Lipid peroxidation: Mechanism, analysis, enzymology and biological relevance. in *Oxidative stress*, edited by H Sies (Academic Press, New York) 1986, 273.
18 Sharma P K & Singhal G S, The role of thylakoid lipids in the photodamage of photosynthetic activity, *Physiol Plant*, 86 (1992) 623.
19 Horvath G, Melis A, Hideg E, Droppa M & Vigh L, Role of lipids in the organisation and function of PS II studies by homogenous catalytic hydrogenation of thyalkoid membanes *in situ*, *Biochim Biophys Acta*, 891 (1987) 283.
20 Chirkova T V, Sinyutina N F, Blyudzin Y A, Barsky I E & Smetanikova S V, Phospholipids fatty acids of root mitochondria and microsomes from rice and wheat seedlings exposed to aeration of anaerobiosis, *Plant Physiol* (Russian), 36 (1989) 126.
21 Samala S, Yan J Y & Baird W V, Changes in polar lipid fatty acid composition during cold acclimation in midiron and U3 bermudagrass, *Crop Sci*, 38 (1988) 188.
22 Logan B A, Baker D H, Adams D & Adams III W W, Acclimation of leaf carotenoid composition and ascorbate levels to gradients in the light environment within an Australian rain forest, *Plant Cell Environ*, 19 (1996) 1083.
23 Edge R, Mc Garvey D J & Truscott T G, The carotenoids as anti-oxidants—A review, *J Photochem Photobiol B: Biol*, 41 (1997) 189.
24 Burton G W & Ingold K U, β-carotene an unusual type of lipid antioxidant, *Science*, 224 (1984) 569.
25 Siefermann-Harms D, The light harvesting and protective function of carotenoids in photosynthetic membrane, *Physiol Plant*, 69 (1987) 561.
26 Noctor G, Rees D, Young A & Horton P, The relationship between zeaxanthin, energy dependent quenching of chlorophyll feluorescence and trans thylakoid *p*H gradient in isolated chloroplast, *Biochim Biophys Acta*, 1057 (1991) 320.
27 Noctor G & Foyer C H, Ascorbate and glutathione: Keeping active oxygen under control, *Annu Rev Plant Physiol Mol Biol*, 49 (1998) 623.
28 Loggini B, Scartazza A, Brugnoli E & Navari-Izzo F, Antioxidative defence system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought, *Plant Physiol*, 119 (1999) 1091.
29 Steenvoorden D P T & Beijersbergen V H G M J, The use of endogenous antioxidants to improve photoprotection, *J Photochem Photobiol B: Biol*, 41 (1997) 1.
30 Mortenssen A, Skibsted L H, Sampson J, Rice Evans C & Evertee S, A comparative mechanism and rates of free radical scavenging by carotenoid antioxidants, *FEBS Lett*, 418 (1997) 91.
31 Mortensen A & Skibsted L H, Relative stability of carotenoid radical cation and homologue tocopheroxy radicals. A real time kinetic study of antioxidant hierachy, *FEBS Lett*, 417 (1997) 261.
32 Biehler K & Fock H, Evidence for the contribution of Mehler-peroxidase reaction in dissipating excess electron in drought stressed wheat, *Plant Physiol*, 112 (1996) 265.
33 Tyystjarvi Esa, Riikonen M, Arisi C M, Rcetta K, Jouanin L & Foyer C H, Photoinhibition of photosynthesis II in tobacco plants overexpressing glutathione reducatse and populars overexpressing superoxide dismutase, *Physiol Plant*, 105 (1999) 409.
34 Slooten I, Capian K, Van camp W, Van montagu M, Sybesma C & Inze D, Factors affecting the enhancement of oxidative stress tolerance in transgenic tobacco over expressing manganese superoxide dismutase in the chloroplast, *Plant Physiol*, 107 (1995) 737.
35 Van Camp W, Capian K, Van Montagum, Inez D & Slooten L, Enhancement of oxidative stress tolerance in transgenic tobacco plants over producing Fe-superoxide dismutase in chloroplasts, *Plant Physiol*, 112 (1996) 1703.
36 Jansen A K J, Shaalhel Y, Kazzes D, Canaani O, Malkin S & Gressel J, Increase tolerance to photoinhibitory light in paraquat resistant *Conyza bonariensis* measured by photoacoustic spectroscopy and $^{14}CO_2$ fixation, *Plant Physiol*, 91 (1989) 1174.
37 Sen-Gupta A, Webb R P, Holaday A S & Allen R D, Over expression of superoxide dismutase protects plants from oxidative stress: Induction of ascorbate peroxidase in superoxide dismutase over producing plants, *Plant Physiol*. 103 (1993) 1067.
38 Perl A, Perl T R, Galili S, Aviv D, Shalgi E, Malkin S & Galun E, Enhanced oxidative stress defence in transgenic potato expressing Cu/Zn superoxide dismutase, *Theor Appl Genet*, 85 (1993) 568.
39 Arisi A C, Cronic G, Joanin L & Foyer C H, Enhanced tolerance to photooxidative stress of transgenic *Nicotiana tobacum* with high chloroplastic glutathione reductase activity, *Plant Cell Physiol*, 34 (1998) 129.
40 Feierabend J & Engel S, Photoinactivation of catalase *in vitro* and in leaves, *Arch Biochem Biophys*, 251 (1986) 567.
41 Streb P, Michael K A & Feierabend J, Preferential inactivation of catalase and photoinhibition of PS II are common early symptoms under various osmotic and chemical stress conditions, *Physiol Plant*, 88 (1993) 590.