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Changes in the Activities of Anti-Oxidant Enzymes during Exposure of Intact Wheat Leaves to Strong Visible Light at Different Temperatures in the Presence of Protein Synthesis Inhibitors

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Changes in activities of the enzymes involved in the metabolism of active oxygen species were followed in homogenates prepared from wheat leaves (*Triticum aestivum* L.) exposed to strong visible light (600 W m^{-2}). The activities of superoxide dismutase (SOD), ascorbate peroxidase, and monodehydroascorbate reductase increased significantly on prolonged illumination of the leaves, indicating an increase in the rate of generation of active oxygen species. This increase was further exacerbated when high light stress was combined with low temperature (8°C). Our results indicate that the increase in activities of SOD and ascorbate peroxidase involved de novo protein synthesis that was sensitive to the nuclear-directed protein synthesis inhibitor cycloheximide. The activity of catalase, on the other hand, decreased on exposure to strong light, which could be due to its photolability, particularly at lower temperatures. Ascorbate and total carotenoid contents also increased on light treatment of the leaves. The induction of the enzymes except for catalase and increase in the levels of ascorbate and total carotenoids in response to the stress conditions indicate that they play an important role in the protection of higher plants from the damaging effects of toxic active species.

Oxidative stress, which arises from the deleterious effects of reduced oxygen species, is an inevitable phenomenon in green plants (McKersie et al., 1988; Steinberg and Rabinowitch, 1991; Mishra and Singhal, 1992). The presence of an active oxygen-generating system in the plants together with the high percentage of polyunsaturated lipids, particularly in the thylakoid membrane, make them susceptible to oxidative injury (Halliwell, 1984). The active oxygen species can be formed in plants either by direct transfer of excitation energy from photosensitized Chl to oxygen or from single-electron reduction of dioxygen (Kaiser, 1987; Cornic and Briantais, 1991). Two main forms of active oxygen, O_2^- (and its products) and $^1O_2$, are apparently involved in the initiation of photooxidative damage in higher plants. O_2^- has been detected in chloroplasts of water-stressed wheat (Price et al., 1989) and chilled spinach plants exposed to high irradiance (Hodgson and Raison, 1991). Although capable of oxidizing various cellular components directly, O_2^- is thought to exert most of its biologically damaging effects by initiating the generation of more reactive and destructive species (Halliwell and Gutteridge, 1989). O_2^- produced in the plants is dismutated either directly (nonenzymically) or via the enzyme SOD. In both cases, the dismutation results in the production of H_2O_2. Although less reactive than O_2^-, H_2O_2 can react with O_2^- in the presence of iron salts and transition metals to form $OH^·$ free radicals. This is one of the most reactive species in biological systems, with a very short lifetime, and may attack and damage almost every molecule in the living cell (Halliwell, 1984).

During the course of evolution, the higher plants have developed an efficient defense system that helps them to survive under environmentally adverse conditions. The protective system of higher plants, through which they survive the oxidative stress, is composed of SOD, ascorbate peroxidase, catalase, MDHAR, DHAR, and GSH reductase (Asada and Takahashi, 1987; Schoner and Krause, 1990). Several anti-oxidants such as ascorbate, GSH, α-tocopherol, carotenoids, flavonoids, etc. that are present in higher plants contribute to the protective system against oxidative stress by deactivation of active oxygen species in multiple ways (Knox and Dodge, 1985; Asada and Takahashi, 1987; Chauhan et al., 1992). Depending upon the efficiency of the anti-oxidant defense systems, plants may differ in resistance to activated oxygen (Krause, 1988).

Under physiological conditions, plants are well adapted for minimizing damage that could occur from misuse of photosynthetic excitation energy. The levels of active oxygen species, which are efficiently removed by natural defense mechanisms of the plants, are kept low; however, oxygen toxicity may arise under environmental stress such as high light and low temperature when the production of these species exceeds the scavenging capacity of the natural defense system (Krause, 1988).

In this paper, we report the changes in the activities of the enzymes SOD, ascorbate peroxidase, catalase, MDHAR, and DHAR involved in the scavenging of active species generated during strong illumination of intact wheat leaves (*Triticum*

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Abbreviations: CAP, chloramphenicol; CHI, cycloheximide; DCIP, 2,6-dichlorophenol-indophenol; DHAR, dehydroascorbate reductase; MDHAR, monodehydroascorbate reductase; O_2^-, superoxide anion radical; $^1O_2$, singlet oxygen; $OH^·$, hydroxyl free radical; SOD, superoxide dismutase.

aestivum) at 19° and 8°C. We have used protein synthesis inhibitors to examine whether the changes in enzyme activities under stress conditions involve de novo synthesis. In addition, a quantitative analysis of ascorbate and pigment content was also undertaken in the control and stressed leaves.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Wheat seeds (*Triticum aestivum* L. var HD 2329, Indian Agricultural Research Institute) were surface sterilized with 0.1% (w/v) $HgCl_2$ solution and were grown on vermiculite supplemented with half-strength Hoagland solution in a plant growth chamber at 25°C under a 14-h photoperiod. Light intensity during growth was 75 W m^{-2}. Six- to 8-d-old seedlings were used for all the experiments.

Light Treatment

Wheat seedlings were placed in a glass tray filled with water and exposed to white light provided by a 1000-W tungsten-halogen lamp. A trough of transparent plexiglass (8 cm in depth) was placed between the lamp and the seedlings; water was continuously circulated through this trough to protect the seedlings from the heat generated by the lamp. Light treatments to the seedlings were carried out at 19° and 8°C. The average light intensity at the upper leaf surface was 600 W m^{-2}.

Light treatments were also carried out after inhibition of protein synthesis in intact leaves. Inhibition of nuclear- and chloroplast-directed protein synthesis was achieved by keeping the seedlings in CHI (10 mg L^{-1}) and CAP (200 mg L^{-1}) solutions, respectively, for 12 h prior to illumination (Filimonov et al., 1990). Plants kept under normal light intensity (75 W m^{-2}) at 19 and 8°C for 20 h were used as controls, and all the results were compared accordingly.

Assay of Enzyme Activities

Washed leaves (10–12 g) were homogenized in a mortar and pestle under ice-cold conditions in 20 mL of 50 mM potassium phosphate buffer, pH 7.0, 0.25% (w/v) Triton X-100, and 1.0% (w/v) insoluble PVP phosphate. The homogenate was filtered through eight layers of cheesecloth and the filtrate was used for enzyme assays. Activities of all the enzymes were expressed on the basis of Chl content because it remained unchanged after illumination.

Catalase (EC 1.11.1.6) was assayed at 25°C in 3 mL of reaction mixture containing potassium phosphate buffer, pH 7.0, 11 mM H_2O_2, and leaf homogenate equivalent to 20 μg of Chl. Activity was determined by UV spectrophotometry at 240 nm by measuring the time required for a decrease in *A* from 0.45 to 0.40 (Aebi, 1983).

SOD (EC 1.15.1.1) was assayed at 25°C following the reduction of Cyt *c* at 550 nm according to McCord and Fridovich (1969) with the modification by Schoner and Krause (1990). The reaction was performed in a total volume of 3 mL containing 50 mM potassium phosphate, pH 7.8, 0.1 mM EDTA, 18 μM Cyt *c*, 0.1 mM xanthine, and leaf homogenate equivalent to 20 μg of Chl. The reaction was initiated by addition of xanthine oxidase to produce a rate of Cyt *c* reduction corresponding to 0.025 *A* units per min in the assay mixture without leaf homogenate. One unit of SOD was defined as the amount of enzyme that inhibited the rate of Cyt *c* reduction by 50% under the specified conditions.

Ascorbate peroxidase (EC 1.11.1.7) activity was determined by following the oxidation of ascorbate as a decrease in A_{290} (2.8 mM^{-1} cm^{-1}) (Nakano and Asada, 1981). Two milliliters of ascorbate was added to the extraction medium to prevent the inactivation of the enzymes. The assay was carried out at 20°C in a reaction mixture containing 50 mM potassium phosphate, pH 7.0, 0.1 mM EDTA, 0.5 mM sodium ascorbate, 0.1 mM H_2O_2, and leaf homogenate equivalent to 20 μg of Chl. The change in *A* was recorded from 10 to 30 s after addition of H_2O_2. Correction was done for the oxidation of ascorbate by H_2O_2 in the absence of the leaf homogenate.

MDHAR (EC 1.6.5.4) was assayed at 25°C according to Hossain et al. (1984) in a reaction mixture containing 50 mM Tris-HCl, pH 7.6, 0.125% (w/v) Triton X-100, 0.2 mM NADH, 2.5 mM ascorbate, 5 μg of ascorbate oxidase, and leaf homogenate equivalent to 20 μg of Chl. The reaction was followed by the decrease in A_{340} due to NADH oxidation.

DHAR (EC 1.8.5.4) activity was assayed according to Nakano and Asada (1981) by following the increase of A_{265} (14 mM^{-1} cm^{-1}) as dehydroascorbate was reduced to ascorbate. The reaction was carried out at 25°C in a final volume of 2 mL containing 20 mM Tricine-KOH, pH 7.0, 0.1 mM EDTA, 3 mM reduced GSH, 2 mM dehydroascorbate, and leaf homogenate equivalent to 20 μg of Chl. Correction was made for low *A* change at 265 nm resulting from the formation of oxidized GSH and for the nonenzymic reduction of dehydroascorbate by reduced GSH. The rate of nonenzymic reduction of dehydroascorbate by reduced GSH was 11 μmol mL^{-1} h^{-1}.

Extraction and Determination of Ascorbate Content

Ascorbate was extracted from the control and stressed leaves as described by Franke (1955). Samples equivalent to 1 g of leaves were ground in a mortar and pestle with some quartz sand and NaCl in 2 mL of freshly prepared 10% (v/v) metaphosphoric acid. The resulting slurry was diluted with distilled water and centrifuged at 4000*g* for 5 min. The volume of the supernatant was adjusted to 10 mL with distilled water to give a final concentration of 2% (v/v) metaphosphoric acid. The extract was kept in the dark on ice until use.

Ascorbate content was determined spectrophotometrically at 524 nm (Tonumura et al., 1978) by measuring the reduction of DCIP by a portion of the sample (Hughes, 1956). An aliquot of 0.5 mL of extract, diluted up to 5-fold with 2% (v/v) metaphosphoric acid, was mixed with 1 mL of citrate-phosphate buffer, pH 2.3, and 1 mL of aqueous solution of DCIP (30 mg L^{-1}). *A* was recorded against a blank containing 2% (v/v) metaphosphoric acid instead of the sample after 30 s of DCIP addition. The amount of ascorbic acid was calculated by reference to a standard curve.

Pigment Estimation

Chl and total carotenoid contents in the 80% (v/v) aqueous acetone extracts of control and stressed leaves were determined spectrophotometrically according to Arnon (1949).

RESULTS AND DISCUSSION

The results of an investigation on the effects of strong visible light at different temperatures on enzymes and other systems involved in processing activated oxygen species in intact wheat leaves are discussed in the following sections.

SOD

SOD catalyzes the dismutation of O_2^- to H_2O_2 and O_2 (McCord and Fridovich, 1969; Monk et al., 1989). A gradual increase in SOD activity was observed in the extracts of illuminated leaves. The activity increased by about 1.5-fold after exposure of the leaves to 600 W m^{-2} for 20 h at 19°C (Fig. 1a). Light treatment of the leaves for 20 h at 600 W m^{-2} at 8°C caused a 2.9-fold increase in the activity (Fig. 1). This increase in activity might signify the production of O_2^- during light treatment of the leaves, particularly at low temperature. The production of O_2^- by thylakoids has previously been reviewed (Elstner, 1982, 1987; Robinson, 1988). When normal pathways and acceptors of photosynthetic electron transport are restricted, O_2 is reduced in the Mehler reaction, providing a pathway for the removal of excess electrochemical energy from the thylakoids (Marsho et al., 1979; Furbank and Badger, 1983; Furbank et al., 1983). In this reaction, O_2 is reduced initially to O_2^- and subsequently to H_2O_2 (Asada et al., 1974; Badger, 1985; Robinson, 1988). Under these conditions, restricted carbon metabolism would lead to a depletion of the natural electron acceptor $NADP^+$, thereby promoting the generation of O_2^- via Fd autooxidation (Schoner and Krause, 1990). Moreover, at low temperature, the concentration of active oxygen species is likely to increase in plants exposed to strong light as the repair processes are slowed down (Oquist et al., 1987).

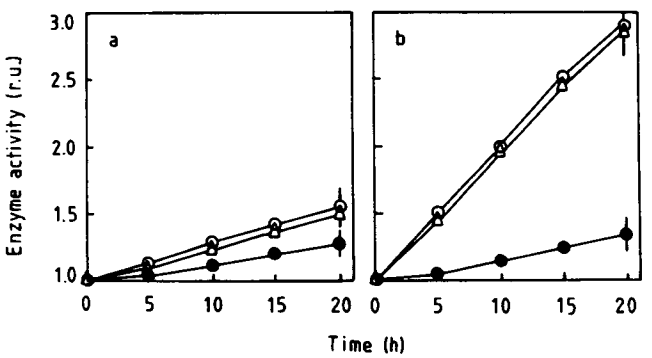

Figure 1. Changes in SOD activity in leaf extracts prepared from unstressed and light-treated leaves. Leaves were illuminated with 600 W m^{-2} at 19° (a) and 8°C (b) for different periods of time without any addition (○) or in the presence of 10 mg L^{-1} of CHI (●) or 200 mg L^{-1} of CAP (△). The seedlings were immersed in CHI and CAP solutions for 12 h prior to illumination. The activities are expressed in fold of the initial rate. Each point represents the mean of three separate sets of experiments. The SD is shown only at 20 h for clarity; the error is approximately the same percentage of the average value at other points. No significant changes in activities were observed in controls run parallel under similar conditions at normal light intensity (75 W m^{-2}). The activity in the homogenates of unstressed leaves was 72 ± 4.6 units mg^{-1} Chl.

High SOD activity has been linked with stress tolerance in plants that survive treatments likely to enhance the production of O_2^- (Tsang et al., 1991; Bowler et al., 1992). Increase in the activity has been reported during exposure of cold-acclimated spinach plants to low-temperature and high-irradiance stress (Schoner and Krause, 1990), drought conditions (Dhindsa and Matowe, 1981; Burke et al., 1985), and chilling of *Chlorella ellipsoidea* (Clare et al., 1984). Tanaka and Sugahara (1980) have suggested that O_2^- produced during SO_2 fumigation of poplar leaves induced the synthesis of SOD. A new Cu/Zn-SOD isoenzyme was also detected in protein extracts from cold-hardened spinach plants (Schoner et al., 1990). We presume that a similar kind of induction of SOD biosynthesis might be occurring in our experiments during illumination of leaves with strong light, particularly at low temperature.

Illumination of CAP-treated leaves at both the temperatures did not result in any further significant change in activity and followed a pattern close to that in which protein synthesis was not inhibited (Fig. 1). Okada et al. (1991) have suggested that CAP acts as an autooxidizing electron acceptor of PSI and may participate in the production of active oxygen species resulting in the inhibition of photosynthesis. However, no such side effects of CAP were observed in our experiments, in accord with the observations of Tyystjarvi et al. (1992). Inhibition of nuclear-directed protein synthesis by CHI resulted in a marked decrease in SOD induction on illumination of leaves at both 19° and 8°C (Fig. 1). Inhibition of SOD induction on light treatment of CHI-treated leaves indicates that the increase in activity was due to biosynthesis of new enzyme directed by the nuclear genome. The precise role of O_2^- in the induction of SOD during light treatment is not clear; however, it cannot be ruled out that an increase in O_2^- may trigger the synthesis of new enzyme, since the amount of SOD present under normal conditions may not be sufficient for the higher concentrations of active species produced during light treatment. The mechanism by which the synthesis of SOD is triggered remains to be established. The increase in SOD activity could be a manifestation of the defense system providing stability to the photosynthetic apparatus to protect it from damage due to strong illumination (Clare et al., 1984).

Ascorbate Peroxidase

The rapid removal of H_2O_2 produced by SOD is important if the generation of highly destructive $OH^\cdot$ is to be avoided. Ascorbate peroxidase is an important enzyme that scavenges H_2O_2 in higher plant chloroplasts (Asada, 1992). H_2O_2 is the first stable product of both monovalent and divalent reduction of molecular oxygen. However, no divalent reduction of O_2 has been observed in normal chloroplasts, and it has been suggested that most of the H_2O_2 in chloroplasts is produced through the dismutation of O_2^- catalyzed by SOD (Asada

and Takahashi, 1987). Ascorbate peroxidase activity increased on illumination of leaves by 1.3-fold with 600 W m^{-2} at 19°C for 20 h (Fig. 2a), whereas similar treatment increased it by 1.8-fold at 8°C (Fig. 2b). The increase in ascorbate peroxidase activity might be due to an increase in H_2O_2 production during light treatment of the leaves. The greater increase in activity during illumination at 8°C is consistent with the proposal of increased rates of active oxygen species generation during light treatments at chilling temperatures (Oquist et al., 1987).

Comparatively slower increase in ascorbate peroxidase activity was observed at both temperatures on light treatment of leaves infiltrated with CHI (Fig. 2). As in the case of SOD, CAP was inefficient in retarding the increase in activity on light treatment (Fig. 2). These results indicate that the increase in ascorbate peroxidase activity during light treatment involved biosynthesis of this nuclear-encoded enzyme. Tanaka et al. (1985) have also demonstrated an increase in the ascorbate peroxidase activity during O_3 fumigation and concluded that H_2O_2, which accumulates in O_3-fumigated leaves, might induce the synthesis of this enzyme. Incubation of leaf discs with low concentrations of paraquat has already been shown to stimulate the activity of ascorbate peroxidase in pea (Gillham and Dodge, 1984). Thus, it seems likely from our observations that the increased production of H_2O_2 during illumination may lead to an increased rate of ascorbate peroxidase biosynthesis.

Catalase

Catalase, which is localized in the peroxisomes of higher plants, functions in the decomposition of H_2O_2, which is also produced outside the chloroplasts by the H_2O_2-generating oxidases present in the peroxisomes (Tolbert, 1971). Despite its restricted localization, it may play a significant role in defending against oxidative stress, since H_2O_2 can readily diffuse across the membranes (Bowler et al., 1992). Illumination of leaves at 19° and 8°C resulted in a decrease in

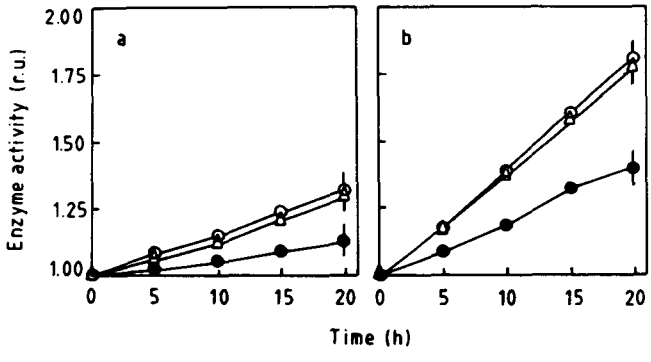

Figure 2. Changes in ascorbate peroxidase activity in leaf extracts prepared from unstressed and light-treated leaves. Leaves were illuminated with 600 W m^{-2} at 19° (a) and 8°C (b) for different periods of time without any addition (○) or in the presence of 10 mg L^{-1} of CHI (●) or 200 mg L^{-1} of CAP (△). Conditions were as described in the legend to Figure 1. The activity in the homogenates of unstressed leaves was 300 ± 32 μmol mg^{-1} Chl h^{-1}.

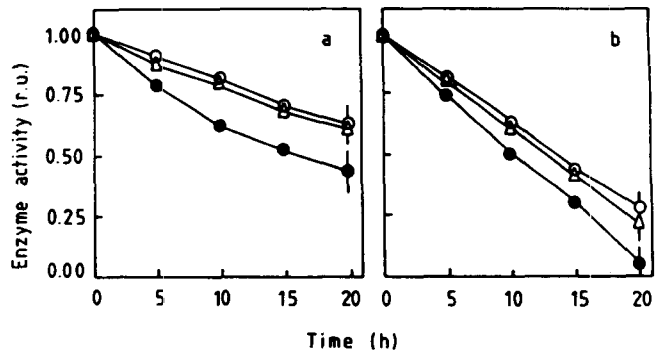

Figure 3. Changes in catalase activity in leaf extracts prepared from unstressed and light-treated leaves. Leaves were illuminated with 600 W m^{-2} at 19° (a) and 8°C (b) for different periods of time without any addition (○) or in the presence of 10 mg L^{-1} of CHI (●) or 200 mg L^{-1} of CAP (△). Conditions were as described in the legend to Figure 1. The activity in the homogenates of unstressed leaves was 74,000 ± 8,100 μmol mg^{-1} Chl h^{-1}.

catalase activity in the leaf homogenates (Fig. 3). Exposure of the leaves to 600 W m^{-2} for 20 h at 19°C resulted in a 30% loss of catalase activity (Fig. 3a), whereas the activity was inhibited by 72% after the same treatment at 8°C (Fig. 3b). No significant change in the activity was observed in extracts of the leaves kept under normal light intensity (75 W m^{-2}) at either temperature for 20 h. These results suggest that the inactivation of catalase observed in our experiments may be mediated through light absorption by both the enzyme-bound heme groups and chloroplast pigments and it is also dependent on the presence of oxygen (Cheng et al., 1981). It has been suggested that this photoinactivation is caused by the active oxygen species and organic peroxides (Elstner, 1982; Feierabend and Kemmerich, 1983; Takahashi and Asada, 1983; Feierabend and Engel, 1986).

The loss of catalase activity was pronounced in the presence of CHI at both 19° and 8°C (Fig. 3). The presence of CAP during illumination did not result in any significant loss of activity at either temperature. The changes in activity on illumination of CAP-treated leaves were similar to those observed in the absence of any protein synthesis inhibitor.

When plants are not exposed to any stress condition, concomitant resynthesis of catalase compensates for the loss of catalase and maintains a constant level in light (Feierabend and Engel, 1986). However, when plants are exposed to strong light, degradation of catalase exceeds the capacity for repair; hence, an apparent loss of activity is observed. Moreover, at chilling temperatures, the rate of protein synthesis is retarded and the plants might not cope with the fast degradation of catalase, resulting in a greater loss of activity. The maximum inhibition of catalase activity in the presence of CHI may indicate an impairment of de novo nuclear-directed synthesis of catalase.

MDHAR

Monodehydroascorbate radicals are produced in the chloroplasts by enzymic reaction through ascorbate peroxidase

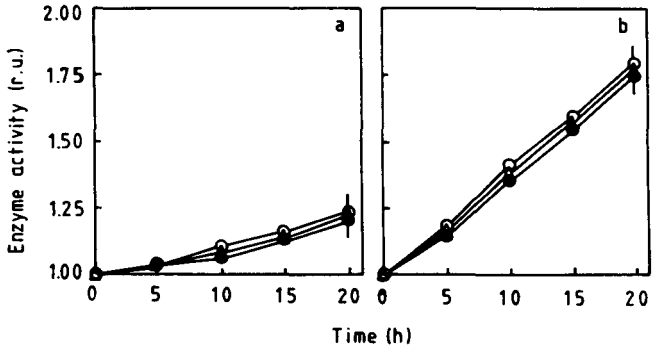

Figure 4. Changes in MDHAR activity in leaf extracts prepared from unstressed and light-treated leaves. Leaves were illuminated with 600 W m^{-2} at 19° (a) and 8°C (b) for different periods of time without any addition (O) or in the presence of 10 mg L^{-1} of CHI (●) or 200 mg L^{-1} of CAP (Δ). Conditions were as described in the legend to Figure 1. The activity in the homogenates of unstressed leaves was 87 ± 9 μmol mg^{-1} Chl h^{-1}.

and by nonenzymic reactions such as oxidation of ascorbate by O_2^- and $OH^\cdot$ (Nakano and Asada, 1981; Asada and Takahashi, 1987). The ascorbate-peroxidase-catalyzed reaction has been suggested as the major source of monodehydroascorbate radical production in higher plant chloroplasts (Asada and Takahashi, 1987). MDHAR reduces the monodehydroascorbate radicals to ascorbate (Hossain et al., 1984), which is useful for the detoxification of active oxygen species (Asada and Takahashi, 1987) and is indirectly required for the activities of several other enzymes such as ascorbate peroxidase (Nakano and Asada, 1987). MDHAR activity in our experiments increased to 1.2- and 1.8-fold during light treatment of leaves at 600 W m^{-2} at 19° and 8°C, respectively (Fig. 4). Because the enzyme MDHAR is known to be regulated by its metabolites (Hossain et al., 1984), an increase in its activity might be correlated with increased production of its substrate, monodehydroascorbate radicals. The protein synthesis inhibitors CHI and CAP did not have any significant effect on the rate of increase in MDHAR activity during illumination of leaves at both 19° and 8°C (Fig. 4). This indicates that the increase in activity during light treatment of leaves did not involve any de novo synthesis of the enzyme.

DHAR

DHAR is suggested to be involved in the H_2O_2 detoxification system in chloroplasts (Dipierro and Borraccino, 1991). A gradual increase in DHAR activity was observed on light treatment of leaves at 19° and 8°C (Fig. 5). Unlike other enzymes in the present study, its activity on illumination of leaves at 8°C was not significantly different than that at 19°C (Fig. 5). Dehydroascorbate is produced nonenzymically in plants by disproportionation of monodehydroascorbate radicals (Hossain and Asada, 1984; Asada and Takahashi, 1987). Because the production of dehydroascorbate is nonenzymic, it is unlikely to be affected by the activities of other enzymes involved in the reduction of ascorbate. The function of DHAR is in recycling to the ascorbate pool those monodehydroascorbate molecules that have escaped the MDHAR reaction and converted to dehydroascorbate (Asada and Takahashi, 1987; Schoner and Krause, 1990). It is also evident from Figure 5 that neither CHI nor CAP could further alter the DHAR activity.

Ascorbate

An increase in ascorbate content of the leaves was observed during illumination. The increase was significantly more on light treatment at 8°C in comparison with that at 19°C (Fig. 6). Ascorbate content increased by about 1.2-fold after 20 h of exposure of the leaves to 600 W m^{-2} at 19°C, whereas the same treatment at 8°C resulted in a 1.8-fold increase. The increase in ascorbate content of illuminated leaves may be related to the increase in MDHAR and DHAR activities, since these enzymes are involved in the recycling of ascorbate from its oxidation products generated through the ascorbate-peroxidase-catalyzed reaction (Shigeoka et al., 1979; Hossain et al., 1984; Hossain and Asada, 1984, 1985). Besides this, the increase in ascorbate content may also be due to higher rates of ascorbate synthesis in light (Hossain and Asada, 1984). Ascorbate functions in several enzymic and nonenzymic systems that scavenge free radicals, remove peroxide, and quench $^1O_2$ (Hossain and Asada, 1984; Larson, 1988), and its role has been studied extensively in relation to environmental stress factors such as frost, chilling, and drought (Levitt, 1972; Chinoy, 1984). Thus, the increase in ascorbate content in leaves may signify one of the mechanisms evolved by the plants for protection against reactive oxygen species generated during illumination, particularly at low temperatures.

Photosynthetic Pigments

Upon absorption of photons, Chl enters the excited singlet state, which normally leads to photochemistry. However, under excessive light, accumulating Chl molecules in the

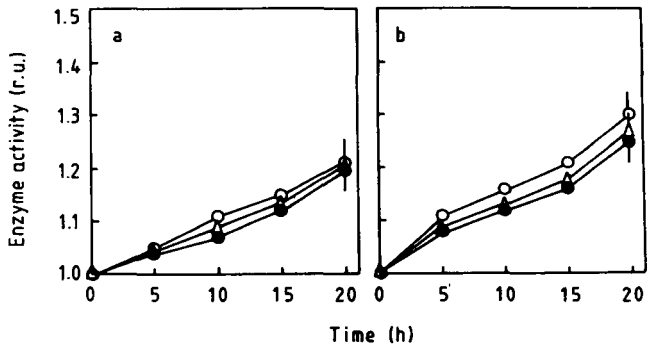

Figure 5. Changes in DHAR activity in leaf extracts prepared from unstressed and light-treated leaves. Leaves were illuminated with 600 W m^{-2} at 19° (a) and 8°C (b) for different periods of time without any addition (O) or in the presence of 10 mg L^{-1} of CHI (●) or 200 mg L^{-1} of CAP (Δ). Conditions were as described in the legend to Figure 1. The activity in the homogenates of unstressed leaves was 43 ± 6 μmol mg^{-1} Chl h^{-1}.

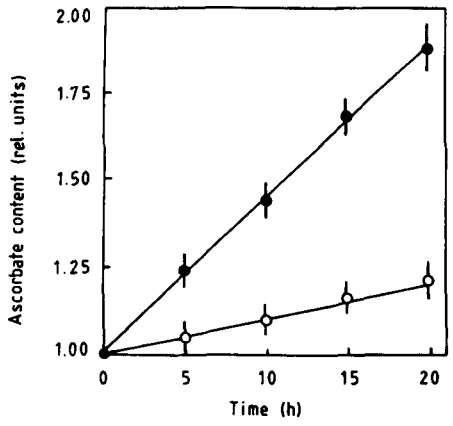

Figure 6. Changes in ascorbate content in leaf extracts prepared from unstressed and light-treated leaves. Leaves were illuminated with 600 W m^{-2} at 19° (O) and 8°C (●) for different periods of time. Ascorbate content in extracts of unstressed leaves was 1.7 ± 0.08 μmol mg^{-1} Chl.

excited singlet state might enter the excited triplet state through intersystem crossing. The interaction of such Chl molecules in the excited triplet state with O_2 leads to the generation of $^1O_2$.

An increase in the total carotenoid content was observed on illumination of leaves at 19° and 8°C (Table I). It is apparent from Table I that there was about a 3-fold increase in the total carotenoid content of leaves illuminated at 8°C. We suggest that this increase in the carotenoid content could be associated with the increased production of $^1O_2$ during illumination. The results are also in agreement with a larger carotenoid pool in the plants grown under strong light (Demmig-Adams and Adams, 1992). Our results, therefore, indicate that the $^1O_2$ is also generated along with other oxyradicals during illumination of leaves. No significant change in Chl content in the leaf extracts was observed on light treatment at either temperature (Table I). These observations indicate that there is no involvement of photooxidation of photosynthetic pigments. The pigment content of the leaves was also not significantly affected by the presence of protein synthesis inhibitors CHI and CAP during light treatment. Photoinhibition of photosynthesis and photooxidation of Chl are known to be two different phenomena, and the former is attributed to the damage occurring without any detectable change in the bulk leaf Chl concentration.

The increased levels of enzymic activities of SOD, ascorbate peroxidase, MDHAR, and DHAR in response to high-light and low-temperature stress may be taken as evidence for an enhanced detoxification capacity of wheat leaves toward reactive oxygen species such as O_2^- or H_2O_2 that might be generated in the stressed leaves. This explanation confirms our observation that despite the severe stress of high light and low temperature, there was no pigment photooxidation in stressed leaves. This may imply that the leaves are not severely damaged and that they possess the ability to augment their anti-oxidant capacity during light treatment at low temperature. However, a significant inhibition of PSII-mediated electron transport activity and a reduction in Chl fluorescence parameters were observed on illumination of leaves at both the temperatures. This damage was further exacerbated in the presence of the chloroplast protein synthesis inhibitor CAP (data not shown). These reductions could be attributed to the photoinhibition damage to the leaves. Radicals derived from the photoreduction of O_2 could act as agents in at least part of photoinhibition of photosynthesis.

The increased defense mechanisms observed in the present study ensure the detoxification of increased levels of active species and presumably prevent the leaves from undergoing photooxidation damage and eventual death. Wheat leaves, when subjected to high-light stress and low temperature, also exhibit an ability for de novo synthesis of some of the antioxidant enzymes in response to prolonged illumination, which may be a manifestation of their natural defense system. Moreover, the mechanism by which the synthesis of these nuclear-encoded enzymes is triggered during high-light stress needs to be investigated further. It may also be added that the level of changes in enzyme activities observed in our studies on wheat leaves are larger in magnitude than those observed previously in spinach by Schoner and Krause (1990), which may be related to constitutive differences between the two species in generation or detoxification of harmful active species.

Table I. *Changes in the total Chl and total carotenoid contents in the extracts of leaves illuminated at 19° and 8°C with 600 W m^{-2} of white light*

Period of Illumination	Chl Content		Carotenoid Content	
	19°C	8°C	19°C	8°C
h	*mg mL^{-1}*		*mg mL^{-1}*	
0	40.3 ± 0.4	43.3 ± 1.3	13.7 ± 0.5	13.2 ± 0.7
20	39.3 ± 0.8	42.1 ± 1.7	22.3 ± 1.1	37.8 ± 0.6

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