Changes in activities of superoxide dismutase, peroxidase and catalase from leaves of *Impatiens flanaganiae* in response to light intensity

N. Lall, R.V. Nikolova and A.J.N. Bosa*

Department of Botany, University of Transkei, Private Bag X1, Umtata, Eastern Cape, 5100 Republic of South Africa

Received 27 April 1998; revised 19 January 1999

Impatiens flanaganiae Hemsl. is a plant species which preferentially grows in shady conditions at an average light intensity of 30–55 microeinsteins m^{-2} sec^{-1} in its natural environment and exhibits strong apical dominance. Previous studies have shown that lateral branching in these plants can be induced by exposing them to a higher light intensity (280 microeinsteins m^{-2} sec^{-1}) under controlled laboratory conditions for up to seven weeks. Prolonged exposure to these light conditions lead to abnormal morphological and biochemical changes probably as a result of photo-oxidative stress. Antioxidative enzymes such as, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), are known to be involved in protecting plants against oxidative stress. Changes in isoenzyme patterns of SOD, POD and CAT from leaves of *Impatiens flanaganiae* in response to different light intensities (30 and 300 microeinsteins m^{-2} sec^{-1}) were investigated over a period of nine weeks. Electrophoretic analysis indicated that the SOD and POD from crude extracts of *I. flanaganiae* leaves were composed of 5 and 10 distinct bands respectively, whereas catalase (CAT) appeared as a single band with low mobility. Four of the SOD's showed sensitivity to cyanide, suggesting they were copper-zinc containing. The fifth SOD band was found to be resistant to both cyanide and hydrogen peroxide and was identified as a manganese containing SOD. The SOD, POD and CAT isoenzymes were found to respond differentially to changes in light intensity. Activities of all SOD's and CAT increased under high light intensity whilst the increase in activities of some isoperoxidases was less pronounced. These results suggest a light dependent induction of SOD and CAT in leaves of *I. flanaganiae* probably in an attempt to protect the plants from the harmfull effect of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) radicals which are considered to be generated at higher levels under photo-oxidative stress. The induced isoperoxidases are considered to be indirectly involved in the regulation of plant growth.

Keywords: catatase (CAT), *I. flanaganiae*, light intensity, peroxidase (POD), photo-oxidative stress, superoxide dismutase (SOD).

* To whom correspondence should be addressed, (E-mail: AJNBOSA@getafx.utr.ac.za).

Introduction

The responses of green plants to a wide variety of unfavourable conditions (e.g. drought, temperature stress, herbicides, UV irradiation, intense light, etc.) have been studied in the past but it still remains an area of intense research (Bowler *et al.* 1992). Most of the injuries observed in plants exposed to stressful conditions are associated with oxidative damage at the cellular level due the overproduction of toxic oxygen species such as, superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) (Allen 1995).

Superoxide radicals are commonly produced in plants when electrons are misdirected and donated to oxygen (e.g. in chloroplast or mitochondrial electron transport chains), then are further converted into hydrogen peroxide by SOD. The accumulation of these Haber-Weiss reaction substrates under prolonged oxidative conditions could result in the production of extremely toxic hydroxyl radicals that are thought to be responsible for serious damage in cellular components. This can frequently lead to irreparable metabolic changes and cell death unless scavenging systems are operating. (Elstner 1982; Monk *et al.* 1989; Miyake *et al.* 1991; Scandalios 1993; Prasad 1997). Therefore, an important emphasis in the literature has been placed upon not only those mechanisms by which radicals are generated, but also on those by which radicals are removed or scavenged. The balance between the two processes is that they either protect cells from, or result in oxidative damage.

Photoproduction of various reactive oxygen species (ROS) is considered inevitable in photosynthetic organisms (Miyake *et al.* 1991). Therefore light, an important environmental factor, might in excess result in increased levels of ROS (Mishra *et al.* 1995). In shade plants the photosynthetic capacity is saturated at low-light intensity and is directly related to the high susceptibility of these plants to photo-inhibition and photo-oxidative damage. Photo-inhibition itself leads to changes in the levels of pigments such as carotenoids and chlorophyll and the subsequent production of highly ROS (Young & Britton 1990). Under normal conditions plants are well adapted for minimizing oxidative damage using numerous defence mechanisms both non-enzymatic and enzymatic (Tsang *et al.* 1991). It is well known that the combined action of non-enzymatic antioxidants such as ascorbate, glutathione and alpha-tocopherol as well as enzymatic antioxidants, such as, SOD, POD and CAT play a key role in controlling deleterious cellular reactions to a minimum.

The capacity of the antioxidative defence system in plants is often increased under stress which may alleviate the injurious effect of ROS generated in excess under environmentally adverse conditions (Bowler *et al.* 1989; Weckx & Clidsters 1996). However, if the response is not sufficient, radical production will exceed scavenging and ultimately lead to the disruption of metabolism and destruction of cellular components (Foyer *et al.* 1994; Orr & Sohal 1994).

The antioxidative enzymes SOD, POD and CAT found in all aerobic organisms examined, convert the potentially dangerous oxygen radicals; $O2^-$ and H_2O_2 to water and molecular oxygen, thus averting cellular damage (Scandalios 1993; Mishra *et al.* 1993; Foyer *et al.* 1994). Three distinct types of superoxide dismutase (SODs) based on their metal co-factor namely Cu/Zn-SOD, Mn-SOD and Fe-SOD, have been found in plants and are characterised with different sub-cellular localisation and *in vitro* sensitivity to KCN and H_2O_2 (Bowler *et al.* 1989). Cu/

Zn-SODs, sensitive to both inhibitors and Mn-SOD which is resistant to both inhibitors have been reported in most plant species studied (Del Rio *et al.* 1985; Bowler *et al.* 1989; Gidrol *et al.* 1994) while H_2O_2 sensitive Fe-SOD has been found in only a few plant species (Salin & Bridges 1982; Sevilla *et al.* 1987).

Hydrogen peroxide, is a bioproduct of SOD, which is scavenged by CAT and POD. CAT that is mostly localised in peroxisomes is considered to have a low affinity for H_2O_2. Ascorbate peroxidase (APOD), a Class I peroxidase found in the chloroplasts and cytosol, is mostly reported to be involved in detoxification of H_2O_2 produced in plant cells in response to various stresses (Cakmak *et al.* 1995). However, it is still unclear whether Class I POD is the only one responsible for stress related H_2O_2 consumption in plant cells. The 'classical' plant peroxidases (Class III) which differ in structure from APOD are also involved in the oxidation of a wide range of organic and inorganic substrates which act as hydrogen donors *in vitro* in the presence of H_2O_2 in plants. The role of different peroxidases with respect to plant growth, development and stress tolerance has been extensively studied but still remains unclear (Lagrimini *et al.* 1997; Cella & Carbonera 1997). There are reports on the changes in activities of antioxidative enzymes in response to high light stress (Schoner & Krause 1990; Cakmak & Marschner 1992). However, the results vary according to plant materials and treatment conditions employed. At present, there is still little information and understanding of the underlying molecular mechanisms for the mobilisation of defences in plants.

Our preliminary studies on optimizing the conditions for propagation of *I. flanaganiae* showed that initially exposing them to high light intensities for a short period of time could be beneficial for purposes of vegetative propagation. However, continuous propagation of these shade loving plants under these conditions led to some abnormal changes such as enhanced senescence, curling, necrosis, chlorosis and reduction of leaf expansion especially in newly developed leaves (Lall *et al.* 1997). Initiation of oxidative stress by high light intensities was considered to be the possible reason for the observed changes in *I. flanaganiae*. However, there have been no reports of changes in enzymes that are involved in the detoxification of active oxygen species that may arise in response to variation of the light regime in *I. flanaganiae*.

The purpose of this study was to investigate the iso-enzyme patterns of SOD, POD and CAT and their differential responses to light intensity in leaves of *I. flanaganiae*.

Materials and Methods

Plant propagation

Tubers of *I. flanaganiae* were collected from the Port St. Johns area of the Eastern Cape and were initially propagated in pots for two weeks as described by Lall *et al.* (1997).

The plants were randomised into two groups of 30 each, transferred to a growth room at 22–25°C with a relative humidity of 65% and exposed to a 16-hour photoperiod at different light intensities, over a period of nine weeks. Metal halide lamps were used as a light source. Plants from variant I were on average exposed to a high light intensity of about 300 microeinsteins m^{-2} sec^{-1} while plants of variant II were grown under shade cloth with a light intensity of 30 microeinsteins m^{-2} sec^{-1}. That is equivalent to the intensity found mid-canopy under forest conditions.

Preparation of enzyme extracts

Randomly selected leaves of *I. flanaganiae* of each variant were collected weekly and used for the preparation of enzyme extracts. Leaves were homogenised with 0.1 M phosphate buffer pH 7.8, containing 0.1 mM EDTA at a w:v ratio of 1:2 in mortar-pestle dish. Homogenates were filtered through 4 layers of muslin cloth and centrifuged at 12.000 g for 20 min. The entire procedure was performed at 4°C. The supernatants containing soluble proteins were then collected and stored at -80°C in 1 ml Eppendorf tubes. These small aliquots were used only once for electrophoretic analysis to avoid the effect of freezing and thawing of the samples that can negatively affect enzyme stability. Preliminary tests showed that extracts stored under these conditions retain their activity well.

The analysis for protein estimation was carried out according to Bradford (1976) using Bovine Serum Albumin as a standard.

Electrophoresis

Polyacrylamide gel electrophoresis of the enzyme extract for isoenzymes separation of SOD, POD and CAT was performed on 7.5% gel according to Davis (1964) using a Gel Hoefer SE 600 electrophoresis unit. Enzyme samples loaded on the gels were equalised on a protein basis.

The gels were run in the vertical dimension in a tank containing Tris-glycine buffer pH 8.3, with the cathode as an upper electrode and anode as a lower electrode. Electrophoresis was conducted at 340 volts, constant voltage at 4°C, for 3–4 hours until the tracking dye almost reached the bottom of the gel. Control gels, without samples, were run in parallel for the detection of possible artificial SOD activity caused by reagents.

The SOD was detected by the photochemical procedure of Beauchamp and Fridovich (1971). Different types of SOD; (Cu/Zn-SOD, Mn-SOD and Fe-SOD) were identified according to their sensitivity to specific inhibitors such as KCN (0.02 M) and H_2O_2 (0.005 M). Gels were stained for peroxidase activity according to Graham *et al.* (1965). Visualization of CAT activity on the gels was done according to Stuber *et al.* (1988).

Photographs of gels were taken immediately after the staining. The isozyme activity of all three enzymes SOD, POD, and CAT, was quantified by recording the transmittance of the gels using a Hoefer GS 300 densitometer. The GS365W Data System was used for acquisition, plotting and analysis of data obtained from the scanning densitometer.

Results

Isoenzyme analysis in the leaf extracts of *I. flanaganiae*

(A) Superoxide dismutase

Electrophoresis of enzyme extracts of *I. flanaganiae*, revealed that SOD activity of the crude extracts is composed of five distinct bands (Figure 1) which are numbered in order of increasing relative mobility.

Bands 2, 3, 4 and 5 showed sensitivity to both cyanide and H_2O_2 and could be classified as Cu/Zn- SODs. Bands 2 and 3 were totally inhibited by 0.02 M KCN and 0.005 M H_2O_2 while bands 4 and 5 were less inhibited by both the inhibitors (Table 1).

Band 1 with relatively low mobility was resistant to both the inhibitors indicating that this SOD probably belongs to the family of Mn containing superoxide dismutases. No Fe containing SOD was identified in this plant.

Four active SOD bands, 1, 2, 4 and 5 showed apparent quantitative differences between the two variants which were evident from the band area (Table 1 and Figure 1). An increase in the band area was recorded in all SODs from plants subjected to high light intensity. The highest band area reflecting high SOD activity was recorded for Cu/Zn containing SOD's (bands 2 and 4) in both variants but was more pronounced for variant I.

Qualitative differences in the SOD isoenzymes pattern between variants were also recorded. An additional band of relatively low area (band 3) was induced in variant I.

(B) Peroxidase

Electrophoretic analysis of enzyme extracts from leaves of *I. flanaganiae* shows that at least 10 peroxidase enzymes are

Table 1 Rf values and band areas of SOD isoenzymes in *I. flanaganiae*; variants I and II, and in the presence of inhibitors

		SOD + inhibitors band area				% Inhibition	
Bands	Rf	SOD		KCN	H_2O_2	KCN	H_2O_2
		Variant 1	Variant 2				
1	0.46	4278	2512	4270	4275	0	0
2	0.53	5581	2874	-	-	100	100
3	0.55	2241	-	-	-	100	100
4	0.63	5156	2628	901	1442	83	72
5	0.65	2960	1291	857	1621	71	45

present in the tissue. Bands 1, 2 and 3 with relatively low mobility were not so distinct and appeared like a diffused zone. Bands 6 and 7 were also with low intensity. Two isozymes, (bands 8 and 9) of Rf 0.68 and 0.74 respectively accounted for most of the peroxidase activity in the variants. The isozyme profiles of POD present in both the variants were similar, showing only quantitative differences between the variants (Figure 2). For example, the two peroxidase isoenzymes depicted by bands 8 and 9 showed a higher band area in variant I (8716 and 8118) than those same isoenzymes in variant II (5010 and 3818).

(C) CAT

Catalase was detected in all the variants as a single diffuse white band on a blue background with a Rf value of 0.14. Higher CAT activity represented by higher band area (16837) was recorded in plants exposed to intense light (variant I) than in plants from variant II (9389), (Figure 3).

Discussion and conclusion

Even though light is required for photosynthesis, exposure of plants to high light intensities leads to reduction of photosynthetic capacity owing to oxidative stress. Detoxifying enzymes have been reported as being induced by diverse stress conditions such as drought, air pollution, fungal attack, low temperature and high light (Marschner & Cakmak 1989; Karpinski *et al.* 1993).

Similarly, exposing the plants of *I. flanaganiae* to higher light

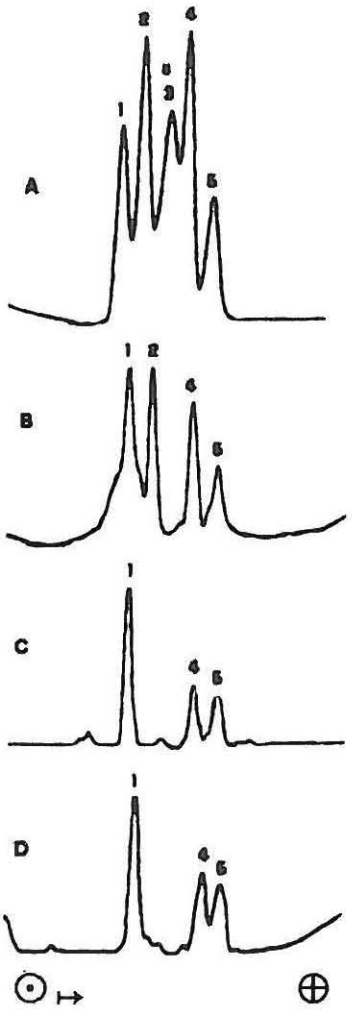

Figure 1 Densitometric scans of SOD from leaves of *I. flanaganiae* subjected to different light intensities for a period of 9 weeks (Graph A: variant I-300 microeinsteins m^{-2} sec^{-1}; and Graph B. variant II-30 microeinsteins m^{-2} sec^{-1}). Graph C. gels stained in the presence of 0.02M KCN; Graph D. gels stained in the presence of 0.005M H_2O_2).

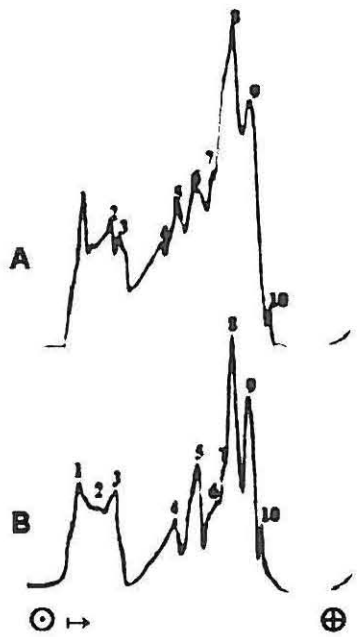

Figure 2 Densitometric scans of POD from leaves of *I. flanaganiae* subjected to different light intensities for a period of 9 weeks (Graph A: variant I-300 microeinsteins m^{-2} sec^{-1} and Graph B: variant II-30 microeinsteins m^{-2} sec^{-1}).

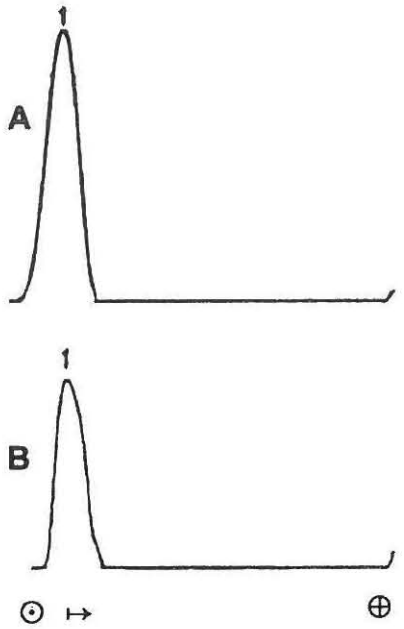

Figure 3 Densitometric scans of CAT from leaves of *I. flanaganiae* subjected to different light intensities for a period of 9 weeks: (Graph A variant I-300 microeinsteins m^{-2} sec^{-1} and Graph B: variant II-30 microeinsteins m^{-2} sec^{-1}).

intensities than in its natural environment resulted in a pronounced increase of SOD and CAT activity in the leaves. It has been documented that stress causes impairment of electron transport, and increased activity of enzymes of the H_2O_2 scavenging system probably as a consequence of superoxide radical generation in damaged chloroplasts (Elstner 1982).

SOD from leaves of *I. flanaganiae* shows a multiple pattern of five closely spaced bands. These were classified as Cu/Zn- and Mn-SODs according to their sensitivity to cyanide and H_2O_2 (Bowler *et al.* 1992). Four of the bands in leaves of *I. flanaganiae* that showed cyanide sensitivity are considered to be Cu/Zn-SODs. These SODs also exhibited a strong sensitivity to hydrogen peroxide indicating their vulnerability to this product of superoxide dismutation *in vivo* (Mavelli *et al.* 1983). H_2O_2 could be expected to accumulate at high levels if the activities of its scavenging enzymes are negatively affected by light induced oxidative stress. One band in *I. flanaganiae* could be classified as Mn-SOD because of its resistance to H_2O_2. The activation of this particular isoenzyme is thought to be important for maintaining SOD activity during light mediated stress episodes when Cu/Zn SODs might be inactivated due to elevated levels of H_2O_2 (Bowler *et al.* 1992).

The pronounced increase in SOD isoenzyme activity after exposure of *I. flanaganiae* to high light intensity is considered to be in response to oxidative stress induced by high light. Enzyme activity can increase significantly either as a result of *de novo* synthesis or by activation of enzymes that are already present (Van Assche and Clijsters 1990). The increase in SOD in the leaves of *Impatiens flanaganiae* appears to be as a result of both qualitative and quantitative changes in the isoenzyme pattern. An induction of O_2^- and H_2O_2 scavenging enzymes by high light is also reported for other plant species (Schoner & Krause 1990; Fuse *et al.* 1993) and could be considered an adaptational reaction in response to oxidative stress. It has been suggested that the induction of SOD under high light intensity is not a direct response to the light but most likely is a response to increased superoxide formation under stress. The higher SOD activity will result in more O_2^- being converted to H_2O_2 which is neutralized by POD and/or CAT.

The results of this study suggest light mediated induction of CAT activity in *I. flanaganiae*. Sensitivity of CAT to light was also documented by other studies (Mishra *et al.* 1995; Hertwig *et al.* 1992). The sharp increase in CAT activity recorded in *I. flanaganiae* plants from variant I was probably in response to the light-induced generation of higher levels of H_2O_2. This response could be important for the plants as it prevents the inactivation of H_2O_2 sensitive SODs. Similarly, stress-induced CAT seems to play a mojor role in inducing chilling tolerance in plants (Prasad 1997).

According to most studies, ascorbate peroxidase (APOD) which is found in the chloroplasts and cytosol, is mostly reported to be involved in detoxification of H_2O_2 produced in plant cells in response to various stresses (Cakmak *et al.* 1995). However, there are also reports that relate 'classical' peroxidases investigated in this study to stress tolerance in plants (Prasad 1997). Numerous peroxidase isoenzymes, between 10 and 20, displaying various degrees of reactivity towards different substrates have been reported in vascular plants. The differential expression of distinct isoenzymes is genetically determined and is considered to be specific to a particular cellular compartment or tissue. However, it has been difficult to assign a specific function to the indivudual isoenzymes due to the broad substrate specificity and the presence of many closely related isofroms (Cella & Carbonera 1997; Guzen 1997). This study revealed at least ten 'classical' POD isoenzymes in leaves of *I. flanaganiae* with only quantitative differences between the treatments, suggesting light mediated induction of some isoperoxidases. The role of these peroxidases with respect to IAA metabolism, plant growth and morphology has been studied but still remains unclear (Lagrimini *et al.* 1997; Cella & Carbonera 1997).

POD is known to exhibit IAA oxidase activity, its involvement in IAA degradation and thus the growth regulation of plants is considered. Our previous morphological studies of *I. flanaganiae* revealed that the length of plants kept under high light was reduced as compared to the plants exposed to low light (Lall *et al.* 1997). The reduction in the top growth of *I. flanaganiae* plants subjected to high light intensity could be related indirectly to the high activity of some of the POD isoenzymes.

From the present study it can be concluded that the leaves of *I. flanaganiae* contain mainly Cu/Zn SOD's and one manganese SOD. The study indicated a light-dependent induction of the antioxidative enzymes such as SOD and CAT in leaves of *I. flanaganiae* which is considered to be in response to photo-oxidative stress. This conclusion can be related to the fact that the induction of a defence mechanism is often triggered by co-ordinated mechanisms to control damage in stressed plants. The role of some isoperoxidases in IAA metabolism and subsequently reduced top growth in the plant grown under high light is also considered.

Acknowledgements

R.V. Nikolova acknowledges the University of Transkei, Umtata, and FRD, South Africa for the financial assistance of this study.

References

ALLEN, RD. 1995. Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiol.* 107: 1049–1054.

BOWLER, C., ALLIOTTE, T., LOOSE, M.D., MONTGU, M.V. & INZE, D. 1989. The induction of Mn-SOD in response to stress in *Nicotiana plumbaginifolia* Embo. *J* 8: 31–38.

BOWLER, C., MONTAGU, M. & INZE, D. 1992. Superoxide

dismutase, and stress tolerance. *Ann. Rev. of Plant Physiol.* 43: 83–116.

BRADFORD, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 48–254.

CAKMAK, I. & MARSCHNER, H. 1992. Magnesium deficiency and high light intensity enhance activities of SOD, ascorbate POD & glutathione reductase in bean leaves. *Plant Physiol.* 98: 1222–1227.

CAKMAK, I., ATLI, M., KAYA, R., EVLIYA, H. & MAIRSCHER, H. 1995. Association of high light and zinc deficiency in grapefiuit and Mandarin trees. *J. Plant Physiol.* 146: 355–360.

CELLA, R. & CARBONERA, D. 1997. Peroxidases and morphogenesis. *Plant Perox. New.* 10: 24–29.

DAVIS, B.J. 1964. Disc gel electrophoresis. *Ann. N. Y. Acad. Sci.* 121: 404–427.

DEL RIO, L.A., SANDALIO, L.M., YANEZ, J. & GOMEZ, M. 1985. Induction of a Manganese-containing superoxide dismutase in leaves of *Pisum sativum* L. by high nutrient levels of zinc and manganese. *J. Inorg. Biochem.* 24: 25–34.

ELSTNER, E.F. 1982. Oxygen activation, and oxygen toxicity. *Ann. Rev. Plant Physiol.* 33: 73–96.

FOYER, C.H., LELANDAIS, M. & KUNERT, K. 1994. Photo-oxidative stress in plants. *Phsiol. Plant.* 92: 696–717.

FUSE, T., IBA, K., SATOH, H. & NISHIMURA, M. 1993. Characterisation of a rice mutant having an increased susceptibility to light stress at high temperature. *Physiol. Plant.* 89: 799–804.

GIDROL, X., DEGOUSEE, N., YIP, S.F. & KUSH, A. 1994. Accumulation of reactive oxygen species and oxidation of cytokinin in germinating Soybean seeds. *Eur. J. Biochem.* 224: 21–28.

GRAHAM, R.C., LUNDHOLM, H. & KARNOVSKY, M.J. 1965. Cytochemical demonstration of peroxidase activity with 3-amino-9-ethylcarbazole. *J. Histochem. Cytochem.* 13: 50–152.

GUZEN, M. 1997. Seed Peroxidases. *Plant Perox. New.* 10: 30–33.

HERTWIG, B., STEB, P. & FEERA, J. 1992. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. *Plant Physiol.* 100: 1547–1553.

KARPINSKI, S., WINGSLE, G., KARPINSKI, B. & HALLGREN, I.E. 1993. Molecular responses to photo-oxidative stress in *Pinus sylvestris* L. *Plant Physiol.* 103: 1385–1391.

LAGRIMINI, M., GINGAS, V., FINGER, F., ROTHSTEIN, S. & LIU, T.T.Y. 1997. Characterisation of antisense transformed plants deficient in the Tobacco anionic peroxidases. *Plant Physiol.* 114: 1187–1196.

LALL, N., BOSA, A. & NIKOLOVA, R.V. 1997. Morphological characteristics of *Impatiens flanaganiae* Hemsl. Grown under different light conditions. *S. Afr. J. Bot.* 63: 216–222.

MARSCHNER, H. & CAKMAK, I. 1989. High light intensity enhances chlorosis and necrosis in leaves of Zn, K and Mg deficient bean (*Phaseolus vulgaris*) plants. *Plant Physiol.* 134: 308–315.

MAVELLI, I., CIRIOLO, M.R. & ROTILIO, G. 1983. Multiple electrophoretic variants of Cu, Zn superoxide dismutase as expression of the enzyme aging. *Biochem. Biophy. Res. Commun.* 117: 677–681.

MISHRA, N.P., MISHRA, R.K., & SINGHAL, G.S. 1993. Changes in the activities of antioxidant enzymes during exposure of intact wheat leaves to strong visible light at different temperatures in the presence of protein synthesis inhibitors. *Plant Physiol.* 102: 903–910.

MISHRA, N.P., FATMA, T. & SINGHAL, G.S. 1995. Development of antioxidative defense system of wheat seedlings in response to high light. *Physiol. Plant.* 95: 7–8.

MIYAKE, C., MICHIHATA, F. & ASADA, K. 1991. Scavenging of hydrogen peroxide in prokaryotic and eukaryotic algae: Acquisition of ascorbate peroxidase during the evolution of Cynobacteria. *Plant Cell Physiol.* 32(1): 33–43.

MONK, L.S., FAGERSTEDDT, K.V. & CRAWFORD, M.M., 1989. Oxygen toxicity and SOD as an antioxidant in physiological stress. *Physiol. Plant.* 76: 456–459.

ORR, C. & SOHAL, R.S., 1994. Extension of life-span by over-expression of superoxide dismutase and catalase in *Drosophila melanogaster. J. Science* 263: 1061–1192.

PRASAD, T.K., 1997. Role of catalase in inducing chilling tolerance in Pre-emergent maize seedlings. *Plant Plysiol.* 114: 1369–1376.

SALIN, M.L. & BRIDGES, M.L. 1982. Isolation and characterisation of an iron containing superoxide dismutase from Waterlily, Naphurteum. *Plant Physiol.* 69: 161–165.

SCANDALIOS, J.G. 1993. Oxygen stress and superoxide dismutase. *Plant Physiol.* 101: 7–12.

SCHONER, S. & KRAUSE, H.G. 1990. Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. *Planta* 180: 383–389.

SEVILLA, F., HELLIN, E., ALCARAZ, C.F. & DEL RIO, L.A. 1987. Induction of iron and manganese superoxide dismutases in *Citrus limonum* leaves by iron. *Plant Physiol. (Life Sci. Adv.).* 6: 133–139.

STUBER, C.W., WENDEL, J.F., GOODMAN, M.M. & SMITH, J.S.C. 1988. Techniques and scoring procedures for starch gel electrophoresis of enzymes for maize (*Zea mays* L.). Tech. Bull. 286. NC Agric. Res. Station, NC State University.

TSANG, W.T., BOWLER, C., HEROUART, D., CAMP, W.V., VILLARROEL, R.D., GENETELLO, C., MONTAGU, V. & INZE, D. 1991. Differential regulation of superoxide dismutase in plants exposed to environmental stress. *Plant Cell* 3: 783–792.

VAN ASSCHE, F. & CLLTSTERS, H., 1990. Effects of metals on enzyme activity in plants. Pl. Cell E,rvir. 13:195-206.

WECKX, J.E.J. & CLIJSTERS, H.M.M. 1996. Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. *Physiol. Plant.* 96: 506–512.

YOUNG, A. & BRITTON, G. 1990. Stress responses in plants: adaptation and acclimation mechanisms. Wiley-Liss, New York.