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# Changes in Hydrogen Peroxide Levels and Catalase Isoforms Expression are Induced with Freezing Tolerance by Absciscic Acid in Potato Microplants

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## 1. Introduction

There is evidence that abscisic acid (ABA) has a protective signaling role in freezing stress in plants (Kobayashi et al., 2008), including mosses (Minami et al., 2003). ABA signaling networks and their actions are not totally understood, but H<sub>2</sub>O<sub>2</sub> has been implicated as an intermediary in several ABA responses, where its roles include induction of the antioxidant system (Cho et al., 2009). Mora-Herrera & López-Delgado (2007), using *in vitro* microplants as employed in potato production programs, found freezing tolerance was enhanced by culture on ABA-containing medium. This ABA treatment tripled survival of a -6°C incubation in the cold-sensitive cv. Atlantic, while in the more cold-tolerant cv. Alpha, survival improved by two-thirds. In the ABA-treated microplants, they found the H<sub>2</sub>O<sub>2</sub>-scavenging enzyme ascorbate peroxidase increased in activity.

Stress tolerance in potato is growing in importance, as increases in potato production by developing countries greatly exceed other major crops (FAO, 2008). The present study used the microplant system to investigate effects of prolonged ABA treatment on catalase, another enzyme important in controlling cellular H<sub>2</sub>O<sub>2</sub>. Catalases are tetrameric, heme-containing oxidoreductases that dismutate H<sub>2</sub>O<sub>2</sub> to water and oxygen. In plants, their peroxisomal location coincides with the cellular site of H<sub>2</sub>O<sub>2</sub> generation by photorespiration or fatty acid  $\beta$ -oxidation (Feierabend, 2005)(Scheme 1). Evidence for catalase involvement in

these processes includes susceptibility of catalase mutants to photorespiration-promoting conditions (Queval et al., 2007), and catalase induction in nutrient stress conditions promoting fatty acid catabolism (Contento & Bassham, 2010). Catalases respond to a wide range of stresses (Du et al., 2008) and, most relevantly here, have been functionally implicated in low-temperature tolerance by transgenic experiments on rice (Matsumura et al., 2002). Moreover, there is evidence that catalase is an integral component of ABA-activated stress protection mechanisms (Xing et al., 2008).

Plant catalases occur in small gene families, whose differential expression reflects different roles (Feierabend, 2005). In *Arabidopsis*, *CAT2*, expressed in photosynthetic tissues (Du et al., 2008), is needed to cope with photorespiration (Queval et al., 2007). *Arabidopsis CAT1* is induced by treatments including cold and ABA (Du et al., 2008). Pharmacological and mutant studies have revealed that *CAT1* induction by ABA involves mitogen-activated protein kinase (MAPK) cascades, in which  $H_2O_2$  is involved (Xing et al., 2008). Among maize catalases, *CAT3* is a chilling-acclimation responsive gene in seedlings, and a long-standing example of regulation by  $H_2O_2$  (Prasad et al., 1994). Maize *CAT1* is highly expressed as seeds dehydrate in late embryogenesis, and its promoter has an ABRE (ABA-responsive) element, while  $H_2O_2$  was also implicated as a signal by Guan et al. (2000) and Zhang et al. (2006) showed *CAT1* induction by ABA in maize leaves involved MAPK cascades and  $H_2O_2$ .

In potato, previous studies have identified two, differentially expressed catalase genes (Santos et al., 2006). In photosynthesizing tissues, where photorespiration occurs, the principal one expressed was *CAT1*. Phylogenetic comparisons by Santos et al. (Santos et al., 2006) found potato *CAT1* was less similar to potato *CAT2* than to *Nicotiana CAT1* genes. Potato *CAT2* shares high identity with *N. plumbaginifolia CAT2*, characteristics of which include inducibility by stressful exposure to ultraviolet light, ozone or  $SO_2$  (Willekens et al., 1994). Consistent with an analogous role in stress conditions, potato *CAT2* was induced in plants suffering nematode or bacterial infection (Niebel et al., 1995). More recently, *CAT2* was found to be induced in potato leaves treated with  $H_2O_2$ , while *CAT1* was not (Almeida et al., 2005).

This study was undertaken with the hypotheses of catalase and  $H_2O_2$  involvement in ABA-induced freezing tolerance in potato microplants. Moreover, catalase isoforms were predicted to show differential patterns of expression and activity in this process.

## 2. Materials and methods

### 2.1 Microplant material

Virus-free microplants of *Solanum tuberosum* L. cv. Alpha and cv. Atlantic, from the Germplasm Bank of the National Potato Program of the National Institute for Forestry Agriculture and Livestock Research (INIFAP), Toluca, México, were micropropagated as nodal cuttings *in vitro* at 20 °C, following previous protocols (Mora-Herrera et al., 2005). In every experiment, 24 microplants were cultured per treatment, and pooled into samples to achieve the weight required for the particular measurement.

### 2.2 Freezing treatments

Microplants cultivated 28 d on medium with 10  $\mu$ M ( $\pm$ )-*cis*, *trans*-ABA (Sigma, USA), or as controls without ABA, were transferred to peat moss (in 3  $\times$  5 cm pots) pre-sterilized for 1 h

at 120 °C. These transplanted microplants were kept for 24 h at 20 °C under a 16 h photoperiod (fluorescent lights, 35  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 400-700 nm), to allow recovery from the stress of transplantation, prior to exposure to  $-6 \pm 1$  °C in darkness for 4 h, as previously (Mora-Herrera et al., 2005).  $\text{H}_2\text{O}_2$  and catalase measurements were performed immediately after this freezing incubation.

### 2.3 Determination of $\text{H}_2\text{O}_2$ content

$\text{H}_2\text{O}_2$  was measured by luminol-dependent chemiluminescence, as in Mora-Herrera et al. (2005), in 3 experiments, with 3 samples per treatment, and each assay replicated 6 times.

### 2.4 Quantification of catalase activity

Frozen shoot tissue (0.5 g) was powdered under liquid  $\text{N}_2$ , and extracted in 2 mL 50 mM potassium phosphate buffer (pH 7.2) containing 5 mM dithiothreitol, 1 mM ethylenediamine tetraacetic acid, and 1% polyvinylpyrrolidone. After clarification by centrifugation (11,000 g, 15 min, 4 °C), catalase activity (EC 1.11.1.6) was determined according to Aebi (1984). The total reaction mixture (3 mL) contained 20  $\mu\text{L}$  extract (100  $\mu\text{g}$  protein) and 30 mM  $\text{H}_2\text{O}_2$  in 50 mM sodium/potassium phosphate buffer (pH 7.0). The reaction was initiated by  $\text{H}_2\text{O}_2$  addition and followed by absorbance decrease at 240 nm (extinction coefficient 39.4  $\text{mM}^{-1} \text{cm}^{-1}$ ) every 20 s for 3 min, at 26 °C. Protein was determined using Bradford reagent. Catalase was measured in 3 experiments, each with 3 samples (assayed in triplicate) per treatment.

### 2.5 Catalase zymograms

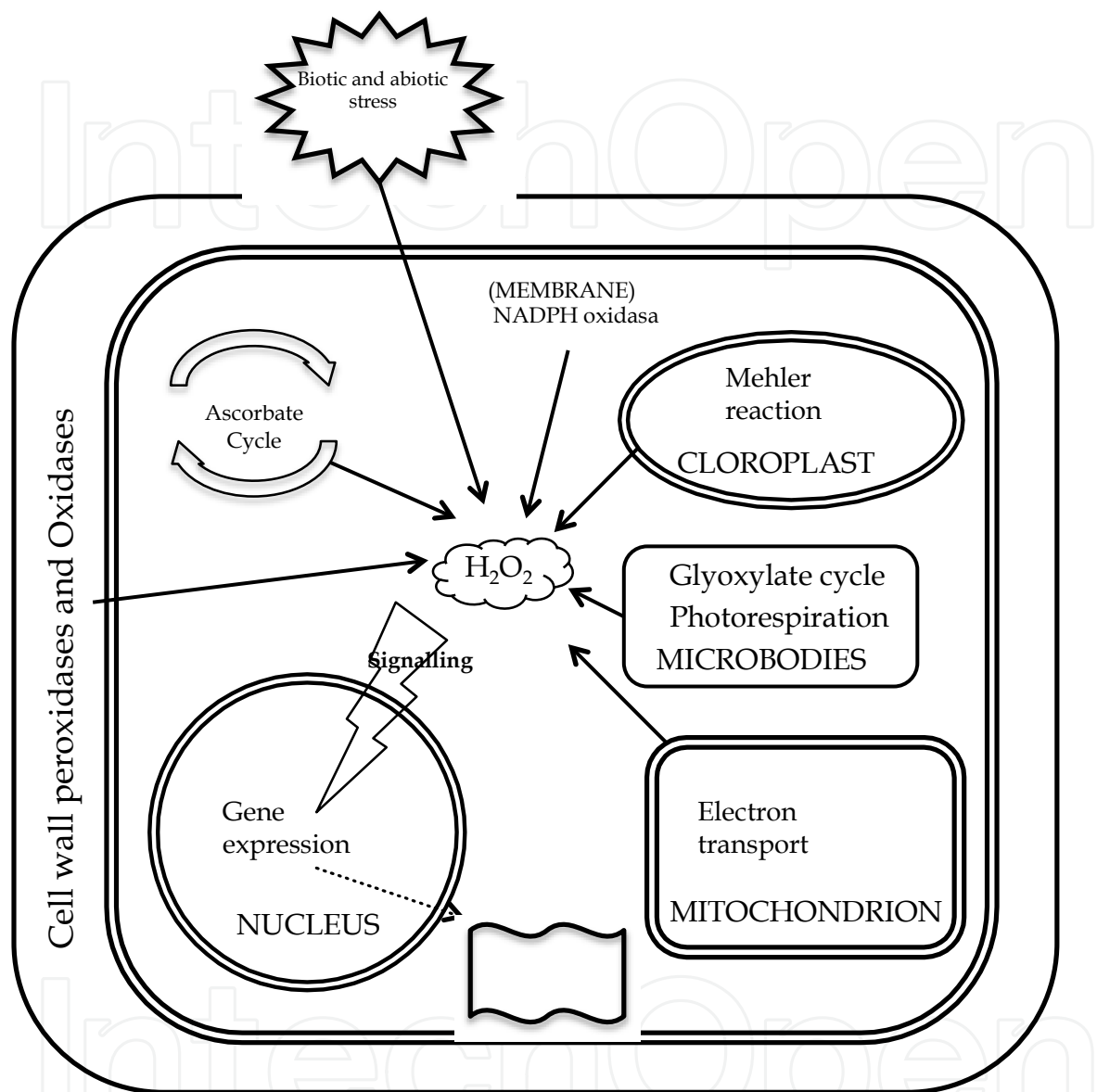
Enzymes were extracted by a similar method to Cruz-Ortega et al. (2002). Frozen tissue (0.1 g) was powdered in liquid  $\text{N}_2$ , then extracted in 100  $\mu\text{L}$  potassium phosphate buffer (pH 7.8, 1 mM ethylenediaminetetraacetate, 1 mM phenylmethanesulfonyl fluoride, 10 mM dithiothreitol, 2% polyvinylpyrrolidone). The extracts were clarified at 11,000 g (10 min, 4 °C). Non-denaturing polyacrylamide gel electrophoresis, as described by Ougham (1987), was performed for 18-20 h at 4 °C. Catalase activity staining used the ferricyanide method of Woodbury et al., 1971. The GE Healthcare Life Sciences HMW Native Marker Kit containing bovine liver catalase (232 kDa) was used as a gel marker. Results shown are representative of 6 experiments.

### 2.6 Analysis of transcripts by RT-PCR

Total RNA extractions used TRIzol isolation reagent, and treatment with DNase I (Invitrogen, USA). cDNAs were synthesized with Oligo(dT)<sub>12-18</sub> primer and SuperScript II reverse transcriptase (Invitrogen). PCR amplifications of potato *CAT1* and *CAT2* used the primers of Santos et al. (2006). As an internal quantitative control, potato actin (NCBI accession X55751) primers (forward, 5'-AGACGCCTATGTGGGAGATG-3'; reverse, 5'-GCGAGCTTTTCTTTCACGTC-3') were used. After 40 cycles at 52 °C, PCR products were electrophoresed in 1% agarose and visualized with ethidium bromide. Images were acquired by a gel documentation system (UVItec, UK), and relative transcript levels estimated with Quantity One v.4.6.5 software (Bio-Rad, USA). Results shown are representative of 4 experiments.

2.7 Statistical analysis

Statgraphics Plus v.5.0 (StatPoint Technologies, USA) was used for *t*-tests, and ANOVA with Tukey *post-hoc* tests (*P*< 0.05).



Scheme 1. H<sub>2</sub>O<sub>2</sub> is produced in chloroplasts via the Mehler reaction, photorespiration in peroxisomes , glyoxylate cycle, and via electron transport in mitochondria. Cell wall peroxidases and NADPH oxidases in the plasma membrane also can increase the H<sub>2</sub>O<sub>2</sub> production when the plant is under biotic or abiotic stress. The signaling role of H<sub>2</sub>O<sub>2</sub> is mediated by enzymatic antioxidants one of them is catalase.

3. Results

3.1 Effects of ABA on H<sub>2</sub>O<sub>2</sub> content of potato microplants

*In vitro* microplants were cultured for 28 d on MS medium supplemented with 10 μM ABA. In the study of Mora-Herrera & López-Delgado (2007), 10 μM was the highest ABA

concentration used, which gave greater improvements in freezing tolerance than lower concentrations. It also caused growth inhibition, but this did not detract from eventual growth and tuber yield of microplants transplanted to compost and glasshouse conditions (Mora-Herrera & López-Delgado, 2007). We investigated H<sub>2</sub>O<sub>2</sub> and catalase in cv. Alpha and Atlantic microplants at three stages: (a) after 28 d of culture in the presence (or absence) of ABA, (b) 24 h after transplanting from *in vitro* culture to compost, and (c) after 4 h of freezing (-6 °C) in microplants transplanted 24 h previously to compost.

Shoot H<sub>2</sub>O<sub>2</sub> contents were on average 24% higher in microplants (of either cv.) that had been cultured for 28 d on ABA-containing medium (Fig. 1). This ABA-induced elevation of H<sub>2</sub>O<sub>2</sub> contents persisted in microplants transplanted for 24 h to compost, and also after these transplanted microplants had been subjected to freezing (Fig. 1). It was also notable that freezing treatment also increased H<sub>2</sub>O<sub>2</sub>, by 23% on average in the transplanted microplants (Fig. 1).

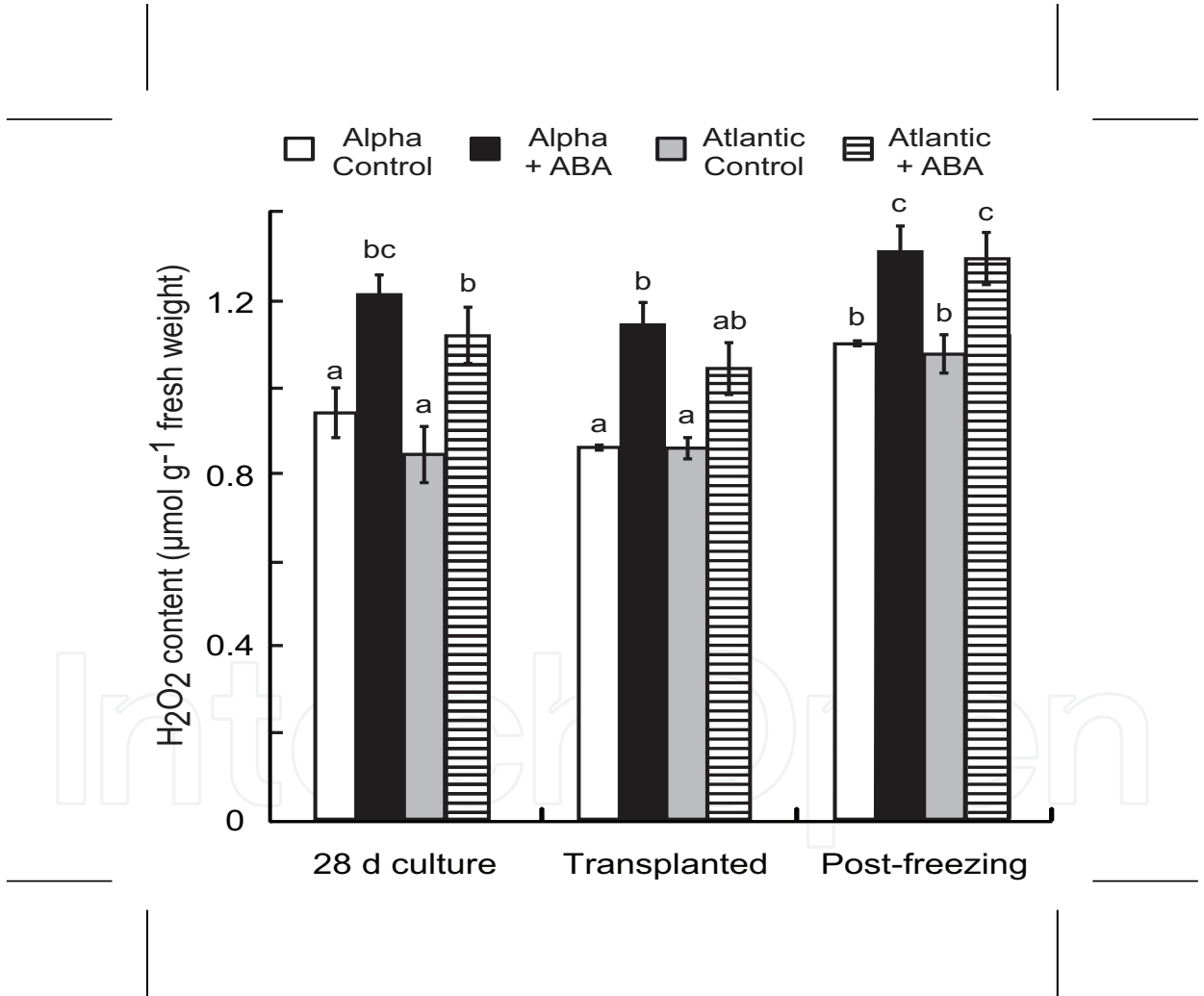


Fig. 1. H<sub>2</sub>O<sub>2</sub> content of microplants (cvs. Alpha and Atlantic) grown in the presence of ABA (10 µM), or its absence (controls), assayed at three stages. '28 d culture', after 28 days of *in vitro* culture. 'Transplanted', 24 h after transfer to compost. 'Post-freezing', immediately after 4 h of freezing (-6 °C). Bars show means (*n* = 3) ± SE, those with different letters differing significantly (ANOVA, *P* < 0.05).



3.2 Effects of ABA on CAT1 and CAT2 transcripts

RT-PCR with primers specific to CAT1 or CAT2 was used to compare the abundance of their transcripts in response to ABA treatment (Fig. 2). The results showed CAT1 and CAT2 were differentially regulated by ABA, in both cvs. Relative abundance of CAT1 transcripts was lower by 25% on average, while CAT2 transcripts increased up to 4-fold, in ABA-treated microplants (Fig. 2). In consequence, CAT2 was the gene predominantly expressed in ABA-treated microplants.

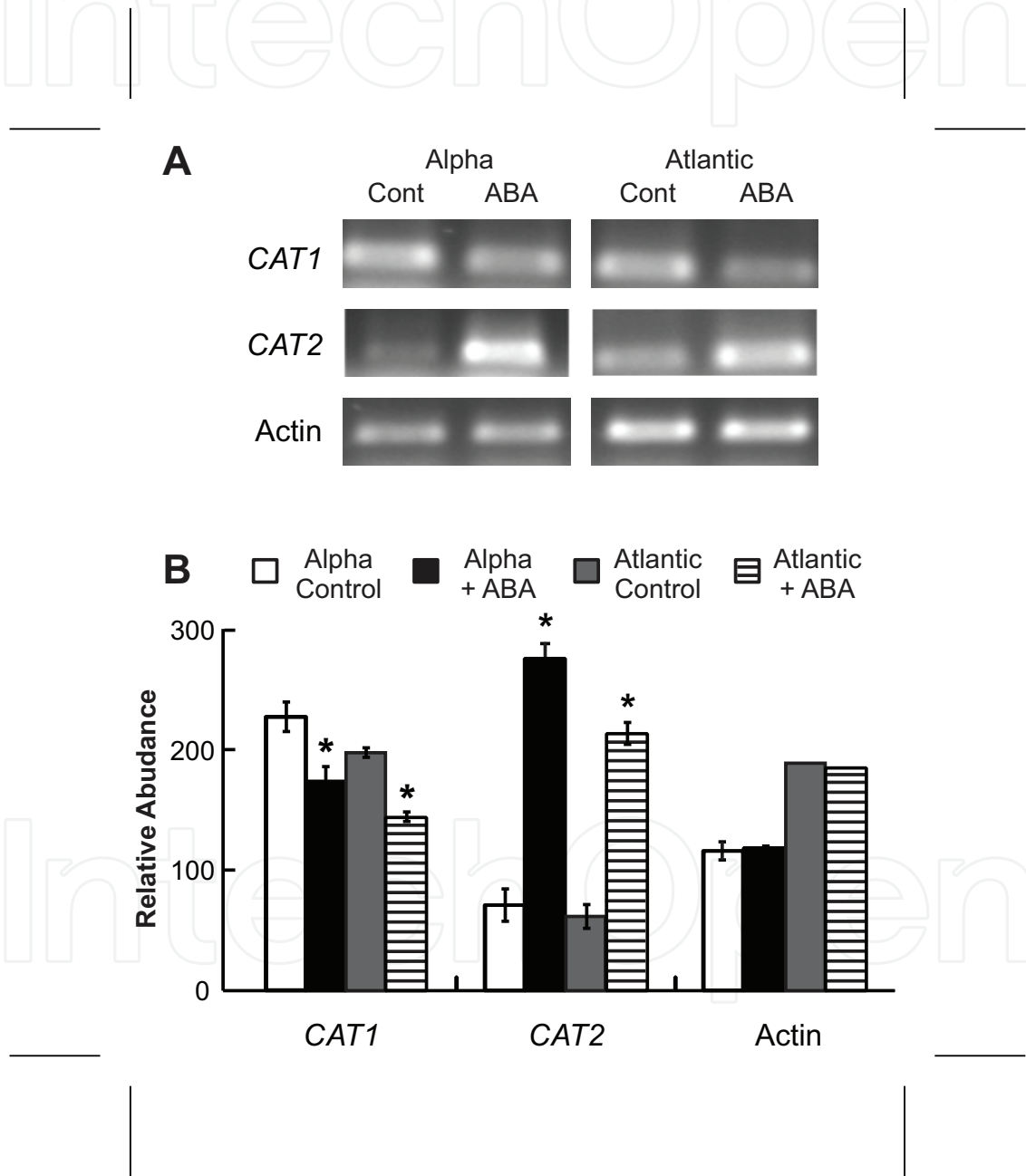


Fig. 2. Effects of ABA (10  $\mu$ M) on CAT1 and CAT2 transcripts in microplants (cvs. Alpha and Atlantic) grown *in vitro* for 28 d. (A) RT-PCR products in agarose gels typical of 4 experiments. (B) Mean relative abundance ( $\pm$  SE) of RT-PCR products in 4 experiments. Actin was the internal control. \*ABA treatments significantly different to controls (*t*-tests,  $P < 0.05$ ).

3.3 Effects of ABA on catalase activities

Native gels stained for enzyme activity ('zymograms') confirmed the occurrence of catalase isozymes (Fig. 3), as would be expected from the expression of more than one gene. The faster-migrating native isozyme was greatly increased in ABA-treated microplants of both cvs (Fig. 3), and was attributed to the CAT2 protein, based on the similar effects of ABA on CAT2 transcripts (Fig. 2) and the immunological evidence of Santos et al. (2006). This isozyme showed similar migration to a 232-kDa standard of bovine liver catalase (Fig. 3). Less expected was the occurrence of more than one slower-migrating isozyme (Fig. 3), since Santos et al. (2006) reported only one, which they assigned as CAT1. The two slower-migrating bands were apparently absent in zymograms of ABA treatments, which represented a more dramatic difference in CAT1 activity than the 25% reduction in CAT1 transcripts seen in RT-PCR.

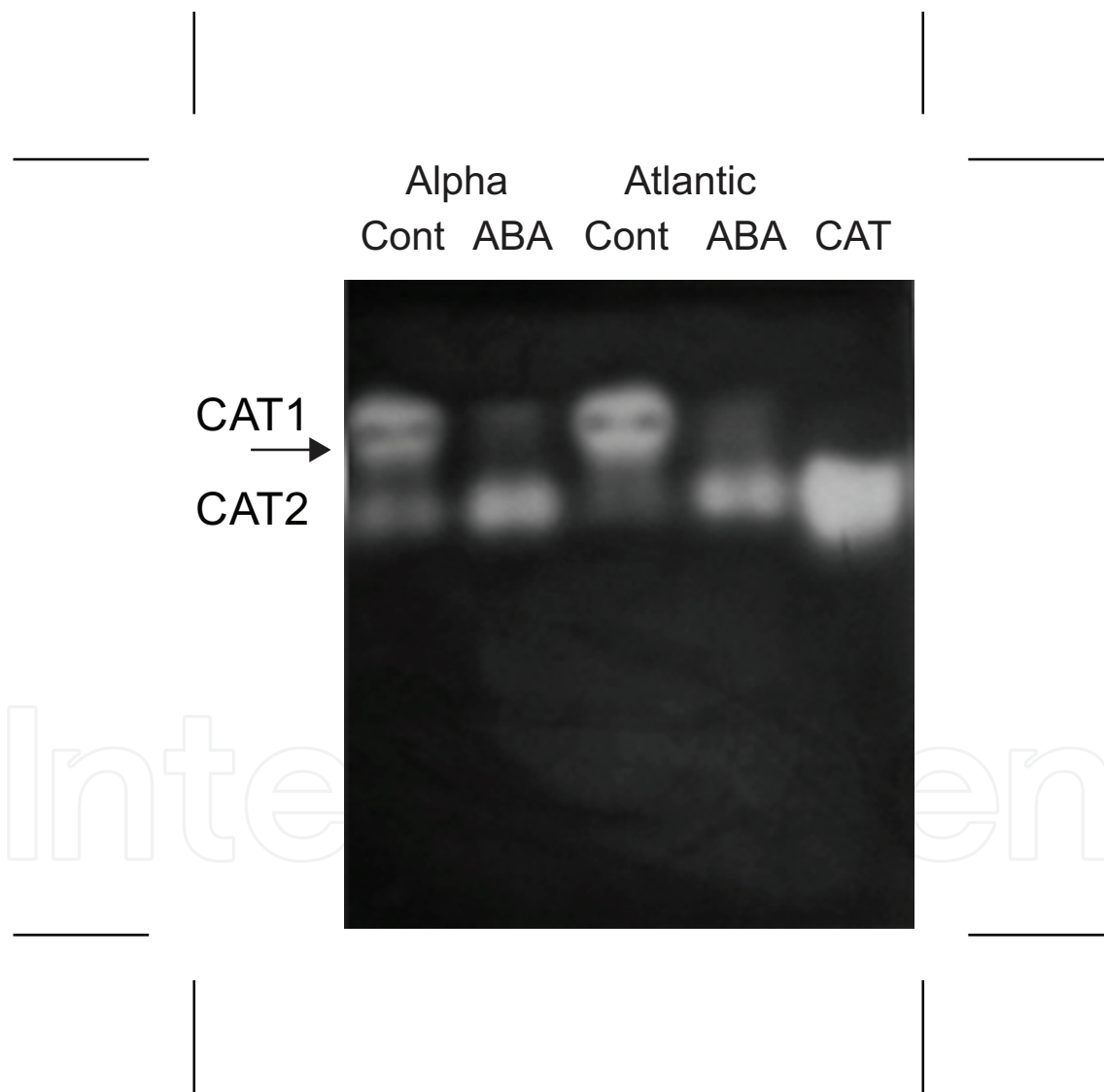


Fig. 3. Catalase zymograms of microplants (cvs. Alpha and Atlantic) grown in vitro in the presence of ABA (10  $\mu$ M), or its absence (controls) for 28 d. 'CAT' lane: bovine liver catalase (232 kDa). Labels on left: attribution of bands to CAT1 or CAT2 isoforms. Arrow indicates possible heterotetrameric form.



The isozymes had different distributions in microplant shoot tissues. In zymograms of leaves, the two slower-migrating bands dominated, though a faint CAT2 band was visible (Fig. 4). Stem zymograms, in contrast, showed the CAT2 band only (Fig. 4).



Fig. 4. Catalase zymograms of leaves, stems or whole-shoots of microplants (cv. Alpha) cultured *in vitro* (without ABA). Labels on left: attribution of bands to CAT1 or CAT2 isoforms. Arrow indicates possible heterotetrameric form.

Quantifications of catalase activity indicated the changed isozyme profiles induced by growth on ABA medium resulted in a net decrease, at least in the enzymic assay conditions used. Significant reductions (of 22% on average) were observed in ABA-treated microplants of either cv., relative to untreated controls, both before and after transplantation from *in vitro* culture to compost (Fig. 5).

Catalase activities in ABA-treated and control microplants showed differential responses to freezing. Post-freezing catalase activities in ABA-treated microplants were not significantly different to pre-freezing levels (Fig. 5). In controls, by contrast, catalase activities were lower after freezing, by 33% on average. The net result was that post-freezing catalase activity was not significantly different in ABA-treated and control microplants (Fig. 5).

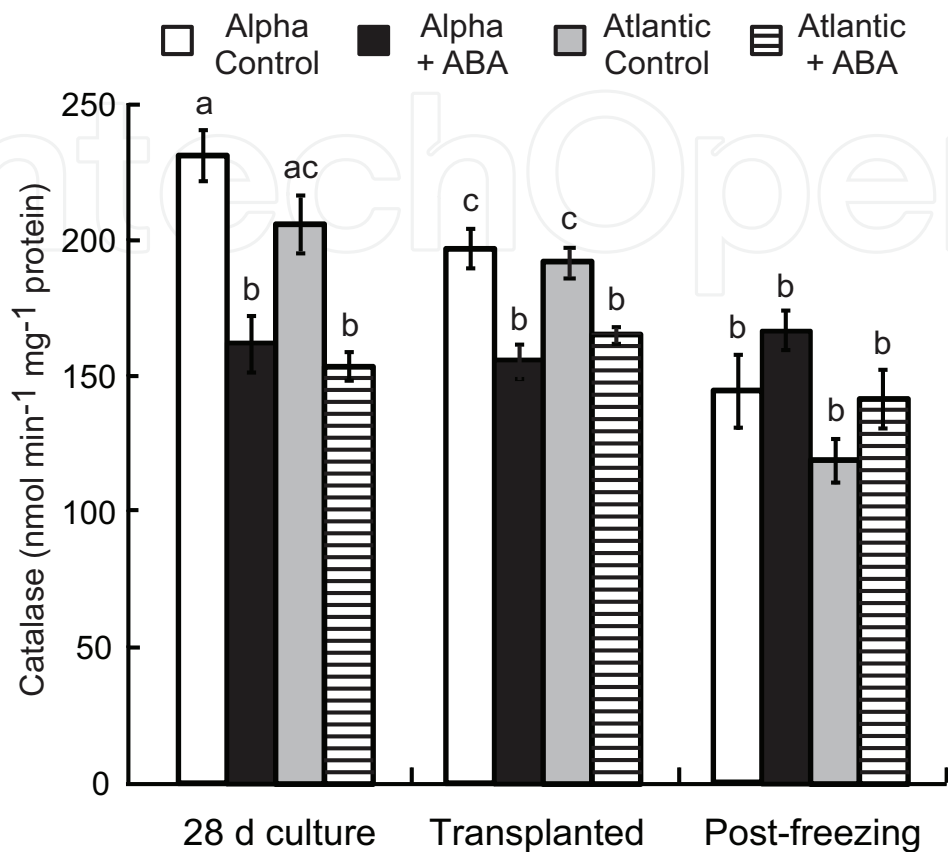


Fig. 5. Catalase activity of microplants (cvs. Alpha and Atlantic), grown in the presence of ABA (10  $\mu$ M), or its absence (controls), assayed at three stages. '28 d culture', after 28 days of *in vitro* culture. 'Transplanted', 24 h after transfer to compost. 'Post-freezing', immediately after 4 h of freezing ( $-6^{\circ}\text{C}$ ). Bars are means ( $n = 5 - 6$ )  $\pm$  SE, those with different letters differing significantly (ANOVA,  $P < 0.05$ ).

#### 4. Discussion

This paper belongs to a series on protection by growth regulators against freezing stress in potato microplants (Mora-Herrera et al., 2005, Mora-Herrera & López-Delgado 2007). One finding was that freezing treatment increased  $\text{H}_2\text{O}_2$  levels. Despite recognition that abiotic stress is likely to promote formation of reactive oxygen species (Jaspers & Kangasjärvi, 2010), direct studies of the effects of sub-zero temperatures on tissue  $\text{H}_2\text{O}_2$  are surprisingly sparse. It is therefore worth aligning our results with the only comparable recent study (Yang et al., 2007), especially since concerns have been expressed about variability of  $\text{H}_2\text{O}_2$  literature data (Queval et al., 2008).

Yang et al. (2007) subjected wheat plants to  $-6^{\circ}\text{C}$  for 6 h, the temperature being changed from, and back to,  $20^{\circ}\text{C}$  over 6 h periods.  $\text{H}_2\text{O}_2$  (measured spectrophotometrically after reaction with KI) increased from ca.  $1.2$  to  $2.1\ \mu\text{mol g}^{-1}$  in this treatment (Yang et al., 2007). These values are comparable to  $\text{H}_2\text{O}_2$  in potato microplants in this and previous papers (López-Delgado et al., 1998; Mora-Herrera et al., 2005). The increase from ca.  $0.87$  to  $1.1\ \mu\text{mol g}^{-1}\text{H}_2\text{O}_2$  in our (ABA-untreated) microplants resulted from a treatment of similar severity ( $-6^{\circ}\text{C}$  for 4 h), but was measured without any post-freezing period.

The present study was prompted by the finding that 28 d culture with ABA protected microplants in freezing (Mora-Herrera & López-Delgado, 2007). In these prolonged exposures to ABA,  $\text{H}_2\text{O}_2$  levels were higher by an average ( $\pm$  SD) of  $24 \pm 7.3\%$  across cvs. and experimental stages ( $n = 6$ ). This was also seen in treatments with another class of protective growth regulators, the salicylates (López-Delgado et al., 1998; Mora-Herrera et al., 2005). The  $\text{H}_2\text{O}_2$  increment in culture with these growth regulators was notably consistent.  $\text{H}_2\text{O}_2$  was 27% higher on  $100\ \mu\text{M}$  salicylate (Mora-Herrera et al., 2005), and 24% on  $1\ \mu\text{M}$  acetylsalicylate (López-Delgado et al., 1998). This may reflect a tight control of maximal  $\text{H}_2\text{O}_2$  in healthy tissues to avoid toxic concentrations (Queval et al., 2008).

Despite the increase in  $\text{H}_2\text{O}_2$  induced by freezing treatment, the difference between ABA-treated and untreated microplants was maintained. Therefore, cellular mechanisms for  $\text{H}_2\text{O}_2$  generation were not saturated by either treatment. The origin of  $\text{H}_2\text{O}_2$  induced by ABA has been identified as superoxide generation by plasma membrane NADPH oxidases, encoded by *Rboh* (respiratory burst oxidase homolog) genes (Cho et al., 2009). Recent work in maize indicates that the ABA-induced expression and activity of NADPH oxidases is further stimulated by the resultant  $\text{H}_2\text{O}_2$  in a MAPK-regulated positive feedback (Lin et al., 2009).

The *Arabidopsis* *RbohD* NADPH oxidase was recently also implicated in a systemic reactive oxygen signal in plants subjected to stresses including ice-water cooling (Miller et al., 2009). This class of enzymes, which have now been characterized in potato tubers (Kobayashi et al., 2007), are therefore candidates for  $\text{H}_2\text{O}_2$  production in both ABA and freezing treatments of the microplants. While it is obviously probable that freezing resulted in  $\text{H}_2\text{O}_2$  generation by cellular processes under stress (Jaspers & Kangasjärvi, 2010), cellular signaling may also have been involved.

The redox state adjustment indicated by higher  $\text{H}_2\text{O}_2$  levels may have been a factor in the growth retardation that was another shared effect of ABA (Mora-Herrera & López-Delgado, 2007) and acetylsalicylate (López-Delgado et al., 1998), since a direct pre-treatment with  $\text{H}_2\text{O}_2$  can itself inhibit microplant growth in culture (López-Delgado et al., 1998). If NADPH oxidases were responsible for the ABA-induced  $\text{H}_2\text{O}_2$ , it could be relevant that certain *Arabidopsis* *atrboh* mutants are defective in ABA inhibition of root growth (Kwak et al., 2003).

We investigated catalase, as a principal  $\text{H}_2\text{O}_2$  scavenger, in ABA-treated microplants. RT-PCR and zymogram analyses revealed contrasting ABA responses for different catalase forms. *CAT2* transcripts and the relevant isozyme were strongly ABA-inducible. Given the increased  $\text{H}_2\text{O}_2$  levels in ABA-treated microplants, and the  $\text{H}_2\text{O}_2$ -inducibility of potato *CAT2* (Almeida et al., 2005), this gene may have an ABA-induction mechanism like *Arabidopsis* *CAT1* (Xing et al., 2008) and maize *CAT1* (Lin et al., 2009). Our data are consistent with potato *CAT2* as the ortholog of the stress-inducible *N. plumbaginifolia* *CAT2* (Willekens et al., 1994; Santos et al., 2006).

CAT1 transcripts, in contrast, showed a 25% reduction in abundance in ABA-treated microplants. Zymograms showed more dramatic difference, with the putative CAT1 band absent in ABA treatments. Almeida et al. (Almeida et al., 2005) found H<sub>2</sub>O<sub>2</sub> treatment reduced CAT1 in immunoblots and zymograms, whereas CAT1 in RNA gel blots did not show the same decline. As in our study, therefore, there was a disparity between the RNA and protein findings, which suggested post-transcriptional effects of ABA and H<sub>2</sub>O<sub>2</sub> on CAT1 expression. Spectrophotometric assays showed a consistent net reduction in catalase activity in ABA-treated microplants at standard temperature. This suggests the zymograms, where the decline in CAT1 appeared more dramatic, were better indicators of enzymic activity than the RT-PCR.

In zymograms of field-grown plants, Almeida et al. (Almeida et al., 2005) saw only one slower-migrating band, attributed to CAT1, whereas our *in vitro* microplants yielded two slower-migrating bands. An extra isozyme could reflect a third, uncharacterized catalase potato gene, since at least three occur in confamilial species (Santos et al., 2006). On the other hand, the coincidental expression patterns (Figs. 3-4) of the two slower-migrating bands suggested at least one (presumably the faster-migrating) may have been a heterotetramer of CAT1 and CAT2 proteins, analogous to those in other species (Feierabend, 2005).

Heterotetrameric isoforms probably depend on the different loci being co-expressed in a given cell type (Feierabend, 2005), and in some respect the distribution of CAT1 and CAT2 expression may have differed *in vitro* and in the field. In microplants under standard conditions, the isoforms did have different tissue distributions. In stem zymograms only the CAT2 band was visible, while leaf zymograms were dominated by the two bands that putatively included CAT1, consistent with an association of CAT1 with photorespiration (Santos et al., 2006). It is furthermore possible that catalase could be differentially distributed in different types of leaf cells, as has been observed for H<sub>2</sub>O<sub>2</sub> and ascorbate peroxidase (Galvez-Valdivieso et al., 2009).

In theory, the reduced catalase activity seen in spectrophotometric assays could have facilitated a controlled H<sub>2</sub>O<sub>2</sub> increase to adjust growth and prime defenses against abiotic stress. Our data suggest the leaf would be the critical site of these events, since it was the leaf-localized isoforms whose decline was evidenced by isozyme results.

Tsai & Kao (2004) also saw a decrease in catalase activity in ABA treatment, of rice roots. On the other hand, other studies have found increased activity in response to ABA (Agarwal et al., 2005, Zhang et al., 2006, Du et al., 2008, Kumar et al., 2008). Our model system was different in that the microplants experienced prolonged growth on ABA-containing medium. This may have brought a different physiological adjustment to those seen in single, brief treatments, whose effects are transient (Du et al., 2008). It may be more pertinent that, on exposure to freezing, catalase activity levels were maintained in ABA-treated microplants, but not in controls.

The potential value for food security of ABA and catalase lies in their association with coping mechanisms for stresses that challenge crop production (Cho et al., 2009). Crop species in which the effects of ABA on catalase had previously been investigated were cereals (Tsai & Kao 2004, Agarwal et al., 2005, Zhang et al., 2006) or legumes (Kumar et al., 2008). We have added potato to this list. Moreover, we suggest that experimental systems like the cultured microplants may have particular biotechnological relevance, because

exploitation of stress tolerance mechanisms are likely to involve the stable changes in physiology seen in prolonged treatments.

## 5. Conclusion

Freezing tolerance-enhancing treatments with ABA caused differential changes in catalase isoforms and activities, in concert with changes in H<sub>2</sub>O<sub>2</sub> levels. At least one isoform may have been a heterotetramer of CAT1 and CAT2 proteins. This may reflect a tight control of maximal H<sub>2</sub>O<sub>2</sub> in healthy tissues to avoid toxic concentrations. Knowledge of stress tolerance mechanisms involve stable changes in physiology during prolonged treatments.

## 6. Acknowledgments

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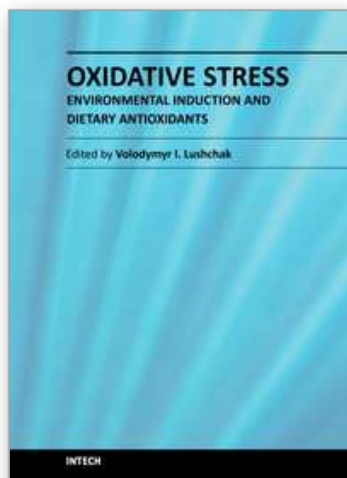
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## **Oxidative Stress - Environmental Induction and Dietary Antioxidants**

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This book focuses on the numerous applications of oxidative stress theory in effects of environmental factors on biological systems. The topics reviewed cover induction of oxidative stress by physical, chemical, and biological factors in humans, animals, plants and fungi. The physical factors include temperature, light and exercise. Chemical induction is related to metal ions and pesticides, whereas the biological one highlights host-pathogen interaction and stress effects on secretory systems. Antioxidants, represented by a large range of individual compounds and their mixtures of natural origin and those chemically synthesized to prevent or fix negative effects of reactive species are also described in the book. This volume will be a useful source of information on induction and effects of oxidative stress on living organisms for graduate and postgraduate students, researchers, physicians, and environmentalists.

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