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Exogenous 4-hydroxybenzoic acid and salicylic acid modulate the effect of short-term drought and freezing stress on wheat plants

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Abstract

Exogenous salicylic acid has been shown to confer tolerance against biotic and abiotic stresses. In the present work the ability of its analogue, 4-hydroxybenzoic acid to increase abiotic stress tolerance was demonstrated: it improved the drought tolerance of the winter wheat (*Triticum aestivum* L.) cv. Cheyenne and the freezing tolerance of the spring wheat cv. Chinese Spring. Salicylic acid, however, reduced the freezing tolerance of Cheyenne and the drought tolerance of Chinese Spring, in spite of an increase in the guaiacol peroxidase and ascorbate peroxidase activity. The induction of cross tolerance between drought and freezing stress was observed: drought acclimation increased the freezing tolerance of Cheyenne plants and cold acclimation enhanced the drought tolerance. The induction of drought tolerance in Cheyenne was correlated with an increase in catalase activity.

Additional key words: antioxidant activity, chlorophyll fluorescence induction, low temperature, water stress.

Introduction

Cold acclimation – the development of freezing tolerance – is a complex biochemical process sharing common components with drought acclimation, since part of the injury at freezing temperature is caused by the dehydration of cells following extracellular ice formation (Thomashow 2001). Most stress conditions impose an oxidative challenge on plants. Therefore, the enhancement of antioxidant capacity is also essential in the induction of freezing and drought tolerance (Janda *et al.* 2003, Baczek-Kwinta *et al.* 2006, Jain *et al.* 2006).

In recent years the role of salicylic acid (SA) in the induction of tolerance against several abiotic stresses, such as heat stress in mustard (Dat *et al.* 1998), chilling in maize (Janda *et al.* 1999, Szalai *et al.* 2000) or paraquat-induced oxidative damage in barley (Ananieva *et al.* 2004) has been described. In the case of drought tolerance, however, the effect of SA is still unclear. SA potentiates the generation of reactive oxygen species in photosynthetic tissues of *Arabidopsis thaliana* during salt and osmotic stresses (Borsani *et al.* 2001). In agreement with these results, it was shown that pre-treatment with 0.5 mM SA for 1 d decreased the drought tolerance of 2-week-old maize plants (Németh *et al.* 2002), though increasing their polyamine content. However, soaking grains in acetylsalicylic acid improved the drought tolerance of wheat plants (Hamada 1998). SA or acetylsalicylic acid increased the drought tolerance of tomato and bean plants (Senaratna *et al.* 2000). Salicylic acid treatment inhibited the ABA-induced closure of the stomata (Rai *et al* 1986), possibly leading to enhanced drought-induced injury. The endogenous SA content was shown to increase in drought-stressed *Phillyrea angustifolia* plants (Munne-Bosch and Penuela 2003), suggesting that SA might have a role in the drought stress response of plants. SA was shown to increase the ABA content of wheat plants, leading to the accumulation of proline (Shakirova *et al.* 2003).

In the case of freezing tolerance, the effect of SA has not been well characterized yet. It was shown to increase the freezing tolerance of a winter wheat cultivars

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Abbreviations: APX - ascorbate peroxidase; CAT - catalase; Chl - chlorophyll; F_m - maximal fluorescence; F_v - variable fluorescence; GPX - guaiacol peroxidase; GR - glutathione reductase; 4-HBA - 4-hydroxybenzoic acid; PAL - phenylalanine ammonialyase; PEG - polyethylene glycol; PPFD - photosynthetic photon flux density; PR - pathogenesis-related; PS 2 - photosystem 2; SA - salicylic acid; SAR - systemic acquired resistance.

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when sprayed on the leaves at 0.01 and 0.1 mM concentrations by increasing the ice nucleation activity of apoplastic proteins (Tasgín *et al.* 2003). In rye, however, the apoplastic proteins induced by salicylic acid did not have antifreeze activity (Yu *et al.* 2001), and only treatment with ethylene induced antifreeze activity in winter rye leaves.

The salicylic acid analogue 4-hydroxybenzoic acid (4-HBA) is usually referred to as a biologically inactive compound as it is not effective in inducing biotic stress tolerance or the synthesis of pathogenesis-related (PR) proteins (Chen *et al.* 1993). It was also unable to stimulate the alternative respiratory pathway in tobacco and thus did not induce heat production (Van der Straeten *et al.* 1995). However, similarly to SA, 4-HBA was able to induce the octopine synthase (ocs) element from the soybean auxin-inducible GH2/4 promoter (Ulmasov *et al.* 1994). 4-HBA was detected in cell wall extracts from *Arabidopsis thaliana* roots and its concentration increased upon infection with *Pythium sylvaticum* (Tan *et al.* 2004). Aromatic cell wall-bound compounds seem to be structurally more uniform than the highly diverse, species-specific soluble aromatic metabolites. 4-HBA accumulated in the phloem fluid of cucumber following infection by *Pseudomonas syringae* pv. *syringae*, and was synthesized *de novo*, together with SA, after the induction of phenylalanine ammonialyase (PAL) (Smith-Becker *et al.* 1998). The physiological role of this 4-HBA accumulation is not clear. It may be only a non-specific consequence of the induction of PAL activity.

The aim of the present work was to determine the effect of salicylic acid and its analogue, 4-hydroxybenzoic acid on the freezing tolerance and drought tolerance of wheat plants (*Triticum aestivum* L. cvs. Cheyenne and Chinese Spring). A correlation was also sought between changes in the activity of antioxidant enzymes and in stress tolerance. As it was proposed that SA may act through an increase in the hydrogen peroxide content, the effect of 0.1 mM H_2O_2 treatment was also tested. Investigations were also made on the effect of drought and cold acclimation on the freezing tolerance and drought tolerance of the plants, in order to determine whether cross-tolerance to these stress factors developed in wheat.

Materials and methods

Plants and treatments: Wheat (*Triticum aestivum* L. cvs. Cheyenne and Chinese Spring) plants were grown in Hoagland hydroponic solution at 20/18 °C, a 16-h photoperiod and photon flux density (PPFD) 200 μmol m^{-2} s^{-1} in a *Conviron PGR-15* plant growth chamber (*Controlled Environments Ltd*, Winnipeg, Canada). Ten-day-old seedlings were treated with 0.5 mM salicylic acid, 0.5 mM 4-hydroxybenzoic acid or 0.1 mM H_2O_2 hydroponically for 1 d, or cold-acclimated for 2 d at 5 °C. Subsequently the plants were either subjected to drought stress induced by 15 % polyethylene glycol (PEG) solution for 1 d, or to freezing stress at -11 °C for 1 d. The freezing stress was performed in a freezing chamber (*National Lab*, Mölln, Germany) in the dark.

Chlorophyll fluorescence induction parameters of the youngest fully developed leaves were determined at room temperature using a pulse amplitude modulated fluorometer (*PAM-2000, Walz*, Effeltrich, Germany) as described by Janda *et al.* (1994). Before the measurements, the plants were dark-adapted for 30 min at room temperature.

Electrolyte leakage measurements were performed on the youngest fully developed leaves of wheat plants. The middle part of the leaves was cut into 1 cm segments, and two such segments were placed in 2 cm^3 *MilliQ* water. After 60 min the conductance of the solution was measured using an *Automatic Seed Analyzer* (*ASA610, Agro Science Inc.*, Ann Arbor, MI, USA). After freezing the samples at -80 °C for 1 d, the conductance was measured again. This latter value was taken as 100 %.

Total chlorophyll content was measured on the second leaves using a *SPAD-502* chlorophyll meter (*Minolta Camera Co., Ltd*, Tokyo, Japan). The SPAD readings were used as relative values for chlorophyll content.

Enzyme extraction: The youngest fully developed leaves were used for protein extraction. One g of plant material was ground using a mortar and pestle with 0.5 g quartz sand in 3 cm^3 of 0.5 mM Tris-HCl buffer (pH 6.8) at 4 °C, then filtered through four layers of cheesecloth and centrifuged for 20 min at 12 000 *g* at 4 °C. The protein concentration of the supernatant was determined according to the Bradford (1976) using bovine serum albumin as a standard.

Enzyme activity assay: The activity of catalase (EC 1.11.1.6) was measured by following the decrease in the H_2O_2 concentration spectroscopically at 240 nm according to Ádám *et al.* (1995). The reaction mixture contained 10 mM H_2O_2 and 0.05 cm^3 plant extract in 0.5 mM Tris-HCl buffer (pH 7.4) in a total volume of 3 cm^3. The activity of guaiacol peroxidase (GPX, EC 1.11.1.7) was determined according to Ádám *et al.* (1995). The oxidation of guaiacol was measured as the increase in absorption at 470 nm. The reaction mixture consisted of 0.1 mM Na-acetate buffer (pH 5.5) containing 10 mM H_2O_2, 1 mM guaiacol and 0.05 cm^3 plant extract in a total volume of 3 cm^3. The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was determined spectroscopically by measuring the decrease in ascorbate at 290 nm (Nakano and Asada 1987). The reaction mixture consisted of 25 mM ascorbic acid and

0.5 mM H_2O_2 in 0.2 mM Tris buffer (pH 7.8) and 0.05 cm^3 plant extract was added to 2.25 cm^3 reaction mixture. The activity of glutathione reductase (GR, EC 1.6.4.2) was measured by following the increase in 5,5'-dithiobis(-2-nitrobenzoic acid) (DTNB) at 412 nm according to Smith *et al.* (1988). The reaction mixture had a total volume of 1 cm^3 and contained 0.15 mM diethylenetriamine-pentaacetic acid, 0.75 mM DTNB, 0.1 mM NADPH, 0.5 mM GSH and 0.05 cm^3 plant extract in 75 mM Na-phosphate buffer (pH 7.5).

Statistical analysis: The results are the means of at least 15 replications for the Chl fluorescence induction parameters and of 3-5 replications for the enzyme activity studies for each treatment. Three independent repetitions were performed for each experiment. The data were statistically evaluated using the standard deviation and *t*-test methods.

Results

Effect of SA, 4-HBA and H_2O_2 pre-treatments on drought tolerance: The effect of a 1-d pre-treatment with 0.5 mM SA, 0.5 mM 4-HBA or 0.1 mM H_2O_2 on the response of wheat to short-term drought (15 % PEG treatment) was compared to the effect of 2-d long cold acclimation at 5 °C. The total chlorophyll (Chl) content of the leaves decreased significantly in the SA pre-treated and cold-acclimated Cheyenne plants and in the SA pre-treated Chinese Spring plants (Fig. 1). The determination of the F_v/F_m ratio revealed that the maximum photochemical efficiency of PS 2 was not altered by any of the pre-treatments (Fig. 2). Membrane integrity, however, was affected by the SA pre-treatment as indicated by an increase in the electrolyte leakage parameter in both genotypes (Fig. 2).

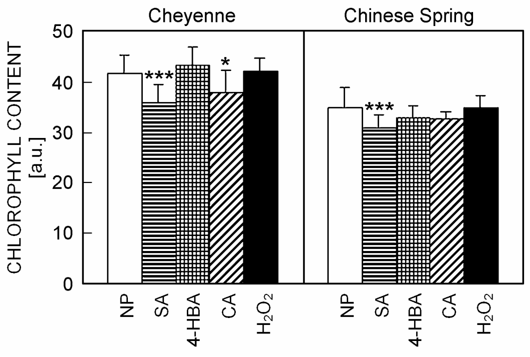

Fig. 1. Relative chlorophyll content of wheat plants (winter wheat cv. Cheyenne and spring wheat cv. Chinese Spring) with no pre-treatment (NP), or pre-treated with 0.5 mM salicylic acid (SA), 0.5 mM 4-hydroxybenzoic acid (4-HBA) or 0.1 mM H_2O_2 for 1 d, or cold acclimated for 2 d at 5 °C (CA). *, *** - significant at the 0.05 and 0.005 levels, respectively, compared to control (NP) plants.

The 1-d drought stress caused a decline in the F_v/F_m value of Cheyenne plants with no pre-treatment (Fig. 2). Cheyenne plants pre-treated with SA or H_2O_2 also exhibited decreased F_v/F_m values after drought stress. After 4-HBA pre-treatment and 2-d cold acclimation, however, there was no decrease in the F_v/F_m ratio in this genotype. In the case of Chinese Spring, there was no significant change in the F_v/F_m values after the 1-d PEG treatment, except for a decrease in plants pre-treated with SA (Fig. 2).

The electrolyte leakage values increased in the case of Cheyenne (Fig. 2) without pre-treatment and with SA or H_2O_2 pre-treatment after the short-term drought stress, though the increase was less pronounced in plants pre-treated with H_2O_2. After pre-treatment with 4-HBA or cold acclimation there was no increase at all. Chinese Spring plants did not show an increase in electrolyte leakage after drought stress, except in the case of SA pre-treatment and cold acclimation.

Effect of SA, 4-HBA and H_2O_2 pre-treatments on freezing tolerance: Cheyenne and Chinese Spring plants were pre-treated with 0.5 mM SA, 0.5 mM 4-HBA or 0.1 mM H_2O_2 for 1 d and their response to short-term freezing was examined and compared to the effect of 1 d drought stress (15 % PEG) and 2 d cold acclimation at 5 °C.

After the short period of freezing, the F_v/F_m values of plants with no pre-treatment (NP) decreased considerably in both genotypes (Fig. 3). Pre-treatment with SA caused an even more pronounced decrease. Pre-treatment with 4-HBA had no effect on Cheyenne plants; however, Chinese Spring plants pre-treated with 4-HBA displayed a significantly smaller decrease in the F_v/F_m parameter than plants with no pre-treatment after freezing stress. Drought and cold acclimation also had an ameliorating effect in both genotypes, with the highest F_v/F_m value being recorded for cold-acclimated plants. H_2O_2 had no appreciable effect on the F_v/F_m value compared to plants without pre-treatment.

The electrolyte leakage increased to 90 - 95 % in plants with no pre-treatment, and after SA or H_2O_2 pre-treatment in both genotypes after the freezing stress (Fig. 3). The 4-HBA pre-treatment caused a similar increase in Cheyenne plants, while the increase was significantly less in Chinese Spring (55 %). Drought prevented the large increase in the electrolyte leakage after freezing in Cheyenne (27 %), but this was not observed in Chinese Spring (80 %). Cold acclimation for 2 d resulted in only a small increase in the electrolyte leakage after freezing.

Antioxidant enzyme activities following the different pre-treatments: Glutathione reductase activity was not affected significantly by 0.5 mM SA, 0.5 mM 4-HBA and 0.1 mM H_2O_2 pre-treatments (Fig. 4). The activity of

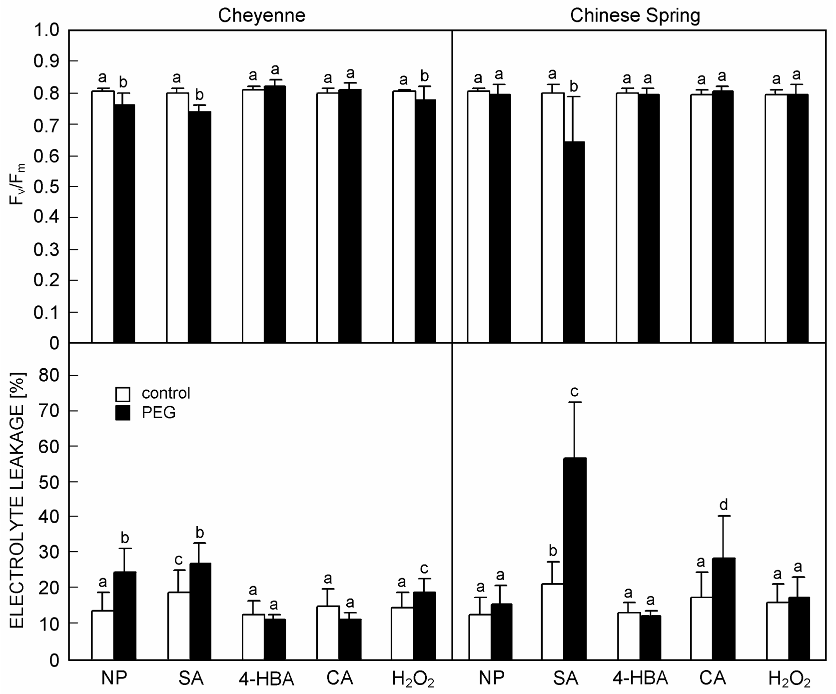

Fig. 2. The chlorophyll *a* fluorescence induction parameter (F_v/F_m) and the electrolyte leakage of wheat cvs. Cheyenne and Chinese Spring with no pre-treatment (NP), or pre-treated with 0.5 mM salicylic acid (SA), 0.5 mM 4-hydroxybenzoic acid (4-HBA) or 0.1 mM H_2O_2 for 1 d, or cold acclimated for 2 d at 5 °C (CA), measured on the youngest fully developed leaves of non-stressed plants (control - *white bars*) and after 1-d drought stress induced by PEG (*black bars*). Values carrying different letters were significantly different at $P < 0.05$.

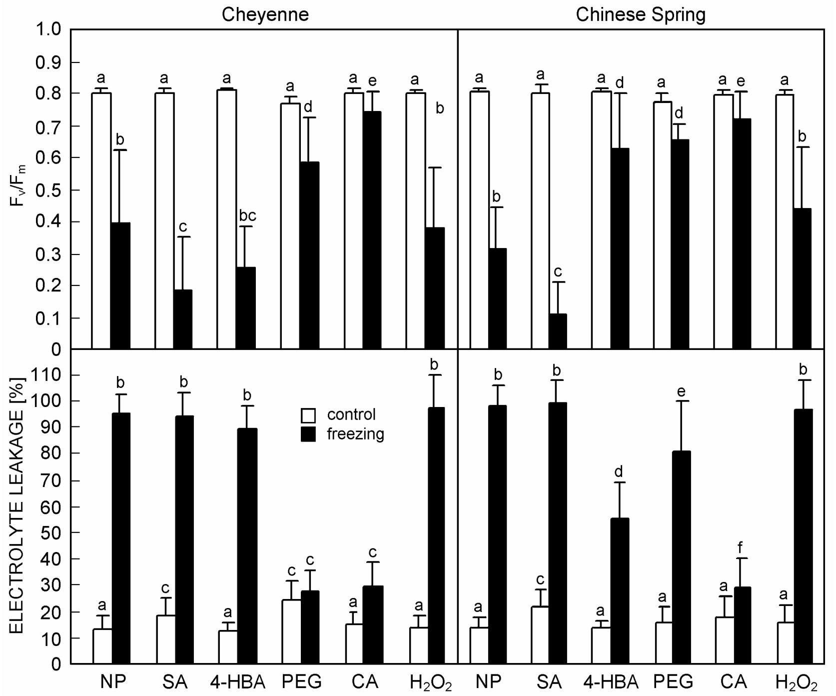

Fig. 3. The chlorophyll *a* fluorescence induction parameter (F_v/F_m) and the electrolyte leakage of wheat cvs. Cheyenne and Chinese Spring with no pretreatment (NP), or pre-treated with 0.5 mM salicylic acid (SA), 0.5 mM 4-hydroxybenzoic acid (4-HBA), 0.1 mM H_2O_2 or 15 % PEG for 1 d, or cold acclimated for 2 d at 5 °C (CA), measured on the youngest fully developed leaves of non-stressed plants (*white bars*) and after 1-d freezing stress (*black bars*). Values carrying different letters were significantly different at $P < 0.05$.

catalase increased after 4-HBA pre-treatment, drought and cold acclimation in Cheyenne. In Chinese Spring, the CAT activity only increased significantly after cold acclimation, while it decreased after SA pre-treatment. Guaiacol peroxidase activity was enhanced in Cheyenne by SA, drought and cold acclimation. In Chinese Spring, SA, 4-HBA and H_2O_2 pre-treatments and drought stress resulted in increased GPX activity. Ascorbate peroxidase activity increased after all the treatments in Cheyenne. In Chinese Spring, only 4-HBA, H_2O_2 and drought treatment caused a slight but significant increase in APX activity, while it was decreased by cold acclimation.

Discussion

The role of SA in inducing tolerance of biotic stress and several forms of abiotic stress is already well established. In the case of drought and freezing stress, however, its role is still ambiguous. The present study examined the effect of 0.5 mM SA and its analogue, 4-HBA on the drought and freezing tolerance of wheat, together with changes in certain antioxidant enzyme activities.

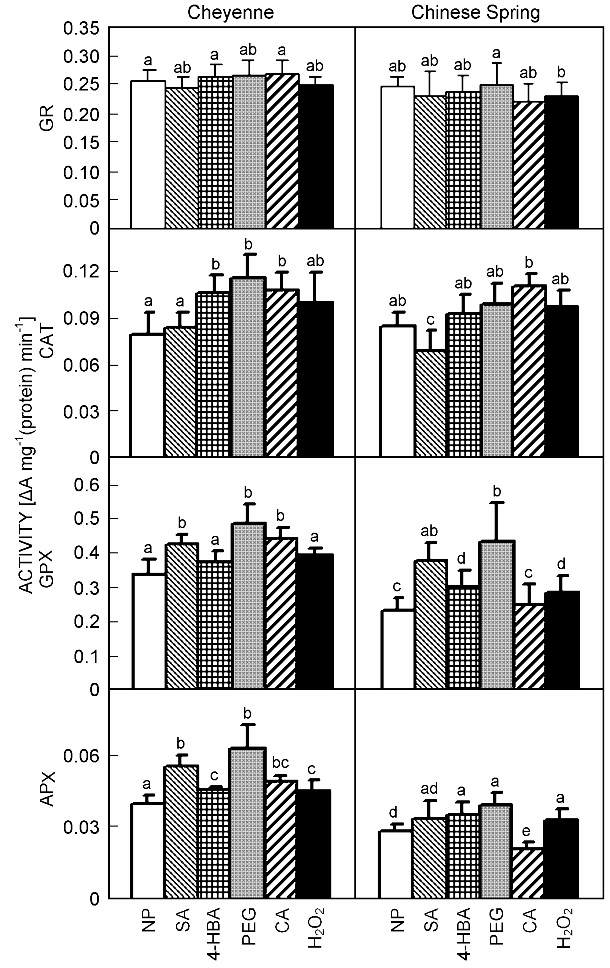

Fig. 4. Antioxidant enzyme activities (glutathione reductase: GR, catalase: CAT, guaiacol peroxidase: GPX and ascorbate peroxidase: APX) of wheat cvs. Cheyenne and Chinese Spring with no pre-treatment (NP), or pre-treated with 0.5 mM salicylic acid (SA), 0.5 mM 4-hydroxybenzoic acid (4-HBA), 0.1 mM H_2O_2 or 15 % PEG for 1 d, or cold acclimated for 2 d at 5 °C (CA). Values carrying different letters were significantly different at $P < 0.05$.

The Chl content of the wheat plants decreased significantly following the 1-d application of SA, in agreement with previous results (Moharekar *et al.* 2003). The Chl fluorescence induction method is widely used to detect low temperature injury in cereals (Janda 1998, Pál and Nagy 2002). The maximum photochemical efficiency of PS 2 indicated by the F_v/F_m parameter, was not affected, while membrane integrity was slightly disrupted, as indicated by an increase in the electrolyte leakage parameter. This suggests that the application of 0.5 mM SA imposes a mild stress on wheat plants.

The drought tolerance of maize and wheat plants has been shown to decrease after hydroponic treatment with 0.5 mM SA, in spite of an increase in the polyamine content (Németh *et al.* 2002). In the present work, SA pre-treatment also decreased the drought tolerance of Chinese Spring, while it had no effect on Cheyenne. However, SA might induce drought tolerance when it is imbibed by seeds (Hamada 1998, Senaratna *et al.* 2000). The effect of SA in the induction of stress tolerance appears to depend greatly on the mode of application.

SA has been implicated in the induction of freezing tolerance in winter wheat (Tasgín *et al.* 2003) when it was sprayed on to the leaves, possible as the consequence of the increased ice nucleation activity of the apoplastic proteins. By contrast, experiments on rye plants showed that the PR proteins accumulating as the result of SA treatment had no antifreeze activity, while ethylene treatment and cold acclimation caused the accumulation of PR proteins with antifreeze activity (Yu *et al.* 2001). According to the present data, SA applied hydroponically decreased whole plant survival after freezing stress in both the winter and the spring wheat genotype. The contradiction between these and previous results (Tasgín *et al.* 2003), which suggested that SA promoted freezing tolerance, might be explained partly by the difference in genotype or by the difference in the method of SA application. This suggests that the effect of SA might be tissue-specific.

At relatively low concentrations, SA seems to act as a moderate stress, having an acclimation-like effect on the oxidative status of the plant. A rapid transient increase in active oxygen species (AOS) is followed by enhanced antioxidative capacity, which protects the plant from the severe damage caused by subsequent stress factors. Most antioxidant enzymes are induced by SA, while the activity of catalase was shown to be inhibited (Dat *et al.* 1998, Janda *et al.* 1999, Agarwal *et al.* 2005, Molassiotis *et al.* 2005). Although the activities of GPX and APX were also stimulated by SA treatment, this did not enhance tolerance against freezing, despite the fact that the activity of these enzymes has been shown to correlate with the freezing tolerance of wheat varieties (Janda *et al.* 2003). This effect of SA on GPX and APX may be counteracted by others, such as the decrease in catalase activity.

The induction of freezing tolerance by cold acclimation is a long and complex process. The activity of most antioxidant enzymes (APX, GPX and GR) was shown to be induced, while catalase activity decreased during cold acclimation (Kocsy *et al.* 2001, Baek and Skinner 2003, Janda *et al.* 2003). The activities of APX and GPX were shown to correlate with the freezing tolerance of various cereal genotypes (Janda *et al.* 2003). In the present work, the effect of 2d cold acclimation against short-term freezing and drought stress was examined, as well as its effect on the activity of antioxidant enzymes. The 2d cold acclimation conferred tolerance against short-term freezing stress and also enhanced the drought tolerance of the winter wheat Cheyenne. The activities of GPX and APX increased in Cheyenne during cold acclimation, in accordance with previous observations (Janda *et al.* 2003). In the spring wheat, however, cold acclimation did not induce GPX activity and decreased APX activity, though it still increased the freezing tolerance of the plants. The catalase activity increased significantly in both genotypes.

The 1-d drought stress also increased the survival of Cheyenne wheat plants after subsequent freezing stress. In the case of Chinese Spring, the effect of drought on the freezing tolerance was ambiguous. It decreased injury to the photosynthetic apparatus, but did not prevent damage to the membranes. Drought stress enhanced the activities of GPX and APX in both genotypes. Catalase is an important factor in maintaining the redox balance of the cell by preventing the oxidation of the ascorbate and glutathione pools (Willekens *et al.* 1997). The increase in catalase activity, however, was only significant in the Cheyenne plants. The weak induction of catalase in Chinese Spring might account for the membrane injury following freezing stress.

4-HBA is usually thought of as a biologically inactive compound, which is unable to induce biotic stress tolerance (Chen *et al.* 1993). The present data demonstrated for the first time the role of 4-HBA in increasing abiotic stress tolerance. 4-HBA ameliorated the freezing tolerance of the spring wheat Chinese Spring, and increased the drought tolerance of the winter wheat Cheyenne. 4-HBA is known to function as a phytoalexine. The excretion of 4-HBA was observed after the elicitor treatment of carrot protoplast culture (Bach *et al.* 1993), and it was shown to bind covalently to the newly synthesized cell wall polysaccharides (Koch *et al.* 1998). It is hypothesized that 4-HBA increases the impermeability of the cell wall, leading to increased resistance against pathogen infection. The reinforcement of the cell wall by 4-HBA may contribute to increased tolerance against freezing stress. The application of 4-HBA reduced the F_v/F_m value of *Dactylis glomerata* plants and also affected the proline content of the plants (Duran-Serantes *et al.* 2002). The increased proline content induced by 4-HBA treatment might cause osmotic adjustment. Among the antioxidant enzymes examined, the activity of catalase increased after 4-HBA treatment in the winter wheat and decreased after SA treatment in the spring wheat. The increased catalase activity may also play a role in the enhancement of drought tolerance by 4-HBA.

Salicylic acid and acclimation both caused a transient increase in the H_2O_2 content (Okuda *et al.* 1991). The role of H_2O_2 as a secondary messenger in several signal transduction pathways is well documented. Similarly to SA, H_2O_2 at 0.1 - 50 mM concentration was shown to increase the heat tolerance of potato plants (Lopez-Delgado *et al.* 1998). Exogenous H_2O_2 did not cause the same degree of lipid peroxidation or oxidative injury to proteins as 0.2 mM SA treatment (Rao *et al.* 1997). Dimethylthiourea (a trap for H_2O_2) decreased the effect of SA, however, suggesting that H_2O_2 contributes to the effect of SA. The SA radical shown to be formed during the inhibition of catalase activity by SA, and the subsequent lipid peroxidation might also be part of the signal transduction pathway of SA (Klessig *et al.* 2000). Since in a previous study (Lopez-Delgado *et al.* 1989) the greatest protective effect of H_2O_2 against heat shock was recorded at 0.1 mM concentration, the effect of 0.1 mM H_2O_2 on drought and freezing tolerance in wheat was also examined. The application of H_2O_2 did not decrease the drought tolerance of Chinese Spring as SA did, nor did it decrease the freezing tolerance. Thus the tolerance-decreasing effect of SA is possibly not mediated by H_2O_2.

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Francis, C.A., Poincelot, R.P., Bird, G.W. (ed.): **Developing and Extending Sustainable Agriculture. A New Social Contract.** - Haworth Food & Agricultural Products Press, An Imprint of The Haworth Press, New York - London - Oxford. 2006. 367 pp. USD 49.95. ISBN 13: 978-1-56022-331-3.

This book starts with a brief information about the editors. A list of all the 21 contributors follows. All the editors and authors of the individual chapters are engaged in the US development of sustainable agriculture as scientists, scholars, managers, extension officers, farmers, *etc*. The authors summarized their experience, expertise and expectation concerning the extension of sustainable agriculture focused on the conditions in the USA. Actually, the book contributions could also be used as valuable source of general information about several aspects of the US agriculture development during the last decades. Such a local limitation need not be considered as a drawback for potential readers outside the USA. In fact, there is much to learn from various attempts to transform conventional agricultural into partly or ultimate sustainable management at both the lowest level of some local farms up to the whole region or state. Similarly, the examples described in the individual chapters point out, that such a transition, its rate and success, depends not only on the elaborated technologies. Experience clearly indicates the need to establish the appropriate study protocols, extension systems, general social climate and economic instruments. From these points of view, the chapters of this book offer an immense amount of information and suggestion. Nevertheless, the reader should not expect a comprehensive discourse on sustainable agriculture or protocols simply applicable for any location and country.

Each of the 16 chapters represents a freestanding contribution surveying and analysing particular topics. Chapter 1 offers an overview involving education and extension as well as information sources available including journals, bulletins, sheets, detailed list of books available, *etc*. Pest and soil management are treated in chapters 2 and 3. Managed grazing, whole-farm planning and economic analysis are dealt with in the chapters 4, 5 and 6. Chapters 7, 10 and 12 contain a factual description of the experience from the sustainable agriculture in Iowa. The need to develop regional training workshops as well as regional research and education is set forward in the chapters 8 and 9. Developing sustainable agriculture will modify the relationships between rural and urban communities (chapter 11). Interesting data on sustainable agriculture funding during the last 15 years are summarized in the chapter 13. Sustainable agriculture refers by far not only to farmers. The role of social scientists and motivation theories has been made clear in the chapter 14. A brief survey of organic farming and detailed characteristics of challenges facing agriculture in the 21st century could be found in the Chapter 15. The last chapter (16) offers suggestion concerning the future development of rural communities and the whole landscape. Each chapter contains its list of references. The book terminates with a detailed index.

The book represents a useful tool of detailed information. It could be of interest to any reader who has been engaged in the development of sustainable forms of agriculture and forestry and needs to compare his or her experience with the expertise of the authors. Let me evidence such a conclusion of mine by quoting from the Preface: ”(this book)…*is not a formula or road map toward the future, but rather a conceptual and practical collection of chapters on the current state of farming and ranching and how it may be improved*”. There is no doubt that mankind on this planet is in need to develop and extend sustainable agriculture. And this book represents a valuable contribution to this urgent goal.

L. NÁTR (*Praha*)