EFFECT OF EXOGENOUS TREATMENT WITH SALICYLIC ACID ON PHOTOSYNTHETIC ACTIVITY AND ANTIOXIDANT CAPACITY OF CHILLED WHEAT PLANTS

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Summary. The effects of salicylic acid (SA) and cold on photosynthesis, activities of carboxylating enzymes ribulose-1,5-bisphosphate carboxylase (RuBPC) and phosphoenolpyruvate carboxylase (PEPC) and activities of photorespiratory enzymes glycolate oxidase (GO) and catalase (CAT), and on the activies of antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and peroxidase (POX) were investigated in winter wheat (*Triticum aestivum,* cv. Dogu-88) leaves. Exposure of wheat plants to a low temperature (3 ^{o}C) for 48 h and 72 h resulted in decreased levels of chlorophyll, CO_2 assimilation and transpiration rates and increased activity of GO and CAT. Treatment with SA alone for 24 h resulted in a lower rate of photosynthesis, decreased transpiration and stomatal conductance accompanied with enhanced rate of lipid peroxidation and peroxides level. Treatment with 500 μmol/L SA for 24 h before exposure to chilling provided protection on RuBPC activity and chlorophyll content. The activities of GO, CAT, POX, and APX additionally increased in SA-treated plants. The impact of SA and cold on photorespiratory and antioxidative

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metabolism is discussed.

Key words: *Triticum aestivum*, antioxidant enzymes, freezing tolerance, photosynthesis, salicylic acid, winter wheat.

Abbreviations: A-CO_2 assimilation, APX-ascorbate peroxidase, CAT-catalase, PEPC-phosphoenolpyruvate carboxylase, GO-glycolate oxidase, POX-peroxidase, GR-glutathione reductase, g_s-stomatal conductance, RuBPC-ribulose 1-5 bisphosphate carboxylase, Tr-transpiration, SOD-superoxide dismutase.

INTRODUCTION

Low temperature is one of the most important stress factors limiting the growth and productivity of cereals. Exposure of plants to suboptimal growth temperatures and high irradiance can disrupt all major components of photosynthesis including thylakoid electron transport, carbon cycle metabolism and control of stomatal conductance (Krause, 1994). The primary target of damage related to photosynthesis caused by low temperature stress is photosystem II (PSII). Low temperatures and high irradiance cause photoinhibiton of photosynthesis resulting in the formation of reactive oxygen species (ROS) in thylakoid membranes (Huner et al., 1998). Low temperatures decline the activity of energy-consuming Calvin cycle enzymes more than energy-transducing light reactions, thus causing leakage of energy to oxygen. To alleviate or prevent low temperature-induced oxidative injury, plants have evolved mechanisms to scavenge these toxic reactive species by antioxidant compounds and by enzymatic antioxidant systems (Tasgin et al., 2006). The chloroplasts also contain high levels of both lipid- and water-soluble antioxidants that act alone or in concert with the antioxidative enzymes to scavenge ROS (Wise, 1995).

The damaging effects of low temperature can result in repression of the activity of many stromal enzymes of Calvin cycle and that of ATP synthase, and also cause a restriction of ribulosebisphosphate regeneration and limitation of photophosphorylation. The enzymes of carbon assimilation are inhibited by exposure to low temperatures (Allen and Ort, 2001). Another impact of low temperature exposure is the decline of carbon export from

leaves witch results in accumulation of soluble carbohydrates (Strand et al., 2003).

Salicylic acid (SA) is part of a signaling pathway that is induced by a number of biotic and abiotic stresses. It has been recognized as an endogenous regulatory signal in plants mediating plant defense against pathogens (Raskin**,** 1992). A lot of data exist on the protective effect of SA against ultraviolet light (Yalpani et al., 1994), salinity (Shakirova et al., 2003), drought (Singh and Usha, 2003), heavy metal toxicity (Metawally at al., 2003), high temperatures (Dat et al., 1998) and paraquat (Ananieva et al., 2002). It was shown that exogenous treatment of young maize plants with SA grown under optimal growth conditions provided protection against subsequent low-temperature stress. Besides the obvious visual symptoms this observation was confirmed by changes in chlorophyll fluorescence parameters and electrolyte leakage measurements (Janda et al., 1999). Further studies proved that benzoic acid, aspirin or coumaric acid also might have protective role against chilling stress in maize plants (Janda et al., 2000; Horváth et al., 2002). Treatment of bean and tomato plants with SA or aspirin increased their tolerance against heat, chilling and drought stress (Senaratna at al., 2000). These observations suggest that the role of SA in chilling tolerance is related with its influence on the antioxidative enzyme activities and hydrogen peroxide metabolism. Kang and Saltveit (2002) reported that SA-induced chilling tolerance in maize and cucumber plants might be associated with an increase in the activity of glutathione reductase (GR) and peroxidase (POX). More recently Wang and Li (2005) showed that SA treatment of grape plants exposed to low temperature stress led to a decrease in the rates of lipid peroxidation and electrolyle leakage and induced cold tolerance. A certain level of cold tolerance was shown to be induced by SA in winter wheat exposed to low temperature stress. The authors supposed that SA could increase the freezing tolerance of winter wheat by affecting apoplastic protein synthesis (Tasgin et al., 2003). Fung et al. (2004) reported that treatment with methyl salicylate and methyl jasmonate increased the resistance against chilling injury in green bell pepper. It was proposed that increased transcript levels of alternative oxidase (AOX) correlated with reduced incidence of chilling injury. It is known that alternative respiratory pathway may mediate chilling injury by

keeping the production of ROS in balance with the levels of antioxidants and active oxygen scavenging enzyme systems, causing an increased heat evolution, especially in chilling tolerant species.

This study was undertaken to determine the physiological and biochemical changes in pretreated with SA wheat plants exposed to cold stress in order to examine whether this plant regulator is involved in the induction of defence responses to low temperature stress.

MATERIALS AND METHODS

Winter wheat plants (*Ttriticun aestivum cv. Dogu-88*) were grown for 10 days in soil in a growth chamber. The environmental conditions were: irradiance - 120 μmol m^{-2} s^{-1} PAR, 12 h-photoperiod, day/night temperature 24/20 °C, and relative humidity of 60 %. When the plants were at the second leaf stage, half of the plants were sprayed with 500 μmol/L SA. Twenty four hours after the treatment half of the control (untreated with SA) and half of the SA-treated plants were placed in camber for cold treatment at 2.8-3.5 °C. Samples were taken 48 h and 72 h after chilling treatment. Each measurement was done independently and experiments were repeated at least three times.

Gas exchange measurements were performed using a portable photosynthesis system LI 6000 at 830 μmol $m^{-2}s^{-1}$ PAR.

Activities of RuBP carboxylase (EC 4.1.1.39) and PEP carboxylase (EC 4.1.1.31) were assayed in the activated crude preparation following the incorporation of $NaH^{14}CO_3$ into acid stable products, as described by Popova et al. (1998). The assay mixture for RuBPC contained in 50 mM HEPES-NaOH (pH 8.0): 20 μmol $MgCl_2$, 1 μmol ditiothreitol (DTT), 20 μmol $NaH^{14}CO_3$ (specific radioactivity 0.38 MBq μmol^{-1}), and the enzyme extract equivalent to 0.3-0.4 mg protein. Reactions, at 25±1 °C, were initiated by addition of 2 μmol RuBP and stopped after 1 min reaction time with 6 M HCl. The assay mixture for PEPC activity contained in 50 mM HEPES-NaOH (pH 8.0): 20 μmol $MgCl_2$, 0.4 μmol NADH, 20 μmol $NaH\ ^{14}CO_3$ (specific radioactivity 0.38 MBq μmol^{-1}), 1 μmol DTT, and enzyme extract equivalent to 0.3-0.4 mg protein. The reaction volume was 1 mL. Reactions, at 30±1 °C, were initiated by addition of 3 μmol PEP.

The reaction time was 1 min. The amount of fixed $^{14}CO_2$ was measured in a liquid scintillation spectrometer.

Activity of GO (EC 1.1.3.1) was measured as described by Kolesnikov (1962). For enzyme extraction, 25 mg of leaves was ground with 20 mL 1/15 M K/Na phosphate buffer, pH 8.0, and centrifuged at 20,000 *g* for 15 min. 0.5 ml of 0.1 M Na-glycollate was added to 5 ml of extract and incubated for 10 min at 25 °C. Extracts were precipitated with 3 % TCA and developed a color reaction with 0.3% phenilhydrazine hydrochloride and 1.5 % $K_3Fe(CN)_6$. The amount of glyoxilic acid was assayed specrophotometrically at 530 nm.

Chlorophyll content was determined according to Arnon (1949).

Superoxide dismutase (SOD, EC1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971) taking into account the notes by Beyer and Fridovich (1987). Leaf samples were homogenized in 4 volumes (w/v) of an ice-cold buffer containing 0.1 M Tris-HCl, (pH 7.8), 0.1 mM EDTA and 0.05 % Triton X-100. The homogenates were filtered through 4 layers of cheesecloth and centrifuged at 4 °C for 30 min at 15,000 *g*. The crude extracts were dialyzed for 24h against half strength extraction buffer without Triton X-100, centrifuged for 20 min at 15,000 *g* and the supernatants were used for SOD assay. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.053 mM NBT, 10 mM methionine, 0.0053 mM riboflavin and an appropriate aliquot of enzyme extract. The reaction was started by switching on the light and allowed to run for 7 min. One unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the reduction of NBT as monitored at 560 nm.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined in soluble fraction and chloroplasts membrane fraction in 2 mL reaction mixture containing 50 mM potassium phosphate (pH 7.0), 0.5 mM ascorbate (extinction coefficient 2.8 mM cm^{-1}), 0.1 mM H_2O_2 and leaf extract causing a linear decrease in absorbance at 290 nm for 20 sec (Nakano and Asada, 1981).

Peroxidase (POD, EC 1.11.1.7) activity was measured by following the change of absorbtion at 470 nm due to guaiacol oxidation. The activity was

assayed for 1 min in a reaction solution (3 ml final volume) composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2 and 50 µl of crude extract (Polle et al., 1994).

Catalase (CAT, EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 (extinction coefficient 39.4 mM cm $^{-1}$) at 240 nm for 30 sec (Aeby, 1984). The assay mixture contained 100 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2 and 50 ml leaf extract in a 3 ml volume.

The level of lipid peroxidation was estimated by the method of Heath and Packer (1968). To an aliquot (0.5 ml) of the supernatant, 0.5 ml buffer and 1 ml of reagent (0.5 %TBA in 20 % TCA, w/v) were added. The test tubes were heated at 95 ^{o}C for 30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was read at 520 nm and the value for nonspecific absorption at 600 nm subtracted. The level of MDA was estimated by using the extinction coefficient of 155 mM cm^{-1}.

Peroxides content was estimated according to Jessup et al. (1994). Fresh material (0.150 g) was homogenized in 2.0 ml 0.1 % cold TCA. The homogenate was centrifuged at 15,000 *g* for 25 min. The endogenous hydrogen peroxides were measured spectrophotometrically (λ=390) by a reaction with 1M KI. The results were calculated using a standard curve prepared with fresh hydrogen peroxide solutions.

RESULTS

Exposure of wheat plants to chilling temperatures for 48 h and 72 h caused a slight decrease in the rate of photosynthesis (A) (Table 1). Treatment of control plants (24 ^{o}C) with SA alone led to a decline in A by approximately 40 %. When plants were pretreated with SA for 24 h and then exposed to chilling the rate of photosynthesis increased.

The changes in the rate of transpiration (Tr) and the values of stomatal conductance (g_s) followed the trend of A.

Exposure of control plants to 3 ^{o}C for 72 h caused a decrease in RuBPC activity by 20 % (Fig. 1). No changes were found in treated with SA plants grown under optimal temperature conditions. Full recovery of the activity of RuBPC was found in plants treated with SA for 24 h before exposure to

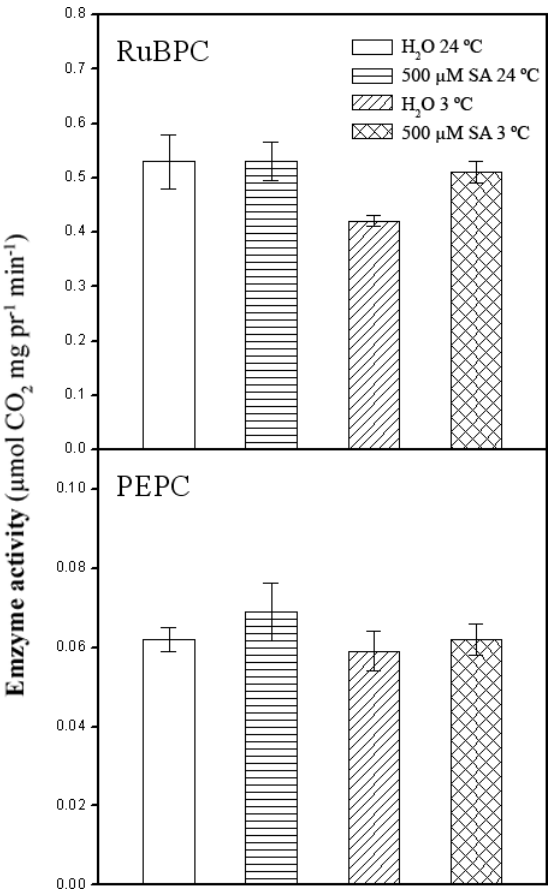

Fig 1. Effect of pretreatment with SA on the activity of RuBPC and PEPC in winter wheat seedlings chilled for 72 h. Means of 4 experiments ± SD.

low temperature. PEPC activity was almost unaffected when plants were exposed to low temperature or treated with SA.

The activities of two photorespiratory enzymes GO and CAT were examined. Cold treatment (72 h at 3 °C) caused an increase by 37 % in GO activity. SA alone led to an increase in GO activity. Treatment of plants with SA for 24 h before exposure to 3° C caused approximately 2-fold increase in GO compared with the control plants (24 °C). A similar trend was observed for catalase activity (Fig. 2).

To detect differences in the antioxidative capacity of wheat plants subjected to low temperature stress the activity of four antioxidant enzymes were measured. The activity of SOD was strongly decreased by the low temperature (nearly 2-fold) compared with the control (Fig. 3A). A slight

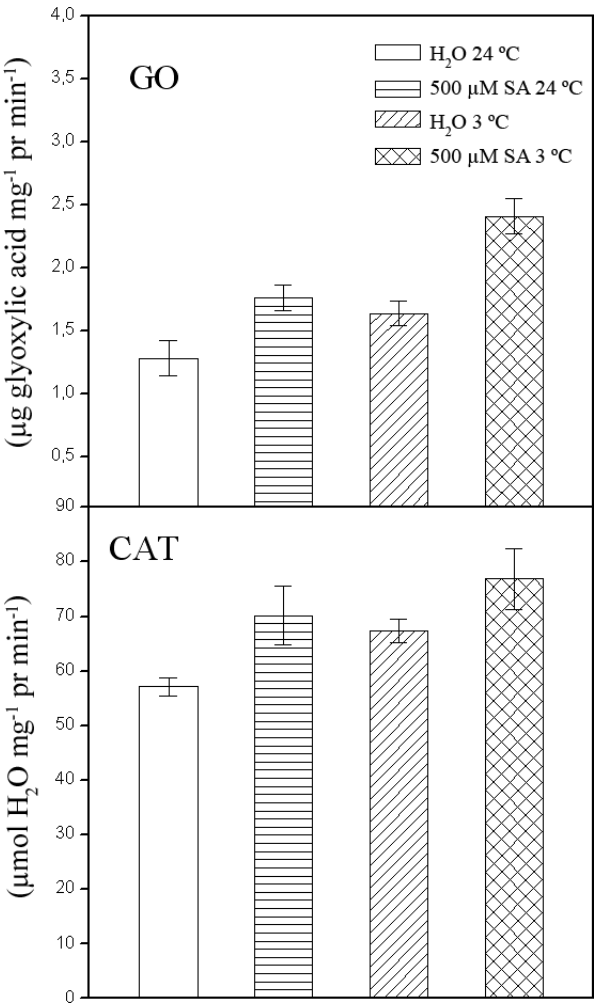

Fig 2. Effect of pretreatment with SA on glycolate oxidase and catalase activities in winter wheat seedlings chilled for and 72 h. Means of 4 experiments ± SD.

positive effect of SA was found only in plants grown at 24 °C. Treatment with SA did not alleviate the inhibitory effect of the cold stress. In contrast to SOD, APX activity showed a significant rise in chilled plants pretreated with SA (Fig. 3B). GR activity was not affected by cold and SA treatments (Fig. 3C). No significant changes in the activity of POD were found (Fig. 3D).

Exposure of plants to chilling or treatment with SA alone or in combination with a low temperature had no significant effect on peroxides accumulation.

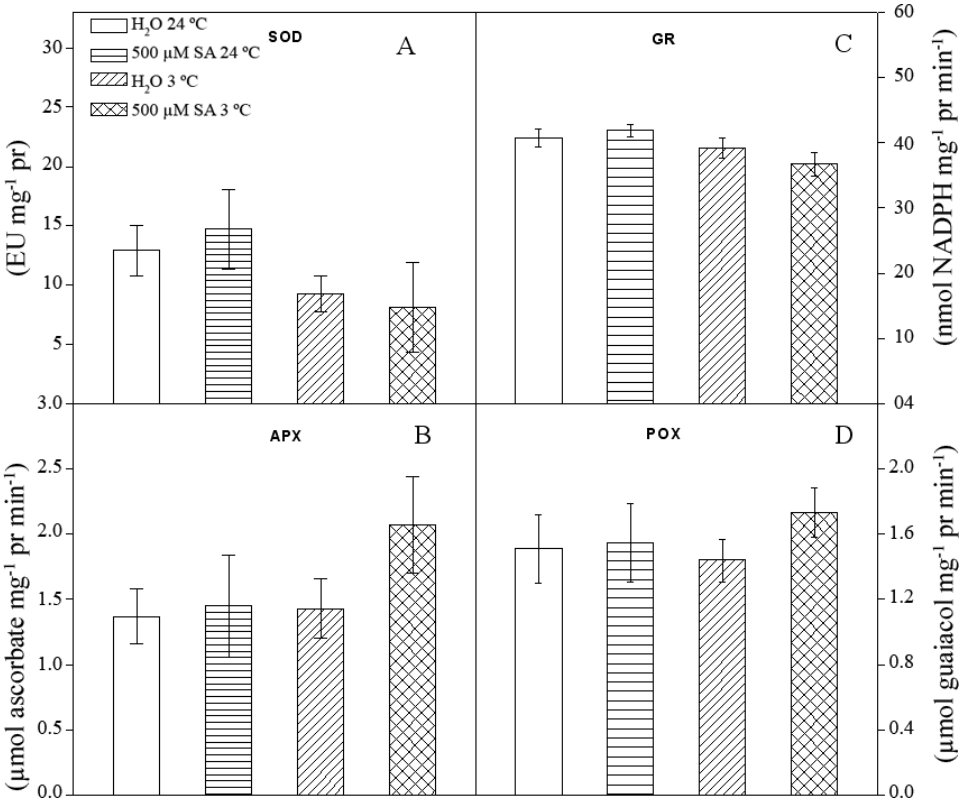

Fig 3. Effect of pretreatment with SA on the activity of SOD, APX, GR and POX in winter wheat seedlings chilled for 72 h. Means of 4 experiments ± SD.

Relative to control, SA and cold-treated plants exhibited a higher rate of lipid peroxidation. MDA content was highest in plants exposed to 3 °C for 72 h. Cold and SA treatment alone decreased the chlorophyll level. Slight protection on chlorophyll was observed in pretreated with SA wheat plants before exposure to low temperature (Table 2).

DISCUSSION

Compounds that reduce the damaging effects of certain stresses, including low temperature stress, may be of great importance for both the

Table 1. Effect of treatment with SA on gas exchange characteristics in winter wheat seedlings chilled for 48 h and 72 h. Means of 4 experiments ± SD.

	Variants	A ($mg\ CO_2\ m^{-2}s^{-1}$)	g_s ($cm\ s^{-1}$)	Tr ($mg\ H_2O\ m^{-2}s^{-1}$)
48 h	H_2O 24 °C	0.340±0.076	0.18±0.03	32.5±0.3
	500 μM SA 24 °C	0.208±0.054	0.08±0.02	24.0±0.6
	H_2O 3 °C	0.328±0.012	0.16±0.03	30.9±0.3
	500 μM SA 3 °C	0.300±0.009	0.15±0.02	26.5±0.4
72 h	H_2O 24 °C	0.355±0.078	0.19±0.02	33.6±0.4
	500 μM SA 24 °C	0.239±0.057	0.11±0.03	24.6±0.5
	H_2O 3 °C	0.318±0.014	0.16±0.04	28.4±0.3
	500 μM SA 3 °C	0.275±0.011	0.14±0.02	25.3 ±0.2

theoretical and practical considerations. Salicylic acid and its derivatives, may mediate the acclimation of plants to environmental stress, and they may interact with other cellular metabolites and environmental factors in the regulation of stress responses.

In the present study, the protective role of exogenous SA against the damaging effect of chilling stress in winter wheat plants was demonstrated. Exposure of winter wheat plants to low temperature (3 °C) for 48 h and 72 h caused a decline in the rate of CO_2 assimilation. A decrease in transpiration also occurred but it was less expressed compared with A (Table 1). The activity of RuBPC and the level of chlorophyll also decreased (Fig. 1, Table 2), while the activity of the photorespiratory enzymes GO and CAT increased in the treated plants (Fig. 2). Relative to control plants, cold-treated plants showed increased levels of MDA (Table 2). Thus, our results confirmed the well-known effect of cold stress on photosynthesis, chlorophyll breakdown

Table 2. Effect of pretreatment with SA on chlorophyll content, level of lipid peroxidation and peroxides content in winter wheat seedlings chilled for 48 h and 72 h. Means of 4 experiments ± SD.

	Variants	Chlorophyll (mg g $^{-1}$FW)	MDA (nmol g^{-1} FW)	Peroxides (μmol H_2O_2 g^{-1} FW)
48h	H_2O 24°C	1.76±0.15	20.1±1.7	0.77±0.08
	500 μM SA 24°C	1.67±0.13	24.1±1.0	0.96±0.06
	H_2O 3°C	1.55±0.11	25.0±1.5	0.73±0.05
	500 μM SA 3°C	1.72±0.20	26.9±1.7	0.84±0.04
72h	H_2O 24°C	1.70±0.13	23.1±2.0	0.75±0.05
	500 μM SA 24°C	1.46±0.15	26.4±1.8	0.84±0.06
	H_2O 3°C	1.40±0.12	29.8±2.1	0.83±0.06
	500 μM SA 3°C	1.54±0.13	27.4±1.8	0.87 ±0.07

and membrane integrity.

However, treatment of winter wheat plants with 500 μM SA alone for 24 h also resulted in a decrease in the rate of photosynthesis, transpiration and stomatal conductance. A similar effect of SA on stomatal closure was observed also for barley (Pancheva et al., 1996; Ananieva et al., 2002) and for maize (Janda et al., 1999). Under our experimental conditions treatment with SA caused an increase in the rate of lipid peroxidation and peroxides level, thus suggesting that the compound itself stressed the plants.

Our experiments showed that cold temperature independently and in combination with SA led to an increase in the activity of photorespiratory enzymes – GO and CAT. The observed higher activity of the photorespiratory enzymes in treated plants was most probably due to enhanced energy dissipation through photorespiration in the stressed plants. Despite the

considerable loss of assimilated carbon as a result of photorespiration, photosynthesis benefits since photorespiration protects the photosynthetic membranes against photon-induced damage at time when carbon assimilation is limited.

It is considered that one of the possible mechanisms of SA action is the inhibition of catalase, resulting in H_2O_2 accumulation (Dat el al., 1998; Horvath et al., 2002). Recently Mora–Herrera et al. (2005) reported that SA-induced freezing tolerance in potato plants was not connected with an inhibition of catalase activity and pertoxides accumulation. Based on these results we can explain the observed rise in CAT activity only with the enhanced photorespiration in the cold-treated plants where CO_2 access was restricted.

Exposure to low temperatures in most cases is related with increased antioxidant capacity (Wise, 1995). As AOS concentration during low temperatures exposure depends on the balance between their production and scavenging, the correct functioning and cooperation of antioxidant systems, and their low temperature stability were supposed to be an important requirement for plants to survive (Scebba et al., 1999).

Our data showed that treatment of wheat plants with SA led to a slight increase in SOD activity, whether exposure to 3 ^{o}C decreased it. The low temperature in combination with SA resulted in a remarkable rise in APX activity. The rest two investigated antioxidant enzymes GR and POX did not show significant changes in their activity. Kang et al. (2002) reported that pretreatment of banana-sensitive plants with SA before exposure to chilling temperatures led to activation of SOD, CAT and APX, but had no effect on POX activity. It was shown that treatment with SA of wheat plants that were grown under optimal temperature conditions resulted in higher activities of SOD and APX (Agarwal et al., 2005). These authors demonstrated also that H_2O_2 accumulation induced by SA was not related to inhibition of CAT and APX activities.

Although some authors (Kocsy et al., 2001) stressed on the important role of GR in low-temperature tolerance in maize plants, others working with chilling tolerant wheat plants (Janda et al., 2003) did not find any significant changes in GR activity even after strong freezing (-15 ^{o}C).

In conclusion, SA is involved in defence reactions of winter wheat

plants to freezing stress. Most probably its beneficial effect is exerted on photorespiratory metabolism and antioxidative defence system. On the other hand, it is difficult to separate any presumable beneficial effect of SA from the adaptive reactions of chilling tolerant plant species like winter wheat.

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References

Aeby, H., 1984. Catalase in vitro, Methods Enzymol., 105,121-126.

Agarwal, S., R.K. Sairam, G.C. Srivastava, A. Tyagi, R.C. Meena, 2005. Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings, Plant Science, 169, 559-570.

Allen, D.J., D.R. Ort , 2001. Impacts of chilling temperatures on photosynthesis in warm-climate plants, Trends Plant Sci., 6, 36-42.

Ananieva, E.A., V.S. Alexieva, L.P. Popova, 2002. Treatment with salicylic acid decreases the effects of paraquat on photosynthesis, J. Plant Physiol., 159, 685-693.

Arnon, D.I., 1949. Cooper enzymes in isolated chloroplasts in *Beta vulgaris*, Plant Physiol., 24, 1-15.

Beauchamp, C, I. Fridovich, 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels, Anal Biochem., 44, 276-287.

Beyer, W, I. Fridovich, 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions, Anal Biochem., 161, 559-566.

Dat, J.F., H. Lopez-Delago, C.H. Foyer, I.M. Scott, 1998. Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings, Plant Physiol., 116, 1351-1357.

Fung, R.W.M., C.Y. Wang, D.L. Smith, K.C. Gross, M.S. Tian, 2004. MeSA

and MeJa increase steady-state transcript levels of alternative oxidase and resistance against chilling injury in sweet peppers (*Capsicum annuum* L.), Plant Sci., 166, 711-719.

Horváth, E., T. Janda, G. Szalai, E. Páldi E, 2002. *In vitro* salicylic acid inhibition of catalase activity in maize: differences between the isozymes and a possible role in the induction of chilling tolerance, Plant Sci., 163, 1129-1135.

Heath, R.L., L. Pacher, 1968. Photoperoxidation in isolated chloroplasts. I. Kineticks and stoichiometry of fatty acid peroxidation, Arch. Biochem. Biophys., 125, 189-198.

Huner, N.P., G. Oquist, F. Sarhan, 1998. Energy balance and acclimation to light and cold. Trends Plant Sci., 3, 224-230.

Janda, T., G. Szalai, I. Tari, E. Paldi, 1999. Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants, Planta, 208, 175-180.

Janda, T., G. Szalai, Zs. Antunovics, E. Horváth, E. Páldi, 2000. Effect of benzoic acid and aspirin on chilling tolerance and photosynthesis in young maize plants, Maydica, 45, 29-33.

Janda, T., G. Szalai, K. Rios–Gonzales, O. Veisz, E. Paldi, 2003. Comparative study of frost tolerance and antioxidant activity in cereals, Plant Science, 164, 301-306.

Jessup, W., R. Dean, J. Gebicki, 1994. Iodometric determination of hydroperoxides in lipids and proteins. Methods Enzimol., 233, 289-303.

Kang, H.M., M.E. Saltveit, 2002. Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid, Physiol. Plant., 115, 571-576.

Kolesnikov, P., 1962. Colorimetric methods for the determination of glycolic acid oxidase and glyoxalic acid reductase , Biochimia (Russ.), 27, 193-196.

Kocsy, G., G. Galiba, C. Brunold, 2001. Role of glutathione in adaptation and signaling during chilling and cold acclimation in plants, Physiol. Plant., 113, 158-164.

Krause, G.H. Photoinhibition induced by low temperatures. – In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of photosynthesis. Pp. 331-

348. Bios. Scintific Publishers. Oxford 1994.

Metwally, A., I. Finkemeier, M. Georgi, K. Dietz, 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings, Plant Physiology, 132, 272-281.

Mora-Herrera, M. E., H. Lopez-Delago, A. Castillo-Morales, C. H. Foyer, 2005. Salicylic acid and H_2O_2 function by independent pathways in the induction of freezing tolerance in potato, Physiol. Plant., 125: 430-440.

Nakano, Y, K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, Plant Cell Physiol., 22, 867-880.

Pancheva, T.V., L.P. Popova, A.N. Uzunova, 1996. Effect of salicylic acid on growth and photosynthesis in barley plants, J. Plant Physiol., 149, 57-63.

Polle, A., T. Otter, F. Seifert, 1994. Apoplastic peroxidases and lignification in needles of Norway (*Picea abies* L.), Plant Physiol., 106, 53-60.

Popova, L., T. Tsonev, S.G. Vaklinova, 1998. Changes in some photorespiratory and photosynthetic properties in barley leaves after treatment with jasmonic acid, J. Plant Physiol., 132, 257-261.

Raskin, I., 1992. Role of salicylic acid in plants, Ann. Rev. Plant Physiol. and Plant Mol. Biol., 43, 439-463.

Scebba, F., L. Sebastiani, C. Vitagliano, 1999. Protective enzymes against activated oxygen species in wheat (*Triticum aestivum* L.) seedlings: responses to cold acclimation, J. Plant Physiol.,155: 762-768.

Senaratna, T., D. Touchell, E. Bunn, K. Dixon, 2000. Acettyl salicylic acids (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants, Plant Growth Regulation, 30, 157-161.

Singh, B., K. Usha, 2003. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regul., 39, 137-141.

Shakirova, F.M., A.R. Sakhabutdinova, M.V. Bezrukova, R.A. Fatkhutdinova, D.R. Fatkhutdinova, 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity, Plant Sci., 164: 317-322.

Strand, A., C.H. Foyer, P. Gustafsson, V. Hurry, 2003. Increased expression

of sucrose-phosphate synthase in transgenic *Arabidopsis thaliana* results in improved photosynthetic performance and increased freezing tolerance al low temperatures. Plant Call Environ., 26, 523-535.

Tasgin, E., O. Atici, B. Nalbantoglu, 2003. Effect of salicylic acid and cold on freezing tolerance in winter wheat leaves, Plant Growth Regul., 41: 231-236.

Tasgin, E., O. Atici, B. Nalbantoglou, L.P. Popova, 2006. Effects of salicylic acid and cold treatments on protein levels and on the activities of antioxidants enzymes in the apoplast of winter wheat leaves, Phytochemistry, 67, 710-715.

Yalpani, N., A.J. Enyedi, J. León, I. Raskin, 1994. Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogenesis-related proteins and virus resistance in tobacco, Planta, 193, 372-376.

Wang, L.J., S.H. Li, 2006. Salicylic acid-induced heat or cold tolerance in relation to Ca^{2+} homeostasis and antioxidant systems in young grape plants. Plant Science, 170, 685-694.

Wise, R.R., 1995. Chilling-enhanced photooxidation: the production, action and study of reactive oxygen species produced during chilling in the light, Photosynthesis Research., 45, 79-97.