Bulgarian Journal of Agricultural Science, 19 (2) 2013, 45–48
Agricultural Academy

STUDY OF PRIMARY PHOTOSYNTHETIC REACTIONS IN WINTER WHEAT CULTIVARS AFTER COLD HARDENING AND FREEZING. EFFECT OF SALICYLIC ACID

P. SASHEVA[1], R. YORDANOVA[2*], T. JANDA[3], G. SZALAI[3] and L. MASLENKOVA[2]
[1] *Medical University of Sofia, Faculty of Pharmacy, BG – 1000 Sofia, Bulgaria*
[2] *Institute of Plant Physiology and Genetics, BAS, BG – 1113 Sofia, Bulgaria*
[3] *Agricultural Institute, Centre for Agricultural Research, HAS, H-2462, Martonvasar, Hungary*

Abstract

SASHEVA, P., R. YORDANOVA, T. JANDA, G. SZALAI and L. MASLENKOVA, 2013. Study of primary photosynthetic reactions in winter wheat cultivars after cold hardening and freezing. Effect of salicylic acid. *Bulg. J. Agric. Sci.,* Supplement 2, 19: 45–48

Thermoluminescence emission in winter wheat cultivar "Sadovo-1", subjected to short period of low positive temperatures (cold hardening) and subzero temperatures (freezing) was measured in order to determinate changes in the redox cycling of Photosystem 2 centers and in the assimilatory potential in chloroplasts. Freezing stress seems to have pivotal a role in the accumulation of bound and free o-HCA forms, while levels of endogenous SA had slight increase. Subjecting the plants on freezing provoked a strong increase in plasma membrane leakage in no hardened plants. Dynamic changes of levels of free proline content were estimated after exposure of plants to low positive temperatures and subsequent freezing. Hardening effect of low positive temperatures was manifested by doubled gap of POD activity in the leaves of nonhardened winter wheat plants.

Key words: winter wheat, cold hardening, freezing, thermoluminescence
Abreviations: o-HCA – ortho-hydroxi-cimanic acid; POD – guaiacol peroxidase; PS2 – photosystem 2; SA – salicylic acid; SOD – superoxyde dismutase

Introduction

Frost tolerant species like winter wheat developed tolerance to subzero temperatures after certain period of growth at low, but not freezing temperatures called hardening. In response to low non-freezing temperatures a wide range of physiological and biochemical changes in plants were induced in order to achieve maximum capacity of frost tolerance (Hunter et al., 1998).

Under conditions of lowered temperature, the primary photosynthetic reactions are unaffected, thus posing risk of imbalance between fast photochemistry reactions and much slower transformation of the light into reducing power (NADPH) and chemical energy (ATP). The impaired energy balance then resulted in increased PS2 excitation pressure, which in turn reflects the relative reduction state of the photosystem. Although different potential mechanisms of photosynthetic adjustments to high PS2 excitation pressure have been discussed (Huner et al., 1998), the exact role of the acceptor side of PS2 in acclimation of the photosynthetic apparatus has not been evaluated directly. Because PS2, especially its acceptor side, has generally been considered the primary target for photo inhibition of photosynthesis (Aro et al., 1993), we have used thermo luminescence (TL) measurements to evaluate more precisely the charge recombination events between the acceptor and donor sides of PS2 during acclimation of winter wheat plants to low temperature and subsequent freezing.

Salicylic acid (SA) is an endogenous signal molecule involved in plant response to biotic and abiotic stress. Exogenous application of SA was found to protect plants against chilling injury (Janda et al., 1999). One possible mechanism of action, discussed by Horvath et al. (2002), is induction of transitory oxidative stress, which enhancing oxidative capacity in cells during hardening process.

**E-mail: r_yordanova@abv.bg*

In this investigation, the effects of cold hardening on PSII reactions and some possible role of salicylic acid in this process were studied by analysis of the TL glow curve parameters in unhardened and cold-hardened winter wheat cultivar Sadovo subjected to freezing. Endogenous levels of SA and its precursor were measured in order to observed dynamic of changes in different temperature regimes. Some parameters, considered like stress markers – the levels of hydrogen peroxide, free proline, rate of electrolyte leakage and the activity of two important antioxidative enzymes were also measured.

Material and Methods

Seeds of winter wheat (*Triticum aestivum* L.) 'Sadovo-1' were grown for 12 days in loamy soil under controlled conditions: PPFD – 210 μmol m^{-2} s^{-1}, photoperiod – 16 h and 20/16°C. Prior the low temperature hardening at 2°C for 7 days, in light half of the plants were sprayed with 0.5 mM SA. Part of cold hardened and control plants were shifted to frost-chamber, where wheat plants were subjected to freezing treatment for 24 hours in dark with gradually decreasing temperature (1°C per hour, final temperature –7°C). TL glow curves of wheat leaf discs were measured using the apparatus and software as described (Miranda and Ducruet, 1995). Salicylic acid and its precursors were measured according to Meuwly and Métraux, (1993). Superoxide dismutase (SOD) was estimated according to the method of Beauchamp and Fridovich, (1971) and Guaiacol peroxidase (POD) was measured after Polle et al., (1994). The endogenous hydrogen peroxides were measured spectrophotometrically (Jessup et al., 1994). The amount of electrolyte leakage was detected conductometrically. Proline concentration was determined after Bates et al. (1973).

Results and Discussion

The illumination of unfrozen dark adapted leaves from non-hardened (control) winter wheat plants cv. Sadovo with two consecutive flashes (2FL) generate a maximal TL emis-

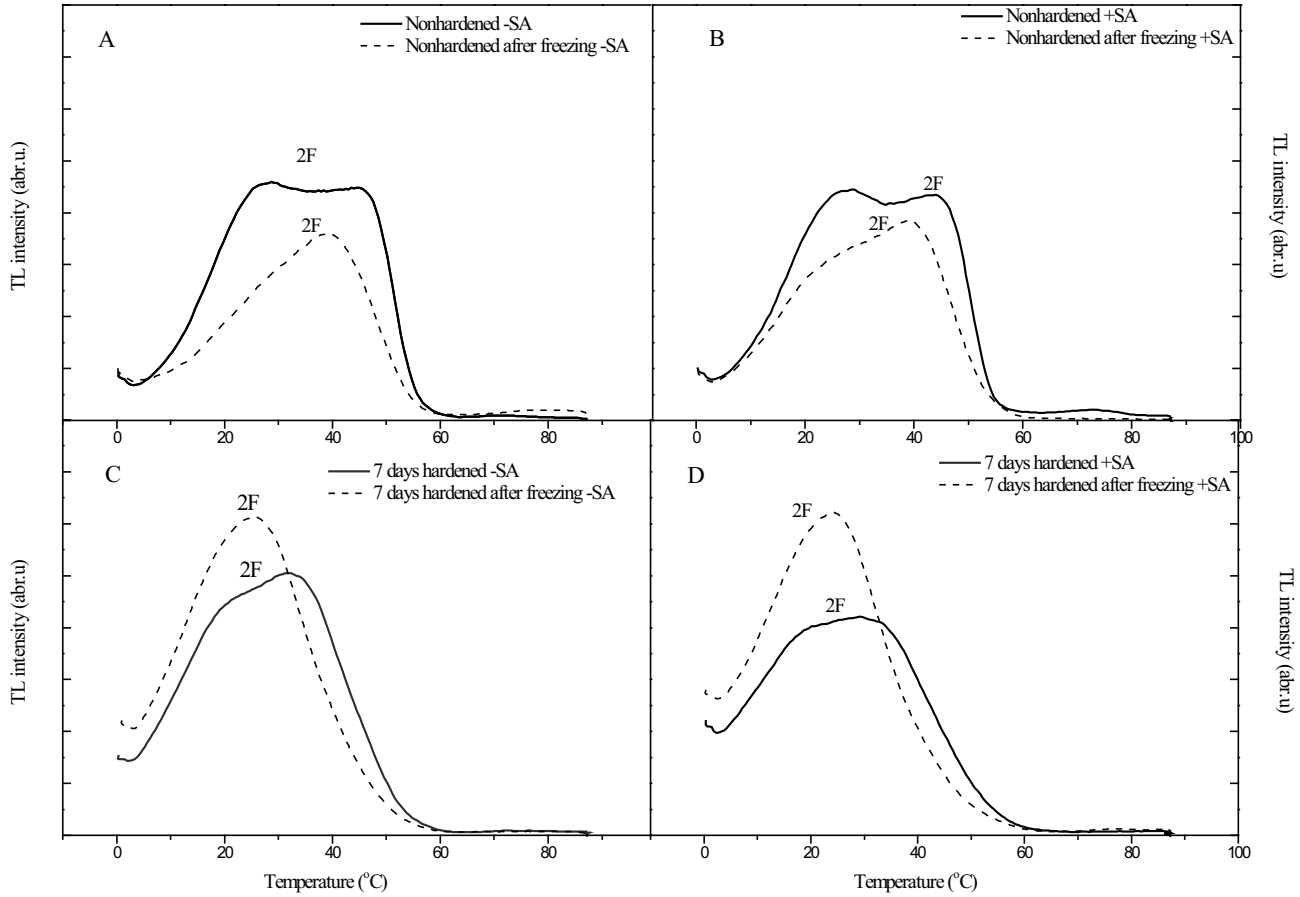

Fig. 1. Termoluminescense glow curves in winter wheat plants, hardened for 7 days and subjected to subzero temperatures, induced by 2 flashes. TL was recorded immediately after corresponding excitation, with a 0.5°C s^{-1} heating rate

Table 1

Endogenous levels of ortho-hydroxycinnamic acid and salicylic acid (free and bound forms) in leaves of winter wheat plants hardened for 7 days and subjected to subzero temperatures. The values are means ± s.e. (n = 4)

Treatment	Free *o*-HCA, ng/g FW		Bound *o*-HCA, ng/g FW		Free SA, ng/g FW		Bound SA, ng/g FW	
	SA–	SA+	SA–	SA+	SA–	SA+	SA–	SA+
NonH	24.95 ± 2.1	42.50 ± 2.4	69.95 ± 2.5	37.44 ± 2.9	25.79 ± 1.2	104.9 ± 7.6	222.36 ± 7.8	219.41 ± 11
Hardened	117.31 ± 2.5	88.36 ± 2.4	1204.89 ± 10	718.98 ± 6.5	29.43 ± 3.5	23.63 ± 4.2	234.20 ± 6.5	214.82 ± 7.8
NonH+Fr	146.80 ± 7.8	39.70 ± 3.7	1262.03 ± 11	425.88 ± 1.9	25.29 ± 3.1	28.53 ± 1.9	187.53 ± 5.0	257.81 ± 4.6
Nardened+Fr	61.35 ± 1.3	166.33 ± 1.7	2134.71 ± 25	2411.77 ± 77	44.20 ± 6.9	50.33 ± 3.0	314.38 ± 4.3	266.41 ± 9.4

Table 2

Rate of electrolyte leakage, hydrogen peroxide and free proline accumulation in leaves of winter wheat plants, hardened for 7 days and subjected to subzero temperatures. The values are means ± s.e. (n = 4)

Treatment	Electrolyte leakage, μS/cm^2		Peroxides, nmol/g FW		Free proline, μmol/g FW	
	SA–	SA+	SA–	SA+	SA–	SA+
NonH	5.1 ± 0.2	4.8 ± 0.7	2.45 ± 0.13	4.2 ± 0.54	25.79 ± 1.2	104.9 ± 7.6
Hardened	5.2 ± 0.7	5.4 ± 0.4	3.41 ± 0.17	3.8 ± 0.20	29.43 ± 3.5	23.63 ± 4.2
NonH+Fr	22.4 ± 1.2	38.3 ± 1.7	3.44 ± 0.81	3.5 ± 0.04	25.29 ± 3.1	28.53 ± 1.9
Nardened+Fr	10.3 ± 0.5	9.1 ± 0.6	3.93 ± 0.09	4.5 ± 0.17	44.20 ± 6.9	50.33 ± 3.0

sion with B-band ($S_{2(3)}Q_B$-) peaking at 29°C and AG at 45°C. The B-band results from the thermal activated recombination of the trapped electrons and positive charges on the reduced quinone acceptor (Q_B-) and the $S_2(S_3)$oxidation state of the water-oxidizing complex of PSII, whereas AG-thermoluminescence emission corresponds to a back electron transfer towards PSII centers initially in $S_{2(3)}Q_B$ state and has been proposed to reflect the [HADPH + ATP] assimilatory potential in chloroplasts when induced by flashes. TL glow curve from control plants subjected to freezing show a strong inhibition of the amplitude of B-band, hardly distinguished as a shoulder at 28–30°C, and an intense AG emission at lower temperature at about 40°C (Figure 1A). Cold-hardening (Figure 1C) lead to a small decrease in B-band and AG emission temperatures concomitantly with changes in peak amplitudes as compared to non-hardened control. The AG/B ratio, estimated from the amplitudes of B- and AG peaks at T_{max} show an increase from the value of 0.96 in control to1.29 in cold-hardened plants. TL glow curve from cold-hardened plants after freezing show disappearance of AG- band whereas the B-band at 28°C looks broadened and with unchanged intensity (Figure 1C). In SA treated hardened plants no significant changes in B-band intensity and T_{max} were observed (Figure 1D).

Exogenous SA treatment of wheat plants caused an initial rise in the endogenous levels of non-bound SA, with a subsequent decrease during hardening. Freezing of non-hardened plants caused dramatic increase in levels of bound o-HCA, whereas levels of this SA precursor in hardened plants were only doubled after freezing (Table 1). Janda et al. (2007) reported similar rate of o-HCA accumulation in wheat plans, hardened in normal light regimes. It is known that not only SA, but also o-HCA could modulate response of young maize plants to low temperatures (Janda et al., 2000) and author suggested an independent SA biosynthesis from the o-HCA accumulation, related with its ability to quench singlet molecular oxygen.

When applied in suitable concentration SA may cause transient oxidative stress in plants and increase the antioxidative capacity of the plants (Dat et al., 1998).Treatment with SA cause significant changes in levels of endogenous hydrogen peroxide only in leaves of no hardened plants, with can only be related with its mediatory role (Agarwal et al., 2005). Freezing itself did not enhanced H_2O_2 accumulation (Table 2). Plasma membrane is the most susceptible membrane structure to freezing damages, and the impaired integrity is connected with leakage of electrolytes (Pearce, 2001). Our data shown that rate of electrolyte leakage was not influenced by cold hardening while subjecting the plants on subzero temperatures provoked a strong increase in plasma membrane leakage in no hardened plants (Table 2). Three-fold increase in free proline levels was measured in hardened and no hardened plants after freezing. Treatment with SA slightly diminished proline accumulation in hardened plants (Table 2).

The only significant change in SOD activity is a decrease in SA treated, but not frost plants in two temperatures re-

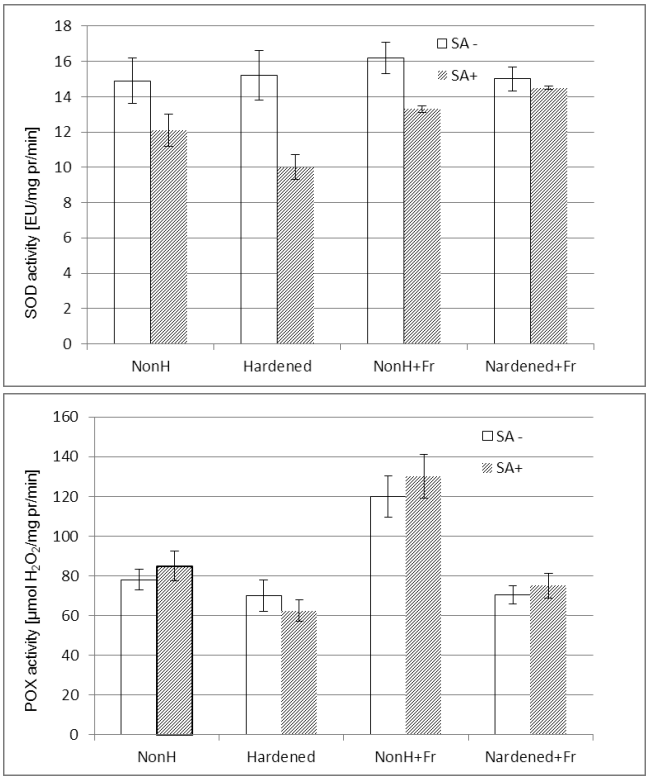

Fig. 2. Total activity of superoxidedismutase (A) and peroxidase activity (B) in the leaves of winter wheat plants, hardened for 7 days and subjected to subzero temperatures. The values are means ± s.e. (n = 3)

gimes (Figure 2A). This results confirmed finding of Tsang et al. (1991) that expression of MnSOD and cytosol Cu/Zn-SOD genes were unaffected by chilling. Total POX activity showed 100% leap in leaves of no hardened freeze plants (Figure 2B), which might be a demonstration how cold hardening "prepared" plants to harmful effect of freezing.

Besides the fact that cold hardening ensures unchanged photochemical activity of PS2, treatment with SA before freezing of unhardened plants affects positively photochemical performance of the plants,observed in changes of AG/B ratio and the investigated parameters of B-band. Changes in the level of some stress markers and the endogenous content of total SA and o-HCA confirm the idea that hardening of the winter wheat plants increased tolerance of plants to freezing temperatures by inducing osmolyte accumulation, membrane stability and mitigate oxidative stress.

References

Aro, E-M., Virgin, I. and Andersson, B., 1993. Photoinhibition of photosystem II: inactivation, protein damage and turnover. *Biochim Biophys Acta,* **1143:** 113–134.

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

Bates, L. S., Waldren R. P. and Teare, I. B., 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, **39:** 205–207.

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

Dat, J. F., Lorenz-Delgado, H., Foyer, C. H. and Scott, I. M., 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.

Horvath, E., Janda, T., Szalai, G. and Paldi, E., 2002. *In vitro* salicylic acid inhibition of catalase activity in maize: differences between the izoenzymes and a possible role in the induction of chilling tolerance. *Plant Science*, **163:** 1129–1135.

Huner, N. P. A., Öquist, G. and Sarhan, F., 1998. Energy balance and acclimation to light and cold. *Trendsin Plant Science*, **3:** 224–230.

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

Janda, T., Szalai, G., Lesko, K., Yordanova, R., Apostol, S. and Popova, L., 2007. Factors contributing to enhanced freezing tolerance in wheat during frost hardening in the light. *Phytochemistry*, **68:** 1674–1682.

Jessup, W., Dean, R. and Gebicki, J., 1994. Iodometric determination of hydroperoxides in lipids and proteins. *Methods Enzymol*., **233:** 289–303.

Meuwly, P. and Métraux J. P., 1993. *Ortho*-anisic acid as internal standard for the simultaneous quantitation of salicylic acid and its putative biosynthetic precursors in cucumber leaves. *Anal. Biochem,,* **214:** 500–505.

Miranda, T. and Ducruet J.-M., 1995. Characterization of the chlorophyll thermoluminescence afterglow in dark-adapted or far-red illuminated plant leaves. *Plant. Physiol. Biochem*., **33:** 689–699.

Pearce, R. S., 2001. Plant freezing and damage. *Annals of Botany,* **87:** 417–424.

Polle, A., Otter, T. and Seifert, F., 1994. Apoplastic peroxidases and lignification in needles of Norway Spruse (*Piceaabies* L.). *Plant Physiology,* **106**: 53–60.

Tsang, E. W. T., Bowler, C., Herouart, D., Van Camp, W., Villarroel, R., Genetello, C., Van Montagu, M. and Inze, D., 1991. Differential regulation of superoxide dismutases in plants exposed to environmental stress. *Plant Cell,* **3:** 783–792.