ISSN 1992-4917. ВІСНИК ХАРКІВСЬКОГО НАЦІОНАЛЬНОГО АГРАРНОГО УНІВЕРСИТЕТУ СЕРІЯ БІОЛОГІЯ, 2015, вип. 2 (35), с. 50-57

УДК 581.1

EFFECT OF COLD HARDENING ON RESISTANCE OF WHEAT SEEDLINGS TO HYDROGEN PEROXIDE AND IRON (II) IONS ACTION.

I. PARTICIPATION OF LOW-MOLECULAR PROTECTORS

© 2015 Yu. E. Kolupaev[1], N. I. Ryabchun[2], T. O. Yastreb[1], A. I. Oboznyi[1], M. V. Shvidenko[1]

[1]V.V. Dokuchaev Kharkiv National Agrarian University (Kharkiv, Ukraine)

[2]V.Ya. Yurjev Plant Production Institute of National Academy of Agrarian Sciences of Ukraine (Kharkiv, Ukraine)

The effect of wheat seedlings cold hardening on their resistance to the agents of oxidative stress (OS) – hydrogen peroxide and iron (II) sulfate was studied. Cold hardening for 6 days at 2°C reduces the sensitivity of wheat seedlings of cold-resistant cultivar Lutescens 329 to 150 mM hydrogen peroxide and 5 mM Fe^{2+}, which was reflected in lower growth inhibition of hardened seedlings and less accumulation of the product of lipid peroxidation malondialdehyde in them as compared to unhardened. A cold-sencitive cultivar Bezostaya 1 was not shown a positive impact of cold hardening on resistance of seedlings to the OS agents. Hardening caused a significant increase in the content of proline and sugars in wheat seedlings of both varieties. Under the influence of hydrogen peroxide there was an increase in the content of proline and sugars in unhardened seedlings of both cultivars, but at Lutescens 329 these effects were more pronounced. Iron sulfate caused a slight increase in proline content in unhardened seedlings of two cultivars and almost no effect on the content of sugars. Under the influence of OS agents the changing in content of proline and sugars in hardened seedlings was less significant. Cold hardening caused a large increase in the content of anthocyanins in seedlings of Lutescens 329 cultivar, but had no effect on this parameter at Bezostaya 1. After exposure to OS agents in hardened seedlings of Lutescens 329 anthocyanins pool maintained at a higher level compared to the unhardened, in seedlings of Bezostaya 1 the positive impact of hardening on anthocyanins pool conservation was less noticeable. The conclusion about the role of primary and secondary metabolites in the basic and induced by cold hardening resistance of seedlings to oxidative stress was made.

Key words: *Triticum aestivum, cold hardening, oxidative stress, hydrogen peroxide, iron, resistance, proline, sugars, anthocyanins*

Question of cross-tolerance mechanisms in living organisms (increasing of their resistance to certain stress factor by pre-moderate influence of a different nature of stressor) attracted the attention of researchers for several decades (Hale, 1969; Cheeseman, 2007; Titov, Talanova, 2009; Zhong, Gong, 2011). To explain this phenomenon, a number of molecular mechanisms were proposed. Thus, it is expected that an important component of cross-resistance may be an effective functioning of the antioxidant system (Shao et al., 2008; Kolupaev, Oboznyi, 2013), since oxidative damage occurs under the action of almost any adverse factors on plants (Scandalios, 2005).

Cold hardening of plants is a complex process, accompanied by a change in expression of many genes, which leads to the synthesis of cold-shock proteins, modifying the composition of membranes, the accumulation of cryoprotectants, the activation of an alternative respiration and other adaptive reactions (Grabelnych et al., 2004; Trunova, 2007; Teocharis et al., 2012). Also hard-

Adress for correspondence: Yu. Kolupaev, V.V. Dokuchaev Kharkiv National Agrarian University, p/o «Communist-1», Kharkiv, 62483, Ukraine;
e-mail: yukolupaev@mail.ru

ening induces many components of the antioxidant system (Kolupaev et al., 2015). It is shown that along with changes in the activity of antioxidant enzymes (Janda et al., 2003) during cold hardening in plant cells there is an accumulation of primary and secondary stress metabolites – sugars, proline, flavonoids and so on (Vagujfalvi et al., 1999; Tantau et al., 2004; Olenichenko et al., 2008). It is known that these compounds exhibit a sufficiently high antioxidant activity (Makarevith et al., 2010; Liang et al., 2013; Keunen et al., 2013).

A number of studies reported on the induction by cold hardening of plant resistance to other stress factors, in particular, to heavy metals able to induce oxidative stress (Titov, Talanova, 2009). At the same time, the participation of specific components of antioxidant system in the basic and cross-induced plant resistance to cold and oxidative stress (OS) agents remains little explored.

The aim of the present study was to investigate the participation of various components of the antioxidant system in response of unhardened and hardened by cold exposure wheat seedlings to OS agents – exogenous hydrogen peroxide and iron (II) sulfate (Gaber et al., 2006). This report presents the results of research on the role of proline, sugars and flavonoids in wheat seedlings resistance to these stressors.

METHODS

The object of study was etiolated seedlings of winter wheat *Triticum aestivum* L. cultivars Lutescens 329 (cold-resistant) and Bezostaya 1 (cold-sensitive) obtained from the collection of The National Centre for Plant Genetic Resources of Ukraine (Kharkiv).

Seeds after 40 minutes in disinfection of 6% hydrogen peroxide solution were germinated on purified tap water at 20°C for 3 days. Then, plantlets were placed for 6 days in the refrigerator compartment Danfoss (Netherlands) for hardening at a temperature of 2°C (Samygin, 1968). As controls, four-day-old seedlings not subjected to hardening were used, since at a low temperature growth of seedlings was slowed and 10-day-old hardened plants corresponded to 4-day ones grown at 20°C.

Control and hardened seedlings were subjected in a two-day exposure to the OS agents – 150 mM hydrogen peroxide or 5 mM iron (II) sulfate (Gaber et al., 2006). The concentration of said compounds was selected based on results of preliminary experiments. After exposure to these substances plantlets were incubated for day (until the end of experiment) on purified tap water.

Before exposure to the OS agents and a day after the mass of seedlings and their length were determined. As an indicator of the stability of seedlings to the OS agents a value of relative inhibition of biomass accumulation and linear growth of seedlings was used, which were calculated by the formula:

$$I = \frac{(C_2 - C_1) - (E_2 - E_1)}{C_2 - C_1} \cdot 100\%,$$

where I – inhibition (%), C_1, C_2, E_1 and E_2 – respectively the initial and final results of measuring the mass or length of sprouts in the control and experimental variants.

The intensity of lipid peroxidation (LPO) in tissues of seedlings was determined by the amount of products that react with 2-thiobarbituric acid (mainly malondialdehyde – MDA) (Merzlyak et al., 1978) using the protocol described previously (Kolupaev et al., 2015).

The content of proline in seedlings was determined by the method of Bates et al. (1973) with minor modifications (Kolupaev et al., 2015). The total amount of sugars were analyzed by Morris-Rohe with anthrone reagent (Zhao et al., 2003) with modifications described previously (Kolupaev et al., 2015).

To determine the flavonoids with an absorption maximum in UV B and anthocyanins the sample of plant material was homogenized in 10 ml of 1% methanolic HCl (Nogues, Baker, 2000). After centrifuging the homogenate at 8000g for 15 min the supernatant was determined by absorbance at wavelengths of 300 and 530 nm (Nogues, Baker, 2000).

The experiments were repeated independently three times at three biological replicates for each of them. In determining the biometric indicators each repetition included 30 seedlings. The average values and their standard deviations are shown. Unless otherwise specified the differences significant at $p \leq 0.05$ are discussed.

RESULTS AND DISCUSSION

Exposure to hydrogen peroxide and iron (II) sulfate caused a significant inhibition of the growth of unhardened seedlings (table 1). Herewith the significant varietal differences in reaction to the action of the OS agents were not observed.

Table 1. Relative inhibition of wheat seedlings growth by the action of hydrogen peroxide and iron (II) sulfate

Treatment	Unhardened seedlings		Hardened seedlings	
	Inhibition of seedlings biomass accumulation, %	Inhibition of plantlets linear growth, %	Inhibition of seedlings biomass accumulation, %	Inhibition of plantlets linear growth, %
Lutescens 329				
H_2O_2 (150 mM)	61,1 ± 2,2	58,6 ± 2,0	50,7 ± 2,5	34,8 ± 3,7
$FeSO_4$ (5 mM)	56,5 ± 1,8	29,7 ± 1,7	33,3 ± 3,1	22,5 ± 1,5
Bezostaya 1				
H_2O_2 (150 mM)	70,8 ± 3,6	55,7 ± 2,7	62,7 ± 4,1	46,5 ± 3,4
$FeSO_4$ (5 mM)	60,6 ± 3,8	33,3 ± 2,6	57,8 ± 2,8	34,4 ± 2,2

Table 2. Effect of agents of oxidative stress (OS) on malondialdehyde content in wheat seedlings (% to corresponding values in control)

Treatment	Unhardened seedlings		Hardened seedlings	
	After 2 days exposure to OS agents	One day after transfer to water	After 2 days exposure to OS agents	One day after transfer to water
Lutescens 329				
H_2O_2 (150 mM)	126 ± 3,6	134 ± 2,7	108 ± 2,8	107 ± 4,2
$FeSO_4$ (5 mM)	143 ± 3,9	129 ± 3,1	114 ± 3,1	112 ± 3,5
Bezostaya 1				
H_2O_2 (150 mM)	137 ± 4,7	135 ± 3,5	133 ± 3,3	139 ± 2,7
$FeSO_4$ (5 mM)	144 ± 3,3	140 ± 2,8	147 ± 4,1	142 ± 3,2

Cold hardening caused a significant increase in the resistance of wheat seedlings of cold-resistant cultivar Lutescens 329 to H_2O_2 and $FeSO_4$ action, resulting in less inhibition of biomass accumulation and linear growth of pre-hardened seedlings compared with unhardened (table 1). At the same time, cold-sensitive Bezostaya 1 after hardening was observed only a slight tendency to increase resistance to hydrogen peroxide, and the change in resistance of iron (II) sulfate do not occurs at all.

Under the influence of two studied OS agents the increase in content of LPO product MDA was happened in unhardened wheat seedlings, and this effect persisted one day after the termination of H_2O_2 and $FeSO_4$ treatment (table 2). Herewith the significant varietal differences were not noted. At the same time a pre-hardened seedlings of Lutescens cultivar, unlike unhardened, were not significant increase in MDA content occurred under influence of hydrogen peroxide, and $FeSO_4$ treatment caused only a slight increase in amount of this OS marker (table 2). The hardened seedlings of cold-sensitive Bezostaya 1 cultivar showed almost the same magnitude in MDA content increasing after exposure to OS agents as in unhardened ones (table 2).

Thus, the cold hardening caused an increase in resistance to the OS agents only a cold-resistant variety, which suggests a more substantial induction of the antioxidant system of this genotype by hardening.

As already noted, proline and sugars are currently being considered as multifunctional protector compounds, one function of which is antioxidant (Sin'kevich et al., 2009; Liang et al., 2013). Within 6 days after the cold hardening the increase in proline content in seedlings of both varieties was happened, while in cold-resistant cultivar Lutescens 329 absolute values were higher than in cold-sensitive Bezostaya 1 (table 3). When transferring seedlings to conventional temperature conditions (20°C) proline content in hardened seedlings of both varieties fell sharply and after 3 days did not differ from the corresponding control. It should be noted that in the course of experiment a content of proline in seedlings was decreasing without undergoing cold hardening that seems to be associated with the use of metabolites to activat-

Table 3. Proline and sugars content in wheat seedlings

Treatment	Proline (μmol/g dry matter)			Sugars (mg/g dry matter)		
	After 6 days of hardening	After 2 days exposure to OS agents	One day after transfer to water	After 6 days of hardening	After 2 days exposure to OS agents	One day after transfer to water
Lutescens 329						
Control	21,6 ± 0,7	15,6 ± 0,8	13,1 ± 0,4	68,1 ± 3,1	66,2 ± 2,2	66,3 ± 3,4
Hardening	63,8 ± 1,6	20,1 ± 0,7	11,2 ± 0,7	104,3 ± 3,8	107,2 ± 4,6	69,5 ± 4,8
H_2O_2 (150 mM)	-	48,0 ± 1,8	60,9 ± 2,1	-	89,4 ± 3,4	76,9 ± 2,2
Hardening + H_2O_2 (150 mM)	-	17,6 ± 1,4	14,7 ± 1,3	-	99,5 ± 5,5	71,5 ± 2,8
$FeSO_4$ (5 mM)	-	20,2 ± 0,9	18,8 ± 1,1	-	64,0 ± 2,9	61,5 ± 1,9
Hardening + $FeSO_4$ (5 mM)	-	17,2 ± 1,3	17,2 ± 0,8	-	105,4 ± 4,5	74,5 ± 4,5
Bezostaya 1						
Control	18,3 ± 0,9	13,2 ± 1,6	12,0 ± 0,6	69,5 ± 2,6	56,9 ± 1,9	58,6 ± 2,2
Hardening	54,4 ± 1,7	19,9 ± 1,2	14,4 ± 0,8	79,0 ± 3,1	74,2 ± 2,0	59,2 ± 1,6
H_2O_2 (150 mM)	-	36,7 ± 2,1	36,4 ± 1,6	-	77,0 ± 1,6	83,7 ± 2,7
Hardening + H_2O_2 (150 mM)	-	24,5 ± 1,4	16,5 ± 1,2	-	68,8 ± 3,2	67,6 ± 2,3
$FeSO_4$ (5 mM)	-	16,1 ± 0,9	16,4 ± 1,5	-	55,4 ± 1,8	61,5 ± 2,5
Hardening + $FeSO_4$ (5 mM)	-	23,8 ± 1,2	14,4 ± 0,8	-	65,2 ± 2,2	63,4 ± 1,6

ing growth processes, as well as flow of reserve materials from grains.

Under the influence of hydrogen peroxide there was a significant increase in proline content in the shoots of unhardened seedling of both varieties (table 3). The absolute value of the cultivar Lutescens 329 was larger. In addition, the increase in content of proline in this cultivar seedlings continued after transfer to medium without hydrogen peroxide.

Increased content of proline in variants with treatment of iron (II) sulfate was low, especially in Bezostaya 1 (table 3).

Reaction of hardened seedlings to the action of OS agents was significantly different from that of unhardened. Under the treatment with hydrogen peroxide hardened Lutescens 329 seedlings showed proline content in them practically not increased, while Bezostaya 1 showed a slight increase of this index (table 3). No significant effect on the proline content in hardened seedlings of both varieties was observed after treatment with $FeSO_4$.

Cold hardening caused a significant increase in the content of sugars in Lutescens 329 cultivar seedlings and less significant in Bezostaya 1 (table 3). After transferring the hardened seedlings to conventional temperature conditions the sugar content in them was gradually decreased, approaching both cultivars to values of control.

Under the influence of hydrogen peroxide in unhardened seedlings of both varieties there was a marked increase in sugar content. At the same time, another OS agent – iron (II) sulfate did not cause such an effect (table 3). Under the action of both OS agents on hardened seedlings of two cultivars changes in the content of sugars in them were comparable with the corresponding values of variants with a single hardening.

It is known that when plants exposed to stressors of different nature, including temperature, the content of anthocyanins and colorless flavonoids having a maximum absorbance in UV B may change (Pietrini, Massacci, 1998; Havaux, Kloppstech, 2001; Gould, Lister, 2006). In our experiments, the content of colorless flavonoids after hardening of both varieties changed insignificantly (table 4). It should be noted that in literature there are reports of increasing the flavonoid compounds content when hardening of green wheat plants (Olenichenko et al., 2008). Probably, the contribu-

Table 4. Flavonoids and anthocyanins content in wheat seedlings

Treatment	Flavonoids (A_{300}/g dry matter)			Anthocyanins (A_{530}/g dry matter)		
	After 6 days of hardening	After 2 days exposure to OS agents	One day after transfer to water	After 6 days of hardening	After 2 days exposure to OS agents	One day after transfer to water
Lutescens 329						
Control	21,2 ± 0,5	20,1 ± 0,6	21,3 ± 0,9	1,46 ± 0,04	1,63 ± 0,06	1,49 ± 0,07
Hardening	19,2 ± 0,8	17,9 ± 0,9	18,2 ± 0,6	2,21 ± 0,07	1,77 ± 0,03	1,83 ± 0,06
H_2O_2 (150 mM)	-	16,4 ± 0,9	16,6 ± 0,8	-	1,42 ± 0,04	1,18 ± 0,03
Hardening + H_2O_2 (150 mM)	-	16,6 ± 1,2	16,2 ± 0,6	-	1,45 ± 0,05	1,63 ± 0,09
$FeSO_4$ (5 mM)	-	22,3 ± 1,3	20,2 ± 0,8	-	1,33 ± 0,06	0,86 ± 0,06
Hardening + $FeSO_4$ (5 mM)	-	18,3 ± 0,8	18,9 ± 0,6	-	1,38 ± 0,04	1,74 ±0,05
Bezostaya 1						
Control	21,6 ± 0,4	21,1 ± 0,7	21,9 ± 0,5	1,16 ± 0,06	1,29 ± 0,06	1,10 ± 0,04
Hardening	18,7 ± 0,7	17,9 ± 0,8	18,0 ± 0,6	1,07 ± 0,03	1,23 ± 0,04	1,18 ± 0,06
H_2O_2 (150 mM)	-	16,2 ± 0,6	15,6 ± 0,9	-	0,85 ± 0,03	0,82 ± 0,06
Hardening + H_2O_2 (150 mM)	-	14,9 ± 1,3	13,9 ± 1,1	-	0,79 ± 0,06	0,98 ± 0,04
$FeSO_4$ (5 mM)	-	17,7 ± 0,7	18,7 ± 1,3	-	0,94 ± 0,08	0,64 ± 0,06
Hardening + $FeSO_4$ (5 mM)	-	15,8 ± 1,0	16,3 ± 0,9	-	0,87 ± 0,03	0,77 ± 0,04

tion of flavonoids to antioxidant protection in the adaptation of green and etiolated plants may vary.

Under the influence of hydrogen peroxide it noted a decrease in the content of flavonoids in hardened and unhardened seedlings of both cultivars, the impact of iron (II) sulfate on their amount was less noticeable. Such changes in the content of flavonoids under severe stress conditions were described by other authors (Zagoskina et al., 2011).

The content of anthocyanins under the influence of hardening was significantly increased in cold-resistant cultivar Lutescens 329 and did not change in unresistant Bezostaya 1 (table 4). After transferring the hardened seedlings to optimal temperature conditions the content of anthocyanins in Lutescens 329 was slightly reduced, but exceeds the corresponding value of control.

Under the influence of hydrogen peroxide and iron (II) sulfate there was a decrease in anthocyanin content in unhardened seedlings of both varieties, wherein the absolute value in cv Lutescens 329 remained at a higher level. The hardened Lutescens 329 seedlings anthocyanin content remained at a level far above the corresponding variants without hardening. At the same time, the differences in content of anthocyanins in exposed to OS agents hardened and unhardened Bezostaya 1 seedlings were not significant (table 4).

Thus, the cold hardening of seedlings of cold-resistant cultivar Lutescens 329 induced the development of resistance to hydrogen peroxide and iron (II) sulfate, which was reflected in lower compared to the non-hardened seedlings growth inhibition after these effects (table 1) and a lower accumulation in seedlings of the LPO product MDA (table 2) which is known to reflect oxidative damage.

The cold hardening and action of H_2O_2 both caused a significant increase in proline and sugars content in seedlings. Should be noted that in available literature we did not find data on induction of sugars accumulation in plant cells by direct action of hydrogen peroxide. In our case, the accumulation of sugars in shoots, apparently, was due to the mobilization of reserve substances from grains.

Under the influence of iron (II) sulfate a slight increase in the content of proline was noted, and the amount of sugar was not significantly changed. Apparently, these differences are related to the specific manifestation of the effects of $FeSO_4$ as a stressor, differing from the action of H_2O_2.

It should be noted that the reaction of hardened and unhardened seedlings for some indicators differed appreciably. Thus, in both varieties of hardened seedlings, unlike unhardened, no significant increase in proline content after exposure to hydrogen peroxide was observed (table 3). Perhaps this is due to the more efficient functioning of other protective systems in the hardened seedlings, for example, a complex of antioxidant enzymes. Naturally, this assumption requires experimental verification.

Another significant difference of hardened seedlings from unhardened ones, manifested only in cold-resistant cultivar Lutescens 329, was in maintaining their pool of anthocyanins after the action of the OS agents. Increasing their content after hardening and preserving the necessary pool in the action of stressors, is probably one of the reasons for greater stability of hardened seedlings Lutescens 329 to the OS agents. It is known that anthocyanins are one of the most effective low-molecular antioxidants (Makarevich et al., 2010).

Overall, the obtained results indicate that the proline, sugars, and flavonoid compounds participate in protecting of etiolated wheat seedlings from oxidative damage. In this case, apparently, the contribution of proline and sugars in the resistance to OS agents in hardened and unhardened seedlings varies. Thus, increasing the amount of these compounds in response to treatment with hydrogen peroxide and iron (II) sulfate was occurred the most in unhardened seedlings of Lutescens 329, whereas the hardened plantlets of this cultivar showed a high resistance to OS agents with no increasing the content of proline and sugars was noted. High resistance of Lutescens 329 cultivar hardened seedlings to OS agents may be due to a significant contribution of other mechanisms, in particular, an increase in the content of anthocyanins recorded in this paper. On the other hand, it is known, that hardening of cereals seedlings causes noticeable changes in the activity of antioxidant enzymes too (Kolupaev et al., 2015). The results of the research of the antioxidant system enzymatic component functioning in hardened and unhardened wheat seedlings under the action of OS agents will be presented in the next report.

REFERENCES

Bates L.S., Walden, R.P. Tear G.D. Rapid determination of free proline for water stress studies // Plant Soil. – 1973. – V. 39. – P. 205-210.

Cheeseman J.M. Hydrogen peroxide and plant stress: a challenging relationship // Plant Stress. – 2007. – V. 1, № 1. – P. 4-15.

Gaber A., Yoshimura K., Yamamoto T., Yabuta Y., Takeda T., Miyasaka H., Nakano Y., Shigeoka S. Glutathione peroxidase-like protein of *Synechocystis* PCC 6803 confers tolerance to oxidative and environmental stresses in transgenic *Arabidopsis* // Physiol. Plant. – 2006. – V. 128. – P. 251-262.

Gould K.S., Lister C. Flavonoid functions in plants // Flavonoids: chemistry, biochemistry, and applications / Eds. O.M. Andersen, K.R. Markham. – Taylor & Francis Group, 2006. – P. 397-442.

Grabelnych O.I., Sumina O.N., Funderat S.P., Pobezhimova T.P., Voinikov V.K., Kolesnichenko A.V. The distribution of electron transport between the main cytochrome and alternative pathways in plant mitochondria during short-term cold stress and cold hardening // J. Therm. Biol. – 2004. – V. 29. – P. 165-175.

Hale H.B. Cross adaptation // Environm. Res. – 1969. – V. 2, № 2. – P. 324.

Havaux M., Kloppstech K. The protective functions of carotenoid and flavonoids pigments against excess visible radiation at chilling temperature investigated in Arabidopsis npq and *tt* mutants // Planta. – 2001. – V. 213. – P. 953-966.

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

Kolupaev Yu.E., Oboznyi A.I. Reactive oxygen species and antioxidative system at cross adaptation of plants to activity of abiotic stressors // Bull. Kharkiv. Nat. Agr. Univ. Ser. Biol. – 2013. – Iss. 3 (30). – P. 18-31. (In Urainian).

Kolupaev Yu.E., Ryabchun N.I., Vayner A.A., Yastreb T.O., Oboznyi A.I. Antioxidant enzyme activity and osmolyte content in winter cereal seedlings under hardening and cryostress // Russ. J. Plant Physiol. - 2015. – V. 62, №. 4. P. 499-506.

Keunen E., Peshev D., Vangronsveld J., Van Den Ende W,. Cuypers A. Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept // Plant Cell Environ. – 2013. – doi: 10.1111/pce.12061.

Liang X., Zhang, L., Natarajan S.K., Becker D.F. Proline mechanisms of stress survival // Antioxid. Redox Signal. – 2013. – V. 19. – P. 998-1011.

Makarevich A.M., Shutova A.G., Spiridovich Ye.V., Reshetnikov V.N. The functions and properties of anthocyanins of plant raw materials // Trudy Belarus. Un-ta. – 2010. – V. 4, iss. 2. – P. 1-11. (In Russian).

Merzlyak M.N., Pogosyan S.I., Yuferova S.G., Shevyreva V.A. The use of 2-thiobarbituric acid in the study of lipid peroxidation in plant tissues // Biol. Nauki. – 1978. – № 9. – P. 86-94. (In Russian).

Nogues S., BakerN.R. Effects of drouht on photosynthesis in Mediterranean plants grown under UV-B radiation // J. Exp. Bot. – 2000. – V. 51. – P. 1309-1317.

Olenichenko N.A., Zagoskina N.V., Astakhova N.V., Trunova T.I., Kuznetsov Yu.V. Primary and secondary metabolism of winter wheat under cold hardening and treatment with antioxidants // Appl. Biochem. Microbiol. – 2008. – V. 44, № 5. – P. 535-540.

Pietrini F. Massacci A. Leaf anthocyanin content changes in *Zea mays* L. grown at low temperature: Significance for the relationship between the quantum yield of PS II and the apparent quantum yield of CO2 assimilation // Photosynthesis Res. – 1998. – V. 58. – P. 213-219.

Samygin G.A. Rapid determination of the relative hardiness of wheat samples by freezing of germinated seeds // Metody opredeleniya morozostoikosti rastenii. – Moscow: Nauka, 1967. – P. 77-84. (In Russian).

Scandalios J.G. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses // Braz. J. Med. Biol. Res. – 2005. – V. 38. – P. 995-1014.

Shao H.B., Chu L.Y., Lu Zh.H., Kang C.M. Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells // Int. J. Biol. Sci. – 2008. – V. 4. – P. 8-14.

Sin'kevich M.S., Deryabin A.N., Trunova T.I. Characteristics of oxidative stress in potato plants with modified carbohydrate metabolism // Russ. J. Plant Physiol. – 2009. – V. 56. – P. 168-174.

Tantau H., Balko C., Brettschneider B., Melz G., Dorffling K. Improved frost tolerance and winter survival in winter barley (*Hordeum vulgare* L.) by in vitro selection of proline overaccumulating lines // Euphytica. – 2004. – V. 139. – P. 19-32.

Theocharis A., Clement C., Barka E.A. Physiological and molecular changes in plants grown at low temperatures // Planta. – 2012. – V. 235. – P. 1091-1105.

Titov A.F., Talanova V.V. Resistance of plants and phytohormones. – Petrozavodsk, 2009. – 206 p. (In Russian).

Trunova T.I. Rastenie i nizkotemperaturnyi stress. 64-e Timiryazevskoe chtenie. – Moscow: Nauka, 2007. – 54 p. (In Russian).

Vagujfalvi A., Kerepesi I., Galiba G., Tischner T., Sutka J. Frost hardiness depending on carbohydrate changes during cold acclimation in wheat // Plant Sci. – 1999. – V. 144. – P. 85-92.

Zagoskina N.V., Olenichenko N.A., Nazarenko L.V. Changes in activity of antioxidant enzymes and content of phenolic compounds in the leaves of spring and winter wheat seedlings under short-term effect of low temperature // Bull. Kharkiv. Nat. Agr. Univ. Ser. Biol. – 2011. – Iss. 3 (24). – P. 25-34. (In Russian).

Zhao K., Fan H., Zhou S., Song J. Study on the salt and drought tolerance of *Suaeda salsa* and *Kalanchoe claigremontiana* under isoosmotic salt and water stress // Plant Sci. – 2003. – V. 165, Is. 4. – P. 837-844.

Zhong-Guang L, Ming G. Mechanical stimulation-induced cross-adaptation in plants: an overview // J. Plant Biol. – 2011. – V. 54. – P. 358-364.

Received
18.05.2015

ВПЛИВ ХОЛОДОВОГО ЗАГАРТУВАННЯ НА СТІЙКІСТЬ ПРОРОСТКІВ ПШЕНИЦІ ДО ДІЇ ПЕРОКСИДУ ВОДНЮ ТА ІОНІВ ЗАЛІЗА (II). I. УЧАСТЬ НИЗЬКОМОЛЕКУЛЯРНИХ ПРОТЕКТОРІВ

Ю. Є. Колупаєв[1], Н. І. Рябчун[2], Т. О. Ястреб[1],
О. І. Обозний[1], М. В. Швиденко[1]

[1]Харьковський національний аграрний університет ім. В.В. Докучаєва
(Харків, Україна)
[2]Інститут рослинництва ім. В.Я. Юр'єва
Національної академії аграрних наук України
(Харків, Україна)
e-mail: yukolupaev@mail.ru

Досліджували вплив холодового загартування проростків пшениці на їх стійкість до дії агентів окиснювального стресу (ОС) – пероксиду водню та сульфату заліза(II). Холодове загарту-

вання протягом 6 діб при 2°С зменшувало чутливість проростків пшениці морозостійкого сорту Лютесценс 329 до 150 мМ пероксиду водню і 5 мМ Fe^{2+}, що виявлялося у менших інгібуванні росту загартованих проростків і накопиченні в них продукту пероксидного окиснення ліпідів малонового діальдегіду порівняно з незагартованими. У неморозостійкого сорту Безоста 1 не виявлялося позитивного впливу холодового загартування на резистентність проростків до агентів ОС. Загартування викликало істотне підвищення вмісту проліну і цукрів у проростках пшениці обох сортів. Під впливом пероксиду водню відзначалося підвищення вмісту проліну і цукрів у незагартованих проростках обох сортів, однак у сорту Лютесценс 329 ці ефекти були більш помітними. Сульфат заліза викликав невелике підвищення вмісту проліну в незагартованих проростках двох сортів і практично не впливав на вміст у них цукрів. За дії агентів ОС на загартовані проростки зміни вмісту проліну і цукрів були менш суттєвими. Холодове загартовування викликало значне підвищення вмісту антоціанів в проростках сорту Лютесценс 329, але не впливало на цей показник у сорту Безоста 1. Після впливу агентів ОС у загартованих проростків сорту Лютесценс 329 пул антоціанів зберігався на більш високому рівні в порівнянні з незагартованими, у сорту Безоста 1 позитивний вплив загартування на збереження пула антоціанів був менш помітним. Зроблено висновок про роль первинних і вторинних метаболітів в базовій та індукованої холодовим загартуванням стійкості проростків до окиснювального стресу.

Ключові слова: *Triticum aestivum, холодове загартування, окиснювальний стрес, пероксид водню, залізо, стійкість, пролін, цукри, антоціани*

ВЛИЯНИЕ ХОЛОДОВОГО ЗАКАЛИВАНИЯ НА УСТОЙЧИВОСТЬ ПРОРОСТКОВ ПШЕНИЦЫ К ДЕЙСТВИЮ ПЕРОКСИДА ВОДОРОДА И ИОНОВ ЖЕЛЕЗА (II). I. УЧАСТИЕ НИЗКОМОЛЕКУЛЯРНЫХ ПРОТЕКТОРОВ

Ю. Е. Колупаев[1], Н. И. Рябчун[2], Т. О. Ястреб[1],
А. И. Обозный[1], Н. В. Швиденко[1]

[1]Харьковский национальный аграрный университет им. В.В. Докучаева
(Харьков, Украина)
[2]Институт растениеводства им. В.Я. Юрьева
Национальной академии аграрных наук Украины
(Харьков, Украина)
e-mail: yukolupaev@mail.ru

Исследовали влияние холодового закаливания проростков пшеницы на их устойчивость к действию агентов окислительного стресса (ОС) – пероксида водорода и сульфата железа(II). Холодовое закаливание в течение 6 сут при 2°С уменьшало чувствительность проростков пшеницы морозоустойчивого сорта Лютесценс 329 к 150 мМ пероксиду водорода и 5 мМ Fe^{2+}, что выражалось в меньших ингибировании роста закаленных проростков и накоплении в них продукта пероксидного окисления липидов малонового диальдегида по сравнению с незакаленными. У неморозоустойчивого сорта Безостая 1 не проявлялось положительного влияния холодового закаливания на резистентность проростков к агентам ОС. Закаливание вызывало существенное повышение содержания пролина и сахаров в проростках пшеницы обоих сортов. Под влиянием пероксида водорода отмечалось повышение содержания пролина и сахаров в незакаленных проростках обоих сортов, однако у сорта Лютесценс 329 эти эффекты были более заметными. Сульфат железа вызывал небольшое повышение содержания пролина в незакаленных проростках двух сортов и практически не влиял на содержание в них сахаров. При действии агентов ОС на закаленные проростки изменения содержания пролина и сахаров были менее существенными. Холодовое закаливание вызывало значительное повышение содержания антоцианов в проростках сорта Лютесценс 329, но не влияло на этот показатель у сорта Безостая 1. После воздействия агентов ОС у закаленных проростков сорта Лютесценс 329 пул антоцианов сохранялся на более высоком уровне по сравнению с незакаленными, у сорта Безостая 1 положительное влияние закаливания на сохранение пула антоцианов было менее заметным. Сделано заключение о роли первичных и вторичных метаболитов в базовой и индуцированной холодовым закаливанием устойчивости проростков к окислительному стрессу.

Ключевые слова: *Triticum aestivum, холодовое закаливание, окислительный стресс, пероксид водорода, железо, устойчивость, пролин, сахара, антоцианы*