Antioxidative system activity as the marker for freezing and drought tolerance in winter barley (*Hordeum vulgare* L.)

Iwona Żur[1], Ewa Surówka[1], Ewa Dubas[1], Anna Nowicka[1], Monika Krzewska[1], Marcin Rapacz[2], Magdalena Wójcik-Jagła[2], Agnieszka Ostrowska[1], Sabina Malaga[1], Tomasz Hura[1], Franciszek Janowiak[1]

[1] Polish Academy of Sciences, The *Franciszek Górski* Institute of Plant Physiology, Niezapominajek 21, 30-239 Kraków, Poland

[2]Department of Plant Physiology, University of Agriculture in Kraków, Podłużna 3, 30-239 Kraków, Poland

INTRODUCTION

Any disturbance in physiological homeostasis of a plant can lead to the overproduction of reactive oxygen species (ROS), often resulting in a cascade of uncontrolled oxidation. For that reason plants evolved a sophisticated defence system, in which the most important role is played by antioxidative enzymes – superoxide dismutase (SOD), catalyse (CAT) and peroxidase (POX) – working in concert with low molecular weight (LMW), non-enzymatic antioxidants to protect plant cells from oxidative damage. In the presented study, the role of the antioxidative system in seedling acclimation to low temperature and drought at the generative phase of plant development was studied with the use of ten doubled haploid (DH) lines of winter barley (*Hordeum vulgare* L.), which showed significant variation in respect of freezing and drought tolerance level (Gołębiowska-Pikania et al. 2017a,b).

MATERIAL AND METHODS

The plant material consisted of ten DH lines of winter barley produced from F1 generation of Polish breeding materials through anther culture method.

Cold hardening and freezing tolerance (FT) test

At the stage of 3-leaves, the seedlings were cold hardened (20 days at 4/2°C, day/night), then cut about 1 cm above the soil and put into a freezer for 8h at -12°C. FT was estimated after 21 days based on the ability of the seedlings to regrow.

Drought treatment and drought tolerance (DT) estimation

Soil drought was started when the flag leaf was fully developed. Water content in the pots was gradually reduced to 33-35% of soil water content and was maintained at that level for the next two weeks. Water content in the control pots was adjusted to 75-78%.

Leaf water content (WC) was measured in control (WC_c) and drought-treated plants (WC_d) by quantitative sampling of leaf fresh mass (L_{FM}), followed by 72-hour lyophilisation. The obtained leaf dry mass (L_{DM}) was then estimated and WC was calculated according to the following equation and expressed as a percentage:

$WC = ((L_{FM} - L_{DM})/L_{FM}) \times 100\%$.

DT was estimated on the basis of leaf water loss (LWL) calculated according to the equation: $LWL= [(WC_c-WC_d)/WC_c] \times 100\%$

Measurements of antioxidative system activity

Activities of antioxidative enzymes were determined spectrophotometrically by the methods of Minami and Yoshikawa (1979) [SOD], Aebi (1984) [CAT] and Luck (1963) [POX]. The total content (activity) of low molecular weight (LMW) antioxidants was measured by DPPH method according to Brand-Williams et al. (1995).

All studied parameters were measured in 5 biological replicates.

RESULTS AND CONCLUSIONS

Freezing/drought tolerance tests divided the studied DH lines of barley into three groups: highly tolerant, moderately tolerant and susceptible to studied stress factors (Table 1, 2).

SOD activity: In response to cold-hardening SOD activity increased significantly in the majority of DH lines with higher level of FT (Fig.1A), whereas drought induced a significant decrease of SOD activity in DH lines with low level of DT (Fig.1B). As a result, significant variation between freezing/drought tolerant and freezing/drought sensitive DH lines could be noticed (Table 3, 4).

CAT activity: A significant decrease of CAT activity was a typical reaction to cold hardening and drought treatment (Fig. 2A, 2B). This effect was particularly dramatic in freezing and drought susceptible DH lines (Table 3, 4).

POX activity: Cold hardening had no effect on POX activity in freezing tolerant seedlings (Fig. 3A), whereas in freezing susceptible seedlings POX activity – significantly lower at the beginning of hardening – increased drastically to a significantly higher value (Table 3). In response to drought treatment, activity of POX increased in the majority of examined DH lines (Fig. 3B). This effect was genotype-dependent but also seemed connected with DT level (Table 4).

LMW antioxidant activity: LMW antioxidant activity and its change in response to cold hardening was genotype-dependent and not associated with FT level (Fig. 4A). In response to drought, LMW antioxidant activity dropped significantly to a very similar value, regardless of DT level (Fig. 4B, Table 4).

Table 1. Percentage (means ± Se) of plants survival evaluated 21 days after freezing test (8h at -12°C).

DH line	Survival rate [%]
DH602	80.0 ± 5.0
DH534	80.0 ± 0.0
DH584	66.7 ± 5.6
DH435	64.3 ± 7.1
DH61	60.0 ± 5.0
DH561	54.5 ± 3.0
DH65	40.0 ± 13.3
DH158	40.0 ± 16.7
DH575	38.7 ± 6.5
DH363	35.7 ± 7.1

Table 2. Changes in leaf water content (WC) induced by drought stress and leaf water loss (LWL) characteristic for the studied DH lines. Means (± Sd) within each column marked with the same letter do not differ significantly according to Duncan test (p≤ 0.05).

DH lines	control [%]	drought [%]	LWL [% of control]
DH534	84.9 ± 0.5 l	78.6 ± 1.2 g	7,4
DH561	81.3 ± 0.7 i	75.1 ± 0.7 ef	7,7
DH584	78.3 ± 0.6 g	72.0 ± 0.7 c	7,9
DH363	81.5 ± 0.5 ij	74.1 ± 0.5 de	9,1
DH65	83.5 ± 0.4 k	75.3 ± 0.8 f	9,8
DH61	83.2 ± 0.7 k	73.7 ± 0.8 d	11,4
DH158	81.7 ± 1.2 ij	72.1 ± 0.7 c	11,8
DH575	83.5 ± 0.7 k	73.3 ± 0.9 d	12,2
DH602	82.6 ± 0.6 jk	70.5 ± 1.3 b	14,7
DH435	79.7 ± 0.5 h	67.6 ± 0.6 a	15,2

Fig. 1-4. Activity of antioxidative system (1. SOD; 2. CAT; 3. POX; 4. LMW antioxidants) and its changes in response to cold-hardening (A) and drought (B) in studied DH lines.

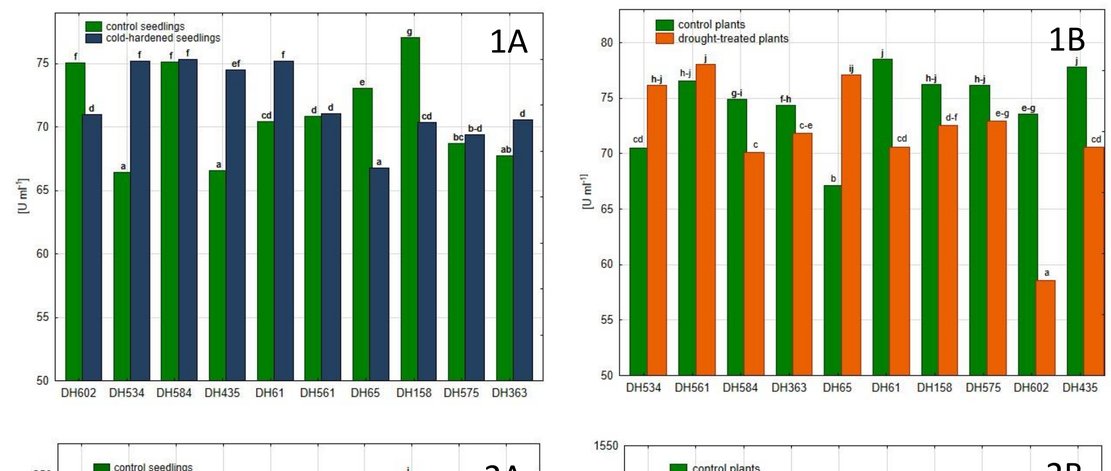

Table 3. Changes in antioxidative system activity induced by cold hardening in the groups of DH lines significantly differentiated in FT level. Mean values (± Se) within each column marked with the same letter do not differ significantly according to Duncan test (p≤0.05).

DH lines	Antioxidative system activity			
	SOD [U ml⁻¹]	CAT [µmol min⁻¹mg⁻¹]	POX [nmol min⁻¹mg⁻¹]	LMW [µM Troloxg⁻¹ DW]
Control seedlings				
Freezing tolerant	70.7 ± 1.7 ab	189.4 ± 7.6 b	3.1 ± 0.0 b	10.5 ± 0.6 a
Moderately tolerant	70.5 ± 0.8 ab	182.6 ± 4.4 b	3.4 ± 0.1 bc	**13.1 ± 0.5** b
Freezing susceptible	71.4 ± 1.0 a-c	**215.1 ± 4.9** c	**2.2 ± 0.1** a	10.9 ± 0.7 a
Cold-hardened seedlings				
Freezing tolerant	73.1 ± 0.8 bc	121.0 ± 2.9 a	3.0 ± 0.2 b	12.6 ± 0.5 b
Moderately tolerant	74.0 ± 0.5 c	120.6 ± 2.0 a	3.1 ± 0.1 b	12.0 ± 0.3 ab
Freezing susceptible	**69.3 ± 0.5** a	106.2 ± 1.8 a	**3.9 ± 0.3** c	12.9 ± 0.4 b

Among them, the ability to sustain high activity of SOD and CAT during stress treatment seems to be the most important.

Appropriate activity of enzymatic counterparts of the antioxidative system in plants is very important for effective freeze/drought adaptation.

Table 4. Changes in antioxidative system activity induced by drought stress in the groups of DH lines differentiated in DT level. Mean valuess (± Sd) marked with the same letter do not differ significantly according to Duncan test (p≤0.05).

DH lines	Antioxidative system activity			
	SOD [U ml⁻¹]	CAT [µmol min⁻¹mg⁻¹]	POX [nmol min⁻¹mg⁻¹]	LMW [µM Trolox g⁻¹ DW]
Control plants				
Drought tolerant	74.0 ± 2.9 b	1364.5 ± 79.9 c	1.44 ± 0.14 b	9.0 ± 3.3 b
Moderately tolerant	74.5 ± 4.2 b	1382.9 ± 86.7 c	1.07 ± 0.32 a	8.9 ± 1.5 b
Drought susceptible	75.7 ± 2.3 b	1350.1 ± 97.8 c	1.47 ± 0.14 b	**10.6 ± 1.4** c
Drought-treated plants				
Drought tolerant	74.8 ± 3.7 b	1294.7 ± 84.4 b	1.90 ± 0.34 c	6.0 ± 0.9 a
Moderately tolerant	73.0 ± 2.9 b	1332.3 ± 81.9 bc	1.67 ± 0.43 bc	6.3 ±1.1 a
Drought susceptible	**64.6 ± 6.4** a	**1184.1 ± 43.1** a	**2.16 ± 0.21** d	6.4 ± 0.3 a

ACKNOWLEDGMENTS: The research was performed in the frame of the project HOR hn-801-Pb-8/16, No. 26 financed by the Ministry of Agriculture and Rural Development within the program Basic Research for Biological Progress in Plant Production.

REFERENCES: Gołębiowska-Pikania, et al. 2017a. J. Proteomics (in press) https://doi.org/10.1016/j.jprot.2017.07.016; Gołębiowska-Pikania et al. 2017b. J. Proteomics (in press) https://doi.org/10.1016/j.jprot.2017.08.019; Minami M., Yoshikawa H., 1979. Clin. Chem. Acta 92: 337–342; Aebi H. 1984. Methods in Enzymol. 105: 121–126; Luck H. 1963. In: Bergmeyer H.U. (ed.), Methods of enzymatic analysis, Academic Press Inc., London, pp. 895–897; Brand-Williams W. et al. 1995. LWT Food Sci. Technol. 28: 25–30.