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COLD-ENHANCED GENE EXPRESSION OF THE FOLIAR THIOL-SPECIFIC ANTIOXIDANT PROTEIN IN TRITICALE (X *TRITICOSECALE* WITTM.) SEEDLINGS RESISTANT TO *MICRODOCHIUM NIVALE* (SAMUELS & I.C. HALLETT) INFECTION

Abstract

Pink snow mould resulting from *Microdochium nivale* (Samuels & I.C. Hallett) infection is one of the most wide spread and serious diseases of winter cereals and amongst them triticale (x *Triticosecale*, Wittm.), the man-made crop with great yielding potential even in poor environmental conditions. Therefore, we aimed to identify snow mould-resistant genotypes and gene/s potentially involved in the increased resistance.

In our experimental design we have used two parental cultivars and four descendent doubled haploid (DH) lines, with the highest and the lowest cold-induced snow mould resistance in our multi-season cold chamber tests. In seedlings leaves of those genotypes we verified the effect of cold-hardening (4 weeks at 4°C) on the relative expression of selected genes: catalase 1 (*Cat1*), pathogen-

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esis-related protein 1 (*PR1*) and thiol-specific antioxidant protein (*Tsa*), using quantitative reverse transcriptase Real-Time PCR (qPCR).

In present analysis, the genotype and the interaction of genotype–hardening had significant effects on the mean relative expression of all genes studied. The relative *Cat1* and *Tsa* genes expression measured in non-hardened plants showed weak positive correlation with triticale seedlings pink snow mould susceptibility. Contrary, in cold-hardened plants we found negative correlation of the relative *PR1* and *Tsa* genes expression with the susceptibility. Based on our results, we suggest that cold-enhanced *Tsa* gene expression in leaves of winter triticale seedlings could be possibly involved in plant preparing against following *M. nivale* infection but it should be verified during further inoculation experiments. According to our knowledge, the expression of *Tsa* gene in triticale was for the first time confirmed by us.

Keywords: cereals, cold-hardening, cross-tolerance, thiol-specific peroxidases

Abbreviations:

Act – mRNA for actin • *Cat1* – mRNA for catalase 1 • Cq – quantitation cycle value • DH line – doubled haploid line • *PR1* – pathogenesis-related protein 1 mRNA • Prx – peroxiredoxin • ROS – reactive oxygen species • SAR – systemic aquired resistance • *Tsa* – mRNA for thiol-specific antioxidant protein • qPCR – quantitative Real-Time polymerase chain reaction • *18s* – 18S ribosomal RNA

Introduction

Pink snow mould resulting from *Microdochium nivale* (Samuels & I.C. Hallett) infection is one of the most wide spread and serious diseases of winter cereals and grasses in moderate and cold climate. Furthermore, fungicides used to control this pathogen are not very efficient and are rather toxic to the environment (Leroux et al. 2006; Bateman 2008; Parry et al. 2008; Nielsen et al. 2013). Resistance to *M. nivale* of triticale (x *Triticosecale*, Wittmack) is also an essential trait for its winter survival (Hudec, Bokor 2002; Sliesaravičius et al. 2006; Zhukovsky, Ilyuk 2010). This man-made hybrid cereal (Ryöppy 1997) is composed of A and B genomes of wheat (*Triticum* sp.), R genome of rye (*Secale cereale*) and

fragments of D wheat genome sometimes present as translocations, with great yielding ability even in severe growth conditions. Therefore, it is important to find resistant genotypes and resolve the processes standing behind the efficient plant defence, in aim to obtain an implement for screening and improving the wide range of new cultivars of this crop and other cereals.

In our previous studies we have shown that a cold-hardening treatment induces the resistance of winter triticale to the infection of *M. nivale* (Gołębiowska, Wędzony 2009; Gołębiowska et al. 2011; Dubas et al. 2011; Szechyńska-Hebda et al. 2011; Żur et al. 2013). Without hardening, seedlings of studied triticale cultivars: Hewo and Magnat were completely damaged by *M. nivale* mycelium. When exposed to the low temperature in cold chamber, they achieved different levels of resistance: seedlings of cv. Magnat remained susceptible, while the resistance of cv. Hewo increased gradually during plant exposition to 4°C and obtained maximum level of resistance after 4 weeks of cold-hardening (Gołębiowska, Wędzony 2009; Gołębiowska et al. 2011). These results are supported by our field experiments (unpublished data). Such outcomes make the above cultivars a good model for further research on the cereal defences to *M. nivale* infection. Moreover, from F_1 hybrid between these two cultivars we developed a population of 92 doubled haploid (DH) lines using an anther culture method (Wędzony 2003). Then, in multi-season's cold-chamber tests, we selected lines that exhibited transgression of resistance in relation to parents and included them in the present study for increasing the resistance variability in the investigated material.

Many other authors have also reported the importance of cold-hardening for the cereal defence responses to the *M. nivale* and other snow mould fungi infection and that individual genotypes differ in their resistance levels (Laroche et al. 1997; Ergon et al. 1998; Browne et al. 2006; Gaudet et al. 2011; Miedaner et al. 2011; Nakajima, Abe 2011). However, those long-term studies didn't point out a cogent marker that could serve for the selection of the snow mould-resistant genotypes of winter triticale or other crops. In general, the increased antioxidative activity in which catalases and peroxidases are involved, neutralizes the overdose of ROS produced under stress conditions so could help plants in surviving both cold (Scebba et al. 1999; Shigeoka et al. 2002) and pathogen infection (Płażek et al. 2003; Ivanov et al. 2004; Király et al. 2007; Kumar et al. 2009; Gołębiowska et al. 2011). Higher plants can also produce special proteins in defence response to fungal attack and other stresses (Van Loon, Van Strien 1999; Okushima et al. 2000). Many of them are now grouped as a Pathogenesis-Related proteins

(PR proteins). They are expressed mainly in leaves, but also in other plant organs (Van Loon 1997). Among others, glucanases and chitinases are suggested to decompose fungal cell wall, while PR1, peroxidases or thaumatins function as antibiotics, membrane permeabilizators of invaders and inhibitors of fungal proteases. Such activity can inhibit pathogenesis/infection course (Van Loon, Van Strien 1999). PR proteins can be a part of first defence barrier since they are targeted to cell wall apposition in early stage of pathogenesis before concise host-pathogen contact; they would also play a role in systemic acquired resistance (SAR) (Tuzun, Somanchi 2006). Amongst them, PR1 is often reported as a marker gene of a biotic stress. PR proteins accumulating in the apoplast of winter rye during cold acclimation were shown to exhibit glucanase and chitinase activity in addition to antifreeze activity unlike PR proteins produced at warmer temperatures (Hiilovaara-Teijo et al. 1999; Yeh et al. 2000). Additionally, winter rye AFPs (Anti-Freeze Proteins) were apparently isoforms of PR proteins specifically induced at low temperature (Yeh et al. 2000).

A potential role in defence response to *M. nivale* infection has previously been proposed for catalase and peroxidase (Gołębiowska et al. 2011) and chitinase (Żur et al. 2013) in winter triticale; chitinase, endochitinase, 1,3–β glucanase in winter rye (Hiilovaara-Teijo et al. 1999; Yeh et al. 2000); chitinase, endochitinase, 1,3–β glucanase, PR1-a protein and peroxidase in winter wheat (Ergon et al. 1998) and thaumatin-like proteins in winter wheat (Kuwabara et al. 2002). Cold-hardening enhanced snow mould resistance since stronger and more rapid transcription of genes encoding different PR proteins were found in hardened compared to unhardened winter wheat plants after *M. nivale* and other snow mould species inoculation (Ergon et al. 1998; Hiilovaara-Teijo et al. 1999; Gaudet et al. 2000; Kuwabara et al. 2002; Gaudet et al. 2003a, 2003b).

Our recent results have shown cold-accumulation of thiol peroxidases, namely thiol-specific antioxidants (TSA), in pink snow-mould resistant triticale seedlings (unpublished data). These enzymes were characterized as heme-free peroxidases, which catalyze the reduction of peroxynitrites and various peroxides by catalytic cysteine residues and thiol-containing proteins as reductants (Rouhier, Jacquot 2005). Thiol-specific antioxidant is a member of the peroxiredoxin (Prx) protein family, which was previously referred to the alkyl hydroperoxide reductase/thiol-specific antioxidant family (Jung et al. 2001). Its removal of hydrogen peroxide *via* thiol peroxidase activity was shown by Netto el al. (1996). The 652 bp linear mRNA for *Tsa* (Acc. No. BAA19099.1) from wheat seedlings chloro-

plasts was submitted to the DDBJ/EMBL/GenBank databases in 1997. The gene *ycf42* encoding TSA protein was found in the pool of protein-coding genes in sequenced chloroplast genome (Stoebe et al. 1999). Thioredoxin homolog was cloned in winter rye (Berberich et al. 1998).

Taking into account the literature cited above, our goal is to dissect the relative expression of *Cat1*, *PR1* and *Tsa* transcripts in triticale seedlings leaves grown under control conditions and exposed to a cold hardening treatment. As the result of our experiments, the gene that may be potentially involved in cold-induced snow mould resistance of winter triticale seedlings could be pointed.

Materials and Methods

Plant material

Two winter triticale (x *Triticosecale* Wittm., 2n = 6x = 42) parental cultivars, differing in their cold-induced resistance to *M. nivale* infection under controlled conditions as previously reported (Gołębiowska, Wędzony 2009) were used in this study: Magnat (Danko Plant Breeders Ltd., Poland) and Hewo (Strzelce Plant Breeding – IHAR Group Ltd., Poland). In 2007, in the frame of the project COST/254/2006, 92 Doubled Haploid (DH) lines were derived from F_1 hybrid of cross between Hewo and Magnat, by the anther culture method according to Wędzony (2003), at the Department of Cell Biology of IPP PAS. Analyses with DArT and SSR markers by Assoc. Prof. Mirosław Tyrka (University of Rzeszów, Faculty of Biology and Agriculture, Department of Biochemistry and Biotechnology) confirmed homozygotic condition of the progeny DH lines that makes this set of material especially suited for further experiments in field and controlled conditions.

For the present analysis, 4 out of 92 DH lines were selected following 3 years of cold-chamber *M. nivale* resistance testing as described in Gołębiowska and Wędzony (2009) with further modification. This include: lines DH1 and DH2 with the highest cold-induced snow mould resistance (mean level of infection index P = 38 ±9.8% and 47 ±7.2%, respectively) as well as lines DH91 and DH92 with the lowest resistance (P = 64 ±7.0% and 71 ±18.5%) in the preceding experiments in the frame of National Science Centre project No. N N310 140239. Those four DH lines, taken together with their parental cultivars with intermediate resistance were used in the present study. Previously described as

resistant and susceptible cultivars Hewo and Magnat (Gołębiowska, Wędzony 2009) showed smaller difference in the level of resistance after triple testing in our present experimental conditions with plants growing singly in multi-pots instead of 16-seedlings pots (P = 49 ±15.5% and P = 59 ±11.3%, respectively), so they were assigned as moderately resistant and moderately susceptible in the present paper.

Plant growth conditions

Well formed kernels were surface-sterilized using 96% ethanol for 3 min, then 25% Domestos – commercial mixture of 1–5% sodium hypochlorite, 1–5% $C_{12–18}$ alkyl dimethylamine and <1% caustic soda (Unilever Polska Sp. z o.o.) for 15 min and finally washed in sterile water. Afterwards they were grown on filter paper soaked with sterile water in plastic Petri dishes at 26°C in darkness for 2 days. Healthy seedlings were planted in multi-pots (60 plants per pot) with 2 : 2 : 1 v/v/v sterile mixture of soil, turf substrate and sand. Six seedlings per genotype were grown in a row, 10 rows per multi-pot of different genotypes in a randomized complete block design with 3 replicates.

The plants were grown in a climatic chamber under light of 100 ±10 μmol (photon) m^{-2} s^{-1} PAR, at 8h/16h (day/night) photoperiod, at temperature 16°C/12°C, RH = 60–67%, for 7 days. On the 7th day, plants were supplemented once with Hoagland and Arnon's (1938) sterile medium, 0.05 m^3 per pot. Starting with the 8th day after potting, one half of plants was subjected to the prehardening at 12°C/12°C, with 8h/16h (day/night) photoperiod for 14 days. Then, they were hardened at 4°C/4°C for 28 days in the same light regime. The remaining control, non-hardened plants were grown constantly at 16°C/12°C, 8h/16h (day/night) photoperiod, until achieving the same morphological developmental stage, considering the number of leaves and shoots as the cold-hardened plants after the hardening on 49th day, i.e. for the next 14 days (total 21 days from potting).

Plant sampling

For present analyses, fully-expanded leaves (2nd in appearance) of 15 different seedlings of each genotype (2 parental cultivars, 2 most resistant and 2 most susceptible DH lines) from the control and the cold-hardened plants were collected, immediately frozen in liquid nitrogen and stored at –80°C. Analyses were per-

formed in 3 biological replicates consisting of 5 leaves each, in a single growth experiment.

Isolation of RNA and quantitative RT-PCR

Total RNA was isolated from triticale leaves using an RNA isolation kit GeneJET™ Plant RNA Purification Mini Kit (Fermentas, Poland), according to the manufacturer's instruction (Fig. 1). Then, the concentration of the obtained RNA was measured with BiospecNANO equipment. Synthesis of the first-strand cDNAs was performed with 1×10^{-9} kg of RNA using the QuantiTect® Reverse Transcription Kit (QIAGEN), in accordance with the manufacturer's protocol. Quantitative real-time PCR analyses were conducted on a CX96 Real Time PCR System (BioRad, USA), using a Maxima SYBRGreen qPCR Master Mix (Fermentas, Waltham, USA), based on the manufacturer's instructions.

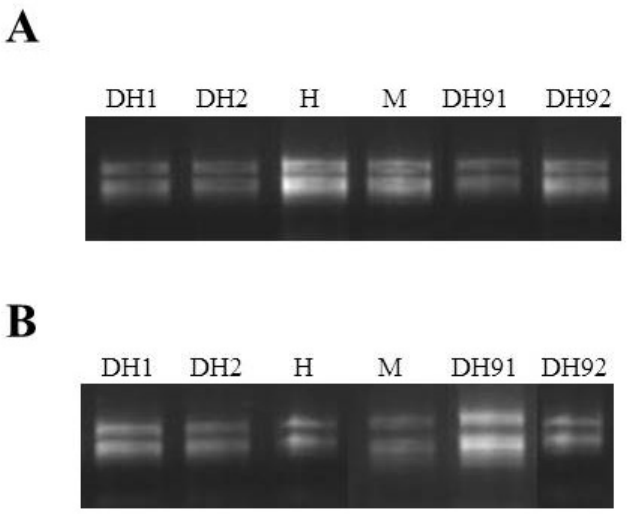


Figure 1. The isolation of total RNA from leaves of triticale control, non-hardened (A) and cold-hardened (B) seedlings, with an RNA isolation kit GeneJET™ Plant RNA Purification Mini Kit (Fermentas, Poland). H – parental cultivar Hewo, M – parental cultivar Magnat, DHs – descendent doubled-haploid lines.

qPCR cycling consisted of three steps that included: 10 min incubation at 95°C, followed by 40 cycles of 15 s at 95°C and 30 s at 60°C; and finally the dissociation curve step of 15 s at 95°C, 60 s at 60°C, and 15 s at 95°C. Fluorescence readings were taken during the annealing/extension step (60°C incubation). The quantification cycle (Cq) values for each reaction were calculated automatically by the CX96 Real Time PCR System detection software. The PCR Master Mix comprised of the primers (1×10^{-9} m^3 primer pair mix of 10 μM primer pair stock), 7.5×10^{-9} m^3 of 2 × Maxima SYBR Green qPCR Master Mix (Fermentas), and sterile nuclease-free water to a final volume of 14×10^{-9} m^3. Finally, 1×10^{-9} m^3 of cDNA was added to this mixture.

The qRT-PCR reactions were performed with the following specific primers (sequences from 5' to 3'): *Triticum aestivum* mRNA for actin, partial cds (*Act*, Acc. No. AB181991.1, forward ggcggagtacgatgagtctg, reverse attcttggcagcaagtcccc, predicted Tm = 64 and 62°C, respectively), *Triticum aestivum* 18S ribosomal RNA (*18s*, Acc. No. M82356.1, forward gtgacgggtgacggagaat, reverse acactaatgcgcccggtat, predicted Tm = 60 and 58°C, respectively); *Triticum aestivum* mRNA for catalase, complete cds (*Cat1*, Acc. No. E16461.1, forward ccaccacaacaaccactacg, reverse tgttgatgaatcgctcttgc, predicted Tm = 62 and 58°C, respectively); *Triticum aestivum* pathogenesis-related protein 1 mRNA, complete cds (*PR1*, Acc. No. AF384143.1, forward ccaagctagccatcttgctc, reverse ttgcagtcgttgatcctctg, predicted Tm = 62 and 60°C, respectively) and *Triticum aestivum* mRNA for thiol-specific antioxidant protein, partial cds (*Tsa*, Acc. No. AB000405.1, forward ttcgggctctgcaatacgtc, reverse gggtcaggcttcatcgactt, predicted Tm = 62 and 62°C, respectively). Accumulation of *Actin* transcripts was used as an external control. The accumulation of each transcript was analyzed in 3 independent repetitions. The raw data were analyzed using the CX96 System Software release v2.0 (Bio-Rad, USA). The accumulation of transcripts was normalized by setting the average ratio of transcripts to 1. All results were compared to the data obtained for cv. Magnat (for this genotype values of both non-hardened and cold-hardened plants were assumed as 1.0).

Statistics

Analysis of variance was carried out using the Fisher test, differences among the means were statistically examined with a Student's t-test as well as correlations between the mean relative gene expression and plants level of infection index

were calculated. Calculations were done with the help of STATISTICA® version 10.0 software at $p \leq 0.05$. When necessary, values were transformed logarithmically before applying a selected test.

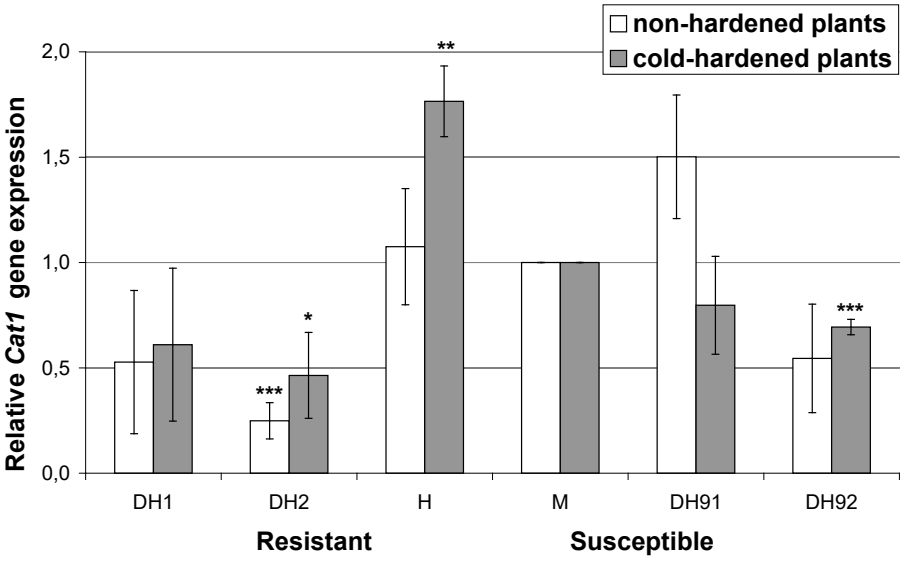
Results

Effect of cold-hardening on relative Cat1 gene expression

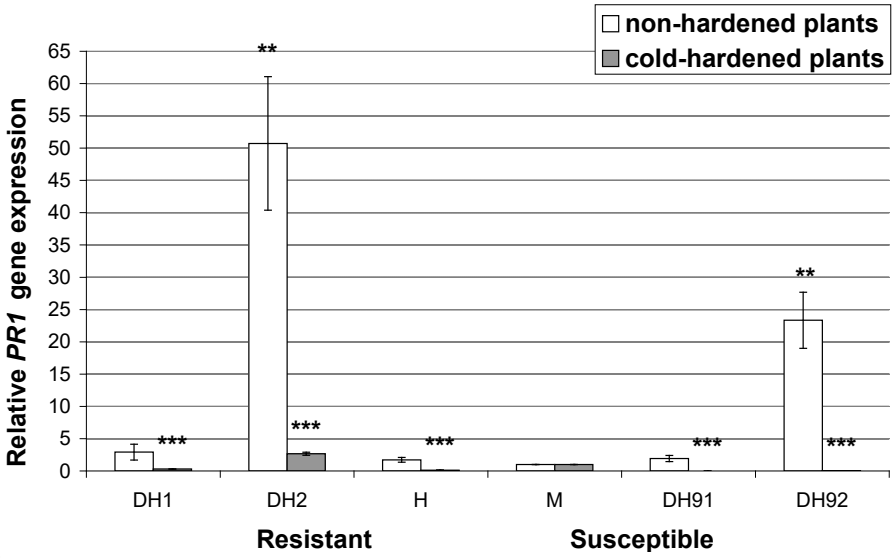
The Cq values for *Cat1* gene in leaves of cv. Magnat reached 23.5 ±0.2 and 24.9 ±0.1 in non-hardened and cold-hardened seedlings, respectively. In non-hardened, control plants, the minimal mean relative *Cat1* gene expression was found in leaves of DH2 seedlings, while the maximal expression in DH91 ones (Fig. 2A). After cold-hardening, significant changes in the relative *Cat1* gene expression were noted only in leaves of 2 genotypes: an increase in cv. Hewo and a decrease in DH91seedlings (Fig. 2A), when comparing to the proper non-hardened control. In hardened plants, the minimal relative *Cat1* gene expression was found again in leaves of DH2 seedlings, but the experimental maximum was noted for cv. Hewo plants (Fig. 2A).

In relation to cv. Magnat non-hardened plants, significant differences were found only in DH2 control seedlings, with decreased ($p < 0.001$) relative *Cat1* gene expression (Fig. 2A). In cold-hardened plants more differences were noted: the decrease in DH2 and DH92 leaves ($p < 0.05$ and $p < 0.001$, respectively) as well as increase ($p < 0.01$) in cv. Hewo leaves (Fig. 2A), in respect to cold-hardened cv. Magnat seedlings.

The analysis of variance showed that genotype and the interaction of genotype-hardening had the significant effect on the mean relative *Cat1* gene expression, in contrast to cold-hardening alone (Tab. 1). The mean relative *Cat1* gene expression measured in non-hardened plants showed weak positive correlation with triticale seedlings pink snow mould susceptibility ($r = 0.35$), while it was not correlated with that trait in leaves of cold-hardened plants ($r = –0.03$).


non-hardened plants
cold-hardened plants
Relative Cat1 gene expression
2,0
1,5
1,0
0,5
0,0
**
*
***
***
DH1
DH2
H
M
DH91
DH92
Resistant
Susceptible

A


non-hardened plants
cold-hardened plants
Relative PR1 gene expression
65
60
55
50
45
40
35
30
25
20
15
10
5
0
**
***
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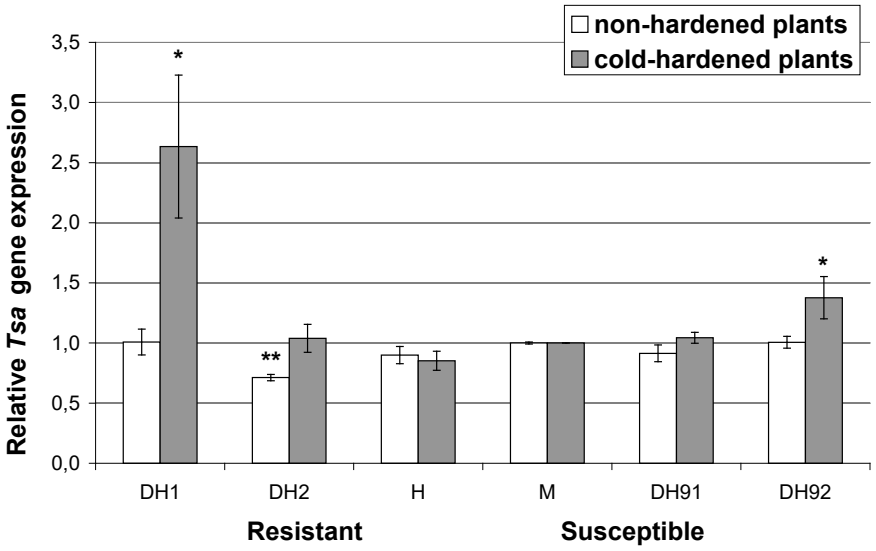
**
***
DH1
DH2
H
M
DH91
DH92
Resistant
Susceptible

B



C

Figure 2. The impact of cold-hardening (4 weeks at 4°C) on the mean relative gene expression of: A) *Cat1* – mRNA for catalase 1; B) *PR1* – mRNA for pathogenesis-related protein 1; C) *Tsa* – mRNA for thiol-specific antioxidant protein, in leaves of winter triticale seedlings
Non-hardened, control plants grew at 16°C/12°C, until acquiring the same morphological stage as the cold-hardened plants after full hardening period. Mean quantitative RT-PCR results from 3 biological replicates (15 leaves) ± St. Dev. Values that significantly differ from the data obtained for cv. Magnat are marked with stars (*** – significant at $p < 0.001$; ** – significant at $p < 0.01$; * – significant at $p < 0.05$; ns – not significant), as a result of the Student's t-test. H – parental cultivar Hewo; M – parental cultivar Magnat; DH – descendant doubled-haploid lines. Snow mould-resistant (DH1, DH2), moderately resistant (H), moderately susceptible (M) and susceptible (DH91, DH92) genotypes were selected after 3 years of the cold-chamber tests with *Microdochium nivale* mycelium.

Effect of cold-hardening on relative PR1 gene expression

The Cq values for *PR1* gene in leaves of cv. Magnat reached 35.2 ±0.2 and 27.9 ±0.1 in non-hardened and cold-hardened seedlings, respectively. In leaves of DH2 non-hardened seedlings, the mean relative *PR1* gene expression was distinctly higher then in all remaining plants, while it was the lowest in cv. Magnat ones (Fig. 2B). The hardening caused significant decrease in the relative *PR1* gene

expression in leaves of all studied genotypes (Fig. 2B) in comparison to their non-hardened controls. After cold-hardening, the maximal relative *PR1* gene expression was found again in leaves of DH2 seedlings, while it decreased in DH1 and cv. Hewo plants as well as disappeared in DH91 and DH92 ones (Fig. 2B).

In relation to cv. Magnat non-hardened plants, significant differences were found in DH2 and DH92 control seedlings, with increased ($p < 0.01$) relative *PR1* gene expression (Fig. 2B). In cold-hardened plants, the decrease ($p < 0.001$) was noted for all remaining genotypes (DH1, cv. Hewo, DH91 and DH92), except DH2 leaves with increased ($p < 0.001$) *PR1* relative gene expression (Fig. 2B), in respect to hardened seedlings of cv. Magnat.

All independent factors: genotype, hardening and the interaction of genotype-hardening, significantly influenced the mean relative expression of *PR1* gene (Tab. 1). In non-hardened plants, the relative *PR1* gene expression was not correlated with triticale seedlings pink snow mould susceptibility ($r = –0.04$), while it showed weak negative correlation with that trait in leaves of cold-hardened plants ($r = –0.34$).

Table 1. The effect of cold-hardening in genotypes of winter triticale. Results from analysis of variance on relative gene expression data

Source	DF	Mean square	*F* value	*P* value
A) Relative *Cat1* gene expression				
Genotype	5	0.076608	12.70607	0.000004
Hardening	1	0.076608	0.63179	0.434492
Genotype-hardening	5	0.076608	4.01305	0.008684
B) Relative *PR1* gene expression				
Genotype	5	15.96079	41.20201	0.000000
Hardening	1	15.96079	94.01347	0.000000
Genotype-hardening	5	15.96079	35.0548	0.000000
C) Relative *Tsa* gene expression				
Genotype	5	0.053672	14.47574	0.000001
Hardening	1	0.053672	26.9041	0.000026
Genotype-hardening	5	0.053672	10.87271	0.000015

Effect of cold-hardening on relative Tsa gene expression

The Cq values for *Tsa* gene in leaves of cv. Magnat reached 19.2 ± 0.1 and 18.9 ± 0.1 in non-hardened and cold-hardened seedlings, respectively. In control plants, the minimal mean relative *Tsa* gene expression was found in leaves of

DH2 seedlings, while it was almost equal in all remaining genotypes (Fig. 2C). Plant exposition to the low temperature caused the increase of relative *Tsa* gene expression in leaves of all DH lines in comparison to their controls, the most remarkable in case of DH1 (Fig. 2C). In leaves of DH1 cold-hardened seedlings, the relative *Tsa* gene expression was distinctly higher then in all remaining plants (Fig. 2C).

In relation to cv. Magnat non-hardened plants, significant differences were found only in DH2 control seedlings, with decreased ($p < 0.01$) relative *Tsa* gene expression (Fig. 2C). In cold-hardened plants, the increase ($p < 0.05$) was noted for DH1 and DH92 leaves (Fig. 2C) in respect to cv. Magnat ones.

Genotype, hardening and the interaction of genotype-hardening significantly effected the mean relative expression of *Tsa* gene (Tab. 1). Without hardening, the relative *Tsa* gene expression showed weak positive correlation ($r = 0.27$) with triticale seedlings pink snow mould susceptibility, while it was negatively correlated with that trait in leaves of cold-hardened plants ($r = –0.49$).

Discussion

In our experiments, the genotype and the interaction of genotype-hardening had a significant impact on the mean relative expression of all genes studied. After cold-hardening, the mean relative *Cat1* gene expression increased only in leaves of moderately resistant cv. Hewo, when comparing to its non-hardened control as well as in respect to cold-hardened seedlings of moderately susceptible cv. Magnat. This result states in accordance with our previous spectrophotometric data on unspecific catalase activity, higher in cv. Hewo seedlings exposed to cold, than in cv. Magnat ones (Gołębiowska et al. 2011). However, such effect was not observed in the offspring of those parental genotypes in the present studies. Moreover, the relative *Cat1* gene expression was the lowest in leaves of both non-hardened and cold-hardened plants of the most resistant lines. Similarly, other authors reported decrease of unspecific catalase activity in cold-hardened plants of the pink snow mould-resistant androgenic genotype of *Festulolium* (Pociecha et al. 2008).

In opposition to results obtained by Ergon et al. (1998), cold-hardening under our own experimental conditions caused significant decrease in the mean relative *PR1* gene expression in leaves of all studied genotypes in relation to controls. Only in leaves of resistant DH2 seedlings its expression was increased in re-

spect to hardened plants of moderately susceptible cv. Magnat. In two remaining genotypes – resistant DH1 and moderately resistant cv. Hewo, the relative *PR1* gene expression was substantially lowered. Nevertheless, we found weak negative correlation between relative *PR1* gene expression and triticale seedlings pink snow mould susceptibility. Such results suggest that the enhanced expression of gene encoding PR1 protein could possibly manifest not before but after fungal infection as well as that it could be necessary to analyse the expression of genes encoding other PR proteins with antifungal potential. Above results could be also partly explained by a difference in Cq values between non-hardened and cold-hardened seedlings of cv. Magnat ($\Delta = 8.3$).

Contrary to the results discussed above, cold-hardening effected the increase of relative *Tsa* gene expression in leaves of all DH lines in comparison to controls. It was the most remarkable in leaves of the most resistant DH1 seedlings, enriched in *Tsa* transcripts in respect to cv. Magnat plants as well. Interestingly, we found the weak positive correlation of the relative *Tsa* gene expression with triticale seedlings pink snow mould susceptibility in non-hardened plants and its negative correlation in cold-hardened ones. When comparing all data obtained, leaves of resistant DH2 seedlings showed the minimal relative *Cat1* gene expression and the maximal relative *PR1* gene expression both in control and hardened plants; it revealed also the minimal relative *Tsa* gene expression in non-hardened seedlings and intermediate in hardened ones. In turn, the most resistant DH1 plants had low relative *Cat1* and *PR1* gene expression in leaves of both control and hardened plants as well as high relative *Tsa* gene expression in leaves of hardened plants, in respect to plants of remaining genotypes studied.

Based on our results, we suggest that cold-enhanced *Tsa* gene expression in seedlings leaves of the most resistant DH line could be potentially connected with better plant condition under stress of low temperature. Additionally, in our knowledge, *Tsa* gene expression in triticale is for the first time confirmed in present paper. There is also little information about this protein from other cereals. Plant peroxiredoxins are regularly shown to act in cellular defence-signalling mechanisms against oxidative stress (Jang et al. 2006). In 1999, Baier and Dietz concluded that the photosynthetic machinery needs high levels of 2-Cys Peroxiredoxin during leaf development of *Arabidopsis thaliana* to protect it from an oxidative damage. Other authors suggested that a chloroplastic NADPH-dependent thioredoxin reductase-2-Cys PRX complex functions in the acquisition of freezing tolerance of *Chlorella vulgaris* (Machida et al. 2009). Horling et al.

(2002) placed an emphasis on the type II PRX C, as highly active in decomposing H_2O_2 when investigated as heterologously expressed protein. In yeast cytosol, Jang et al. (2004) documented two PRXs that could act alternatively as peroxidases and molecular chaperones.

In out results we didn't observe strong correlations or similar trends with the three more resistant lines. The studies of other authors also indicate that it could be difficult to identify a single informative marker related to the multigenic composition and low heritability of snow mould resistance in cereals and grasses species. Our present research suggests that the promising molecule could be thiol-specific antioxidant protein, a member of thiol-specific peroxidases, possibly involved in plant cold-mediated preparation to the effective fungal defence, but it is necessary to undertake further analysis on its expression after inoculation with fungus as well as function in winter triticale and other cereals.

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WZMOCNIONA NISKĄ TEMPERATURĄ EKSPRESJA GENU KODUJĄCEGO TIOL-SPECYFICZNE BIAŁKO ANTYOKSYDACYJNE W LIŚCIACH SIEWEK PSZENŻYTA (X *TRITICOSECALE* WITTM.) ODPORNYCH NA INFEKCJĘ *MICRODOCHIUM NIVALE* (SAMUELS & I.C. HALLETT)

Streszczenie

Różowa pleśń śniegowa wywołana przez fitopatogenny grzyb *Microdochium nivale* (Samuels & I.C. Hallett) jest jedną z najbardziej rozpowszechnionych i znacznie obniżających plon chorób zbóż ozimych. Patogen ten poraża także pszenżyto (x *Triticosecale*, Wittm.), gatunek zboża wytworzony w warunkach laboratoryjnych, o dużym potencjale plonowania przejawiającym się nawet w trudnych warunkach środowiskowych.

Celem przedstawianych badań była identyfikacja genotypów pszenżyta ozimego o podwyższonej odporności na infekcję *M. nivale* oraz genu/-ów potencjalnie z nią powiązanych. Do analiz użyto dwóch odmian rodzicielskich oraz czterech potomnych linii podwojonych haploidów (DH) o najwyższej i najniższej indukowanej niską temperaturą odporności na pleśń śniegową, obserwowanej w wieloletnich testach komory chłodniowej. W liściach siewek wymienionych genotypów badano wpływ hartowania niską temperaturą (4 tygodnie w 4°C) na względną ekspresję genów: katalazy 1 (*Cat1*), białka związanego z patogenezą 1 (*PR1*) oraz tiol-specyficznego białka antyoksydacyjnego (*Tsa*), przy wykorzystaniu ilościowej reakcji odwrotnej transkryptazy Real-Time PCR (qPCR).

Otrzymane wyniki wskazują na istotny wpływ genotypu oraz interakcji genotyp–hartowanie na średnią względną ekspresję wszystkich badanych genów. Względna ekspresja genów *Cat1* i *Tsa* mierzona w liściach kontrolnych niehartowanych siewek pszenżyta wykazała słabą dodatnią korelację z podatnością roślin na różową pleśń śniegową. Natomiast w liściach hartowanych niską temperaturą siewek obserwowano ujemną korelację względnej ekspresji genów *PR1* i *Tsa* z podatnością roślin na *M. nivale*.

Wyniki przeprowadzonych analiz sugerują, że wzmocniona chłodem ekspresja genu *Tsa* w liściach pszenżyta ozimego może być potencjalnie powiązana z lepszym przygotowaniem rośliny do obrony przed infekcją *M. nivale*, jednakże konieczna jest weryfikacja tej tezy w dalszych eksperymentach z zastosowaniem inokulacji grzybnią. Zgodnie z naszym stanem wiedzy, jest to pierwsza praca dokumentująca ekspresję genu *Tsa* u pszenżyta.

Słowa kluczowe: zboża, hartowanie niską temperaturą, tolerancja krzyżowa, peroksyredoksyny

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