Proceedings of the 7th Hungarian Congress on Plant Physiology, 2002
S2-07

Volume 46(3-4):67-69, 2002
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Correlation between frost tolerance and antioxidant activities in cereals

Tibor Janda*, Gabriella Szalai, Krisztina Rios-Gonzalez, Ottó Veisz, Emil Páldi

Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary

ABSTRACT Frost-tolerant and frost-sensitive cereal species were hardened at low, non-freezing temperature. Changes in the activity of antioxidant enzymes (catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione-S-transferase and glutathione reductase) in the crown and leaves were determined in the control and in hardened plants. The highest correlation between enzyme activity and frost tolerance was found in the case of guaiacol peroxidase and ascorbate peroxidase from hardened leaves. Enzyme activities in the crown and in unhardened leaves showed no significant positive correlation.

Acta Biol Szeged 46(3-4):67-69 (2002)

KEY WORDS

antioxidant enzymes
cereal
crown
freezing stress
leaf

Low temperature is one of the most important stress factors limiting the growth and productivity of cereals. Only a few data are available on changes in antioxidant activity during cold hardening, and its role in the development of frost tolerance is not clearly understood. The aim of the present work was to investigate which antioxidant enzyme activities show the highest correlation with the frost tolerance of the plant.

Materials and Methods

Hardened plants were grown in a growth chamber where the temperature progressively decreased weekly from 15°C to 3°C. The enzyme activities in the crown and leaf of 11 cereal genotypes with different frost tolerance were measured as described by Janda et al. (2000).

Results and Discussion

After freezing 11 hardened cereal genotypes at –13°C two main groups could be created. While CS, Gerald, CS/Ch 5D, CS/Ch 5A and Hardy showed less than 30% survival (frost-sensitive group), this figure was higher than 80% for Mv Magvas, Presto, Cheyenne, Martondur 1, B 1201 and Motto (frost-tolerant group) To differentiate within the groups the sensitive genotypes were also frozen at –11°C and the tolerant ones at –15°C. The chromosome substitution lines CS/Ch 5D and CS/Ch 5A had significantly better frost tolerance than CS and Gerald, while Hardy was significantly better than all the others within the group. In the frost-tolerant group the rye variety Motto had significantly higher frost tolerance than Mv Magvas or B 1201. Martondur 1 was also better than Mv Magvas, but the difference between Martondur 1 and the other genotypes in the group was not statistically significant. Plants grown under non-hardening conditions all died even after freezing at –11°C.

The catalase activity in the crown was highest in the rye variety Motto, and was also relatively high in the winter wheat genotypes Mv Magvas and B 1201 and in the winter oat variety Gerald. Ascorbate peroxidase activity was substantially lower in Gerald than in the other species and was also relatively low in the winter barley Hardy, while the activity of this enzyme was nearly the same in the other genotypes. Gerald also showed very low guaiacol peroxidase activity, but in Hardy the activity of this enzyme, in contrast to ascorbate peroxidase, was the highest of all the genotypes tested, comparable only with that of Motto.

Compared to a growth temperature of 15°C, hardening growth temperature did not cause any significant difference in the activity of catalase, ascorbate peroxidase and guaiacol peroxidase in the crown.

The activity of the glutathione reductase enzyme was the lowest in Gerald and the highest in Motto, and had similar values in all the other genotypes. There was usually a slight difference between the activity of glutathione reductase in control and hardened plants. This difference was not statistically significant in the genotypes Chinese Spring, CS/Ch 5A, Mv Magvas, Martondur 1 or B 1201, while there was a slight increase in Gerald, CS/Ch 5D, Hardy and Motto. In Presto and Cheyenne the glutathione reductase activity was 9% and 22%, respectively, lower in hardened than in unhard-ened plants.

The activity of glutathione-S-transferase was the lowest in the barley variety Hardy, and was the highest in hardened plants of the rye variety Motto. With the exception of Hardy and B 1201 its activity was significantly higher in plants grown under hardening conditions. The relative increase was the highest in Motto.

The activity of none of the antioxidant enzymes isolated from the crown showed a close correlation with the frost tolerance of the plant (Table 1).

The same antioxidant enzymes were also investigated in the leaves. Catalase activity was relatively low in the barley variety Hardy compared to the other genotypes tested in these experiments. In plants grown at low, hardening temperature the catalase activity was approx. 65% (on average) of that in plants grown under control conditions. The only genotype

*Corresponding author. E-mail: jandat@mail.mgki.hu

Table 1. Correlation coefficients between the frost tolerance of hardened plants, expressed as survival after freezing at –13°C, and the activity of antioxidant enzymes isolated from the crown and leaf of control and frost-hardened plants.

Enzyme	Correlation coefficient with frost tolerance			
	Crown		Leaf	
	Unhardened	Hardened	Unhardened	Hardened
Catalase	0.442	0.389	0.249	0.631
Ascorbate peroxidase	0.305	0.396	0.341	0.791
Guaiacol peroxidase	0.451	0.586	0.431	0.820
Glutathione-S-transferase	-0.098	-0.178	-0.329	-0.520
Glutathione reductase	0.587	0.280	-0.481	0.314

where this difference was not statistically significant was the winter wheat Cheyenne, which had a relatively low value in the control. The decrease was generally more pronounced in frost-sensitive plants than in frost-tolerant ones, so although there was no significant difference between the two groups in the catalase activity of the control plants, this activity was slightly higher in frost-tolerant plants than in sensitive ones after hardening.

Both ascorbate peroxidase and guaiacol peroxidase exhibited much higher activity in hardened plants compared to the controls. For both enzymes the control values were in the same range in all the genotypes, while the hardened plants showed large differences: activity was usually significantly higher in the tolerant group than in the sensitive one. The exception was guaiacol peroxidase in Presto, but here the control value was also significantly lower than in most of the other genotypes. Interestingly, the guaiacol peroxidase activity was extremely low in both control and hardened plants of the winter oat variety Gerald.

Glutathione-S-transferase activity was also substantially higher in the hardened plants than in the controls, but its value did not show any correlation with frost tolerance either in the controls or in the hardened plants.

Glutathione reductase activity did not change in genotypes CS, Gerald, CS/Ch 5D, CS/Ch 5A, Cheyenne or Martondur 1. It was a little higher in hardened than in unhardened plants in Hardy, Mv Magvas, Presto, B 1201 and Motto. Its value was very similar in all the genotypes, and there was no correlation between glutathione reductase activity and frost tolerance.

Exposure to low temperature may increase the amount of active oxygen species not only in cold-sensitive, but also in cold-tolerant plants. A rapid, transient increase in the hydrogen peroxide level was detected in wheat plants after cold treatment (Okuda et al. 1991). Cold-hardened plants grown at low temperature contained increased levels of both antioxidants and antioxidant enzymes (Kocsy et al. 2001). In cereals a correlation was found between the development of tolerance to freezing and oxidative stress, which suggests that freezing tolerance at the subcellular level may be influenced by the ability to detoxify activated forms of oxygen (Bridger et al. 1994). In the present experiment a significant decrease in the catalase activity could be observed in the leaves of cold-hardened plants, while in the crown it did not change significantly. There may be several explanations for this. It was earlier reported that catalase may suffer photo-oxidative damage in non-hardened winter rye leaves when the plants are exposed to low temperature (Streb et al. 1999). The occurrence of photoinhibition during low temperature hardening was reported by several authors (Hurry and Huner 1991; Hurry et al. 1992; Janda et al. 1994), but it is not generally directly connected with the development of freezing tolerance, as found earlier using chromosome substitution wheat lines (Janda et al. 1994).

Salicylic acid inhibited a substantial portion of the catalase activity in several plant species (Raskin 1992; Sánchez-Casas and Klessig 1994). It was recently shown that not only salicylic acid, but also its precursor benzoic acid and its derivative acetyl-salicylic acid (aspirin) caused a decrease in the catalase activity in maize plants when added to the hydroponic solution (Janda et al. 2000). However, the role of endogenous salicylic acid during the development of frost tolerance is unknown.

The highest correlation between enzyme activity and frost tolerance was found in the case of guaiacol peroxidase and ascorbate peroxidase from hardened leaves. Enzyme activities in the crown and in unhardened leaves showed no significant positive correlation. Interestingly, guaiacol peroxidase from Gerald winter oat showed practically zero activity.

The changes in most of the antioxidant enzymes investigated suggest that they may play an important role in the development of frost tolerance in cereals. However, the responses of these enzymes to the hardening conditions are different in various species.

Acknowledgments

The authors are gratefully indebted to Zsuzsa Kóti and Edit Kövesdi for their technical assistance. This work was supported by grants from the Hungarian National Scientific Research Foundation (OTKA T32653, T33000, T37195) and a J. Bolyai Scholarship, which are gratefully acknowledged.

References

Bridger GM, Yang W, Falk DE, McKersie BD (1994) Cold-acclimation increases tolerance of activated oxygen in winter cereals. J Plant Physiol 144:235-240.

Hurry VM, Huner NPA (1991) Low growth temperature effects a differential inhibition of photosynthesis in spring and winter wheat. Plant Physiol 96:491-497.

Hurry VM, Krol M, Öquist G, Huner NPA (1992) Effect of long-term photoinhibition on growth and photosynthesis of cold-hardened spring and winter wheat. Planta 188:369-375.

Janda T, Kissimon J, Szigeti Z, Veisz O, Páldi E. (1994) Characterization of cold hardening in wheat using fluorescence induction parameters. J Plant Physiol 143:385-388.

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

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

Okuda T, Matsuda Y, Yamanaka A, Sagisaka S (1991) Abrupt increase in the level of hydrogen-peroxide in leaves of winter-wheat is caused by cold treatment. Plant Physiol 97:1265-1267.

Raskin I (1992) Role of salicylic acid in plants. Annu Rev Plant Physiol Plant Mol Biol 43:439-463.

Sánchez-Casas P, Klessig DF (1994) A salicylic acid-binding activity and a salicylic acid-inhibitable catalase activity are present in a variety of plant species. Plant Physiol 106:1675-1679.

Streb P, Shang W, Feierabend J (1999) Resistance of cold-hardened winter rye leaves (*Secale cereale* L.) to photo-oxidative stress. Plant Cell Environm 22:1211-1223.