Abscisic acid content during cold hardening of barley and wheat cultivars with different freezing tolerance

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ABSTRACT

Endogenous content of abscisic acid was studied in a set of two winter cultivars of barleys (Lunet, Cenader), one spring cultivar (Akcent) and five winter cultivars of wheat (Mironovská, Samanta, Šárka, Zdar, Apache) and one spring cultivar (Leguan) in the course of cold hardening of hydroponically grown plants. Freezing tolerance was also determined in all barley and wheat cultivars under study. In none of the barley varieties did cold hardening of plants induce any significant change in abscisic acid content. In wheat plants exposed to cold hardening, the cultivars Apache and Leguan showed a slight transitory increase in abscisic acid content. Abscisic acid content in leaves was very similar in the other wheat cultivars. Neither in barley nor in wheat was the level of freezing tolerance associated with endogenous abscisic acid content or with its transitory changes during cold hardening.

Keywords: abscisic acid; cold hardening; freezing tolerance; barley; wheat

Similarly to other plants, crop plants possess different abilities to overcome adverse environmental conditions. Low temperature is one of the adverse environmental conditions. The effect of below zero temperatures on grain crops is negative. Therefore, different levels of freezing tolerance in grain crops and their cultivars play an important role.

Currently, many authors focus their attention on a biochemical marker of freezing tolerance of plants. Abscisic acid (ABA), a phytohormone designated as a stress one, is mentioned as one of the possible markers of such tolerance (Hartung and Davies 1991, rew. Kadlecová 1999). This phytohormone is attributed the role of a mediator of plant response to a stress, e.g. drought, salinization or low temperatures (rew. Kadlecová 1999). The participation of ABA in the process of increasing the freezing tolerance of plants is derived from the finding that the endogenous content of ABA sometimes increases during cold hardening. An increase in the endogenous ABA content during cold hardening of plants is assumed to be possibly associated with subsequent increase in freezing tolerance of plants exposed to cold hardening. Observations during cold hardening of grain crops confirmed a transitory increase in the endogenous content of ABA (Veisz et al. 1996, Bravo et al. 1998), ABA accumulation (Macháčková et al. 1989) or no changes in ABA content (Dallaire et al. 1994, Kadlecová et al. 2000). The results of investigations into the endogenous content of ABA during cold hardening are not so unambiguous as the effect of drought that causes an increase in the endogenous content of ABA (Walker-Simmons et al. 1989, Grossi et al. 1995). This fact evokes an assumption that an increase in ABA content after cold hardening of plants could be induced by secondary drought stress. Therefore it is advantageous to study the endogenous content of ABA during cold hardening of plants that are grown in hydroponics to minimize potential losses of water after the plants were exposed to low temperature (Faltusová-Kadlecová et al. 2002). Endogenous content of ABA during cold hardening can be studied in one cultivar or it is possible to compare ABA responses in several cultivars with different freezing tolerance. The results of our investigation into ABA endogenous content during cold hardening of freeze-tolerant barley cultivar Lunet suggested a conclusion that cold hardening of these plants did not increase the endogenous content of ABA because the hydroponically grown plants of barley did not show any signs of water deficiency after exposure to low temperature (Kadlecová et al. 2000). Correlations between the achieved level of freezing tolerance of wheat varieties with different tolerance and ABA content accumulation were proved by Macháčková et al. (1989). Lalk and Dörffling (1985) described a tendency to higher ABA accumulation in a wheat cultivar with higher freezing tolerance. Bravo et al. (1998) found that ABA was strongly correlated with freezing tolerance of non-acclimated barley cultivars. The goal of our study was to determine whether cold hardening of plants would induce any changes in the endogenous content of ABA in three barley cultivars and six wheat cultivars with different levels of freezing tolerance. A comparison of ABA content in these two species of grain crops was to show whether the potential change in ABA content induced by a low temperature was species specific.

MATERIAL AND METHODS

Five-day seedlings of barley (*Hordeum vulgare* L.), winter cultivars Lunet and Cenader and spring cultivar Akcent and seedlings of wheat (*Triticum aestivum* L.), winter cultivars Mironovská, Samanta, Šárka, Zdar,

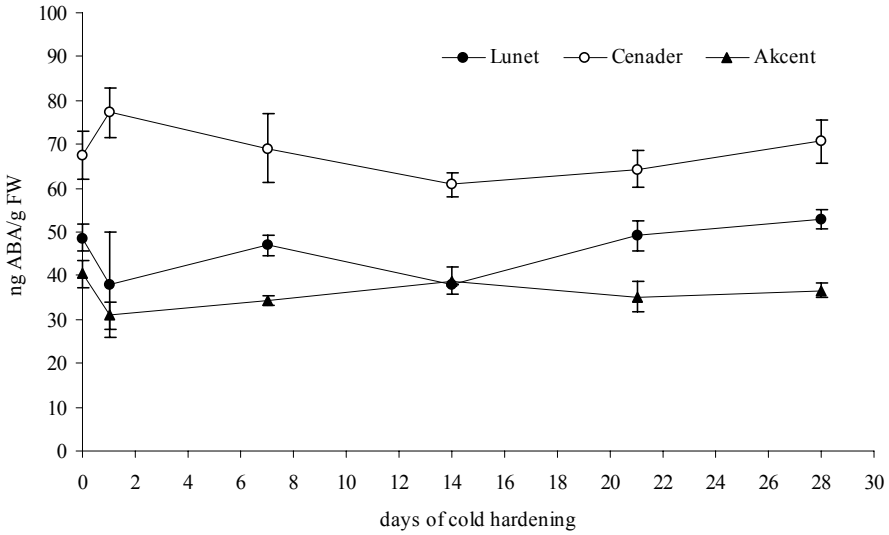

Figure 1. Changes in leaf ABA content during cold hardening of barley cultivars; means of three replications; the vertical bars give *SE*

Apache and spring Leguan were cultivated hydroponically (Hoagland 3) in controlled conditions to minimize possible water stress: 16 h photoperiod, light intensity of 400 µmol.m^{-2}.s^{-1}, relative humidity 65 ± 5% and 90 ± 5% (D/N), temperature 17 ± 1°C. After 15 days of cultivation, the plants were divided into two groups. Control plants remained in the same conditions and plants for cold hardening were exposed to 3°C for a period of 28 days (barley) and 21 days (wheat). Samples for ABA determination were collected on the first day and every week at the end of dark period. Freezing tolerance as the lethal temperature (LT_{50}) was determined before hardening and after three weeks of cold hardening. Second leaves were used for finished expanding growth in all experiments (Prášil and Faltus 1994).

For ABA analysis six leaves from each cultivar of barley or wheat plants were sampled and fresh mass of this amount of leaves was weighed. The samples were homogenized in liquid nitrogen and extracted with distilled water at a ratio 1:10 (1 g FW:10 ml H_2O) and shaken overnight in the dark at 4°C. After then the samples were centrifuged at 10 000 g for 10 min at 4°C and 100 µl of the supernatant in three replications was used for ABA determination. ABA content was determined by the ELISA method (Hansen 1996, Kadlecová and Prášil 2000). The monoclonal ABA-antibody MAC252 obtained from Dr. S.A. Quarrie (John Innes Centre, Norwich, UK) and ABA-BSA conjugate obtained from Prof. Dr. K. Dörffling (Hamburg University) were used for the ELISA analysis.

The level of freezing tolerance was determined by a laboratory freezing test in a set of ten 1cm leaf segments per tube exposed in two replications to five different freezing temperatures. The rate of cooling and thawing was 3°C/h. After thawing these segments 14 ml deionised water was added to each tube and degree of freezing injury was evaluated by a conductivity method (Prášil and Zámečník 1998). The lethal temperature (LT_{50}) (i.e. the freezing temperature at which 50% of leaves were killed) was calculated from S-shaped curve between freezing injury and test freezing temperature by the method of Janáček and Prášil (1991).

Student's *t*-test was used to detect differences between the values of ABA content and LT_{50} of each cultivar (at 5% significance level).

Table 1. Freezing tolerance as LT_{50} of barley and wheat leaves before cold hardening (control) and after three weeks of cold hardening (hardened)

	Variety	Control	*SE*	Hardened	*SE*
Barley	Lunet	–4.7	0.4	–14.2	0.2
	Cenader	–3.4	0.5	–9.6	0.3
	Akcent	–3.7	0.1	–8.4	0.4
Wheat	Mironovská	–5.7	0.2	–19.0	0.3
	Samanta	–5.4	0.1	–18.9	0.2
	Šárka	–5.0	0.1	–17.7	0.2
	Zdar	–4.4	0.1	–16.0	0.2
	Apache	–4.3	0.3	–15.4	0.1
	Leguan	–3.5	0.2	–12.3	0.3

RESULTS

The investigation of ABA endogenous content within 4 weeks of cold hardening of three barley cultivars demonstrated that in none of the cultivars under study did ABA content significantly increase (Figure 1). The highest freezing tolerance was determined in Lunet cultivar when compared with less tolerant cultivars Cenader and Akcent (Table 1). The results indicated that the freezing tolerance of the cultivars under study was not apparently associated with absolute content of ABA because the highest content of ABA in leaves before plant exposure to low temperature and during cold hardening was observed in Cenader cultivar, which is less freeze-tolerant than Lunet cultivar.

The study of ABA endogenous content in six wheat cultivars demonstrated an increase in ABA content only

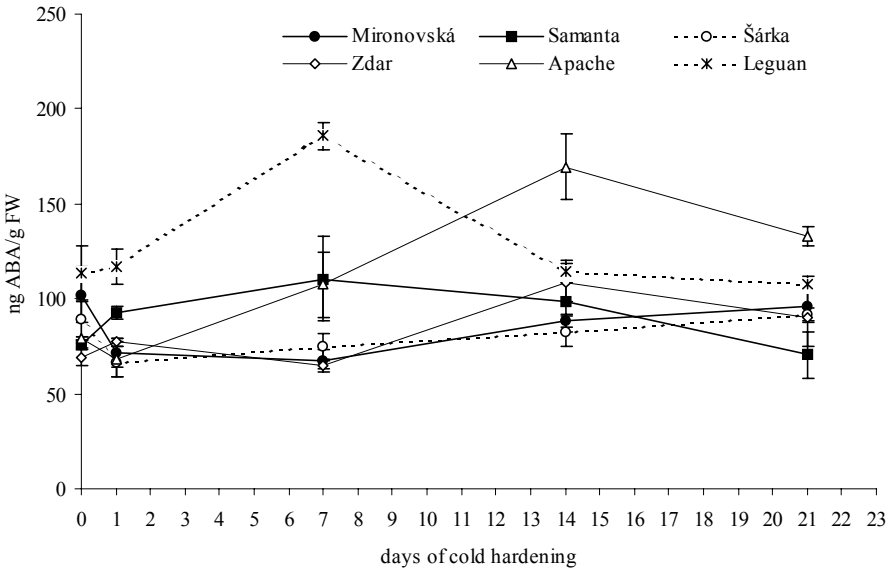

Figure 2. Changes in leaf ABA content during cold hardening of wheat cultivars; means of three replications; the vertical bars give *SE*

in Leguan cultivar after a week and in Apache cultivar after two weeks of cold hardening. Similarly, like in barley cultivars no significant change in the endogenous content of ABA during cold hardening was observed in the other wheat cultivars (Figure 2). When the cultivars were compared for their endogenous contents of ABA detected in leaves before the plants were exposed to low temperature, no differences and no correlations with the achieved freezing tolerance of wheat cultivars were established. The highest content of ABA during cold hardening of plants was determined because of its transitory increase in Apache and Leguan cultivars. The ABA contents of other wheat cultivars were quite similar. The leaves of Mironovská and Samanta cultivars exhibited the highest freezing tolerance, the leaves of Leguan cultivar the lowest (Table 1). Not even in the course of cold hardening was any relationship proved between the endogenous content of ABA and the level of freezing tolerance in the wheat cultivars under study.

DISCUSSION

Contrary to the results published by Bravo et al. (1998), the measurements of ABA endogenous content in barley and wheat leaves before they were exposed to low temperature did not prove any relationship between the content and freezing tolerance of cultivars of these non-acclimated plants. Analogically to our results, Lalk and Dörffling (1985) did not find any significant difference between the endogenous contents of ABA in two wheat varieties with different freezing tolerance grown at an unreduced temperature.

The three barley and four wheat cultivars did not show any great changes in the endogenous content of ABA in the course of cold hardening contrary to the results of the authors who reported a transitory increase in ABA content after the plants were exposed to low temperature (Bravo et al. 1998, in one barley cultivar, Veisz et al. 1996, in three wheat cultivars with different freezing tolerance). Dörffling et al. (1990) described a correlation between the maximum value of transitorily increased ABA content during cold hardening of nine differently freeze-tolerant wheat cultivars and their freezing tolerance determined at the end of cold hardening. Macháčková et al. (1989) detected ABA accumulation during cold hardening of three wheat cultivars with different freezing tolerance that correlated with freezing tolerance of particular cultivars. In our study only a slight transitory increase in ABA content was observed in two wheat cultivars. Most barley and wheat cultivars included in our study did not exhibit any transitory increase in ABA endogenous content in leaves induced by cold hardening. It is in agreement with the results of Dallaire et al. (1994) in wheat plants, and with our previously published results of investigation into the endogenous content of ABA during cold hardening of plants of barley cultivar Lunet when it was found that no changes in ABA endogenous content occurred if the plants did not suffer from high water losses during hardening (Kadlecová et al. 2000, Faltusová-Kadlecová et al. 2002). Accordingly, Capell and Dörffling (1989) did not observe any increase in ABA content in barley leaves after the plants were exposed to low temperature nor any change in water content in plants. The endogenous content of ABA sometimes increases after the cultivation temperature of plants has decreased (Dörffling et al. 1990); we are convinced that it could be associated with potential secondary drought stress induced by a low temperature that was minimized by hydroponics in our study. We assume that the changes in ABA endogenous content can be connected with the method of cultivation of plants exposed to cold hardening. Our results indicate that the endogenous content of ABA cannot be used as an unambiguous marker of freezing resistance of plants.

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ABSTRAKT

Porovnání obsahu kyseliny abscisové u odrůd ječmene a pšenice s různou mrazuvzdorností

U souboru dvou ozimých odrůd (Lunet, Cenader) a jedné jarní odrůdy (Akcent) ječmene a u pěti ozimých odrůd (Mironovská, Samanta, Šárka, Zdar, Apache) a jedné jarní odrůdy (Leguan) pšenice byl sledován endogenní obsah kyseliny abscisové během otužování hydroponicky pěstovaných rostlin nízkou teplotou. Současně byla stanovena mrazuvzdornost sledovaných odrůd ječmene a pšenice. Otužování rostlin nízkou teplotou nevyvolalo ani u jedné ze sledovaných odrůd ječmene významnou změnu obsahu kyseliny abscisové. V případě otužování rostlin pšenice nízkou teplotou vykazovaly mírný přechodný nárůst obsahu kyseliny abscisové pouze odrůdy Apache a Leguan. Ostatní odrůdy pšenice měly obsah kyseliny abscisové v listech velmi podobný. Úroveň dosažené mrazuvzdornosti nesouvisela ani v případě ječmene, ani u pšenice s endogenním obsahem kyseliny abscisové či s jeho přechodnými změnami.

Klíčová slova: kyselina abscisová; otužování; mrazuvzdornost; ječmen; pšenice

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