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Water and ABA content in fully expanded leaves of cold-hardened barleys

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SUMMARY

Changes in water and ABA content during cold-hardening of plants under controlled conditions were investigated in four barley cultivars with different freezing tolerance. Plants were cultivated in a nutrient solution and had good water supply all the time. Mature leaves were sampled for analyses at the end of the darkness period. These fully-expanded leaves increased their dry matter weight, without a corresponding increase in water, during cold hardening. The increase in dry matter weight was related to improved freezing tolerance of cultivars. ABA content did not increase significantly during cold-hardening. There were differences in ABA contents between cultivars, but no significant changes were observed between the hardened and control plants. Our experiments demonstrate that dry weight accumulation is important for the induction of freezing tolerance in barley during cold-hardening while an increase in endogenous ABA is not required at good water supply.

Key words: abscisic acid, barley, cultivars, freezing tolerance, water content.

YFIRLIT

Vatns- og ABA-innihald fullþroskaðra blaða af hertum byggplöntum

Breytingar í innihaldi plantna á vatni og ABA meðan á hörðnun stendur voru kannaðar við stýrðar aðstæður á fjórum byggstofnum með mismunandi frostþol. Plönturnar voru ræktaðar í næringarlausn og því með góðan aðgang að vatni. Fullþroskuðum laufblöðum var safnað til efnagreiningar í lok næturmyrkurs. Þurrefnismagn þessara fullvöxnu laufblaða óx, án samsvarandi aukningar í vatnsmagni við hörðnun. Aukið þurrefni tengdist auknu frostþoli stofnanna. ABA magnið jókst ekki raunhæft á meðan á hörðnun stóð. Munur var á ABA magni stofnanna, en enginn raunhæfur munur var á hörðnuðum og óhörðnuðum plöntum. Rannsóknirnar sýna að aukning í þurrefni er mikilvæg fyrir aukið frostþol í byggi, en aukning í innra ABA magni er ónauðsynlegt við nægan aðgang að vatni.

INTRODUCTION

Cold-hardening of winter crops is necessary for induction of freezing tolerance. This induction is connected with the activation of some genes and regulatory mechanisms (Thomashow, 1999). They cause changes in concentrations of some metabolites and cellular component structures. In the process of plant hardening, osmotically active substances and osmoprotectants (e.g. sugars, proline, glycine-betaine) are accumulated and characteristics of the plant water regime are influenced. The ratio of water to dry matter weight decreases and endogenous content of abscisic acid (ABA) was often found to vary (Lång *et al.*, 1994). Recently, a transitory increase in ABA endogenous content has been observed most frequently at the beginning of plant hardening (Dörffling *et al.*, 1997). It is a well-known fact that ABA content considerably increases in plant tissues at water deficit (Davies and Jones, 1995). Under soil drought, ABA transport from roots to above-ground parts was described as a chemical signal and the resulting increase in ABA content in leaves causes stomata to close. The relationship between decrease in water content and increase in ABA content can be described on the basis of water deficit.

The objective of the present paper was to elucidate in greater detail a relationship between variations in water, dry weight and ABA content during barley cold-hardening. Mature leaves of intact barley plants were used for this study. In the stage of fully expanded blade the mature leaf represents a model of plant organs with terminated growth and differentiation processes (Prášil *et al.*, 2000). If exposed to cold, it reflects the changes connected with plant hardening (Prášil and Faltus, 1994).

MATERIAL AND METHODS

Experiments were conducted on plants of six-row winter barley (*Hordeum vulgare* L.) cultivars Lunet and Cenader, two-row winter barley cultivar Marinka and spring cultivar Akcent. After 6-day germination of barley seeds at 17±1°C, seedlings were grown in culture boxes under a long day of 16 h light (400 μmol m^{-2} s^{-1}) and temperature 18°C by day and 16°C by night in Hoagland 3 nutrient medium. In 15 days, when the full expansion of the second leaf terminated, plants were exposed to hardening under the same cultivation conditions but at 3°C. For all analyses we used only the second (mature) leaves, which we sampled from the plants prior to hardening and repeatedly during hardening. They were removed from the plants at the end of the darkness period while relative air humidity exceeded 90%. These conditions, including plant cultivation in nutrient medium, were supposed to provide sufficiently high water supply after the plants were exposed to cold.

These leaf analyses were made: Fresh weight and the dry matter weight (DMW) after drying at 85°C for 24 h was measured on ten second leaves. The level of freezing tolerance was determined by a laboratory freezing test. A set of ten 1 cm leaf segments per tube (cut from ten leaves) was exposed in two repetitions to five different freezing temperatures. The rate of cooling and thawing was 3°C/h. After thawing, 14 ml deionized water was added to each tube and the degree of freezing injury was evaluated by conductivity methods (Prášil and Zámecník, 1998). The lethal temperature (LT_{50}) (i.e. the freezing temperature at which 50% of leaves were killed) was calculated from an S-shaped curve representing the relationship between freezing injury and test freezing temperature (Janácek and Prášil, 1991). For ABA analysis three replicates, each 0.5 g of fresh weight of second leaves, were used. RIA (radioimmunoassay) according to Quarrie *et al.* (1988) was used to determine abscisic acid content in co-operation with the Division of Radiobiology at Mendel Agricultural and Forestry University at Brno. Monoclonal ABA-antibody MAC252 was obtained from dr S.A. Quarrie (John Innes Institute, England).

A Student's t-test was used to detect differences between values LT_{50}, DMW, and ABA of each treatment.

RESULTS

After the plants of winter barley cv. Lunet were exposed to cold, their water content per unit dry weight was reduced (Figure 1a). At first, the reduction in water content per dry weight was very fast, and then it gradually slowed down in the course of 3-week hardening of plants at a constant low temperature. As mature leaves, just fully expanded, were used for the analysis, it was possible to determine to what extent this reduction was related to a decrease in absolute water content or an increase in absolute dry matter weight. The ratio of water content (in grams per leaf) to initial water content (in grams per leaf) was not found to vary during hardening at all (Figure 1b), whereas dry matter weight, expressed in the same way, increased substantially during hardening. The reduction in water content per dry weight or fresh weight (Figure 1a) was caused by an increase in dry matter weight, not reduction in water content. Dry weight amount increased from the beginning of hardening, at first rapidly, then, after a fortnight, considerably more slowly.

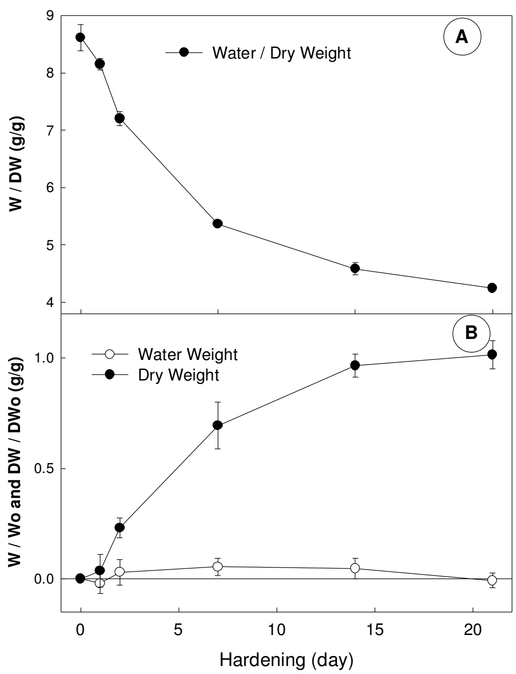

Figure 1. Water and dry matter weights during barley hardening. Water content is expressed per unit dry weight (A), and water content and dry matter weight as ratio of the initial value before plant hardening (Wo, DWo) (B).
1. mynd. Vatnsinnihald og þurrefni við hörðnun byggs. Vatnsinnihald er sýnt sem hlutfall af þurrefni (A) og vatnsinnihald og þurrefni sem hlutfall af upphafsgildi fyrir hörðnun (B).

Table 1. ABA content in fresh weight (FW) of the second mature leaf during plant cold-hardening.
1. tafla. ABA-innihald í votvigt á öðru þroskaða laufblaði byggs eftir mislanga hörðnun.

Hardening, day	ABA/FW, ng/g	SE
0	71.9	6.3
1	85.8	10.3
2	75.1	12.5
7	73.5	11.9

Another experiment was conducted to study ABA endogenous content during the first week of hardening of cv. Lunet (Table 1). No statistically significant changes in ABA content in the second mature leaves were found in the course of hardening.

Target characteristics before and after 3-week hardening were compared in a cultivar experiment. While cultivar differences in freezing tolerance were insignificant before hardening (LT_{50} ranged between –3 and –4°C), the freezing tolerance of mature leaves increased after plant hardening and cultivar differences were confirmed. The most tolerant was winter barley cv. Lunet (LT_{50}=–14.2°C), followed by cv. Marinka with LT_{50}=–11.9°C. The least tolerant were cv. Cenader (–10°C) and spring barley cv. Akcent with LT_{50}=–9.2°C. An increase in dry matter weight in the second mature leaf was highly significant in all cultivars after 3-week hardening (Figure 2). The only significant differences in dry matter weight after hardening are between Lunet and Cenader. The ABA content did not significantly increase in any cultivar after hardening.

DISCUSSION

Cold-hardening of plants and induction of freezing tolerance was accompanied by a reduction in water content. Water content can be determined in organs at diverse growth and differentiation stages that during hardening modify the growth, size and structure of cells in the particular tissues. Only mature, just fully expanded leaves were used in our experiment. Hardening was connected with an increase in dry matter weight of these leaves, rather than loss of water. Gusta *et al.* (1982) reported that the percent decrease in water content in the tillering crowns of grain crops during hardening was related rather to dry weight accumulation than to water reduction. Stout (1980) drew a similar conclusion for alfalfa crowns. Hence the reduction in water content is not a necessary condition for plant hardening against freezing.

No large variations in ABA content during barley hardening were observed under our experimental conditions, when plants were grown in a nutrient medium and samples of mature leaves were taken at the end of the darkness period. Detailed investigations revealed ABA content variations in both the hardened and control treatment while the respective variations were not significant (Kadlecová *et al.*, 2000). An increase in ABA content in mature barley leaves was experimentally induced in other experiments by exogenous applications of ABA or drought stress. Neither of these treatments resulted in improved freezing tolerance of leaves, as did cold-hardening (Kadlecová *et al.*, 2000). Exogenous applications of ABA or drought stress, used for induction of freezing tolerance in intact plants, did not always bring unambiguous results (e.g. Dallaire *et al.*, 1994). Exogenous application of ABA was necessary for cold induction of freezing tolerance in ABA-mutants of *Arabidopsis* (Heino *et al.*, 1990).

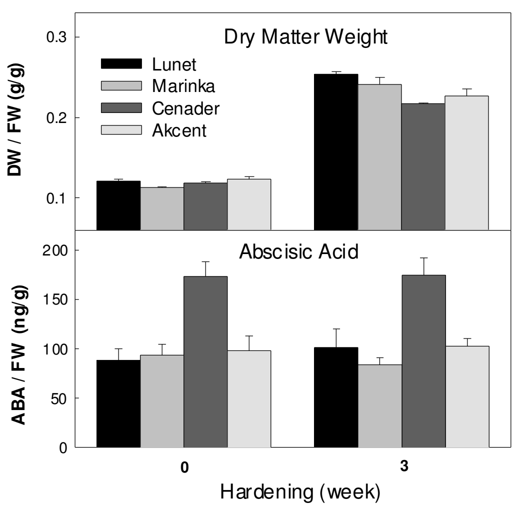

Figure 2. Dry weight and ABA contents before and after 3-week hardening of four barley cultivars.
2. mynd. Þurrefni og ABA-innihald fyrir og eftir þriggja vikna hörðnun fjögurra stofna af byggi.

Barley cultivar trial confirmed that increase of freezing tolerance in the cultivars was not related to ABA accumulation, but to accumulation of dry weight during plant hardening. Cultivar differences in ABA endogenous content in barleys before hardening were reported by Bravo *et al.* (1998). Contrary to our results, they found a correlation between freezing tolerance and ABA content in their cultivars.

Generalisation of the results should take into account possible differences in reactions of different species to cold as mentioned by Capell and Dörffling (1989). Differences in measurements of the constitutive and inducible component of freezing tolerance should also be considered (Stone *et al.*, 1993). Many experiments, including the use of ABA mutants, demonstrate the existence of a certain function of ABA in the course of plant cold-hardening, e.g. induction of cold-regulated proteins. Nevertheless, our experiments indicated the importance of dry weight accumulation for induction of freezing tolerance in barley, while an increase ABA content is not required when water supply is sufficient.

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