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BRIEF COMMUNICATION

Comparison of barley response to short-term cold or dehydration

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Abstract

Abscisic acid (ABA) content and relative water content (RWC) in second fully expanded leaves of cold hardened plants and in dehydrated leaves of freezing tolerant barley (*Hordeum vulgare* L. cv. Lunet) were compared. ABA content and RWC in leaves did not change during the first day of cold hardening. On the contrary, dehydration of leaves led to a decrease of RWC and to an increase of ABA content.

Additional key words: abscisic acid, cold hardening, frost, *Hordeum vulgare*, low temperature, relative water content.

Changes in abscisic acid (ABA) content in plants are usually connected with the occurrence of some type of stress (Hartung and Davies 1991, Walton and Li 1995). For example, it is the case when plants are cold hardened in order to achieve freezing tolerance (Dörffling *et al.* 1990, Murelli *et al.* 1995, Veisz *et al.* 1996, Bravo *et al.* 1998). The question is whether ABA content changes could play any role in acclimation of plants to frost and whether they are induced by the low temperature *per se* or due to parallelly developed drought stress. We measured the endogenous ABA content and relative water content (RWC) in hydroponically cultivated plants of barley after their exposure to a low temperature. We compared ABA content and RWC in leaves of cold hardened plants with these parameters during dehydration of detached barley leaves, because the dehydration of leaves after their detaching leads to distinct increase of ABA content (Stewart and Voetberg 1985, Walker-Simmons *et al.* 1989, Grossi *et al.* 1995, Kadlecová *et al.* 2000, Yang *et al.* 2000).

Plants of barley (*Hordeum vulgare* L.), freezing tolerant cv. Lunet, were cultivated hydroponically (Hoagland 3) in controlled conditions: 16-h photoperiod, irradiance of 400 μmol m^{-2} s^{-1}, temperature 17 ± 1 °C. For cold hardening experiments the plants were divided into two groups after 15 d of cultivation. The control plants stayed in the same conditions and the plants for cold hardening were exposed to 3 ± 1 °C for one day either *1*) during the dark, or *2*) during the light, or *3*) during the light while the plants were covered with a plastic foil to prevent water loss. For dehydration experiments the second leaves of 15-d-old plants were cut off. One half of the detached leaves was put into tubes with distilled water (control) and the second half of the leaves was put into tubes without water (dehydrated) for 1 d.

In all experiments second fully expanded leaves were used for measurements of ABA content and RWC. For ABA analysis three replications of 0.5 g fresh mass were used. The samples were homogenised in liquid nitrogen then extracted with distilled water (5 cm^3) and shaken overnight in the dark at 4 °C. After that the samples were centrifuged at 5 000 g for 10 min at 4 °C and 0.05 cm^3 of the supernatant was used for ABA determination. The ABA content was determined by the RIA method according to Quarrie *et al.* (1988). The monoclonal anti-ABA antibody MAC252 was obtained from Dr. S.A. Quarrie (John Innes Centre, Norwich, UK). RWC was calculated according to the equation:

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Abbreviations: ABA - abscisic acid; f.m. - fresh mass; RWC - relative water content.

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RWC = 100 (FM - DM) / (SM - DM), where FM is fresh mass, DM is dry mass and SM is the mass of 1 cm segments saturated with distilled water at room temperature for 3 h (saturated mass).

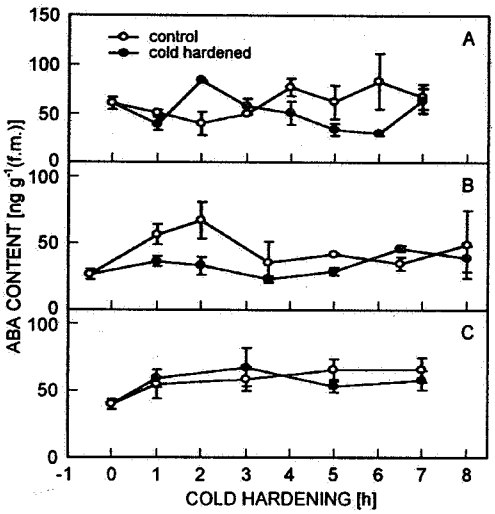

Fig. 1. Changes in ABA content in second fully expanded leaves during the first day of cold hardening of barley cv. Lunet. *A* - cold hardening during the dark, *B* - cold hardening during the light, *C* - cold hardening of covered plants during the light (*open circles* - leaves from the control plants, *filled circles* - leaves from the cold hardened plants). Means from three repetitions. The *vertical bars* indicate SE.

The treatment of hydroponically cultivated barley plants by a low temperature during 1 d in either dark or light phase was connected only with small fluctuations in ABA content. Some fluctuation was observed in control plants, too (Fig. 1*A*,*B*). Covering of plants by a plastic foil prevented this fluctuation in control as well as in cold hardened plants (Fig. 1*C*). The RWC did not change significantly during this first day of cold hardening; neither the uncovered nor the covered plants showed any signs of water deficit. These results led us to a suggestion that hydroponic system was suitable for the study of the relationship between ABA and low temperature treatment due to minimizing water loss during low temperature treatment. Wilen *et al.* (1998) also denote the advantages of hydroponic cultivation for similar studies. On the other hand, rapid dehydration of detached leaves at 17 °C led to a decrease of RWC. The RWC decrease by 29 % (from 97 % to 68 %) after 24 h of leaf dehydration was connected with a distinct increase of ABA content (Fig. 2). The 24-h incubation of control detached leaves in distilled water did not induce changes in ABA content (Fig. 2*A*). Yang *et al.* (2000) described a similar situation in control detached rice leaves. After 1 h of leaf dehydration RWC decreased by 6 % (from 98 % to 92 %) (Fig. 2*B*), the leaves were wilted, but ABA content did

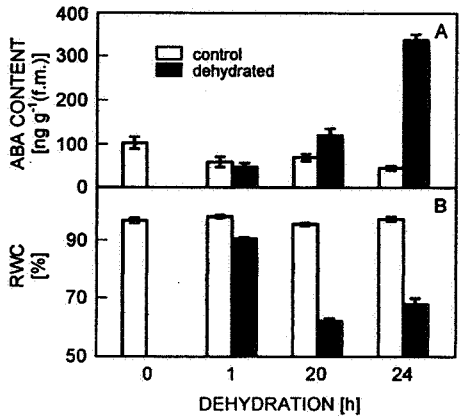

Fig. 2. Changes in ABA content (*A*) and RWC (*B*) in second fully expanded detached leaves of barley cv. Lunet after leaf dehydration. (*open columns* - control leaves, *filled columns* - dehydrated leaves). Means from three repetitions (ABA content) or from ten repetitions (RWC). The *vertical bars* indicate SE.

not increase (Fig. 2*A*). The fact that the 6 % decrease of RWC caused by dehydration did not lead to ABA content increase could explain the unchanged ABA content in cold hardened plants where RWC fluctuated only in the range of 1 % (between 97 % and 98 %). These results are in agreement with the findings of Capell and Dörffling (1989), who observed no change in ABA content together with no changes in water status in the first fully expanded barley leaf after exposing the plants to low temperature. Dallaire *et al.* (1994) also observed no changes in ABA content during the initial phase of cold hardening in comparison with 10-fold ABA content increase after 4 d of water stress in the leaf tissue of freezing tolerant wheat cultivar. Similarly, Lång *et al.* (1994) found only minor decrease of RWC after cold treatment of *Arabidopsis*, while drought induced a substantial RWC decline. Moreover, cold-exposed *Arabidopsis* plants showed only a temporary elevation of ABA content in contrast to considerable increase of ABA content in drought-stressed plants (Lång *et al.* 1994). Therefore, we suggest that changes in ABA content in plants are related rather to water status than to low-temperature treatment and that the initial phase of barley cold hardening is not necessarily accompanied with an increase of ABA content.

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