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Implication of ABA and proline on cell membrane injury of water deficit stressed barley seedlings

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Abstract

The aim of this work was to examine the ability of ABA and proline to counteract the deleterious effect of water deficit stress on cell membrane injuries.

Six-day-old seedlings of two barley genotypes (*cv.* Aramir, line R567) were treated with ABA ($2 \cdot 10^{-4}$ M) or proline (0.1 M) for 24 h, and then subjected to osmotic stress for 24h, by immersing their roots in polyethylene glycol (PEG 6000) solution of osmotic potential of -1.0 MPa and -1.5 MPa or by submerging the leaf pieces in PEG solution of osmotic potential of -1.6 MPa.

Pretreatment of plants with ABA and proline caused an increase of free proline level in the leaves. Plants treated with ABA exhibited a lower membrane injury index under water stress conditions than those untreated even when no effect of this hormone on RWC in the leaves of stressed plants was observed. Pretreatment of plants with proline prevented to some extent membrane damage in leaves of the stressed seedlings, but only in the case when stress was imposed to roots. Improvement in water status of leaves was also observed in seedlings pretreatment with proline. The protective effect of both ABA and proline was more pronounced in line R567 that exhibited higher membrane injury under water deficit stress conditions.

List of abbreviations: ABA - abscisic acid, PEG - polyethylene glycol, RWC - relative water content

Introduction

Water deficit stress often causes an increase in abscisic acid content and proline accumulation in plant leaves (Dashek and Ericson 1981, Quarrie 1991, Ristic *et al.* 1992). The enhancement level of these compounds is believed to be of adaptative significance. Resistance to water deficit stress occurs when plants withstand the imposed stress, and may arise from either tolerance or avoidance of dehydration (Levitt 1980).

It is well documented that ABA plays a vital role in stress avoidance by reducing stomata opening and thus lowering transpiration (Creelman and Mullet 1991, Hetherington, Quatrano 1991). Furthermore, ABA elicits the synthesis of proline and proteins, which have been implicated to have a role in protecting cellular structures during dehydration (Stewart 1980, Singh *et al.* 1989, Ristic *et al.*1992) and it enables plants to survive cellular water deficits. ABA coordinates plant responses to water deficit and the regulation of gene expression plays here an important role (Bray 1997).

Proline is a non-toxic compatible osmolyte which may alleviate the deleterious effects of stress on enzyme activity and the structure of cell membranes (Rudolph *et al.* 1986, Blum 1988, Schwab and Gaff

1992, Nikolopoulos and Manetas 1991). It has been indicated that proline lowers the generation of highly destructive free radicals species (Smirnoff and Cumbes 1989). Moreover, it has been proposed that proline contributes to osmotic adjustment, the process of net accumulation of solutes in cell, which in turn attracts water into cell and tends to maintain turgor, and avoid dehydration (Voetberg and Sharp 1991, Delauney and Verma 1993, Bohnert *et al.* 1996). Genetically engineered tobacco plants with overproduction of proline were able to grow better compared to wild-type during osmotic stress (Kavi Kishor *et al.*1995). So, it is likely that proline may enable crop plants to tolerate water stress.

The aim of the present work was to find out whether the pretreatment of plants with ABA or proline may modify water-stress induced membrane injury in the leaves of two barley genotypes, one exhibiting lower (cultivar Aramir) and one exhibiting higher membrane injury (line R567) under water deficit (Bandurska 1995). The extent of water loss was determined by measuring the relative water content (RWC) in leaves from control and stressed plants.

Materials and Methods

Plant material and treatment

The experiment was carried out on two spring barley (*Hordeum vulgare*) genotypes, cultivar Aramir and line R567. The plants were grown in aerated nutrient solutions as described earlier and used for the experiment (Bandurska and Gniazdowska-Skoczek 1995). The root system of 6-day-old seedlings was immersed in solution of proline (0.1 M) or ABA ($2 \cdot 10^{-4}$ M) for 24 hours. The untreated plants (control) were immersed in nutrient solution. The samples of leaves were then collected and used for determination of proline (Bates *et al.* 1973).

Water deficit stress imposition and samples collection

The sets of seedlings pretreated with proline or ABA and non-treated (control) were subjected to water stress. The stress was imposed either to leaves (Experiment I) or to roots (Experiment II and III).

Experiment I

Nine pieces (2 cm) from 3 leaves were placed in a 50 ml flask, washed three times with 10 ml deionized water and then floated in 10 ml polyethylene glycol (PEG 6000) solution of osmotic potential of -1.6 MPa or in deionized water (control) and kept for 24 h at 10 °C. After this time PEG solution was decanted and the membrane injury index was determined.

Experiment II and III

The root system of plant was immersed in PEG solution of osmotic potential -1.0 MPa (Exp. II) and -1.5 MPa (Exp. III) for 24 hours. The roots of control seedlings were exposed to nutrient solution for the same period. The seedlings were kept under controlled conditions at 21 °C, 70 % humidity and continuous light of 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photon flux density. After exposure to stress, the leaves from seedlings of each set were harvested and used for the analysis. The middle portions of the leaves were cut into 2 cm pieces immediately after sampling. Nine pieces (2 cm) from 3 leaves were placed in a 50 ml flask, and the membrane injuries were determined. Two fragments of leaf were collected for determination of the RWC.

Determination of relative water content (RWC)

The level of leaves dehydration was estimated on the basis of RWC determined by the method of Weatherly (1950) according to Bandurska (1991). The samples of leaf were weighed, then placed in water for four hours (full turgor), weighed again, dried at 60 °C, and weighed once more. The relative water content was calculated according to the following formula:

RWC= (fresh weight - dry weight) / (fresh weight of full turgor - dry weight) x 100 %

Determination of cell membrane injury

Leaf pieces were washed quickly three times in 10 ml of deionized water, then immersed in 10 ml of deionized water, and kept for 24 h at 10 °C. The electrical conductivity was measured and then the leaf tissues were killed by autoclaving for 15 min, cooled to 25 °C, and the electrical conductivity was measured for the second time. Membrane injury was evaluated as the percentage injury index fol-

lowing the formula of Sullivan (1971).
$I = [\ 1-(1-T_1/T_2)/(1-C_1/C_2)] \times 100\ \%$
where C_1 and C_2 represent conductivity measurements of control samples before and after autoclaving, respectively; T_1 and T_2 represent conductivity measurements of water-stressed samples before and after autoclaving, respectively.

Proline determination

Plant material (0.1-0.2 g fr. wt of leaf) was freeze-dried and stored at -20 °C until estimation. The proline content was estimated using the method of Bates *et al.* (1973). The amount of proline was calculated from a previously plotted standard curve and expressed in $mg^{-1} \cdot g^{-1}$ of dry leaf weight.

Presentation of data

The experiments were conducted twice and in each case identical trends were observed. The data presented in Figures are means from at least three independent replications of one representative experiment. Tukey's method was applied to determine the significance of differences between means. The confidence coefficient was set at 0.05. In the Figures, plots bars followed by the same letters are not significantly different.

Results

Effect of pretreatment with ABA and proline on free proline content

Pretreatment of plants separately with ABA and proline caused an increase in the content of free proline in leaves of both genotypes (Fig. 1 A-F). This promotive effect of treatments on proline level was more prominent in the proline treated plants. ABA treated plants exhibited twice or three times higher proline level as compared with the untreated ones (Fig. 1A-C). But in plants treated with proline the level of this amino acid increased in relation to non-treated from two to several times, depending on the experiment (Fig.1 D-F).

Effect of pretreatment with ABA and proline on RWC in water deficit stressed plants

The immersing of root system of plant in PEG solution reduced significantly RWC of leaves in both genotypes (Fig. 2 A-D). The extent of reduction

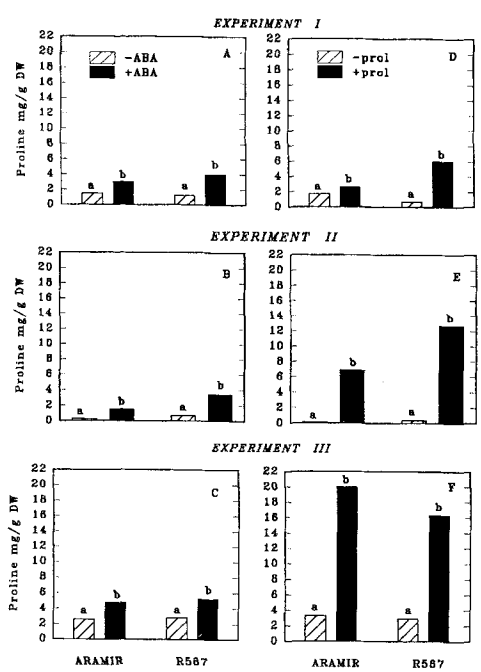

Fig. 1. The effect of plants pretreatment with ABA (A,B,C) and proline (D,E,F) on free proline contents in the leaves of two barley genotypes (*cv.* Aramir, line R567).

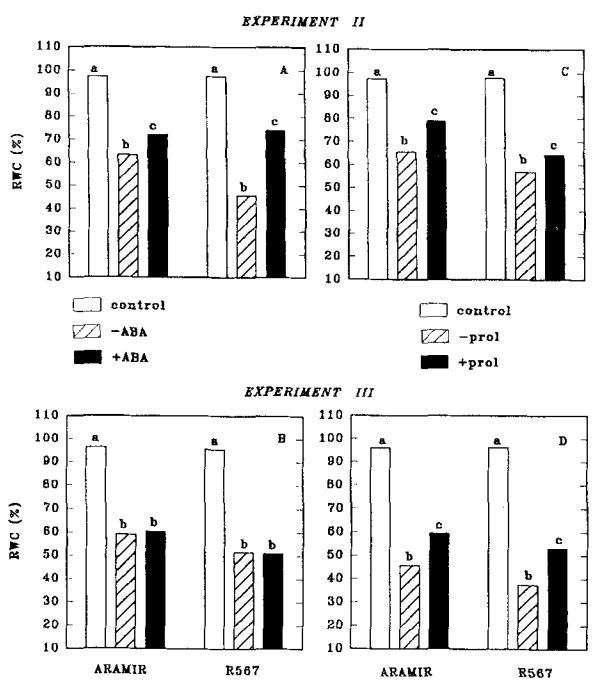

Fig. 2. The effect of plants pretreatment with ABA (A,B) and proline (C,D) on the relative water content (RWC) in the leaves of two barley genotypes (*cv.* Aramir, R567) under water deficit stress imposed to roots (Exp. II. PEG -1.0 MPa, Exp. III PEG -1.5 MPa).

was greater at higher degree of stress factor (-1.5 MPa, Fig. 2 B,D) Application of ABA (Fig. 2 A,B) improved water status in leaves of seedlings subjected to a lower degree of stress factor (-1.0 Mpa, Fig. 2 A) but not in seedlings subjected to higher degree of stress factor (-1.5 MPa, Fig. 2B). The treatment with proline reduced the fall in water status of leaves in seedlings subjected to both degrees of water stress (Fig. 2 C,D).

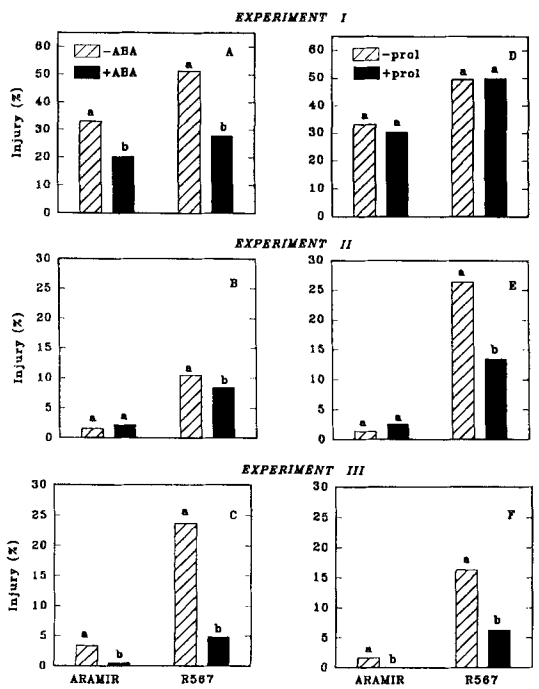

Fig. 3. The effect of plants pretreatment with ABA (A,B,C) and proline (D,E,F) on membrane injury index (in %) in the leaves of two barley genotypes (*cv.* Aramir, R567) under water deficit stress imposed to leaves (Exp. I -1.6 MPa) and to roots (Exp II PEG -1.0 MPa, Exp. III PEG -1.5 MPa).

Effect pretreatment with ABA and proline on cell membrane injury index under water stress conditions

In general, water deficit stress induced membrane injury in the leaves of both studied genotypes. Cultivar Aramir showed a lower membrane injury compared with line R567. This difference was more pronounced under the conditions of stress imposed to roots (Fig. 3 B,C,E,F). In turn, water stress imposed to leaves caused several times higher membrane injury in cultivar Aramir and at most two times higher membrane injury in line R567 (Fig. 3 A,D) as compared with stress imposed to roots (Fig. 3 B,C,E,F). But we did not observe any clear relationship between the value of stress factor imposed to roots and the value of the percentage membrane injury index (exp. II and III).

The treatment of seedlings with ABA was found to decrease the level of membrane injury index in plants subjected to water stress (Fig. 3 A-C). The most pronounced decrease of the analyzed parameter took place in line R567. In the case of the Aramir genotype, this effect was detectable only at higher level of the stress induced to roots (-1.5 MPa, Fig. 3C) and under water stress imposed to leaves (Fig. 3A).

The treatment with proline did not bring about decrease in the value of membrane injury index in the plants subjected to water stress imposed to leaves (Fig. 3 D). The application of proline to the seedlings prior to water stress imposed to roots led to a decrease in the percentage membrane injury index (Fig. 3 E,F). The most pronounced effect was observed also in this case in the line R567 suffering higher membrane injury under water stress conditions.

Discussion

According to Blum (1988) the ability to maintain cell membrane stability under conditions of water deficit is a major component of plant resistance to this stress. The stability of cell membrane under the stress conditions depends on the properties of membrane proteins, lipid composition and the activity of mechanisms countering membrane degradation (Sgherri *et al.* 1993). Degradation of both proteins and lipides in the conditions of water deficit is often due to high content of active oxygen species which is a consequence of a lowering activity of enzymes taking part in removing them (Pastori and Trippi 1993). The present study shows, that similarly as was stated in previous papers line R567 exhibited higher membrane injury than cultivar Aramir under water deficit stress conditions (Bandurska and Gniazdowska-Skoczek 1995, Bandurska *et al.* 1997). But, no evidence was seen that lower membrane injury in *cv.* Aramir is due to a better defense mechanism against harmful metabolite like H_2O_2 (Bandurska *et al.* 1997).

Kacperska (1995) claims that abscisic acid functions as a regulator in processes enabling a plant to reduce the damage that can be caused by different environmental stress factors. In the present paper it was shown that treatment of plant with ABA solution decreased the membrane injury under water deficit in the leaves of both studied genotypes of barley (Fig. 3A-C). The effect was most pronounced under severe water stress (Fig. 3 A,C).

Abscisic acid is a hormone responsible for improvement of plant water management, as, on the one hand, it reduces stomata openings (Cornish and Zeevaart 1985), which lowers transpiration, and on the other hand, it fosters water absorption (Ludwig *et al.* 1988). In this work it was shown that ABA considerably improved leaves hydration in the conditions of water-deficit stress imposed to roots but only for lower values of the stress factor (-1.0 MPa, Fig. 2A).

But, the observed improvement of water status under water deficit stress did not save the plants from membrane injury (Fig. 3B).

In view of the above it can be said that a considerable influence of ABA on lowering membrane injury indicates that the apparent effect of this hormone consisted in alleviating the negative effects of the dehydration on membrane functioning . This effect was observed under water stress imposed to leaves (Fig. 3A), when portions of tissue were subjected to direct treatment with PEG, as well under water-stress imposed to roots at higher level of stress factor (Fig. 3C), when no effect of this hormone on reduction of leaf dehydration was noted (Fig. 1B).

Increase in drought-resistance and reduction of the leakage of ninhydrin reacting substance were observed in germinating embryos of *Haplopapuus gracilis* treated with ABA (Galli and Levi 1982). In the study of *Polypodium virginianum* (Reynolds and Bewley 1993) it was observed that treatment of cut-off fern leaves with ABA led to reduction of electrolyte leakage from the tissue and improved the survival rate of leaves subjected to desiccation. This effect was not however accompanied by the synthesis of proteins which might be somehow associated with the ABA-induced tolerance of dehydration in the plant. Low membrane injury under water-deficit stress was also noted in Vigna (Mukherjee and Choudhuri 1985) and in jute (Chowdhury and Choudhuri 1989) treated with ABA prior to stress. In these experiments pretreatment of plants with ABA improved RWC of leaves. When conducting a study on tobacco, Rensnsberg *et al.* (1993) demonstrated that drought-resistant cultivars were able to accumulate more free proline under water deficit stress and showed lower membrane injury. Previous experiment was also shown that lower membrane injury in drought stressed plants of *cv.* Aramir correlated positively with higher proline accumulation (unpublished data). A water stress-induced accumulation of free proline is a result of its *de novo* synthesis from glutamic acid (Rhodes 1986) and may be induced also by ABA, independently of stress (Stewart and Voetberg 1985).

In the studies presented here an increase of the content of free proline in plant leaves treated with ABA was noticed (Fig. 1A-C). One can concluded therefore that lowered degree of membrane injury observed in dehydrated plants pretreated with ABA is connected with an increased amount of this amino acid in tissues. As follows from the experiments of other authors, proline protects membranes by reducing the content of free radicals formed during stress (Smirnoff and Cumbes 1989, Alia 1993, Bray 1993). Works of other authors suggest that proline may form bonds with membrane proteins and thus stabilize their structure in stress (Schobert and Tschesche 1978, Paleg *et al.* 1984).

Administration of proline applied exogenously caused a significant increase of the content of free proline in leaves (Fig. 1 D,E,F), apparently greater than in the case of plants treated with ABA (Fig. 1 A,B,C). Better leaves hydration was noticed under water deficit stress imposed to roots in proline treated plants relative to the untreated (Fig. 2 C,D). This, in consequence, led to a decrease of the level of membrane injury (Fig. 3 E,F). However, in water stress imposed to leaves when portions of tissue were directly treated with PEG (Fig. 3D) no effect of proline on the degree of membrane injury was noticed. In this experiment the proline accumulated in tissue (Fig. 1D) was not able to counteract the strong osmotic influence of PEG solution on leaf tissue and protect the membranes (Fig. 3D).

A positive effect of proline on water management under water stress conditions was also found in other studies performed on barley (Sinha and Rajagopal 1980) and *Vicia faba* (Rajagopal 1981).

Summarizing the obtained results, it could be said that in the performed experiments the abscisic acid induced in cells such changes which alleviates the negative effect of dehydration on membranes. However, it does not follow from the results obtained that proline could be of any significance in the processes. The role of proline was rather to osmotically maintain water and enhance tissue hydration, or, in other words, to help to avoid water deficit, thus alleviating the injury of cell membrane.

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