WATER DEFICIT-INDUCED MEMBRANE INJURY AND OXIDATIVE STRESS IN TWO BARLEY GENOTYPES

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ABSTRACT

The purpose of this paper was to examine the effect of water deficit on membrane injuries, superoxide radical generation and lipid peroxidation in the leaves of two barley genotypes. Six-day-old seedlings of the cv. Aramir and line R567 were used in the experiments. According to our earlier work these genotypes significantly differ in the level of membrane injuries under water deficit conditions. Water stress was applied directly to leaves or to roots. The stress caused considerable membrane injuries in the leaves of all genotypes investigated. The percentage membrane injury was higher in the line R567 than in the cv. Aramir. Water stress imposed on leaves caused higher membrane injuries than water stress imposed on roots. The water stress treatment followed by an oxidative stress in the leaves. Line R567 having noticeably larger membrane injuries also exhibited a higher level of superoxide radical generation than the cv. Aramir. The level of lipid peroxidation increased in the both genotypes under the conditions of water stress imposed on leaves, but not on roots.

KEY WORDS: barley, lipid peroxidation, membrane injury, superoxide anion, water deficit.

INTRODUCTION

In plants exposed to water deficit stress the ability of membranes to function as a semipermeable barrier is impaired and, as a consequence, an increased leakage of cytoplasmic solutes is observed (Bray 1997). There are a number of metabolic events taking place at the sub-cellular level, which should be responsible for membrane damage under water deficit conditions (Pirce and Hendry 1991; Smirnoff 1993). Limitation of photosynthesis caused by stomata closure and exposure to irradiation during water deficit or the disruption of metabolism during dehydration can result in generation of partially reduced oxygen forms, such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen (Smirnoff 1993). All these forms are far more reactive than the ground state of oxygen and can be potentially highly destructive to all biological systems (Halliwell and Gutteridge 1984; Price et al. 1989). Oxidative stress ensues when the plant defensive capacity is overwhelmed by the formation of active oxygen species. Plants are endowed with an array of enzymes and small molecules to cope with activated oxygen forms (Noctor and Foyer 1998). Dehydration resulting from water deficit could directly hinder antioxidant defence and leave the tissue exposed to oxidative damage (Smirnoff 1993). We have previously reported differences in the level of membrane injuries between two barley genotypes – the cv.

Aramir and line R567 under water deficit stress conditions (Bandurska and Gniazdowska-Skoczek 1995). However, there was no evidence to consider that membrane injuries were due to an increase in the content of harmful H_2O_2 (Bandurska et al. 1997). The current report describes the effect of water deficit on superoxide radical generation, membrane injury and lipid peroxidation in the leaves of these two barley genotypes.

MATERIALS AND METHODS

Plant material and growth conditions

Barley seedlings (*Hordeum vulgare* L. cv. Aramir and line R567) were grown in aerated nutrient solution under controlled conditions according to the method applied by Bandurska and Gniazdowska-Skoczek (1995). Six-day old seedlings were used in the experiments.

Water deficit stress imposition and sample collection

Stress was imposed on roots (Experiment I) or on leaves (Experiment II).

Experiment I

The root system of plant was immersed in polyethylene glycol (PEG 6000) solution of osmotic potential -1.5 MPa for 24 hours. The roots of control seedlings were left in a

nutrient solution. The seedlings were kept under controlled conditions at 21°C, 70% humidity and continuous light of $140 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of photon flux density. At the end of the stress period, leaves from the seedlings of each set were collected and used for 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 membrane injuries were determined. Other nine pieces of leaves were used for the estimation of superoxide anion generation. Two leaf fragments were collected for determination of RWC. The samples of leaves (100–200 mg) collected for determination of lipid peroxidation were freeze-dried and stored at -20°C until further use.

Experiment II

Nine pieces (2cm) from 3 leaves were placed in a 50 ml flask, washed three time with 10 ml deionized water and then floated in 10 ml of PEG solution of water potential of -1.6 MPa or in deionized water (control) and kept for 24 hours at 10°C and continuous light of about $50 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of photon flux density. After exposure to water stress, PEG solution was decanted and samples of each set were collected and used to estimate membrane injury index, superoxide anion generation and lipid peroxidation.

Determination of relative water content (RWC)

RWC was determined by the method of Weatherly (1950) according to Bandurska (1991). The RWC was calculated by the following formula: $\text{RWC} = (\text{fresh weight} - \text{dry weight}) / (\text{fresh weight of full turgor} - \text{dry weight}) \times 100\%$.

Determination of cell membrane injury

After the stress treatment the leaf pieces, collected as described above, 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. 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 following 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.

Assay of superoxide anion generation

The detection of superoxide anion was based on its ability to reduce nitroblue tetrazolium (NBT) in the presence of NADPH according to Doke (1983). Leaf pieces were immersed in 3 ml of 10 mM potassium phosphate buffer (pH 7.8) containing 0.05% NBT and 10 μM NADPH. The amount of reduced NBT was calculated from the increase in absorbance at 580 nm. The results were expressed as $\Delta E \cdot \text{h}^{-1}$ per 10 fragments of leaves.

Determination of lipid peroxidation

The leaf samples were ground in 4 ml of 5% trichloroacetic acid. The homogenate was centrifuged at 5000 g for 15 min. Two ml of supernatant were mixed with 50 mg of activated charcoal and centrifuged at 15 000 g for 30 min. The supernatant was used for lipid peroxidation.

The level of lipid peroxidation was estimated by determination of malondialdehyde (MDA, a product of lipid peroxidation) content in thiobarbituric acid reaction as described by Dhindsa and Matowe (1981).

Presentation of data

Each investigated parameter had 3–5 replications in each experiment and combination. The results are means of all the replications. The data is presented as a mean (standard deviation, which is marked in plots as vertical bars).

RESULTS

The effect of water stress imposed on roots

The immersing of the plant root system in PEG solution significantly reduced RWC in the leaves of the both investigated genotypes (Fig. 1). The extent of reduction was larger in the line R567 than in the cv. Aramir. Water deficit in leaves has its influence on membrane damage (Fig. 1). The percentage membrane injury in the leaves of the line R567 was at the level of 18% and was about four times higher than that in the leaves of cv. Aramir. Following the water stress treatment oxidative stress appeared in the leaves of the both genotypes (Fig. 2). Line R567 having noticeably larger membrane injuries also exhibited a higher level of superoxide radical generation than cv. Aramir. The level of lipid peroxidation decreased somewhat in the leaves of the both genotypes. However, the observed decrease was not statistically significant (Fig. 2).

Fig. 1. The effect of water stress imposed on roots on relative water contents (RWC) and membrane injury in leaves of two barley genotypes.

Fig. 2. The effect of water stress imposed on roots on superoxide radicals generation and lipid peroxidation in leaves of two barley genotypes.

The effect of water stress imposed on leaves

Severe water stress imposed on leaves caused a several times higher membrane injury in cv. Aramir and twofold higher membrane injury in the line R567 (Fig. 3) than the water stress imposed on roots (Fig. 2). It is worth of mentioning that membrane injury index was still larger in the line R567 than in the cv. Aramir. Figure 4 shows the effect of stress on superoxide radical generation measured 24 hrs after leaf incubation in PEG solution (-1.6 MPa). The level of superoxide radical generation in the cv. Aramir remained unchanged, whereas in the leaves of the line R567 it increased after PEG treatment. The level of lipid peroxidation increased in the both genotypes at the end of the stress duration (Fig. 5).

Fig. 3. The effect of water stress imposed on leaves on membrane injury in leaves of two barley genotypes.

Fig. 4. The effect of water stress imposed on leaves on superoxide radicals generation in leaves of two barley genotypes.

Fig. 5. The effect of water stress imposed on leaves on lipid peroxidation in leaves of two barley genotypes.

DISCUSSION

Water deficit has caused significant cell membrane injuries in the leaves of the studied barley genotypes. The cv. Aramir in comparison to the line R567 was characterized by noticeably smaller injuries independently of stress origin (roots, leaves). Under conditions of water stress induced by root immersion in PEG solution, the relative water content in the leaves was about 10% higher in 'Aramir' than that in R567 (Fig. 1). It, however, seems little probable that such relatively small differences in the hydration level could determine an almost fourfold lower membrane injury in that cultivar than that in the line R567. The obtained results have supported earlier studies (Bandurska and Gniazdowska-Skoczek 1995; Bandurska et al. 1997) showing that the cv. Aramir distinguishes by a higher stress resistance, which is at least partly expressed in its better dehydration tolerance.

Water deficit causes changes in membrane structure and composition (Crowe et al. 1987). Some of these changes in membrane components may bring about adaptability to unfavourable conditions, while others may result in functional impairment (Prabha et al. 1985). In lupin studies it was found that water stress in the susceptible genotype induced a larger membrane injury and a larger decrease in the total lipid content than in the resistant genotype (Hubac et al. 1989). Water stress in wheat leaves caused an increase in the level of phosphatidyl choline in the chloro-

plast membrane of the resistant cultivar and a decrease of that substance in the susceptible cultivar (Prabha et al. 1985). A larger decrease in the content of polar lipids without more spectacular changes in the fatty acid composition has been reported for more stress-sensitive varieties compared to resistant varieties of crop species (Liljenberg 1992). Thus, it may be suggested that differences in the degree of cell membrane injury between the studied genotypes of barley under stress conditions were primarily caused by different susceptibility of cell membranes to changes in the content of membrane lipids leading to an increase of their permeability.

The rapidly increasing water deficit in leaves resulting from a direct action of PEG on the tissue caused a significantly larger injury of cell membranes than a milder stress acting indirectly through root treatment with PEG solution (Figs 1 and 3). This indicates that under the influence of a stress factor acting indirectly via root the induction of the occurred changes enabled alleviation of injuries caused by water deficit in leaves. The capacity to control the disruption of cell membranes is well correlated with stress tolerance and to certain extent is genotype-specific. It seems that in the present paper the cv. Aramir was characterized by a larger acclimating possibilities than the line R567. This follows from the fact that under conditions of a slow stress, the cell membrane injury in 'Aramir' was several times lower, while in the line R567 it was only half lower than that under conditions of a severe stress. A partial explanation of this observation may be a lower water deficit in the leaves of the cv. Aramir (Fig. 1) and results from earlier studies in which 'Aramir' accumulated more proline than R567 under drought conditions at a similar decrease in the leaf RWC (Bandurska 2000).

Water stress has contributed to the occurrence of oxidative stress manifesting itself in overproduction of superoxide radicals ($O_2^{\cdot -}$) in the leaves of the studied genotypes (Figs 2 and 4). The line R567, which was characterized by a larger injury of cell membranes, had also a higher $O_2^{\cdot -}$ overproduction in comparison to the cv. Aramir. A considerably higher increase in $O_2^{\cdot -}$ level in comparison to that resulting from a direct PEG action on leaf was found under conditions of water stress induced by root treatment with PEG solution. In the cv. Aramir under conditions of stress acting directly on leaf no occurrence of oxidative stress has been found. It seems to be caused by differences in light intensity used in separate experiments for illumination of plants during treatment with PEG (Exp. I and Exp. II). Light intensity in the experiment with entire plants (Exp. I) was over twofold larger than that in the experiment with leaves alone (Exp. II). Illuminated chloroplasts prepared from drought-stricken wheat plants generated superoxide as a result of impaired electron transport (Price et al. 1989). During water deficit stomata close to limit water loss and there is a CO_2 limited carbon fixation and the pool of oxidized nicotinic adenine dinucleotide ($NADP^+$) as electron acceptor for photosystem I is reduced. Electrons from the excited state of chlorophyll in the reaction centre are transferred rather to oxygen than through the chloroplast electron transport chain to $NADP^+$ and an enhanced generation of superoxide radicals occur (Halliwell 1987, Anderson et al. 1990). The major source of activated O_2 in plant are chloroplast and this reaction is light-dependent (Nakano and Asada 1980, Elstner et al. 1988). However, it cannot

be excluded that violent tissue dehydration under the conditions of stress imposed on leaves has contribute to a rapid and essential increase of superoxide radicals already in the first hours of the stress. This is supported by a large membrane injury (Fig. 3) and by an increase in the level of lipid peroxidation in the leaves of studied genotypes under these conditions (Fig. 5). Superoxide radical is one of the links in oxygen reduction reactions leading to the production of hydrogen peroxide and hydroxyl radicals. All these oxygen-containing components may initiate lipid peroxidation. There is a considerable piece of evidence in the literature that at least part of membrane damage in stressed plants is caused by an uncontrolled increase in free radicals which are very toxic and highly reactive (Senaratna et al. 1987; Leprince et al. 1990; Price and Hendry 1991; Smirnoff 1993). In some case, however an increased permeability of plasma membrane and free radical generation under water stress conditions is not correlated with lipid peroxidation. Under conditions of water stress acting through plant roots no increase in the level of lipid peroxidation has been found (Fig. 2), despite a rise in the level of superoxide radicals (Fig. 2). It is possible that in this experiment under the applied stress conditions, the plants were able to oppose lipid peroxidation due to free radical production. These results are in accordance with those of the studies on sunflower seedlings treated with PEG solution (-1.0 MPa), where no increase was observed in the level of lipid peroxidation, despite RWC decrease, membrane permeability increase and free radical overproduction (Qartacci and Navari-Izzo 1992).

In summary, the observations conducted during these experiments show that the response of plants to water stress depended on the degree of water deficit experienced by them and on the rapidity of the progressing stress. The differences between the investigated barley genotypes in membrane injury were dependent on the capacity to oppose a harmful effect of dehydration.

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WPŁYW DEFICYTU WODY NA USZKODZENIE BŁON KOMÓRKOWYCH ORAZ STRES OKSYDACYJNY U DWÓCH GENOTYPÓW JĘCZMIENIA

STRESZCZENIE

W pracy badano wpływ deficytu wody na stopień uszkodzenia błon komórkowych, poziom rodnika ponadtlenkowego oraz peroksydację lipidów w liściach dwóch genotypów jęczmienia (odm. Aramir, linia R5567). Badania prowadzono na sześciodniowych siewkach. Stres wprowadzano przez umieszczenie korzeni całych roślin lub przez inkubację liści w roztworze PEG. Niezależnie od sposobu wprowadzania stresu odmiana Aramir cechowała się mniejszym uszkodzeniem błon komórkowych niż linia R567. Deficyt wody w liściach przyczynił się do powstania stresu oksydacyjnego. Większą nadprodukcję rodnika ponadtlenkowego stwierdzono w liściach linii R567 co było skorelowane z większym uszkodzeniem błon komórkowych. Poziom peroksydacji lipidów zwiększył się u obu genotypów podobnie, ale jedynie w warunkach stresu wodnego działającego bezpośrednio na liść.

SŁOWA KLUCZOWE: deficyt wody, jęczmień, peroksydacja lipidów, rodnik ponadtlenkowy, uszkodzenia błon.