EFFECTS OF WATER DEFICIT STRESS ON MEMBRANE PROPERTIES, LIPID PEROXIDATION AND HYDROGEN PEROXIDE METABOLISM IN THE LEAVES OF BARLEY GENOTYPES

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ABSTRACT

Two barley genotypes, cultivar Aramir and line R567, were subjected to water stress for 24 hours by immersing their roots in polyethylene glycol (PEG 6000) solution of osmotic potential -1.0 MPa. The relative water content (RWC) in the leaves of both genotypes lowered under the stress conditions. Reduction of RWC led to membrane disorganization, which is evidenced by the injury index data. The injury index was lower in the leaves of cv. Aramir as compared with line R567. The level of lipid peroxidation decreased under stress conditions in the leaves of both genotypes. Hydrogen peroxide content also decreased in the leaves of stressed plants relative to the control ones. The decrease was higher in line R567 and was accompanied by a decrease in ascorbic acid level in the leaves of that genotype. In the leaves of cv. Aramir water stress did not affect the ascorbic acid content. The examined genotypes showed little or no changes in the activity of ascorbic acid peroxidase in the response to water stress. No evidence of an increase in the level of oxidative stress during water depletion in studied genotypes was seen. Water stress-induced decrease in the level of hydrogen peroxide without an increase in the activity of ascorbic acid peroxidase indicate that other mechanisms may be involved in the detoxification of hydrogen peroxide.

KEY WORDS: ascorbic acid, barley, hydrogen peroxide, membrane injury, water stress.

INTRODUCTION

Water deficit stress is known to have deleterious effect on the structure and function of cell membranes. The function of the membrane as a semipermeable barrier is impaired, and consequently, an increased leakage of cytoplasmic solute is observed. The electrolyte leakage from plant tissues is taken as a measure of membrane injury index under stress conditions (Blum, Ebercon 1981, Krishnamani et al. 1984, Venkateswarlu, Ramesh 1993).

Plant tissues subjected to water deficit stress enhance formation of active oxygen species which, in turn, damage cell membrane (Smirnoff 1993). Several authors showed the accumulation of hydrogen peroxide in plant tissues under water stress (Mukherjee and Choudhuri 1983, Chowdhury and Choudhuri 1985). Hydrogen peroxide metabolism within plant is the main source of more aggressive activated oxygen species such as hydroxyl radical and singlet oxygen that cause membrane damage (Elstner 1982). The earliest symptom of oxidative damage is lipid peroxidation (Dhindsa, Matowe 1981, Irigoyen et al. 1992).

Ascorbic acid is an important plant metabolite functioning as an oxygen radicals scavenger (Halliwell and Gutteridge 1989). It was found that in the "resurrection" plant, *Boa hygroscopica*, ascorbic acid was accumulated during dehydration (Sgherri et al. 1994).

In plant cells ascorbic acid also participates in removing hydrogen peroxide through the action of ascorbate peroxidase (Asada 1992).

The present study was undertaken to characterize the possible relationship between lipid peroxidation, hydrogen peroxide and ascorbic acid contents, and the activity of ascorbic acid peroxidase, in the leaves of two barley genotypes differing in membrane injury under water deficit stress (Bandurska, Gniazdowska-Skoczek 1995).

MATERIAL AND METHODS

Plant material, growth conditions.

Barley seedlings (*Hordeum vulgare* cv. Aramir and line R567) were grown under controlled conditions in aqueous cultures, according to the methods applied by Bandurska, Gniazdowska-Skoczek (1995). For the experiments six day-old seedlings were used.

Stress conditions.

Seedlings were subjected to water stress by immersing the root system in polyethylene glycol (PEG 6000) solution of osmotic potential -1.0 MPa (29.8% w/v) for 2, 10, 24 hours. The roots of control seedlings were immersed in distilled water for the same period. In both sets of seedlings the root

system was continuously aerated. The seedlings were kept under controlled conditions at 21°C, 70% humidity and continuous light of 140 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photon flux density. After exposure to water stress, the leaves from seedlings of each set were harvested and used for experimental analyses.

Determination of relative water content (RWC).

The level of water stress in the leaves was estimated on the basis of RWC determined by the method of Weatherly (1950) according to Bandurska (1991). Two fragments from the central part of leaf were weighed, then put into water for four hours (full turgor), weighed again, dried at 60°C and weighed once more. The relative water content was calculated according to 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.

The middle portions of the leaves were cut into 2 cm pieces immediately after sampling. Ten leaf pieces were then washed quickly three times in deionized water, immersed in 10 ml deionized water, and kept for 24 h at 10 °C. The electrical conductivity was measured and membrane injury was evaluated as the percentage injury index following the formula of Sullivan (1972).

$I = [1 - (1 - T_1/T_2) / (1 - C_1/C_2)] \times 100$ where C_1 and C_2 represent conductivity measurements of control samples before and after autoclaving respectively, T_1 and T_2 stand for conductivity measurements of water-stressed samples before and after autoclaving, respectively.

Lipid peroxidation, H_2O_2 and ascorbic acid (AsA) determination.

The leaf samples (100- 200 mg) were ground in 4 ml of 5% trichloroacetic acid. The homogenate was centrifuged at 5000 $\times g$ for 15 min. One ml of the supernatant was taken for ascorbic acid determination following the method described by

Mukherje and Choudhuri (1983). The other 2 ml of supernatant were mixed with 50 mg of activated charcoal and centrifuged at 15000 $\times g$ for 30 min. The supernatant was used for determining of lipid peroxidation level and H_2O_2 content. 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). The H_2O_2 content was determined according to Becana et al. (1986).

Ascorbic acid (ASC) peroxidase activity.

The leaf samples (100-200 mg) were ground in 2 ml of 50 mM phosphate buffer pH 7,8 with 1 mM ascorbate and 20 mg polyclar AT. The homogenate was centrifuged at 15000 $\times g$ for 15 min. All operations were carried out at 0-4 °C. The supernatant was used for enzyme assays and protein measurement. ASC peroxidase activity was estimated by measuring the oxidation of ascorbate at 290 nm according to the method of Nacano and Asada (1981). Protein was determined according to the method applied by Bradford (1976), using bovine serum albumin as a standard.

Presentation of data.

The experiments were done twice for each barley genotype, in each case identical trends were observed. For each investigated parameter, 4-5 analyses were performed both for the control and stressed plants. The presented results of one representative experiment express the mean values of 4-5 replications. Each results equals a mean $\pm$ standard deviation of the mean, which is marked in figures as vertical bars.

RESULTS

The water stress induced changes in RWC and the injury index of cell membrane of two barley genotypes are shown in Figure 1. The successive decrease of RWC in the leaves of

Fig. 1. Relative water content (RWC) and injury index in seedlings of two barley genotypes subjected to water stress.

stressed plants was observed. The fall in the water content during the 24-hr stress period proceeded in three steps. During the first 2 hrs of the stress imposition the RWC markedly decreased and differences between the studied genotypes were noticed. Cultivar Aramir lost water more rapidly than the line R567. Within the next 8 hrs, the RWC remained on the same level in the leaves of line R567 and increased in cv. Aramir. After the next 14 hrs, the RWC in cv. Aramir gradually fell to 60%, and in line R567 – to 52%. The observed differences were within the error limit.

During the whole stress period the percentage membrane injury in the leaves of cultivar Aramir was at the level of 1%. Leaves of line R567 showed about 2% injury after 2 and 10 hrs of stress, which increased to about 7% after 24 hours.

The stress induced changes in the level of lipid peroxidation are shown on Figure 2. The results indicate that water stress affected the decrease in the level of lipid peroxidation. The rate of decrease was higher in the leaves of line R567 than in cultivar Aramir. The loss of MDA content in line R567 was observed after 10 hrs, and in cultivar Aramir – after 24 hrs of stress application.

The effect of water stress on H_2O_2 content is shown in Figure 3. The level of this metabolite decreased in leaves of both genotypes and this decrease was a function of stress duration. But a more pronounced loss was observed in line R567 than in cultivar Aramir.

Figure 4 shows the effect of water stress on ascorbic acid content. The results indicate that water stress treatment brought about a decline in the content of this metabolite in the leaves of line R567. In the case of cultivar Aramir, the level of ascorbic acid in stressed plants did not change in relation to non-stressed plants.

The activity of ASC peroxidase in leaves of stressed and non-stressed plants is shown in Figure 5. As follows from the data the activity of this enzyme decreased in cultivar Aramir due to water stress. The loss of activity was dependent on stress duration. The greatest decline was observed after 24 hrs

of stress duration. In the leaves of line R567 water stress brought about an insignificant decline in ASC peroxidase activity.

DISCUSSION

The present study shows that water stress significantly affected the water status of the leaves in both studied genotypes. The RWC in the leaves decreased with increased duration of stress. It was noticed, however, that between 2 and 10 hrs of stress duration the fall in water status in the leaves was stopped. Schonfeld (1980) suggests that the RWC in the leaves may be a good indicator of plant ability to avoid dehydration by osmotic adjustment. Osmotic adjustment refers to the maintenance of turgor by lowering the osmotic potential arising from net accumulation of solutes. Several authors reported that osmotic adjustment contributed to the drought tolerance of maize (Premachandra et al. 1992, Guei, Wassom 1993) and barley (Blum 1989). But, in our experiments, after 24 hrs of stress duration RWC in the leaves of cv. Aramir and line R567 was lowered by 38% and 45% respectively, which refers to severe water stress (Hsiao 1973). So, the maintenance of turgor at the beginning of stress was temporary, and was rather caused by the closure of stomates.

The data of injury index indicate that at the same level of water stress in the leaves, the value of membrane injury index in cv. Aramir was lower than in line R567. This difference was the most pronounced after the 24 hrs of stress duration. The injury index signifies the degree of membrane damage, and as was demonstrated by several authors, is well correlated with susceptibility of genotypes to water stress (Blum and Ebercon 1981, Krishnamani et al. 1984, Choudhury and Choudhuri 1985). In the present study a higher injury index in line R567 than in cv. Aramir suggests that the former is more susceptible to water shortage.

Fig. 2. Lipid peroxidation ($\mu\text{mol MDA g}^{-1}$ dry weight) in seedlings of two barley genotypes subjected to water stress.

Fig. 3. Hydrogen peroxide content ($\mu\text{mol g}^{-1}$ dry weight) in seedlings of two barley genotypes subjected to water stress.

Fig. 4. Ascorbic acid content (mg g^{-1} dry weight) in seedlings of two barley genotypes subjected to water stress.

Some authors reported that water stress induces the increase in lipid peroxidation. The level of lipid peroxidation increased two times in several grass species exposure to drought for 4 days (Price and Hendry 1991). The increased level of lipid peroxidation was also observed in sugar cane leaves when plants were subjected to water stress in drying soil (Venkataramana et al. 1987). Chowdhury and Choudhuri (1985) demonstrated that drought induced membrane damage is a result

of increased lipid peroxidation in two jute species. Our results show a 40% and 42% decrease in the level of lipid peroxidation after 24 hrs of stress duration in the leaves of cv. Aramir and line R567 respectively. However, one should note that membrane injury index in these barley genotypes was about 15-20% lower than in experiment on jute species. The decrease in the level of lipid peroxidation under water stress conditions was also reported to occur in wheat plants, which

Fig. 5. Ascorbic acid peroxidase activity (nmol of ascorbic acid peroxidized mg^{-1} protein min^{-1}) in seedlings of two barley genotypes subjected to water stress.

– when subjected to rather severe water deprivation (RWC about 50%) – showed a 50% decrease in the level of lipid peroxidation (Floryszak-Wieczorek et al. 1992).

The metabolism of H_2O_2 within plant is the main source of activated oxygen species that cause lipid peroxidation. The water stress induced accumulation of H_2O_2 was observed in leaves of two jute species differing in membrane injury (Chowdhury and Choudhuri 1985). The leaves of jute species having lower membrane damage exhibited lower H_2O_2 accumulation and lower lipid peroxidation under water stress conditions. The author stated that H_2O_2 metabolism can be directly correlated with the stress induced membrane damage. The presented results show the decrease in the level of H_2O_2 due to water stress in barley genotypes. The decrease is sooner and higher in the genotype more susceptible to stress and is correlated with the decline in the level of ascorbic acid in that genotype.

The removal of H_2O_2 in plant tissue can be achieved through the action of ASC peroxidase (Asada 1992). In our experiment, ASC peroxidase activity decreased in leaves of cv. Aramir and did not change in leaves of line R567 under water stress. The decline in the content of ascorbic acid and no change in the activity of ASC peroxidase as a result of water stress was shown by Buckland et al. (1991) in two different herb species. These authors concluded that protection from active oxygen species in biological systems is maintained through the enzymatic processing and also through radical scavengers (antioxidants). Ascorbic acid may act under water stress conditions directly as an antioxidant reducing the level of H_2O_2 in plant tissue (Smirnoff 1993, Bartosz 1995).

The decrease in the ascorbic acid pool as a result of water deprivation was also reported in *Vigna* seedlings (Mukherjee and Choudhuri 1985). This decrease was ascribed to a rise in the activity of ascorbic acid oxidase in stressed seedlings observed in that study.

It was shown by Grag and Singh (1971) that in rice ascorbic acid plays some role in inducing drought resistance, because in the leaves of drought resistant variety, a higher ascorbic acid content was noticed as compared with the drought sensitive one, when plants were subjected to wilting. In our studies, water stressed leaves of cv. Aramir, having lower membrane injury, exhibited higher ascorbic acid content than leaves of line R567, having a higher membrane injury index.

The effect of water stress on ASC peroxidase activity and the content of ascorbic acid and H_2O_2 has been also investigated in the leaves of pea (Moran et al. 1994). These authors observed the decrease of ASC peroxidase activity and no effect on the level of ascorbic acid and H_2O_2 in stressed leaves, and explained that H_2O_2 is removed by ASC peroxidase and monodehydroascorbate reductase (MDHA reductase) acting in a cyclic manner. The functioning of this cycle is supported by the high correlation between the activities of MDHA reductase and ASC peroxidase, and by constant of ascorbic acid content.

In summary, the presented results show that no evidence of the presence of oxidative stress under the applied water stress conditions was seen. Consequently, although the plants have suffered to severe water deficit, the effect of the stress on membrane injury was rather little and was not accompanied by lipid peroxidation. No evidence was seen that lower membrane injury in cv. Aramir is due a better defense mechanism against harmful H_2O_2 . Taking into account the evidence from the literature cited above that ascorbic acid is an important plant metabolite related to drought tolerance of plants, we suppose that the lower membrane injury in cv. Aramir under stress treatment may be connected with lower decrease in ascorbic acid content under these circumstances. One would note that the decrease of H_2O_2 content in water stressed plant without an increase in ASC peroxidase activity suggests that other enzymatic or (and) non-enzymatic defense mechanisms might be involved in detoxification of this harmful metabolite.

LITERATURE CITED

- ASADA K., 1992. Ascorbate peroxidase – hydrogen peroxide-scavenging enzyme in plants. *Physiol. Plant.* 85: 235-241.
- BANDURSKA H., 1991. The effect of proline on nitrate reductase activity in water stressed barley leaves. *Acta Physiol. Plant.* 13: 5-13.
- BANDURSKA H., GNIAZDOWSKA-SKOCZEK H., 1995. Cell membrane stability in two barley genotypes under water stress conditions. *Acta Soc. Bot. Pol.* 64: 29-32.
- BARTOSZ G., 1995. *Druga twarz tlenu*. Wydawnictwo Naukowe PWN pp. 176-178.
- BECANA M., APARICIO-TEJO P., IRIGOYEN J.J., SANCHEZ-DIAZ M., 1986. Some enzymes of hydrogen peroxide metabolism in leaves and root nodules of *Medicago sativa*. *Plant Physiol.* 82: 1169-1171.
- BLUM A., EBERCON A., 1981. Cell membrane stability as measure of drought and heat tolerance in wheat. *Crop Sci.* 21: 43-47.
- BLUM A., 1989. Osmotic adjustment and growth of barley genotypes under drought stress. *Crop Sci.* 29: 230-233.
- BRADFORD M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.
- BUCKLAND S.M., PRICE A.H., HENDRY G.A.F., 1991. The role of ascorbate in drought-treated *Cochleria atlantica* Pobed. and *Armeria maritima* (Mill.) Willd. *New Phytol.* 119: 155-160.
- CHOUDHURY S.R., CHOUDHURI M.A., 1985. Hydrogen peroxide metabolism as an index of water stress tolerance in jute. *Physiol. Plant.* 65: 503-507.
- DHINDSA R., MATOWE W., 1981 Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. *J. Exp. Bot.* 32: 79-91.
- ELSTNER E.F., 1982. Oxygen activation and oxygen toxicity. *Annu. Rev. Plant Physiol.* 33: 73-96.
- FLORYSZAK-WIECZOREK J., GRABIKOWSKI E., KUBIS J., KRZYWAŃSKI Z., 1992. The effect of spermidine on lipid peroxidation in wheat leaves during water stress. *Acta Physiol. Plant.* 14: 3-10.
- GUEI R.G., WASSOM C.E., 1993. Genetic of osmotic adjustment in breeding maize for drought tolerance. *Heredity* 71: 436-441.
- GRAG O.P., SINGH B.P., 1971. Physiological significance of ascorbic acid in relation to drought resistance in rice (*Oryza sativa* L.) 30: 219-223.
- HALLIWELL B., GUTTERIDGE J.M.C., 1989. *Free radicals in biology and medicine*, 2nd Edn. Oxford University Press, London.
- HSIAO T.C., 1973. Plant responses to water stress. *Ann Rev. Plant Physiol.* 24: 519-570.
- IRIGOYEN J., EMERICH D.W., SANCHEZ-DIAZ M., 1992. Alfalfa leaf senescence induced by drought stress: photosynthesis, hydrogen peroxide metabolism, lipid peroxidation and ethylene evolution. *Physiol. Plant.* 84: 67-72.
- KRISHNAMANI M.R.S., YOPP J.H., MYERS O.JR., 1984. Leaf soluble leakage as a drought tolerance indicator in soybean. *Phyton* 44: 43-49.
- MORAN J.F., BECANA M., ITURBE-ORMAETXE I., FRECHILLA S., KLUCAS R.V., APARICIO-TEJO P., 1994. Drought induces oxidative stress in pea plants. *Planta* 194: 346-352.
- MUKHERJEE S.P., CHOUDHURI M.A., 1983. Implications of water stress-induced changes in the level of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plant.* 58:166-170.
- MUKHERJEE S.P., CHOUDHURI M.A., 1985. Implication of hydrogen peroxide-ascorbate system on membrane permeability of water stressed *Vigna* seedlings. *New Phytol* 99: 355-360.
- NAKANO Y., ASADA K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22: 867-880.8
- NAVARI-IZZO F., MILONE M.T., QUARTACCI, M.F., PINZINO C., 1993. Metabolic changes in wheat plants subjected to water-deficit stress programme. *Plant Sci.* 92: 151-157.
- PIRCE A.H., HENDRY G.A.F., 1989. Stress and the role of activated oxygen scavengers and protective enzymes in plants subjected to drought. *Biochemical Society Transactins* 17: 493-494.
- PRICE A. H., HENDRY G.A.F., 1991. Iron – catalysed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. *Plant, Cell and Environment* 14: 477-484.
- PREMACHANDRA G.S., SANEOKA H., FUJITA K., OGATA S., 1992. Osmotic adjustment and stomatal response to water deficits in maize. *J. Exp. Bot.* 43: 1451-1456.
- QUARTACCI M.F., NAVARI-IZZO F., 1992. Water stress and free radical mediated changes in sunflower. *J. Plant. Physiol.* 139: 621-625.
- SCHONFELD M.A., JOHNSON R.C., CARVER B.F., MORNHINWEG D.W., 1988. Water relations in winter wheat as drought resistance indicators. *Crop Sci.* 28: 526-531.
- SGHERRI C.L., LOGGINI B., BOCHACCHIO A., NAVARI-IZZO F., 1994. Antioxidant system in *Boea hygrosopica*: changes in response to rapid or slow desiccation and rehydration. *Phytochemistry* 37: 377-381.
- SMIRNOFF N., 1993. The role of active oxygen in the response of plants to water deficits and desiccation. *New Phytol.* 125: 27-58.
- SULLIVAN C.Y., 1971. Technique for measuring plant drought stress. In: *Drought injury and resistance in crops*. (ed.) K.I. Larson & J.D. Eastin, Madison, Wisconsin; Crop Science Society of America, 1-18.
- VENKATARAMANA S., NAIDU K.M., SINGH S., 1987. Membrane thermostability and nitrate reductase activity in relation to water stress tolerance of young sugar cane plants. *New Phytologist* 107: 335-340.
- VENKATESWARLU B., RAMESH K., 1993. Cell membrane stability and biochemical response of cultured cells of groundnut under polyethylene glycol – induced water stress. *Plant Sci.* 90: 179-185.
- WEATHERLY P.E., 1950. Studies in water relation of cotton plants. I. The field measurement of water deficits in leaves. *New Phytol.* 49: 81-97.

WPLYW STRESU DEFICYTU WODY
NA WŁAŚCIWOŚCI BŁON KOMÓRKOWYCH ORAZ METABOLIZM NADTLENU WODORU
W LIŚCIACH DWÓCH GENOTYPÓW JĘCZMIENIA

STRESZCZENIE

Badano reakcję dwóch genotypów jęczmienia, odmiany Aramir i linii R567, na trwający 24 godziny stres wodny wprowadzony poprzez zanurzenie korzeni roślin w roztworze glikolu polietylenowego (PEG 6000) o potencjale osmotycznym -1.0 MPa. Efektem działania stresu było podobne obniżenie względnej zawartości wody w liściach badanych genotypów oraz różny stopień uszkodzenia błon komórkowych. Procentowy indeks uszkodzenia błon komórkowych wynosił u odm. Aramir 2% a u linii R567 7%. Poziom peroksydacji lipidów obniżył się w efekcie działania stresu w liściach obu genotypów. Podobnie zawartość nadtlenu wodoru w liściach obniżyła się pod wpływem stresu. Obniżenie to było większe u linii R567 i było zsynchronizowane z obniżeniem zawartości kwasu askorbinowego przy braku zmian w aktywności peroksydazy askorbinianowej. W liściach odmiany Aramir nie zaobserwowano zmian w zawartości kwasu askorbinowego pod wpływem stresu, a aktywność peroksydazy askorbinianowej obniżyła się w niewielkim stopniu. Podsumowując, można stwierdzić, że deficyt wody w liściach nie przyczynił się do powstania stresu oksydacyjnego. Znaczne obniżenie poziomu nadtlenu wodoru oraz brak wzrostu aktywności peroksydazy askorbinianowej sugerują uaktywnienie się innych mechanizmów zapobiegających nagromadzeniu się tego szkodliwego metabolitu.

SŁOWA KLUCZOWE: jęczmień, kwas askorbinowy, nadtlenek wodoru, indeks uszkodzeń.