CELL MEMBRANE STABILITY IN TWO BARLEY GENOTYPES UNDER WATER STRESS CONDITIONS

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ABSTRACT

The effect of water stress induced in vitro by polyethylene glycol 6000 (PEG) and under drought stress on cell membrane stability was examined in two barley genotypes, the cultivar Aramir and line R567. The injury of cell membranes was markedly influenced by leaf age, leaf position on the stem, and the degree of drought stress. The differences in percentage of injury to cell membrane between these genotypes were also found. The cv. Aramir exhibited a lower percentage injury value as compared to line R567, measured in vitro by PEG test. Similarly, under drought stress the cv. Aramir showed higher cell membrane stability than the line R567, even in severe stress. The results indicated also that both genotypes differ in the ability to adjust their cell membranes to water stress.

KEY WORDS: barley, cell membrane stability, water stress, drought.

INTRODUCTION

It is generally accepted that the integrity and stability of cell membranes under water stress conditions is a major component of drought tolerance in plants (Bewley 1979). The rate of injury to cell membranes by water stress may be estimated by measuring electrolyte leakage from cells. The method, introduced by Sullivan (1971), is based on desiccation in vitro of leaf tissues by polyethylene glycol (PEG) and subsequent measurement of electrolyte leakage into a deionized aqueous medium. The amount of electrolyte leakage, evaluated by electrical conductivity measurement, is a function of cell membrane stability (CMS). This technique was used by a few researchers (Blum and Ebercon 1981, Krishnamani et al. 1984, Premachandra and Shimada 1987, Premachandra et al. 1992). All of them concluded that the degree of CMS was well correlated with tolerance of genotypes to water stress conditions. In our previous experiments (Gniazdowska-Skoczek, Bandurska in press) it was shown that two barley genotypes cv. Aramir and line R567 differ in their physiological reaction to water stress. This study was performed to evaluate CMS under water stress induced in vitro by PEG, and under drought stress in those two barley genotypes.

MATERIAL AND METHODS

The experiments were carried out on two barley genotypes, cv. Aramir and line R567. Two different experiments were made to measure CMS.

Experiment I

CMS measurement in six day-old seedlings under osmotic stress.

The seeds were germinated in the dark for 24 h in continuously aerated deionized water at 21°C. Then they were placed on cheesecloth stretched over a plastic frame. The frame was put onto a 300 ml beaker with continuously aerated nutrient solution containing: 0.0843g Ca(NO₃)₂ x 4 H₂O, 0.0507g MgSO₄ x 7 H₂O, 0.0114g KH₂PO₄, 0.0745g KNO₃ per liter. Eighteen germinated seeds were placed on cheesecloth on one beaker. After one day of growth in the dark the seedlings were illuminated with fluorescent light at intensity of 90 µmol m⁻² s⁻¹ for 12 h daily. Six day-old seedlings were used for the experiment. Nine fragments (2 cm) from 3 leaves were put into a 50 ml flask, washed three times with 10 ml deionized water, and then submerged in 10 ml 40 % PEG 6000 solution or in deionized water (control) and kept for 24 h at 10°C. The leaf pieces were then washed quickly three times with deionized water, finally submerged in 10 ml deionized water and kept again for 24 h at 10°C. Then the flask was adjusted to 25°C, shaken well and the electrical conductivity was measured. Following the conductivity measurement, the leaf tissues were killed by autoclaving for 15 min, cooled to 25°C and electrical conductivity was measured for the second time. Three replications were made for the desiccation treatment (T) and non-desiccated control (C). The experiment was performed three times. The results are presented as mean values ± S.D. (n=9) from all experiments.

The CMS of leaf tissues was evaluated as percentage injury from the following equation.

$I = [1 - (1 - T_1 / T_2) / (1 - C_1 / C_2)] \times 100$, where T_1 and C_1 = first conductivity measurement, T_2 and C_2 = second conductivity measurement of desiccation treatment and control, respectively (Premachandra, Shimada 1988).

Experiment II

CMS measurement in pot-grown plants.

Plants were planted in soil pot culture under glasshouse conditions. Six plants were grown in each pot containing 5.5 dm³ of soil. The soil was fertilized with N (2.29 g as NH₄NO₃), P (0.77 g as KH₂PO₄), K (0.85 g as KCl) per pot at the beginning of experiment. Soil moisture was maintained at 60% of water capacity. In 65 day-old plants drought was induced by withholding water from half of the pots. The other half treated as control was watered regularly. After nine days the stress was relieved by rewatering to 60% of water capacity. During experiment CMS was measured by drought and PEG test.

The CMS by drought test

The uppermost fully expanded leaves were collected after 7 and 9 days of stress duration. The middle portion of the leaves were cut into 2 cm pieces immediately after sampling. They 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 CMS was determined as in Experiment I.

The level of water stress in leaves was estimated on the basis of relative water content (RWC) determined by the method of Weatherly (1950) adapted by Bandurska (1991)

CMS by PEG test

Measurements were carried out three times during experiment II as in Exp I. The first time in 49 day-old plants, the uppermost fully expanded leaf (the fifth) was sampled for measurement, the second time in 65 day-old plants, three uppermost fully expanded leaves (the fifth, sixth and seventh) were sampled, and the third time in 76 day-old plants, three days after stress relieving, the uppermost fully expanded leaf (the seventh) from drought stress and control plants was sampled for estimation.

Three replications were made in all determinations. Data are expressed as mean $\pm$ S.D. (n=3).

RESULTS AND DISCUSSION

The results presented indicated the differences in percentage injury of cell membrane under water stress between the studied genotypes (Fig. 1-2). Cultivar Aramir showed lower injury values compared to line R567 in all experiments. The injury values obtained in PEG test was dependent on the growth stage of plants (Fig. 1A) as well as leaf position on the stem (Fig. 1B) in both genotypes. The first leaf from six day-old seedlings had higher injury percentage than the fifth and seventh leaves from older plants. A decrease in percentage injury was observed with the increase of plant age in the case of the fifth leaf (Fig. 1A). The results confirm the report of Premachandra and Shimada (1987), where they found a decrease in percentage injury with increase of plant age in wheat. But this relationship was not observed in the case of the seventh leaf in which percent injury increased during ageing. The differences of percentage injury values among three different leaves (Fig 1B) for a given plant stage (65 day-old) were found. The younger leaves (upper) exhibited lower injury values than the older (lower). The results are compatible with those of Blum and Ebercon (1976, 1981) and indicate that the younger leaf tissues are more tolerant to drought than older.

The Fig. 2A shows the effect of magnitude of drought stress on relative water content and injury index. The RWC in leaves of control plants was about 85% in both genotypes. Cultivar Aramir lost water more rapidly under drought stress conditions than the line R567. In spite of this at the same level of water stress (RWC about 40%) percentage injury values in Aramir were lower than in line R567. It was shown in our previous paper that at the same level of water stress cv. Aramir has higher proline accumulation compared to line R567 (Gniazdowska-Skoczek, Bandurska in press). Proline is considered to play an important role in the ability of some organisms to survive low intracellular water content. It was shown that proline can protect biological membranes against

Fig. 1. CMS in the leaves of two barley genotypes measured by PEG test

A. The effect of plant age on injury index;

B. The effect of leaf position on the stem on injury index in 65 day-old plants.

Fig. 2. CMS in the leaves of two barley genotypes from drought stressed plants

A. The effect of drought stress on injury index and RWC during stress;

B. The effect of drought stress on injury index measured by PEG test three days after stress relief.

the consequens of dehydration (Crow 1983). Proline seems to affect the hydration layer surrounding phospholipids which may possibly involve intercalation between phospholipid head groups (Rudolph et al. 1986). Membrane deterioration upon water stress is associated with formation and accumulation of toxic, reactive free radical forms (Elstner 1982). Proline is reported to reduce free radical activity (Flyd and Zs-Nag 1984, Smirnoff, Cumbes 1989).

It was found by several workers that the degree of injury of cell membrane measured by PEG test decreased in plants subjected earlier to a period of drought stress (Blum et al. 1981, Krishnamani et al. 1984, Premachandra, Shimada 1987, 1988). This phenomenon is so-called cell membrane adjustment to water stress. The studied genotypes differ in this adjustment phenomenon because we observed that only pre-stressed plants of line R567 exhibit lower percentage injury than the control (Fig. 2B).

Our results show that examined barley genotypes differ in their reaction to water stress and probably possess other mechanisms of drought tolerance. The line R567 shows relatively lower tolerance to drought in terms of membrane stability, but possess the ability to adjust cell membrane stability to stress. We suppose that the ability to withstand severe dehydration without significant increase in membrane injury in cv. Aramir is connected with higher proline accumulation (Gniazdowska-Skoczek, Bandurska in press).

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STABILNOŚĆ BŁON KOMÓRKOWYCH U DWÓCH GENOTYPÓW JĘCZMIENIA W WARUNKACH STRESU WODNEGO

STRESZCZENIE

Badano stabilność błon komórkowych w warunkach stresu wodnego indukowanego *in vitro* za pomocą glikolu polietylenowego (PEG) oraz w warunkach stresu suszy w liściach dwóch genotypów jęczmienia, odmiany Aramir i linii R567. Stopień uszkodzeń błon komórkowych zależny był od wieku liścia, umiejscowienia liścia na pędzie, a także od stopnia odwodnienia liścia w następstwie suszy. Niezależnie od tego stwierdzono różnice pomiędzy badanymi genotypami jęczmienia w poziomie uszkodzeń błon komórkowych w warunkach zastosowanych czynników stresowych. Odmiana Aramir miała niższy procent uszkodzeń błon komórkowych w porównaniu z linią R567 w warunkach *in vitro*, z zastosowaniem PEG. Podobnie w warunkach stresu suszy odm. Aramir cechowała się większą stabilnością błon komórkowych niż linia R567. Otrzymane wyniki wskazują również, że badane genotypy jęczmienia różnią się zdolnością do utrzymywania zwiększonej stabilności błon komórkowych po przejściu okresu suszy.

SŁOWA KLUCZOWE: jęczmień, stabilność błon komórkowych, stres wodny, susza.