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EVALUATION OF THE REACTION OF TWO CONTRASTING BARLEY (*HORDEUM VULGARE* L.) CULTIVARS IN RESPONSE TO OSMOTIC STRESS WITH PEG 6000

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Summary. Two barley cultivars were subjected to water stress by immersing their root system in PEG 6000. The relative water content (RWC) of the leaves fell under these conditions and membrane disorganisation was evidenced by the injury index data. The two genotypes differed in their leaf water content under the applied stress. The aim of this work was to examine the relationship between proline accumulation and membrane injury in barley plants suffering from the effects of water deficit. The possible role of proline in membrane protection under conditions of osmotic stress is discussed.

Key words: barley, proline, PEG stress, membrane injury

Introduction

The accumulation of free proline in response to osmotic stress is a widely distributed adaptive reaction not only in plants but in other organisms as well (McCue and Hanson, 1990; Delauney and Verma, 1993). This imino acid plays a number of important functions (Hare and Cress, 1997). Of considerable importance among them is its interaction with membrane proteins which is probably involved in the maintenance of the cell membrane structure (Bohnert and Jensen, 1996). It is known that under stress conditions the primary object of injury is the plasmalemma (Levitt, 1980). A consequence of the altered membrane integrity is the increase of the cell permeability which is accompanied by electrolyte leakage from the cell (Blum and Ebercon, 1981). An important strategy for the development of drought resistance in plants is the maintenance of cell membrane integrity after the imposition of water stress (Vasquez-Tello et. al.,

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1990). Test-systems for detecting the so called cell membrane stability (CMS) were used to characterise the drought-resistance of different plants (Gavuzzi et al., 1997; Bandurska, 2000; Venkateswarlu and Ramesh, 1993; van Rensburg et al., 1993). The aim of this work was to study the relationship between the accumulation of free proline, CMS and the relative water content of the tissues after the imposition of osmotic stress.

Materials and methods

Two barley cultivars were used in the experiments – Odeskii and Housters, differing in their reaction to low temperatures. Of them the more tolerant is Odeskii. Seeds were germinated for 48 hours in the dark, in a thermostat (28ºC), on wet filter paper in Petri dishes. The seedlings were grown for 7 days in water culture of 0,25 strength Knop solution in a phytostat chamber (12 hours photoperiod, 25ºC, 60% RH). For the induction of water stress the nutrient solution was replaced by PEG 6000 solutions in distilled water in three different concentrations for the variants and distilled water for the control plants. The treatment lasted 48 hours after which the following parameters were determined: relative water content (RWC), free proline content and CMS in the leaves of the plants. The relative water content was estimated according to Turner, 1981 and was evaluated from the equation:

$$RWC = (FW - DW)/(TW - DW),$$

where FW is the fresh weight of the leaves, TW is the weight at full turgor, measured after floating the leaves for 24 hours in water in the light at room temperature and DW is the weight estimated after drying the leaves for 4 hours at 80ºC or until a constant weight is achieved. Free proline content was measured according to Bates et al. (1973). For the CMS 20 leaf discs (2 cm each) of stressed and unstressed plants were washed with distilled water to remove the solution from the injured cells and tissue particles after which the samples were immersed in 20 ml distilled water at room temperature. After 24 hours the conductivity of the solutions was read. The samples were autoclaved for 15 minutes, cooled to room temperature and the conductivity of the solutions was read again. The electrolyte leakage was measured with a coductometer Radelkis TYPE OK-102/1. CMS or the so called injury index was estimated from the formula:

$$I = 1-(1- T_1/T_2)/(1- C_1/C_2)\times 100,$$

where T_1 and T_2 are the first and second (after autoclaving) measurement of the conductivity of the solutions in which the treated samples were immersed and C_1 and C_2 are the respective values for the controls.

Results and discussion

The RWC in the leaves of the two cultivars decreased with the increase of concentration of PEG solution in which the roots were immersed (Fig. 1c). A slight difference was observed between the cultivars. The one with greater RWC of the control retains a higher amount of water after the stress as well. Moderate stress (6,25% PEG) causes greater injury on the membranes of cv. Housters compared to Odeskii (Fig. 1b). The

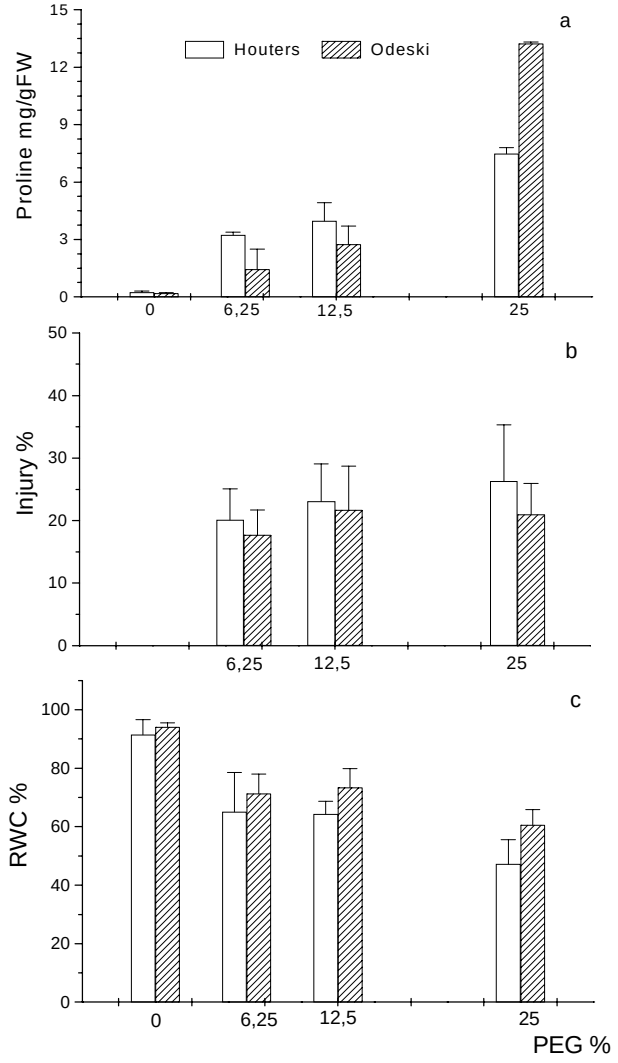

Fig. 1. Changes in: a) proline content, b) injury index (I%) and c) relative water content (RWC%) in the leaves of two barley cultivars subjected to osmotic stress.

difference becomes larger for the severest stress imposed (25% PEG). Although the initial amount of free proline in the leaves of the two cultivars is similar, after imposition of osmotic stress the amount in the cv. Odeskii was twice the amount in Housters (Fig. 1a). The analysis of the free proline content in the leaves of the two cultivars shows that the mild osmotic stress of 6,25% PEG causes an increase in the proline content of Housters as high as 13,5 times that of the control. The maximal imposed stress of 25% PEG shows a 31-fold increase of the proline in the leaves of Housters. The respective rise in Odeskii is 7 times in the case of 6,25% PEG and 70 times in 25% PEG in comparison with the control. The percent of injury of the membranes was estimated from the electrolyte leakage data of the stressed plants. It was found that the injury for both of the cultivars was about 20% of the control. The analysis of the results presented in the figures shows that the use of laboratory test systems simulating drought by the application of agents such as PEG can reveal many characteristics of the resistance of the leaf cells to dehydration. In our experiments, the cultivar more tolerant to low temperatures (Odeskii) shows lower dehydration when treated with 25% PEG which correlates positively with the higher proline content and the lower percent of membrane injury established. This finding suggests the existence of a certain relationship between the genetic factors defining the higher tolerance towards low temperature of the cultivar and its resistance to drought or dehydration. In the literature the opinions on the importance of proline are contradictory (Hare and Cress, 1997). Proline accumulation in great amounts is an established fact for many plant and animal species which most probably is not occasional. However, the exact mechanisms of its action in the adaptation to stress are not completely understood yet. Nevertheless, the accumulation of proline could be regarded as an indicative reaction in response to stress at the cellular level (Delauney and Verma, 1993). The opinion shared by many authors that free proline might be involved in the stabilisation of the membranes during water stress is confirmed in our experiments. Based on the represented data the following conclusions can be made: A correlation is found between the cold- and drought-tolerance of two barley cultivars. The more cold-tolerant cultivar (Odeskii) accumulates more proline and retains more water in the leaves when subjected to dehydration. At the same time lesss damage of the cell membranes is observed.

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