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BRIEF COMMUNICATION

Osmotic stress increases alcohol dehydrogenase activity in maize seedlings

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Abstract

Maize (*Zea mays* L.) seedlings were exposed to osmotic stress, and alcohol dehydrogenase (ADH) activity and abscisic acid (ABA) concentration were determined. The osmotic stress increased ADH activities in both roots and shoots, whereas the increase was 2-fold greater in roots than the shoots. The stress also increased ABA concentration in both roots and shoots and the increase was greater in the roots than in the shoots.

Additional key words: abscisic acid, *Zea mays*.

Alcohol dehydrogenase (ADH) was induced by low oxygen stress in many higher plants and known to play an important role to survive the adverse condition (Kennedy *et al.* 1992, Ricard *et al.* 1994, Drew 1997, Kato-Noguchi 1999). Other environmental stresses, including low temperature and dehydration stresses, also induced the expression of the ADH gene in *Arabidopsis* (Jarillo *et al.* 1993, Dolferus *et al.* 1994, Conley *et al.* 1999). However, induction of ADH by these environmental stresses in other higher plants remains unknown. The present paper described the effect of osmotic stress on the induction of ADH in maize seedlings.

Seeds of maize (*Zea mays* L. cv. Jelly Bantam) were sterilized in a 20 % (m/v) solution of sodium hypochlorite for 15 min, rinsed four times in distilled water, and germinated on two sheets of moist filter paper (No 1; *Toyo Ltd*, Tokyo, Japan) at temperature of 25 °C and 12-h photoperiod for 3 d. Light was provided with a white fluorescent lamp (*FL40SSW, National*, Tokyo, Japan; irradiance of 3.2 W m^{-2} at plant level). Then, uniform seedlings were selected and transferred to 9-cm Petri dishes each containing two sheets of filter paper moistened with 10 cm^3 of distilled water. After 24 h, the seedlings were treated with 10 cm^3 of 12, 28, 55 and 105 g kg^{-1} mannitol solutions (corresponding 75, 150, 300 and 600 mmol kg^{-1}, respectively) and incubated for 24 h in the same condition. Osmotic potential of the solutions was determined by a *Vapor Pressure Osmometer 5500* (*Wescor*, Logan, UT, USA). Then, the seedlings were divided into shoots and roots, frozen immediately with liquid N_2 and stored at -80 °C until extraction.

Frozen roots and shoots of the seedlings were ground to a fine powder in liquid N_2 with a mortar and pestle. The powder was homogenized in four volumes of ice-cold solution containing 100 mM Tris-HCl (pH 8.0), 10 mM Na-ascorbate, 10 mM DTT, 50 mM bovine serum albumin and 5 % (v/v) glycerol. The homogenate was centrifuged at 30 000 *g* for 30 min and the supernatant was used immediately for the measurements of ADH activity (Hanson *et al.* 1984).

ADH activity was measured spectrophotometrically by monitoring the oxidation of NADH at 340 nm (Kato-Noguchi and Watada 1997). The 1-cm^3 assay mixture contained 85 mM MES (pH 6.5), 0.15 mM NADH, 0.02 cm^3 sample, and 5 mM acetaldehyde. The overall recovery of ADH activity through the quantification process was 84 ± 6 % according to five repeated assays with pure enzyme in the extract. Protein was determined by the method of Bradford (1976) using bovine γ-globulin as a standard.

According to the ABA extraction method of Walker-Simmons (1987), root and shoot powder was prepared as described above and suspended in methanol containing 2.5 mM citric acid monohydrate and 0.5 mM butylated hydroxytoluene. Then, extract was stirred for 36 h in

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Abbreviations: ABA - abscisic acid; ADH - alcohol dehydrogenase; DTT - dithiothreitol.
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the dark at 4 °C and centrifuged at 3 000 *g* for 15 min at 4 °C. After passing the supernatant through a C_{18} cartridge (*Sep-Pak*, *Waters*, Tokyo, Japan) with aqueous 70 % (v/v) methanol, the eluate was dried and ABA concentration was determined by ABA immunoassay detection kit (*Sigma*, St. Louis, USA).

The ADH activity in roots and shoots of non-stressed maize seedlings (osmotic potential 0 mmol kg^{-1}) was 1.4 and 1.2 nmol mg^{-1} protein s^{-1}, respectively (Fig. 1). Osmotic stress increased the activity in roots and shoots at greater than 75 and 150 mmol kg^{-1}, respectively. At 600 mmol kg^{-1} of osmotic potential, the activity was 5.4 and 3.5 nmol mg^{-1} protein s^{-1} for roots and shoots, respectively. Thus, osmotic stress increased the ADH activity in both roots and shoots although the sensitivity to osmotic stress and the increase in ADH activity was greater in the roots than shoots. This study was the first to identify that osmotic stress increased the ADH activity in the maize seedlings.

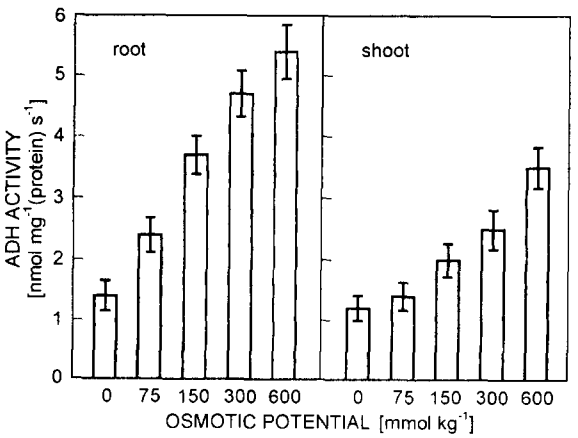

Fig. 1. Effect of osmotic stress on ADH activity in roots and shoots of 3-d-old maize seedlings. Means ± SE from three experiments with at least three assays for each determination.

ABA concentration in maize seedlings was also increased significantly by osmotic stress (Table 1). The concentrations in the seedlings subjected to 600 mmol kg^{-1} of osmotic stress were 4.4- and 3.6-fold greater than those of non-stressed seedlings for roots and shoots, respectively. ABA is known to play an important role in mediating plant tolerance to many environmental stresses, and osmotic stress increased ABA concentration in many plant species (*e.g.* Zeevaart *et al.* 1988, Sánchez-Serrano *et al.* 1991).

Table 1. Effect of osmotic potential (0 or 600 mmol kg^{-1}) on ABA concentration [nmol g^{-1}(f.m.)] in roots and shoots of maize seedlings. Means ± SE from three independent experiments.

Osmotic potential	0	600
Root ABA	0.21 ± 0.3	0.92 ± 0.8
Shoot ABA	0.18 ± 0.2	0.65 ± 0.7

Exogenous applied ABA induced expression of ADH gene in *Arabidopsis*, and this induction was mediated by the same signal transduction pathway as dehydration stress (Dolferus *et al.* 1994, De Bruxelles *et al.* 1996, Conley *et al.* 1999). From the finding it was concluded that, although the biological involvement of ADH to dehydration stress is unknown, dehydration stress including osmotic stress leads to increase in ABA concentration in *Arabidopsis* and the increased ABA plays the role of a stress signal, which results in ADH gene expression (Dolferus *et al.* 1994, de Bruxelles *et al.* 1996). Thus, the present results suggest that the osmotic stress may increase the ADH activity in maize seedlings through the induction of ADH gene expression subsequent to increase in ABA concentration.

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