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BRIEF COMMUNICATION

Preferential induction of alcohol dehydrogenase in coleoptiles of rice seedlings germinated in submergence condition

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Abstract

Difference in the growth response to submergence between coleoptiles and roots of rice (*Oryza sativa* L.) was investigated in 9-d-old rice seedlings. The coleoptile length in the submergence condition was much greater than that in aerobic condition, whereas the root length in the submergence condition was less than that in the aerobic condition. Alcohol dehydrogenase (ADH) activity in the coleoptiles in the submergence condition was much greater than that in the aerobic condition, but ADH activity in the roots in the submergence condition increased slightly. These results suggest that the preferential ADH induction in rice seedlings may contribute to the difference in the growth response between the coleoptiles and roots under low oxygen conditions.

Additional key words: Oryza sativa, flooding, tolerance.

Among cereals, only rice is adapted to flooded-prone environments. Rice seeds are able to germinate and grow in submergence condition where the partial pressure of dissolved oxygen is considerably less than in air (Perata *et al.* 1997, Setter *et al.* 1997, Vartapetian and Jackson 1997). The tolerance of rice to oxygen deficiency was related to the ability to maintain an active glycolysis leading to ATP production (Kennedy *et al.* 1992, Ricard *et al.* 1994, Drew 1997).

The coleoptile elongation of rice seedlings is stimulated in submergence conditions compared with in aerobic conditions, whereas submergence inhibited root elongation (Kordan 1974, Tsuji 1974, Alpi and Beevers 1983). Although there is an extensive literature on some aspects of growth and anaerobic metabolism in rice (*e.g.* Rivoal *et al.* 1989, Guglielminetti *et al.* 1995, Perata *et al.* 1997, Gibbs *et al.* 2000), the biochemical basis for the difference in the growth response to submergence between coleoptiles and roots is unknown. In the present research, effects of submergence on the growth and activity of alcohol dehydrogenase (ADH) were determined in coleoptiles and roots of rice seedlings.

Rice seeds (*Oryza sativa* L. cv. Nipponbare) were surface sterilized in an aqueous solution of 25 mM sodium hypochlorite for 15 min and rinsed four times in distilled water. Then, the 10 seeds were dropped into a jar (300 cm^3) filled with sterile distilled water and allowed to germinate and grow in darkness at 25 °C in a growth chamber (submergence condition). Control seeds were germinated and grown on two sheets of moist filter paper. (*Whatman No. 1*) in darkness at 25 °C in the growth chamber (aerobic condition). After 9 d, length and dry mass of coleoptiles and primary roots of the rice seedlings were determined. For determination of ADH activity, the coleoptiles and primary roots of the seedlings were harvested, frozen immediately with liquid N_2 and stored at -80 °C until extraction.

Frozen coleoptiles and roots of rice seedlings were placed in a mortar containing liquid N_2 and ground to a fine powder using a pestle. Then, the powder was homogenized with five 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, and the homogenate was

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centrifuged at 30 000 *g* for 30 min. The supernatant was used immediately for measurements of ADH activity. ADH activity was measured spectrophotometrically by monitoring the oxidation of NADH at 340 nm as described by Kato-Noguchi (2000). Protein was determined by the method of Bradford (1976) using bovine γ-globulin as a standard.

The germination in the submergence condition was characterized by a rapid elongation of the coleoptiles and leisurely elongation of the roots (Fig. 1). The length of the coleoptiles in the submergence condition was 2.9-fold greater than that in the aerobic condition, whereas the length of the roots in the submergence condition was only 7.2 % of that in the aerobic condition. However, the dry mass of the coleoptiles and roots in submergence condition was 77 and 1.6 % of those in the aerobic condition, respectively. Thus, the submergence increased only the coleoptiles elongation. Root elongation, dry mass gain of coleoptiles and roots were inhibited by the submergence.

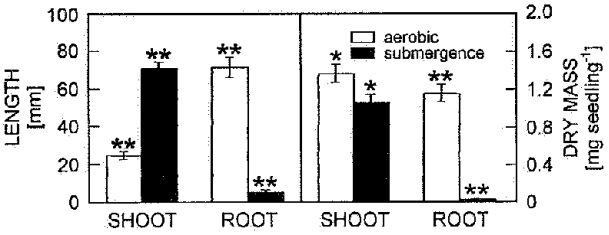

Fig. 1. Elongation and dry mass of rice seedlings in submergence and aerobic conditions. Means ± SE from 3 independent experiments with 10 plants for each determination. *Asterisks* indicate significant differences between treatments as determined by Student's *t* test: * - $0.02 < P < 0.05$; ** - $P < 0.001$.

ADH activity in the coleoptiles of rice in submergence condition was 7.0-fold greater than that in aerobic condition, but ADH in the roots in the submergence condition was only 1.4-fold greater than that that in aerobic condition (Fig. 2). ADH, which is enzyme of the ethanolic fermentation, has been induced by low oxygen stress in many plants including rice (Kennedy *et al.* 1992, Ricard *et al.* 1994, Drew 1997). The importance of ADH is supported by studies of ADH deficient mutants of several plants, which had reduced tolerance to oxygen deficiency (Johnson *et al.* 1994, Ellis *et al.* 1999). In addition, germination of rice ADH deficient mutant was suppressed in a submergence condition (Matsumura *et al.* 1995). The induction of ADH under the low oxygen allows continuing the glycolysis for production of metabolic energy owing to pyruvate consumption (Kennedy *et al.* 1992, Ricard *et al.* 1994, Drew 1997). Thus, the induction of ADH in rice seedlings may be essential for the germination and growth in the submergence condition and preferential ADH induction in the rice coleoptiles may contribute to the growth differences between coleoptiles and roots under the submergence.

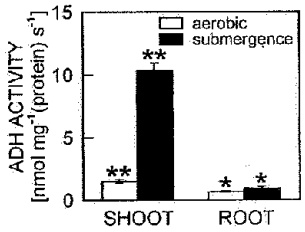

Fig. 2. ADH activity in rice seedlings in submergence and aerobic conditions. Means ± SE from three independent experiments with four assays for each determination. * - $0.02 < P < 0.05$; ** - $P < 0.001$.

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