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Ethanolic fermentation and anoxia tolerance in four rice cultivars

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Summary

The relationship between coleoptile elongation and ethanolic fermentation was investigated in rice (*Oryza sativa* L.) coleoptiles of four cultivars subjected to a 48-h anoxic stress. The coleoptile elongation of all cultivars was suppressed by anoxic stress; however, the elongation of cvs Yukihikari and Nipponbare was much greater than that of cvs Leulikelash and Asahimochi. The stress did not significantly increase lactate dehydrogenase (LDH) activity or lactate concentration, but increased alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) activities, as well as ethanol concentration in the coleoptiles of all cultivars. The elevated ADH and PDC activities and ethanol concentration in cvs Yukihikari and Nipponbare were much greater than those of cvs Leulikelash and Asahimochi, suggesting that ethanolic fermentation is likely more active in cvs Yukihikari and Nipponbare than in cvs Leulikelash and Asahimochi. ATP concentration in cvs Yukihikari and Nipponbare in anoxia was also greater than that in cvs Leulikelash and Asahimochi in anoxia. The ethanol concentration in the coleoptiles was correlated with anoxia tolerance with respect to the ATP concentration and coleoptile elongation. These results suggest that the ability to increase ethanolic fermentation may be one of the determinants in anoxia tolerance of rice coleoptiles.

Introduction

Plants often suffer hypoxic or anoxic environments when they are submerged by heavy rain or flood (Crawford and Braendle, 1996; Vartapetian and Jackson, 1997; Das and Uchimiya, 2002). Anaerobic carbohydrate catabolism is a

Abbreviations: ADH, alcohol dehydrogenase; LDH, lactate dehydrogenase; PDC, pyruvate decarboxylase

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fundamental component of the adaptation of plant tissue to hypoxia and anoxia. A high rate of glycolysis, which is linked predominantly to ethanolic fermentation, occurs in some plants under these conditions (Drew, 1997; Tadege et al., 1999).

Rice is very resistant to anoxia, and rice coleoptiles are one of the few plant organs that can grow in absolute anoxia (Alpi and Beevers, 1983; Cobb and Kennedy, 1987). It has also been shown that rice coleoptiles display a strong ethanolic fermentation system in anoxia (Setter et al., 1997; Boamfa et al., 2003). However, there is a difference in ability to grow in anoxia between rice cultivars (Setter et al., 1994). The objective of this study was to investigate the relationship between anoxia tolerance and ethanolic fermentation. Therefore, alcohol dehydrogenase (ADH), pyruvate decarboxylase (PDC) and lactate dehydrogenase (LDH) activities, and ATP and ethanol concentrations were determined in coleoptiles of four rice cultivars subjected to anoxic stress.

Materials and methods

Plant material

Seeds of rice (*Oryza sativa* L.), cvs Asahimochi, Leulikelash, Nipponbare and Yukihikari, were surface sterilized in an aqueous solution of 25 mmol/L sodium hypochlorite for 15 min and rinsed four times in distilled water. The seeds were then germinated on two sheets of moist filter paper (No. 1; Toyo Ltd., Tokyo) in darkness at 25 °C in a growth chamber. After 3 days, uniform seedlings were transferred in groups of 10 to 9-cm Petri dishes, each containing two sheets of filter paper moistened with 10 mL distilled water, and subjected to anoxic treatment.

Anoxic treatment

The Petri dishes were placed into 5-L jars at 25 °C. Distilled water (200 mL) was placed at the bottom of the jar to maintain humidity and the Petri dishes were elevated above the water. A stream of N_2 was passed continuously through the jar at a rate of 200 mL/min for 48 h. Non-stressed seedlings were supplied with air flowing at 200 mL/min.

Measurements of coleoptile length

The length of coleoptiles was measured with a ruler at the start and the end of the 48-h anoxic treatment, and elongation of the coleoptiles during the treatment was determined. The experiments were repeated three times with 50 plants each.

Extraction and determination of ATP

After anoxic treatment, the seedlings were killed with liquid N_2 in anoxia and stored at −80 °C until extraction. For one determination, 10 coleoptiles of the seedlings were placed in a mortar containing liquid N_2 and ground to a fine powder using a pestle. Powdered tissue was homogenized with five volumes of ice cold 0.4 mol/L $HClO_4$, and the mixture was centrifuged at 30,000 × *g* for 15 min at 4 °C and the supernatant was neutralized with 5 mol/L K_2CO_3. The precipitated potassium perchlorate was removed by centrifugation (30,000 × *g*, 5 min) and the supernatant was used for analysis of adenine nucleotides (Bergmeyer, 1985).

Adenine nucleotides were quantified spectrophotometrically according to the methods described by Mohanty et al. (1993). The overall recoveries of ATP added to the extraction medium containing coleoptile powder before homogenization were 83 ± 6% (mean ± SE) as calculated from five replications.

Extraction and assay of ADH, PDC and LDH

Coleoptile powder was prepared as described above and homogenized with five volumes of ice cold solution containing 100 mmol/L Tris-HCl (pH 8.0), 10 mmol/L Na-ascorbate, 10 mmol/L DTT, 50 mmol/L bovine serum albumin and 5% (v/v) glycerol (Hanson et al., 1984). The homogenate was centrifuged at 30,000 × *g* for 20 min and the supernatant was used immediately for measurements of ADH, PDC and LDH activities.

ADH and PDC activities were measured spectrophotometrically by monitoring the oxidation of NADH at 340 nm as described by Kato-Noguchi (2000). LDH activity was measured for the reaction from pyruvate to lactate by monitoring NADH oxidation in the presence of 4-methylpyrazole in order to inhibit interference from the coupled action of PDC and ADH (Hoffman et al., 1986). The 1-mL assay mixture contained 100 mmol/L TES (pH 7.0), 0.15 mmol/L NADH, 1 mmol/L 4-methylpyrazole, 3 μmol/L NaCN, 10 mmol/L sodium pyruvate and 0.2 mL of sample. The overall recovery of ADH, PDC and LDH activity through the quantification process was 90 ± 6%, 83 ± 7% and 84 ± 6% (means ± SE) for ADH, PDC and LDH, respectively, according