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A Rice Mutant Lacking Alcohol Dehydrogenase

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Summary

A rice mutant lacking alcohol dehydrogenase (ADH) was isolated by screening the M₂ lines of the rice cultivar Kinmaze that had been treated with N-methyl-N-nitrosourea. The mutant seedling lacked all of three isozymes of ADH, and was estimated to carry a single recessive mutation. Germination of mutant seeds was severely suppressed when seeds were submerged. However, mutant seedling survived under submergence for seven days and grew normally when transferred to aerobic conditions. These results indicate that ADH is essential for germination under anaerobic conditions but is not a prerequisite for growth under aerobic conditions.

Key Words : *Oryza sativa*, mutant, alcohol dehydrogenase, anaerobic condition, submergence.

Introduction

Most plants are critically damaged by hypoxia or anoxia when submerged or flooded. Their viability depends mainly on the production of energy by anaerobic respiration, namely, glycolysis and fermentation. Since rice is a plant adapted to anaerobic conditions, an understanding of the mechanism of such adaptation should provide valuable information relevant to possible improvement of tolerance of species that are sensitive to anaerobic conditions. Glycolytic and fermentative enzymes of rice, such as alcohol dehydrogenase (ADH; E. C. 1. 1. 1. 1), have been analyzed in terms of their expression under anaerobic condition (Ricard *et al.* 1986, Rivoal *et al.* 1989, Xie and Wu 1989), in attempts to clarify their function or contribution to tolerance of anaerobic conditions.

We isolated an ADH-less mutant of rice from mutagenized seeds. The seedlings were characterized with respect to the activity and isozymes of ADH and their viability during seed germination under water.

Materials and Methods

The seeds of about 1,000 M₂ lines, derived from the rice (*Oryza sativa* L.) cultivar Kinmaze by treatment of

fertilized egg cells with N-methyl-N-nitrosourea (MNU), were provided by Dr. H. Satoh, Faculty of Agriculture, University of Kyushu. They were submerged for two days and their germination rate was determined. Each line with a germination rate of less than 75 % was assayed for ADH activity. Ten seeds from each of these selected lines were sterilized with a 10 % solution of NaOCl and allowed to germinate under aerobic conditions for four days. Each of the seedlings was homogenized with liquid nitrogen. Soluble proteins were extracted by the addition of extraction buffer (Xie and Wu 1989) and the extract was centrifuged. The supernatant was used as crude enzyme.

Enzymes were also extracted from leaves of M₂ plants or the organs of M₃ seedlings with the same procedure as mentioned above.

Non-denaturing PAGE was performed as described by Hoffman *et al.* (1986), and the gels were stained for ADH activity by the method of Xie and Wu (1989). ADH activity was measured spectrophotometrically by monitoring the production of NADH at 340nm. The assay mixture (1 ml) contained 0.15 M Tris-HCl pH 8.0, 0.3 mM NAD, 30 mM ethanol and 30 μ l of crude enzyme.

Results

Seedlings of M₂ lines were screened and an ADH-less mutant line (92K-2S-40) was isolated. Segregation of eleven 92K-2S-40 M₂ plants for leaf ADH activity is shown in Table 1. Two plants were ADH-less mutants and the other nine showed a normal activity. Among these nine normal plants, three were homozygous and six were heterozygous, based on the ADH activity of M₃ seeds derived from these M₂ plants. In a panicle of a heterozygous M₂ plant, 90 normal and 29 mutant M₃ seeds were found. The segregation of the M₂ plants and the M₃ seeds fitted to the expected ratios of 1 : 2 : 1 and 3 : 1, respectively, suggesting the defect was due to a recessive mutation at a single locus.

Under aerobic conditions, there were no phenotypic differences between wild-type and mutant seedlings (Fig. 1 A and 1 B). Submerged seeds of both types were able to germinate, but the elongation of the coleoptile of the mutant seedlings was strongly suppressed (Fig. 1 C and 1 D). In spite of their reduced growth, the seedlings survived under submerged conditions for seven days

Table 1. Segregation for ADH activity in M₂ plants and M₃ seeds of 92K-2S-40

Source	Normal phenotype	Mutant phenotype	χ^2	P
M ₂ plants	3			
	(homozygous genotype) ¹⁾			
		2	0.273 (1 : 2 : 1)	0.75~0.90
M ₃ seeds from a heterozygous M ₂ plant ²⁾	6			
	(heterozygous genotype) ¹⁾			
	90	29	0.036 (3 : 1)	0.75~0.90

¹⁾ Genotypes were identified, based on the ADH activity of M₃ seeds derived from M₂ plants.

²⁾ M₃ seeds were derived from a panicle of a heterozygous M₂ plant of 92K-2S-40.

Fig. 1. Growth of 92K-2S-40 mutants.

A and B : 7-days-old aerobically grown seedlings, C and D : 7-days-old anaerobically grown seedlings, E : aerobically grown seedling for 5-days after anaerobic treatment for 7-days. A and C : wild-type, B, D and E : mutant.

(Fig. 1 E). Green leaves and roots emerged and grew similarly to those of the wild-type plant when seedlings were transferred to aerobic conditions.

ADH isozymes of the wild-type plant can be sepa-

Fig. 2. ADH isozymes of wild-type and 92K-2S-40 mutant seedlings. Rt : root, Sc : scutellum, Sh : shoot.

rated into three bands by starch gel electrophoresis or PAGE on non-denaturing gels (Xie and Wu 1989). No isozymes were detected in any of the mutants (Fig. 2). Samples in each lane contained the same amount of protein as in the case of wild-type samples and no bands of isozymes were seen even when larger amounts of sample were analyzed (data not shown).

Discussion

Mutants lacking ADH have been isolated in maize (Freeling and Bennett 1985), barley (Harberd and Edwards 1982), *Arabidopsis* (Jacobs *et al.* 1988) and tomato (Wisman *et al.* 1991). Most of the reported mutants lacked one of the ADH isozymes, and in several maize mutants the tissue specificity of the expression of ADH differed from that of the wild type (Kloeckner-Gruissem *et al.* 1992). These mutants are very useful for the analysis of the structure and expression of genes for ADH. No ADH-less mutants of rice have been reported

and ours is the first, being isolated from chemically mutagenized material.

Three ADH isozymes can be detected in rice seedlings even under aerobic conditions and it is suggested that they correspond to three dimers of the products of two genes for ADH (Xie and Wu 1989). Electrophoresis and activity staining of extracts of the mutant seedlings revealed the disappearance of all the isozymes. In the analysis of *Adh1* mutants of barley and tomato, the band of a single homo-dimeric protein, the product of another gene for ADH (*Adh2*), could be detected. Segregation in the M_2 or M_3 generation suggested that the mutation had occurred in a single gene. Since the two rice genes for ADH are known to be independent, it is suggested that the mutation was located in a region separate from the coding sequence of the genes for ADH.

The growth and fertility of the mutant were indistinguishable from those of the wild type even when both were cultivated in a paddy field (data not shown). ADH-less mutants of other plants have also been reported to be fertile and the mutations could be maintained in homozygous plants. It is suggested that ADH is not indispensable for the growth of rice seedlings under aerobic conditions. However, submergence suppressed seed germination of the mutant. Rice seeds are able to germinate under anaerobic conditions, in contrast to those of other cereals. Therefore, ADH appeared to be essential for the growth of submerged rice seedlings. However, since mutant seedlings that were submerged for seven days survived, it is assumed that physiological mechanism for tolerance of anaerobic conditions may be present in our ADH-less mutant.

Further analysis of this mutant should reveal the mechanisms of regulation of expression of the ADH gene and of alcoholic fermentation in response to anaerobic stress.

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