Note

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Decreased Transcription of a Gene Encoding Putative Mitochondrial Aldehyde Dehydrogenase in Barley (*Hordeum vulgare* L.) under Submerged Conditions

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Abstract

The cDNA encoding a putative mitochondrial aldehyde dehydrogenase (ALDH2) of barley (*Hordeum vulgare* L.) was characterized and its predicted amino acid sequence was compared with those of ALDH proteins from various plant species. Southern hybridization revealed that barley ALDH is encoded by more than two *ALDH* genes. The steady-state level of barley *ALDH2* mRNA, unlike that of rice *ALDH2a* mRNA, decreased under submerged conditions.

Accession nos. : AB055519

In higher plants, during oxygen deprivation, energy metabolic pathways switch from respiration to fermentation such as alcoholic fermentation. In alcoholic fermentation, pyruvate is converted to acetaldehyde by pyruvate decarboxylase (PDC), and subsequently acetaldehyde is reduced to ethanol with the concomitant oxidation of NADH to NAD^+ by alcohol dehydrogenase (ADH). This fermentation functions as the principal catalytic pathway for recycling NAD^+ to maintain glycolysis and the ATP level under anaerobic conditions (Perata and Alpi, 1993; Vartapetian and Jackson, 1997). Alternatively, acetaldehyde is oxidized to acetate by aldehyde dehydrogenase (ALDH) and acetate converted to acetyl-CoA by acetyl-CoA synthetase (ACS) (op den Camp and Kuhlemeier, 1997).

Aldehydes are highly reactive molecules that are important in various biological systems. Aldehyde dehydrogenases (ALDHs; EC 1.2.1.3) are a group of $NAD(P)^+$-dependent enzymes that metabolize a wide variety of aliphatic and aromatic aldehydes (reviewed by Vasiliou and Pappa, 2000). In human, the *ALDH* genes have been identified and characterized in detail (reviewed by Yoshida *et al.*, 1998). Among them, it is known that mitochondrial ALDH2 proteins play an important role in the detoxification of acetaldehyde, which is produced during metabolism of dietary ethanol (reviewed by Vasiliou and Pappa, 2000). In 1996, the first gene encoding a plant mitochondrial ALDH was identified as the maize *restorer of fertility 2 (rf2)* gene, which is one of nuclear restorer genes of Texas-type cytoplasmic male sterility (Cui *et al.*, 1996). Subsequently, *ALDH2* genes were identified from tobacco (op den Camp and Kuhlemeier, 1997), rice (Nakazono *et al.*, 2000) and *Arabidopsis thaliana* (Nakazono and Hirai, unpublished data; accession no. AB030820). We previously found that expression of the rice *ALDH2a* gene is induced under anaerobic conditions (Nakazono *et al.*, 2000), implying that ALDH2a is involved in the submergence tolerance of rice. In order to examine this possibility, it is useful to investigate the expression of the *ALDH2* genes in a submergence-intolerant plant species, such as barley and maize, under anaerobic conditions. Thus, in this study, we characterized a cDNA encoding putative mitochondrial ALDH2 protein from barley (*Hordeum vulgare* L.), and investigated the copy number and expression of the barley *ALDH2* under submerged conditions.

As a first step in determining the gene for aldehyde dehydrogenase (ALDH) in barley, we searched the barley EST clone database for genes that share sequence identity with the rice *ALDH2a* gene. As a result, the amino acid sequences of rice ALDH2a protein was found to share homology with a putative protein encoded by the EST clone Hv_CEb0003M11f from green leaves of barley (cv. CI16151) seedlings. The Hv_CEb0003M11f clone was provided by Dr. R. A. Wing (Clemson Univer-

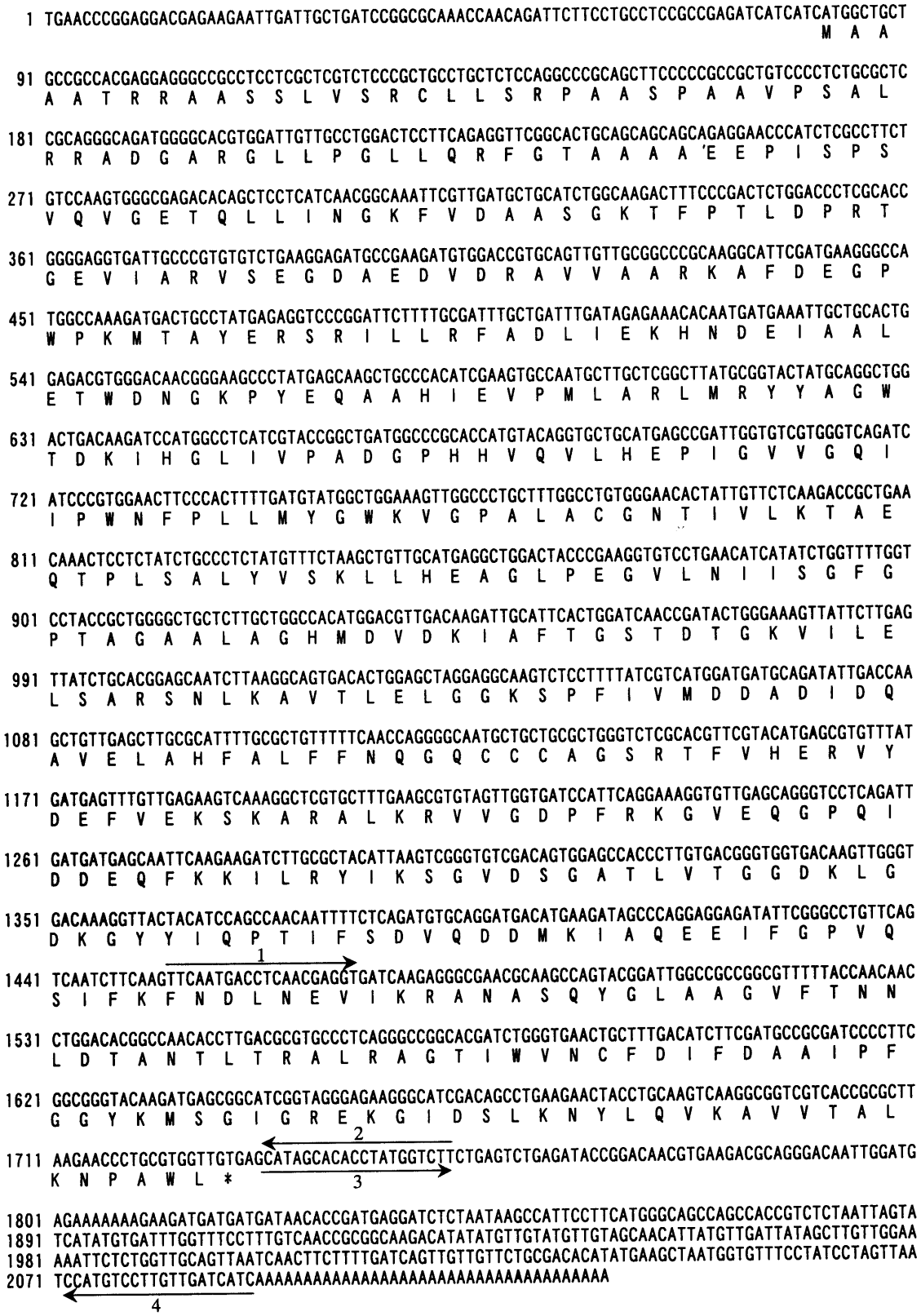

Fig. 1 Nucleotide sequence and deduced amino acid sequence of a cDNA encoding the barley *ALDH2* gene. The positions and the directions of primers are indicated by horizontal arrows. Primers 1 and 2 were used to amplify part of the coding region, and primers 3 and 4 were used to amplify part of the 3' – untranslated region.

sity Genomics Institute, Clemson University, Clemson, SC, USA), and its nucleotide sequence was completely determined with an automatic DNA sequencer (model 310; PE Applied Biosystems, Foster City, CA, USA). DNA sequencing data was analyzed with SEQMAN software (DNASTAR Inc., Madison, WI, USA), GENETYX Software (Software Development, Tokyo, Japan) and the CLUS-

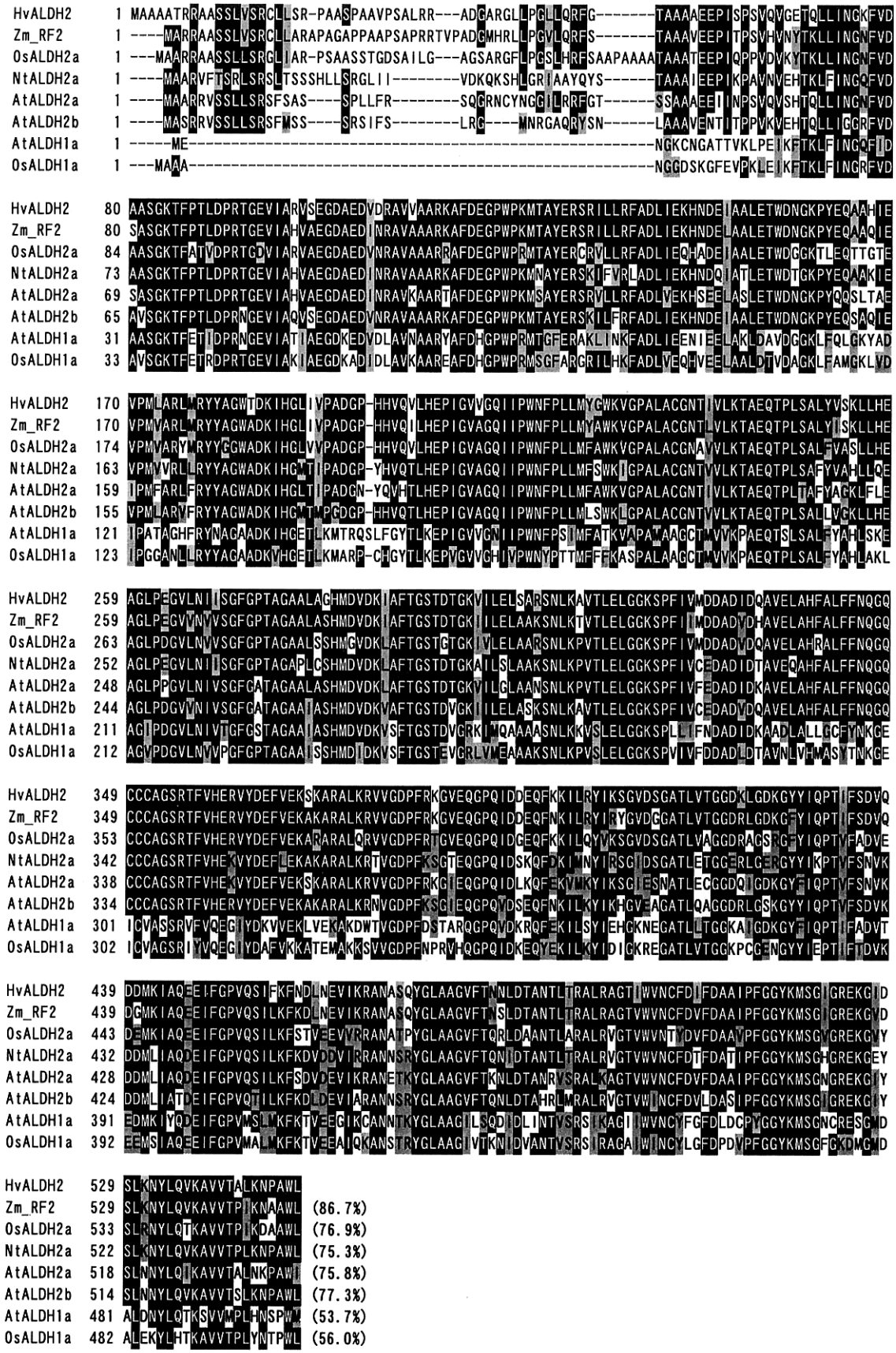

Fig. 2 Alignment of deduced amino acid sequences of mitochondrial ALDH2 proteins and cytosolic ALDH1 proteins from several plant species. Black boxes indicate identical amino acids and gray boxes indicate homologous amino acids. ALDH proteins are from barley (HvALDH2, this study); rice (OsALDH2a, Nakazono *et al.*, 2000; OsALDH1a, Li *et al.*, 2000); maize (Zm_RF2, Cui *et al.*, 1996); *Arabidopsis* (AtALDH2a, accession no. AB030820, Nakazono and Hirai, unpublished data; AtALDH2b, accession no. AC005990; AtALDH1a, accession no. AB020746); tobacco (NtALDH2a, op den Camp and Kuhlemeier, 1997). Percent identities of ALDH proteins from various plants with the barley ALDH2 protein are indicated.

TAL W algorithm (Thompson *et al.*, 1994). The nucleotide sequence of the cDNA was 2,128 bp long (DDBJ, EMBL, NCBI and GSDB DNA databases, accession no. AB055519) (**Fig. 1**), and the predicted amino acid sequence, which consists of 549 amino acid residues, of the barley ALDH is more homologous to those of mitochondrial ALDH2 proteins than those of cytosolic ALDH1 proteins from other plant species (**Fig. 2**). The amino-terminal portion of the predicted ALDH protein contains a typical mitochondrial targeting signal, which can form an amphiphilic α-helical structure and is rich in serine, arginine, alanine and leucine residues (reviewed by Glaser *et al.*, 1998). Therefore, the gene that encodes an ORF in the Hv_CEb0003M11f clone was designated as a barley mitochondrial *ALDH2* gene.

In order to estimate the copy number of the barley *ALDH* genes, genomic Southern hybridization was performed using a PCR-amplified fragment corresponding to part of the coding region of *ALDH2* as a probe (see **Fig. 1**). Total DNA was extracted by the method of Shure *et al.* (1983) from 7-day-old barley (cv. Amagi Nijo) seedlings, and 10 μg of total DNA was digested with *Hin*dIII or *Dra*I. As shown in **Fig. 3**, the probe hybridized to five bands in the *Hin*dIII-digest of barley total DNA, and hybridized to four bands in the *Dra*I-digest, suggesting that barley ALDH is encoded by more than two *ALDH* genes.

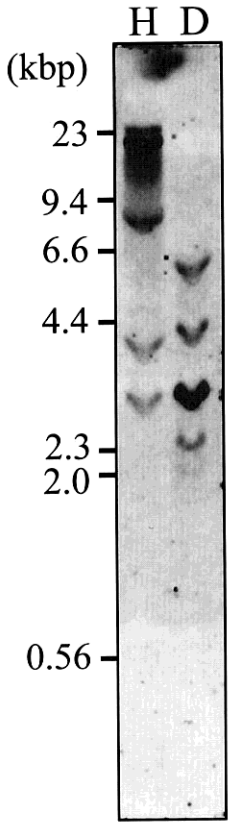

Fig. 3 Genomic Southern hybridization analysis of the barley *ALDH2* gene. Ten μg of total DNA isolated from 7-day-old seedlings was digested with *Hin*dIII (H) or *Dra*I (D). Hybridization was performed with part of the coding region of the barley *ALDH2* gene as a probe. The numbers given on the left indicate the sizes of the fragments in kilobase pairs (kbp).

To determine whether the transcript level of the barley *ALDH2* gene, like the transcript level of the rice *ALDH2a* gene (Nakazono *et al.*, 2000), increase under anaerobic conditions, 7-day-old seedlings grown under aerobic conditions were submerged for 12, 24 and 48 hours and were subsequently transferred to aerobic conditions where they were kept for 12, 24 and 48 hours, and then subjected to a Northern hybridization analysis. Total RNA was extracted by the standard guanidine thiocyanate/CsCl method (Kingston, 1991). A Northern hybridization was carried out. Three fragments from cDNA clones were amplified by PCR and used as probes. The three fragments were from (1) the coding region of the barley *ALDH2* gene, (2) the 3'-untranslated region (3'UTR) of the barley *ALDH2* gene (see **Fig. 1**), and (3) the coding region of the barley *ADH1* gene (accession no. X07774; Good *et al.*, 1988). Following submergence of the barley seedlings, the transcript level of the anaerobic-inducible *ADH1* gene increased, reaching a maximum after 12 hours of submergence, and then decreased from 12 to 48 hours of submergence (**Fig. 4C**). After transfer to aerobic conditions, it continued to decrease. The rapid increase in expression in the first 12 hours of submergence showed that the barley seedlings were in a state of oxygen deprivation. After submergence of the seedlings, the steady-state level of barley *ALDH2* mRNA dramatically decreased (**Fig. 4A-B**). When the submerged seedlings were transferred to an aerobic environment (for 12, 24 and 48 hours), the amount of the barley *ALDH2* transcript recovered (**Fig. 4A-B**).

Although submergence caused a decrease in the amount of the barley *ALDH2* mRNA, it induced an increase in the rice *ALDH2a* mRNA level (Nakazono *et al.*, 2000). This suggests that changes in the accumulations of the rice *ALDH2a* mRNA and the barley *ALDH2* mRNA in response to changes in the oxygen status are antiparallel to each other. However, it is possible that the steady-state level of the other barley *ALDH2* transcripts increase under submerged conditions. The probe corresponding to part of the coding region of the barley *ALDH2* hybridized to four fragments containing the *ALDH2* genes in the genomic Southern hybridization (**Fig. 3**). If submergence-inducible *ALDH2* genes exist in barley, we should have detected an increase of the

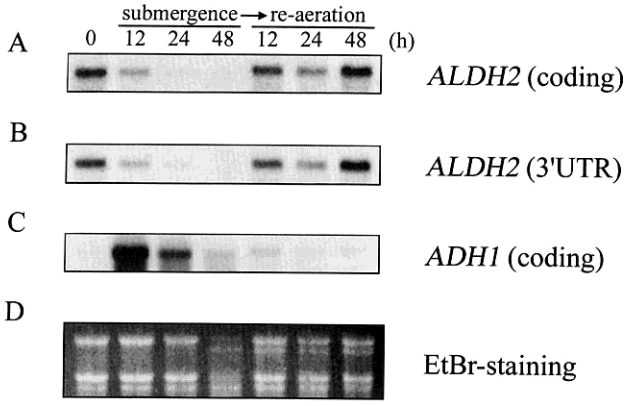

Fig. 4 Northern hybridization analysis of transcripts of the *ALDH2* gene and *ADH1* gene in barley by submergence treatments. Seven-day-old barley seedlings grown in an aerobic environment in the light were submerged in the dark for 12, 24 and 48 hours (submergence). After 48 hours, the seedlings were returned to aerobic conditions in the darkness for 12, 24 and 48 hours (re-aeration). Five μg of total RNA was loaded onto each lane. Hybridization was carried out with part of the coding region (A) and the 3'-untranslated region (B) of the barley *ALDH2* gene and part of the coding region of the barley *ADH1* gene (C) as probes. Equal loadings of total RNA were checked by ethidium bromide staining (EtBr-staining; D).

mRNA using a probe corresponding to the coding region of the *ALDH2* gene. However, the expression pattern of the barley *ALDH2* transcripts following submergence and re-aeration obtained with the probe corresponding to part of the coding region of the *ALDH2* gene (**Fig. 4A**) was similar to that obtained with the probe corresponding to part of the 3'UTR (**Fig. 4B**). Thus, barley appears to either lack submergence-inducible *ALDH2* genes, or if they are present, the amounts of mRNA appear to be very low. It is known that rice is more tolerant to anaerobiosis than barley. Therefore, the difference in the patterns of expression of the *ALDH2* genes between rice and barley under anaerobic conditions may be due to the difference in the anaerobiosis-tolerances of these two plants. Our hypothesis is that rice may have a greater ability than barley to detoxify acetaldehyde, which is produced during alcoholic fermentation in anaerobiosis and is oxidized from ethanol just after re-aeration, by ALDH2. This is supported by the finding that the *ALDH2* mRNA levels in tobacco, an anaerobiosis-intolerant plant, are also reduced by anaerobiosis (op den Camp and Kuhlemeier, 1997). To understand the physiological roles of ALDH2 proteins in plants under anaerobic conditions, a more comprehensive comparison of the functions of anaerobiosis-tolerant and anaerobiosis-intolerant species is needed.

Acknowledgements

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