S24-014

Gene cloning and characterization of salt-inducible aldehyde oxidase in barley

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keywords: *salt stress, differential display, aldehyde oxidase, barley*

Introduction

To study how plants respond and adapt to salt stress, we cloned hundreds of salt-inducible genes in barley (*Hordeum vulgare* L. cv. Haruna-nijyo) by differential display method (Ueda et al, submitted). One of them is a cDNA clone (*HvAO*), encoding aldehyde oxidase (AO).

AO (EC 1.2.3.1) has been extensively investigated in animals and microorganisms (Hall et al., 1986; Yoshihara et al., 1986). The enzyme has been implicated in the detoxification of various xenobiotics. In plants two AO catalyze the final step in biosynthesis of two phytohormones, through the oxidation of abscisic aldehyde to abscisic acid (ABA) (Walker-Simmons et al., 1989; Leydecker et al., 1995) and indole-3-acetaldehyde to indole-3-acetic acid (IAA) (Koshiba et al., 1996). Four Arabidopsis cDNAs were cloned and one of them encodes AAO3, which catalyzes the final step in the biosynthesis of ABA (Seo et al., 2000), whereas barley cDNAs have not been cloned and characterized yet. Three and four AO protein isoforms were detected in Arabidopsis (Seo et al., 2000) and barley (Omarov et al., 1999), respectively. ABA is synthesized by environmental stresses such as salt, drought and cold stress, and stomatal closure is triggered by ABA. To understand the relationship between *HvAO* and ABA synthesis, we studied the expression pattern of *HvAO* under various stresses.

Materials and methods

Plant materials

Barley (*Hordeum vulgare* L. cv. Haruna-nijyo) was hydroponically grown with Hoagland nutrient solution in a growth chamber (13-h light period, 100μmol m^{-2} s^{-1}, 25 °C, humidity 70% ; 11-h dark period, 22 °C, humidity 75%). Three-week-old plants were subjected to 200 mM NaCl and 50μM ABA.

Gene cloning and sequencing of barley AO *gene*

A partial fragment of *HvAO* was obtained by differential display (Ueda et al, submitted). RNA was extracted from salt-stressed barley roots and reverse transcription was carried out with poly $(A)^+$ RNA by RACE-PCR (SMART™ RACE cDNA Amplification Kit, CLONTECH).

Northern blot analysis

Fifteen μg of total RNA was separated on 1.2% agarose gel containing 0.66 M formaldehyde and transferred onto a nylon membrane (Hybond-N; Amersham Pharmacia Biotech). Hybridization was carried out in a solution containing 6×SSC, 5×Denhardt's solution, 0.1%

SDS and 0.1 g L^{-1} denatured herring sperm DNA. The membrane was washed in 6×SSC twice for 30 min. All hybridization and washing were performed at 65 °C. 3'-UTR of *HvAO* was used as a probe.

Southern blot analysis

Genomic DNA was digested with restriction enzymes, *BamHI*, *EcoR*I and *Hind*III, and separated on 0.8% agarose gel. After transferring onto a nylon membrane (Hybond-N^+; Amersham Pharmacia Biotech), hybridization was carried out as described above. The membrane was washed in 2×SSC, 0.1% SDS, and then in 1×SSC, 0.1% SDS and further in 0.1×SSC, 0.1 % SDS for 15 min per each washing. All hybridization and washing were performed at 65 °C.

Results and Discussion

Cloning and sequencing of HvAO *cDNA*

We isolated the candidates of salt-inducible genes in barley by differential display. One of them was judged to be *AO* gene in barley (*HvAO*) by sequencing and searching to databases by the BLASTx algorithms. It was about 1.2 kb DNA fragment and had 69% identity with *AO* gene in *Z. mays* (T01698). We carried out 5'-and 3'-RACE to obtain a full-length cDNA of *HvAO*. *HvAO* was more similar to *AO* genes of monocotyledon (maize) than those of dicotyledons (Fig. 1.).

Expression of HvAO *gene under various stresses*

To examine the inducibility by salt stress and exogenously applied ABA, we carried out Northern blot analysis (Fig. 2.). Under all conditions tested, *HvAO* mRNA was detected at negligible levels in stems and leaves and at a low level in roots of control plants (data not shown). The expression level of *HvAO* was then increased dramatically around 6-12 h under salt stress (Fig. 2-A). In ABA-treated plants, the expression level of *HvAO* was increased around 3-6 h (Fig. 2-B).

Southern blot analysis

To determine the copy number of *HvAO*, we carried out Southern blot analysis using 3'-UTR of *HvAO* cDNA as a probe. Barley was found to have several copies of *HvAO* using this probe (data not shown).

Expression of *HvAO* was enhanced in barley roots by both salt stress and exogenously applied ABA. ABA is a plant hormone that plays important roles in many aspects of plant growth and development, including stomatal closure and adaptation to environmental stresses. So induction of *HvAO* mRNA by exogenous ABA application may suggest the existing of the regulation system of ABA synthesis in root. Substrate specificity of HvAO has to be examined urgently in a future study. Localization of its mRNA in root tissues is also an interesting point to be studied.

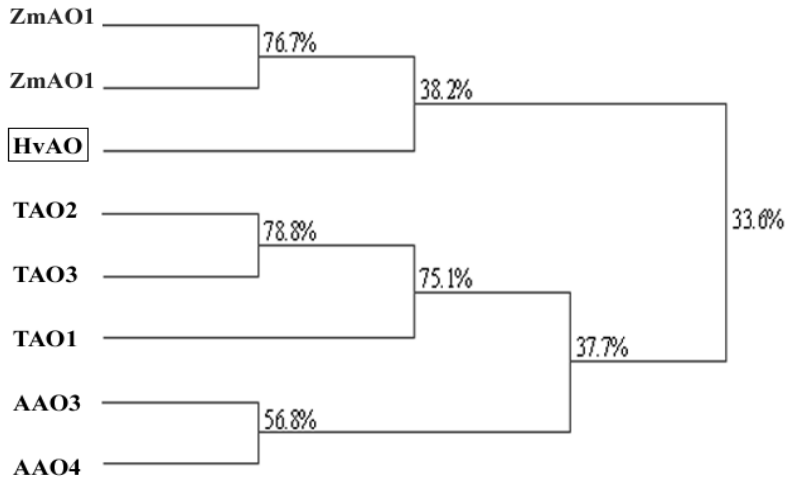

Fig. 1. Phylogenic tree of AOs in higher plants; ZmAO1 and ZmAO2 from maize; TAO1, TAO2 and TAO3 from tomato; AAO3 and AAO4 from *Arabidopsis.*

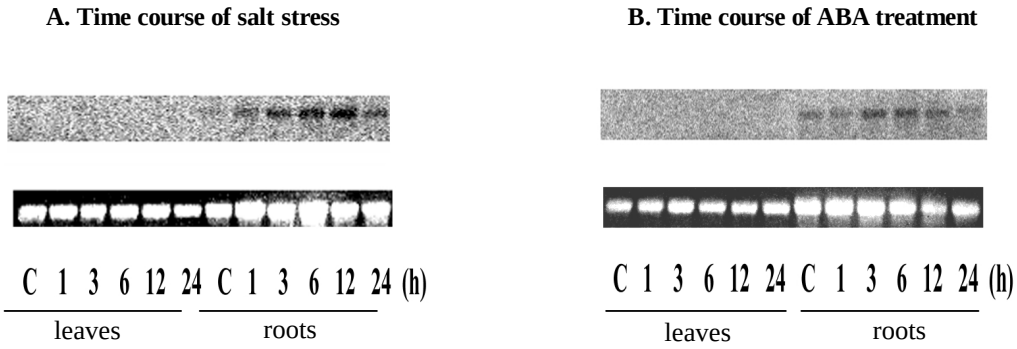

Fig. 2. Northern blot analysis of HvAO leaves and roots under salt stress and ABA treatment ; C, control

Acknowledgements

This research was supported by a JSPS project " Research for future " to T.T.

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