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Tissue specificity of glycinebetaine synthesis in barley

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ABSTRACT

Glycinebetaine is a major compatible solute accumulated in response to salt stress in barley plants. In this study, we investigated the glycinebetaine content and tissue localization of mRNA of two betaine aldehyde dehydrogenase (BADH) genes, *BBD1* and *BBD2*, and of BADH proteins in barley plants grown under control and saline conditions. Glycinebetaine was increased by salt treatment, and accumulated more in younger leaves than in older ones under both control and saline conditions. While *BBD1* and *BBD2* genes were constitutively expressed in mesophyll cells of leaves under both control and saline conditions, the signal of *BBD2* transcripts increased strongly in vascular parenchyma cells in salt-stressed leaves. In roots under saline conditions, *BBD1* transcripts were detected in epidermal cells, and *BBD2* transcripts were detected in the pericycle. Moreover, BADH proteins were detected around the xylem vessels of leaves, and in the pericycle and epidermal cells of roots grown under a saline condition. These results suggested that glycinebetaine is synthesized in vascular tissues of leaves and the pericycle of roots in barley plants grown under salt stress.

1. Introduction

Plant growth is greatly affected by abiotic stresses such as salinity, drought and low temperature. In particular, salinity is one of the major constraints that limits global agriculture and affects about one-third of the world's irrigated land [1]. Therefore it is important to understand the mechanism of salt tolerance in order to breed salt tolerant plants.

One of the metabolic adaptations to salinity in plants is the accumulation of compatible solutes [2]. In many plant species, glycinebetaine is an effective compatible solute for maintaining osmotic balance [3] and protecting enzyme activity [4]. Exogenous application of glycinebetaine to leaves or roots has been shown to improve abiotic stress tolerance of glycinebetaine nonaccumulating plants [5,6].

Glycinebetaine is synthesized by two-step oxidation of choline via betaine aldehyde in higher plants. In Chenopodiaceae, the first step is catalyzed by choline monooxygenase (CMO) which was identified as a ferredoxin-dependent iron–sulfur enzyme localized in the chloroplast stroma [7]. The second and the last step of glycinebetaine biosynthesis is catalyzed by betaine aldehyde dehydrogenase (BADH). BADH determines tissue specificity of glycinebetaine synthesis in barley. The genes encoding BADH have been found in various plants such as spinach [8], sugar beet [9], sorghum [10], rice [11] and barley [12,13]. In spinach, the major isozyme of BADH is localized in chloroplast stroma, and glycinebetaine is synthesized and accumulated mainly in chloroplasts [14,15]. It was also reported that glycinebetaine protects the oxygen-evolving of photosystem II and the activity of ribulose-1,5-bisphosphate carboxylase from high concentrations of NaCl [16,17]. These data indicated that glycinebetaine plays an important role in protecting photosynthetic tissues in spinach leaves under saline conditions.

Barley plants also synthesize and accumulate glycinebetaine in response to various abiotic stresses [18–21]. Two *BADH* genes (*BBD1* and *BBD2*) have been isolated from barley. BBD2 is a more abundant isoform than BBD1 in barley plants [13]. In addition, *BBD2* mRNA is greatly increased in leaves of barley plants by treatment of NaCl, PEG and ABA [13]. Moreover, *in vitro* measurement of the activity of recombinant BBD proteins using *E. coli* indicates that BBD2 protein has a 2000-fold higher affinity for betaine aldehyde than BBD1 [22]. These suggest that BBD2 is mainly involved in glycinebetaine biosynthesis. At the whole plant level, the distribution of glycinebetaine and salt such as sodium and chloride ions are different in barley plants grown under saline conditions [23]. Therefore it might be possible that the accumulation pattern and physiological role of glycinebetaine are also different at the tissue level in barley plants grown under saline conditions.

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Abbreviations: BADH, betaine aldehyde dehydrogenase; BBD, barley betaine aldehyde dehydrogenase; TBS, Tris-buffered saline.

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In order to support this hypothesis, we have investigated the distribution of glycinebetaine, and tissue localization of BADH mRNA and its protein in barley plants under a saline condition. In this study, we have found evidence for unprecedented tissue-specific biosynthesis and accumulation of glycinebetaine in barley plants grown under salt stress.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of barley (*Hordeum vulgare* L. cv. Haruna-nijyo) were surface sterilized and germinated in the dark at 25 °C for 2 d. Seedlings were hydroponically grown with Hoagland nutrient solution [24] in a growth chamber under a 13-h light period (25 °C, 200 μmol m^{-2} s^{-1}, 70% humidity)/11-h dark period (22 °C, 75% humidity). For salt treatment, 3-week-old barley plants were treated with Hoagland solution containing 200 mM NaCl for 72 h. Then, the second (L2), third (L3), fifth (L5) and sixth (L6) leaf blades from bottom up and whole roots (R) were harvested and stored at −80 °C.

2.2. Determination of glycinebetaine content

The glycinebetaine content was determined as described previously [25].

2.3. Northern blot analysis

Total RNA was extracted from individual leaf blades and roots as described previously [26]. Total RNA was electrophoresed on a 1.2% (w/v) agarose gel containing formaldehyde and blotted onto a nylon membrane (Hybond-N; GE Healthcare Biosciences). The membranes were hybridized with ^{32}P-labeled probes. The 3′-untranslated regions of *BBD1* and *BBD2* were used as specific probes. The specific probes were amplified by PCR using the following primer sets (*BBD1* sense primer, 5′-CTGGAGGACGGGATGAATCCTG-3′; *BBD1* antisense primer; 5′-GCAGATGAGCTCTCATTAAAAGGAC-3′; *BBD2* sense primer, 5′-GGACCTGTATCTGTTCCATCAGG-3′; *BBD2* antisense primer, 5′-GCTTCCTTTACATCCTCAATTTGCG-3′).

2.4. In situ hybridization

In situ hybridization for *BBD1* and *BBD2* transcripts was performed as described previously with some modifications [27]. Leaves and roots from barley plants were fixed in FAA [10% (v/v) formaldehyde, 50% (v/v) ethanol and 4% (v/v) acetic acid] at 4 °C overnight. After dehydration in ethanol/butanol series, the samples were embedded in paraffin (Paraplast Plus; New England Co.), and sliced to 10 μm in thickness. RNA probes were labeled using a digoxigenin (DIG) RNA labeling kit (Roche Diagnotics). Sense and antisense probes were synthesized from the 3′-untranslated regions of *BBD1* and *BBD2* cDNA.

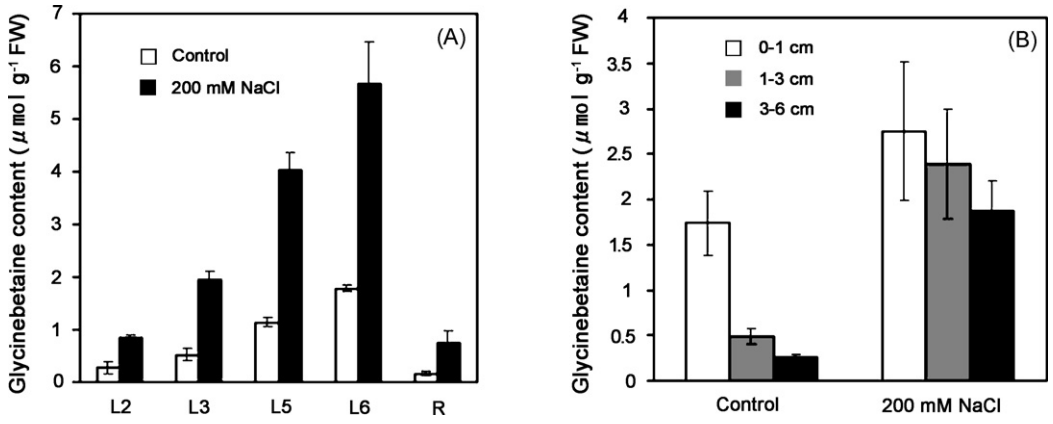

Fig. 1. Effect of NaCl on the glycinebetaine content in individual leaves and roots of barley plants. Barley plants were grown hydroponically for 3 weeks and treated with 0 and 200 mM NaCl for a further 72 h. (A) The second (L2), third (L3), fifth (L5) and sixth (L6) leaf blades from bottom up and whole roots (R) were sampled. (B) Three sections of the root tips were dissected from the root apex [0–1 cm (white bars), 1–3 cm (gray bars) and 3–6 cm (black bars)] after salt treatment for 72 h. The glycinebetaine contents were determined as described in Section 2. Vertical bars on each symbol represent S.E. ($n = 3$).

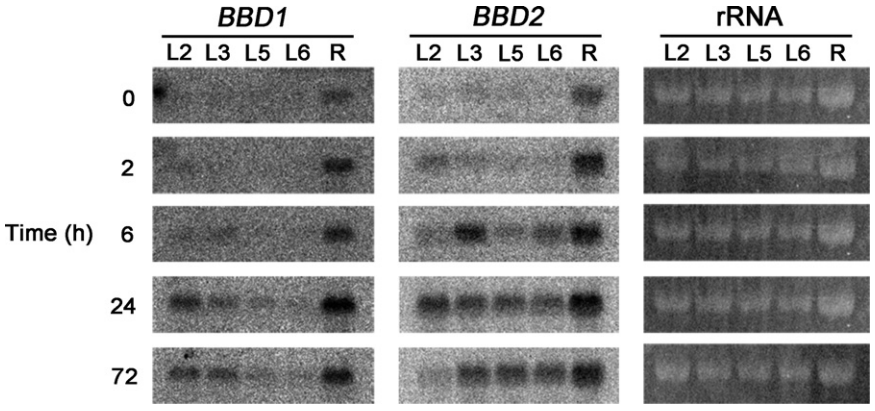

Fig. 2. Expression of *BBD1* and *BBD2* in barley plants. Barley plants were grown in fresh culture medium for 3 weeks, and then transferred into salinized culture medium containing 200 mM NaCl. Each lane was loaded with 10 μg total RNA, extracted from leaves and roots, which were collected at 0, 2, 6, 24 and 72 h after the salt treatment.