Plant Biotechnology 23, 317–320 (2006)

Gene Note

Transcriptional response of glycinebetaine-related genes to salt stress and light in leaf beet

Tomoki Tabuchi[1]*, Tomoyuki Okada[1], Yuhei Takashima[1], Tetsushi Azuma[2], Takashi Nanmori[2], Takeshi Yasuda[1,2]

[1] Laboratory of Tropical Plant Science, Faculty of Agriculture, Kobe University, Nada-ku, Kobe 657-8501 Japan;
[2] Graduate School of Science and Technology, Kobe University, Nada-ku, Kobe 657-8501 Japan
*E-mail: totabuchi@yahoo.co.jp Tel: +81-78-803-5854 Fax: +81-78-803-5857

Received November 22, 2005; accepted March 6, 2006 (Edited by D. Shibata)

Abstract *Beta vulgaris* L. accumulates a large amount of glycinebetaine (betaine), a metabolite that is related to salt-tolerance. Production of betaine involves expression of many genes that encode enzymes such as betaine aldehyde dehydrogenase, choline monooxygenase, phosphoethanolamine *N*-methyltransferase, *S*-adenosyl-L-methionine synthetase, *S*-adenosyl-L-homocysteine hydrolase, and methionine synthase. We examined transcriptional regulation of the betaine-related genes in leaf beet (*Beta vulgaris* L. var cicla, cv. shirogukifudanna). Transcript expression of these betaine-related genes in leaf tissue had the following common features: (1) a similar pattern of transcript induction under salt stress, (2) reduced induction under salt stress in a dark condition, and (3) diurnal rhythms of transcript levels under a photoperiod of 16 h light/8 h darkness. The co-regulation of transcripts may contribute to the effective betaine production without disturbing the biosynthesis of other products in leaf beet.

Key words: *Beta vulgaris*, glycinebetaine, light condition, salt stress.

Many chenopods, including *Beta vulgaris* L., accumulate a large amount of glycinebetaine (betaine), and this metabolite is considered to have various protective effects on plant cells under stress conditions (Storey et al. 1977; Hanson and Wyse 1982; Sakamoto and Murata 2002). In chenopods, betaine is synthesized from choline by the action of choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) (Hanson and Hitz 1982; Sakamoto and Murata 2002). The choline precursor, phosphocholine, is synthesized from phosphoethanolamine by the action of phosphoethanolamine *N*-methyltransferase (PEAMT) that catalyzes successive *S*-adenosyl-L-methionine (SAM)-dependent *N*-methylations of phosphoethanolamine (Smith et al. 2000). In tobacco, phosphoethanolamine is derived from ethanolamine, which is mainly produced by serine decarboxylase (SDC) activity (Rontein et al. 2003). A tracer experiment showed that in spinach leaves most of the ethanolamine and choline are metabolized to betaine (Coughlan and Wyn Jones 1982).

Following transmethylation, SAM is regenerated via *S*-adenosyl-L-homocysteine (SAH), homocysteine, and methionine by the activities of SAH hydrolase (SAHH), methionine synthase (MS), and SAM synthetase (SAMS) (Moffatt and Weretilnyk 2001). Giovanelli et al. (1985) showed that the synthesis of SAM is a major pathway involved in methionine metabolism in *Lemna paucicostata*, and we had previously suggested that the synthesized SAM is consumed mainly during betaine synthesis as a methyl group donor in the leaves of the chenopod *Atriplex nummularia* (Tabuchi et al. 2005). Therefore, it may be assumed that the methionine synthesized in *B. vulgaris* leaves may be used primarily as a methyl donor for betaine synthesis.

Transcripts for (or specific activities of) these betaine-related enzymes, with the exception of MS, are reported to be upregulated by salt stress in chenopod leaves (McCue and Hanson 1992; Russell et al. 1998; Rontein et al. 2001; Weretilnyk et al. 2001; Tabuchi et al. 2005). We previously reported that the regulation pattern of CMO transcripts resembles that of PEAMT and SAMSs in *A. nummularia* leaves under salt stress, after relief from salt stress and on a diurnal rhythm (Tabuchi et al. 2005). In order to further examine the co-regulation of betaine-related genes in chenopods, we compared the

Abbreviations: BADH, betaine aldehyde dehydrogenase; betaine, glycinebetaine; CMO, choline monooxygenase; MS, methionine synthase; ORF, open reading frame; PEAMT, phosphoethanolamine *N*-methyltransferase; SAH, *S*-adenosyl-L-homocysteine; SAHH, SAH hydrolase; SAM, *S*-adenosyl-L-methionine; SAMS, SAM synthetase; SDC, serine decarboxylase; UTR, untranslated region.
This article can be found at http://www.jspcmb.jp/

patterns of transcript expression in a broader range of betaine-related genes in *B. vulgaris*, which is one of the most important agricultural chenopod species. The transcript levels for many betaine-related genes in leaf beet increased under salt stress in the presence of light and showed a relative reduction in darkness, and many genes showed a similar pattern of transcript expression.

Seeds of leaf beet (*Beta vulgaris* L. var cicla, cv. shirogukifudanna) were purchased from Takii Shubyo (Kyoto, Japan). These were germinated on wetted vermiculite for one week, and the seedlings were grown hydroponically (Tabuchi et al. 2003) at 22°C under continuous light (100 μmol m^{-2} s^{-1}) from fluorescent tubes (FPL55EX-N, Matsushita Electric Co., Osaka, Japan) in a growth chamber. The 19-day-old plants were used for salt treatments. For diurnal rhythm experiments, the one-week-old seedlings of leaf beet were transferred onto new vermiculite and grown in the nutrient solution under the condition of 16 h light/8 h darkness at 25°C. The leaves obtained from the 22-day-old plants were used as samples.

Leaf beet grown by hydroponic culture was salt-treated by applying 200 mM NaCl to the nutrient solutions for one day. λTriplEx2 cDNA library was constructed from the poly(A) RNA of the leaves using a SMART cDNA Library Construction Kit (Clontech, Palo Alto, CA, U.S.A.) as described earlier (Tabuchi et al. 2005). The primary library contained 5×10^5 plaque-forming units.

cDNAs for *BADH* and *CMO* genes of sugar beet were previously cloned (McCue and Hanson 1992; Russell et al. 1998). We obtained the open reading frame (ORF) regions of cDNAs for *BADH* and *CMO* in leaf beet by PCR and named their nucleotide sequences *BvBADH1* and *BvCMO1*, respectively. The full-length cDNA clone for *BvCMO1* was obtained by library screening using the ORF fragment of the *BvCMO1* cDNA as a probe. Additionally, we screened the cDNA library of leaf beet using the *PEAMT* gene of spinach (Nuccio et al. 2000), *metE* gene of *Catharanthus roseus* (Eichel et al. 1995), *SAHH1* gene of *Arabidopsis* (Rocha et al. 2005), and *AtSDC* gene of *Arabidopsis* (Rontein et al. 2001) as probes; obtained the corresponding cDNA clones; and named them *BvPEAMT1*, *BvMS1*, *BvSAHH1*, and *BvSDC1*, respectively. The cDNA library was also screened using the *AnSAMS1* gene of *A. nummularia* (Tabuchi et al. 2005), and two types of cDNA clones were obtained. These genes were named *BvSAMS1* and *BvSAMS2*. The obtained clones are listed in Table 1.

RNA gel blot hybridization was performed as described earlier (Tabuchi et al. 2005). The ORF fragment of the cDNA clone corresponding to *BvBADH1*, and the cDNA fragments of the 3′ untranslated region (UTR) corresponding to the other betaine-related genes were amplified by PCR and then used as templates for probe synthesis. Their sequences corresponded to that of the nucleotides from +1 to +1503 (*BvBADH1*), from +1382 to +1770 (*BvCMO1*), from +1752 to +2030 (*BvPEAMT1*), from +1320 to +1720 (*BvSAMS1*), from +1246 to +1570 (*BvSAMS2*), from +2431 to +2720 of *BvMS1*, from +1579 to +1900 (*BvSAHH1*), and from +1616 to +1970 (*BvSDC1*).

We examined the transcript expression of the betaine-related genes in the leaf under salt stress in light and dark conditions (Figure 1A). The plant samples for this experiment were grown under continuous light until the salinity treatments. The levels of mRNAs for *BvBADH1*, *BvCMO1*, *BvPEAMT1*, *BvSAMS1*, *BvMS1*, and *BvSAHH1* hardly increased at 3 h under 100 mM salt stress and began to increase at 6 h under 100 mM or 3 h under 200 mM salt stress in light conditions. In the dark, the levels of mRNA for these genes gradually decreased for 24 h, and increased under 200 mM salt treatment, but the induction by salt was lower in the dark than in the light. The transcript level for *BvSDC1* began to increase at 3 h of salt stress. In the dark, the transcript level of *BvSDC1* was not suppressed and was induced gradually under 200 mM salt stress. The level of mRNA for *BvSAMS2* decreased at 3 h and 6 h under salt stress in light conditions. In the dark, the transcript level of *BvSAMS2* decreased gradually and was not induced under 200 mM salt stress.

We also examined the daily regulation patterns of the transcript levels for these genes under conditions of 16 h light/8 h darkness in the leaf (Figure 1B). The transcript

Table 1. Characteristics of cDNA clones of leaf beet.

Name	GenBank accession number	Homology			Reference
		Annotation	Source	%*	
BvBADH1	AB221006	BADH	*Beta vulgaris*	99.3	McCue and Hanson 1992
BvCMO1	AB221007	CMO	*Beta vulgaris*	99.6	Russell et al. 1998
BvPEAMT1	AB221008	PEAMT	*Spinacia oleracea*	90.7	Nuccio et al. 2000
BvSAMS1	AB221009	SAMS	*Atriplex nummularia*	97.0	Tabuchi et al. 2005
BvSAMS2	AB221010	SAMS	*Catharanthus roseus*	96.3	Schröder et al. 1997
BvMS1	AB221011	MS	*Arabidopsis thaliana*	88.6	Eichel et al. 1995
BvSAHH1	AB221012	SAHH	*Arabidopsis thaliana*	90.9	Rocha et al. 2005
BvSDC1	AB221013	SDC	*Arabidopsis thaliana*	79.0	Rontein et al. 2001

* Identity between the deduced amino acid sequence of the obtained clone and that of the referenced gene.

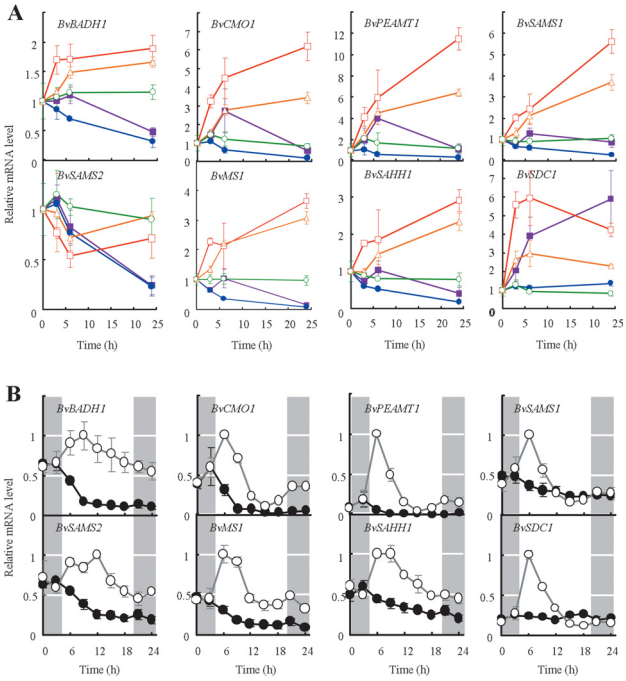

Figure 1. Expression patterns of transcripts of the betaine-related genes. Total RNAs (2 μg lane^{-1}) from leaves were separated by electrophoresis and hybridized with a cDNA probe of the genes or 18S rDNA. The data show the results of quantification of the levels of the mRNAs after normalization to those of 18S rRNA. Each value is the mean and standard error (SE) of three replicates. (A) Transcript responses to salt stress under light or dark condition. Plants were grown under continuous light and then treated with 100 mM (open triangles) or 200 mM (open squares and closed squares) NaCl under a light (open circles, open triangles, and open squares) or dark (closed squares and closed circles) condition. Control treatments are shown by open circles and closed circles. (B) Expression patterns of transcripts under a diurnal photoperiod of 16 h light/8 h dark. Plants were grown in light from 4 AM to 8 PM, and harvested during the photoperiod (open circles). Half of the plants were kept in the dark from 8 PM of the previous day and harvested thereafter (closed circles).

levels for *BvCMO1*, *BvPEAMT1*, *BvSAMS1*, and *BvSDC1* peaked at 2 h after the samples were exposed to light conditions and decreased gradually thereafter. Further, the transcript levels for the other genes peaked after the samples were exposed to light, but the peaks were obtained later than those of the above mentioned four genes at 3 h to 6 h, and then the levels decreased gradually. The peaks of the transcript levels for the genes that we investigated were not detected when the sample was exposed to the dark conditions. This result indicates that the diurnal rhythms of the transcript levels were regulated by the ambient light condition.

Transcript regulation patterns of *BvCMO1*, *BvPEAMT1*, and *BvSAMS1* under salt stress and on a diurnal rhythm were similar to each other (Figures 1A, B) as those of *AnCMO*, *AnPEAMT*, and *AnSAMS1* in *A. nummularia* (Tabuchi et al. 2005). Moreover, the transcript expression of *BvBADH1*, *BvMS1*, and *BvSAHH1* was also regulated in a manner similar to that of *BvCMO1*, *BvPEAMT1*, and *BvSAMS1* (Figures 1A, B). Betaine aldehyde is a toxic metabolite (Rathinasabapathi et al. 1994) and choline, methionine, and SAM are consumed not only for betaine synthesis but also for synthesis of other metabolites (Giovanelli et al. 1985); moreover, superfluous SAH concentrations inhibit various methyltransferase activities including PEAMT activity (Smith et al. 2000; Moffatt and Weretilnyk 2001). Therefore, substantial changes in the pool sizes of these substances may have negative effects on the plant cell. Leaf beet may co-regulate the transcript levels of the genes in order to maintain the specific pool sizes of the metabolites as previously discussed in the case of *AnCMO*, *AnPEAMT*, and *AnSAMS1* in *A. nummularia* (Tabuchi et al. 2005). Moreover, the co-regulation would accelerate accumulation of betaine under salt stress since the supply of the substrates for these gene products would be more than that under normal conditions.

PEAMT activity in spinach is also upregulated by salt stress in the light but not in dark conditions (Weretilnyk et al. 1995) similar to the case of these six betaine-related genes in leaf beet (Figures 1A, B). Since CMO requires reduced ferredoxin as the electron donor that is produced by photosynthesis in the leaf (Brouquisse et al. 1989), the transcript regulations of the betaine-related genes are probably dependent on light similar to the PEAMT activity in spinach as discussed by Weretilnyk et al. (1995).

In barley leaves, protein expression for MS is also seemed to be regulated for betaine synthesis, and this regulation is similar to that of BADH under salt stress (Narita et al. 2004). Therefore, betaine-related genes could be co-regulated not only in leaf beet and *A. nummularia* but also in other plants.

The transcript expression of many stress-inducible genes in *Arabidopsis* is regulated by the transcription factor DREB/CBFs (Fowler and Thomashow 2002; Maruyama et al. 2004). The transcript levels of the downstream genes of DREB1A have been suggested to increase similarly under cold stress (Maruyama et al. 2004). Since the transcript levels of many betaine-related genes in leaf beet (Figures 1A, B) and *A. nummularia* (Tabuchi et al. 2005) were also regulated similarly under salt stress and ambient light conditions, some of the betaine-related genes are likely to be regulated by one transcription factor. Because the *BvSDC1* transcript was induced earlier than those of other betaine-related genes under 100 mM salt stress and not suppressed at 24 h of salt stress in the dark (Figure 1A), the regulation system of *BvSDC1* mRNA may be different from those of other betaine-related genes under salt stress.

Acknowledgements

This study was supported in part by the Scientific Research Funds

of the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

Brouquisse R, Weigel P, Rhodes D, Yocum CF, Hanson AD (1989) Evidence for a ferredoxin-dependent choline monooxygenase from spinach chloroplast stroma. *Plant Physiol* 90: 322–329

Coughlan SJ, Wyn Jones RG (1982) Glycinebetaine biosynthesis and its control in detached secondary leaves of spinach. *Planta* 154: 6–17

Eichel J, Gonzalez JC, Hotze M, Matthews RG, Schroeder J (1995) Vitamin-B_{12}-independent methionine synthase from a higher plant (*Catharanthus roseus*). Molecular characterization, regulation, heterologous expression, and enzyme properties. *Eur J Biochem* 230: 1053–1058

Fowler S, Thomashow MF (2002) Arabidopsis transcription profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. *Plant Cell* 14: 1675–1690

Giovanelli J, Mudd SH, Datko AH (1985) Quantitative analysis of pathways of methionine metabolism and their regulation in *Lemna*. *Plant Physiol* 78: 555–560

Hanson AD, Hitz WD (1982) Metabolic responses of mesophytes to plant water deficits. *Annu Rev Plant Physiol* 33: 163–203

Hanson AD, Wyse R (1982) Biosynthesis, translocation, and accumulation of betaine in sugar beet and its progenitors in relation to salinity. *Plant Physiol* 70: 1191–1198

Maruyama K, Sakuma Y, Kasuga M, Ito Y, Seki M, Goda H, Shimada Y, Yoshida S, Shinozaki K, Yamaguchi-Shinozaki K (2000) Identification of cold-inducible downstream genes of the *Arabidopsis* DREB1A/CBF3 transcriptional factor using two microarray systems. *Plant J* 38: 982–993

McCue KF, Hanson AD (1992) Salt-inducible betaine aldehyde dehydrogenase from sugar beet: cDNA cloning and expression. *Plant Mol Biol* 18: 1–11

Moffatt BA, Weretilnyk EA (2001) Sustaining *S*-adenosyl-L-methionine-dependent methyltransferase activity in plant cells. *Physiol Plant* 113: 435–442

Narita Y, Taguchi H, Nakamura T, Ueda A, Shi W, Takabe T (2004) Characterization of the salt-inducible methionine synthetase from barley leaves. *Plant Sci* 167: 1009–1016

Nuccio ML, Ziemak MJ, Henry SA, Weretilnyk EA, Hanson AD (2000) cDNA cloning of phosphoethanolamine *N*-methyltransferase from spinach by complementation in *Schizosaccharomyces pombe* and characterization of the recombinant enzyme. *J Biol Chem* 275: 14095–14101

Rathinasabapathi B, McCue KF, Gage DA, Hanson AD (1994) Metabolic engineering of glycine betaine synthesis: plant betaine aldehyde dehydrogenases lacking typical transit peptides are targeted to tobacco chloroplasts where they confer betaine aldehyde resistance. *Planta* 193: 155–162

Rocha PS, Sheikh M, Melchiorre R, Fagard M, Boutet S, Loach R, Moffatt B, Wagner C, Vaucheret H, Furner I (2005) The Arabidopsis *HOMOLOGY-DEPENDENT GENE SILENCING1* gene codes for an *S*-adenosyl-L-homocysteine hydrolase required for DNA methylation-dependent gene silencing. *Plant Cell* 17: 404–417

Rontein D, Nishida I, Tashiro G, Yoshioka K, Wu WI, Voelker DR, Basset G, Hanson AD (2001) Plants synthesize ethanolamine by direct decarboxylation of serine using a pyridoxal phosphate enzyme. *J Biol Chem* 276: 35523–35529

Rontein D, Rhodes D, Hanson AD (2003) Evidence from engineering that decarboxylation of free serine is the major source of ethanolamine moieties in plants. *Plant Cell Physiol* 44: 1185–1191

Russell BL, Rathinasabapathi B, Hanson AD (1998) Osmotic stress induces expression of choline monooxygenase in sugar beet and Amaranth. *Plant Physiol* 116: 859–865

Sakamoto A, Murata N (2002) The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant Cell Environ* 25: 163–171

Schröder G, Eichel J, Breinig S, Schröder J (1997) Three differentially expressed S-adenosylmethionine synthetases from *Catharanthus roseus*: molecular and functional characterization. *Plant Mol Biol* 33: 211–222

Smith DD, Summers PS, Weretilnyk EA (2000) Phosphocholine synthesis in spinach: characterization of phosphoethanolamine *N*-methyltransferase. *Physiol Plant* 108: 286–294

Storey R, Ahmad N, Wyn Jones RG (1977) Taxonomic and ecological aspects of the distribution of glycinebetaine and related compounds in plants. *Oecologia* 27: 319–332

Tabuchi T, Kumon T, Azuma T, Nanmori T, Yasuda T (2003) The expression of a germin-like protein with superoxide dismutase activity in the halophyte *Atriplex lentiformis* is differentially regulated by wounding and abscisic acid. *Physiol Plant* 118: 523–531

Tabuchi T, Kawaguchi Y, Azuma T, Nanmori T, Yasuda T (2005) Similar regulation patterns of choline monooxygenase, phosphoethanolamine *N*-methyltransferase and *S*-adenosyl-L-methionine synthetase in leaves of the halophyte *Atriplex nummularia* L. *Plant Cell Physiol* 46: 505–513

Weretilnyk EA, Smith DD, Wilch GA, Summers PS (1995) Enzymes of choline synthesis in spinach: response of phosphobase *N*-methyltransferase activities to light and salinity. *Plant Physiol* 109: 1085–1091

Weretilnyk EA, Alexander KJ, Drebenstedt M, Snider JD, Summers PS, Moffatt BA (2001) Maintaining methylation activities during salt stress. The involvement of adenosine kinase. *Plant Physiol* 125: 856–865