Preliminary

Structural Analysis of Δ^1-Pyrroline-5-carboxylate Synthetase Gene from *Bruguiera gymnorrhiza*[*1]

Yuko MORIOKA[*2,3], Ayako MORIYA[*2,4] and Fukumi SAKAI[*2]

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Mangrove plants differ in an ability growing in high salt concentration from any other plants. On the mechanism of their salt tolerance, there are many physiological and morphological reports so far, but few reports on the mechanism at molecular level. In some mangrove species, proline is accumulated in cells in response to salt stress[1]. Proline can be synthesized by a pathway from glutamate in higher plants under the condition of salt stress. The key enzyme in the pathway is Δ^1-pyrroline-5-carboxylate synthetase (P5CS) which catalyzes the first two steps of proline biosynthesis[2,3]. In this paper, we describe the cloning and sequence analysis of P5CS gene *(BgP5CS)* fragments isolated from mangrove *(Bruguiera gymnorrhiza)* genome by polymerase chain reaction (PCR).

Four grams of mangrove leaves *(B. gymnorrhiza)* were pulverized with liquid nitrogen to fine poweder. Genomic DNA was extracted from the pulverized tissue with CTAB (Cetyltrimethl ammonium bromide) extraction solution (2% CTAB, 0.1 M Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% 2-mercaptoethanol) and purified. The purified DNA was amplified with specific primers for P5CS gene by PCR. The primers were constructed on the basis of the sequence of P5CS genes of *Arabidopsis*[4], mothbean[2], grape[5], tomato[6], rice[7], kiwifruit[8] and alfalfa[9]. The condition of PCR was thirty-five cycles of 30 sec at 95°C, 30 sec at 58°C, 2 min at 68°C. The PCR products were separated on agarose gel and successively were subcloned into a plasmid pGEM-T Vector System (Promega). The insert DNA in plasmids were sequenced by dideoxy chain termination method[10].

Among the DNA fragments amplified by PCR, two clones were selected as *BgP5CS*-related fragments (2.0 kbp and 2.5 kbp in length). The full-length P5CS genomic DNA *(AtP5CS)* from *Arabidopsis* has been reported to be organized into 20 exons interrupted by 19 introns[4]. According to nucleotide sequence analysis, the two *BgP5CS* fragments cloned in this study harboured 13 exons and 12 introns and corresponded to the truncated region from the second to the fourteenth exon of *AtP5CS*, overlapping on the sixth exon. It was found that *BgP5CS* genomic gene is about 1 kbp longer than that of *Arabidopsis* in the region. Each exon of *BgP5CS* was the same size as that of *AtP5CS*, but most of the introns in mangrove were longer than those in *Arabidopsis*. It was conceivable that genes of woody plants have extremely long introns with large genome size.

Although all the junction dinucleotides of *AtP5CS* are constituted of GT-AG splice sequence, there were two locations of GC-AG in splicing region of *BgP5CS*. The GC-AG non-canonical splice sequences are found to be in the equivalent introns of P5CS gene from the other mangrove species, *B. sexangula* reported previously[11].

The deduced amino acid sequence of *BgP5CS* showed partially high identity with that in various plants[4–6] (Fig.1). The deduced amino acid sequence from *BgP5CS* third exon contains putative ATP-binding domain (GAVGLGR) for γ-glutamyl kinase activity and shows higher similarity (71–74%) with those of known plants than the other exons. While, the eleventh and the twelfth exons contained putative NADPH-binding domain for γ-glutamyl phosphate reductase activity and was similarity between 75% and 97% at the level of deduced amino acid sequence. On the sixth exon, there were putative leucine zipper motif and γ-glutamyl kinase motif with highly conserved sequence (81–88% amino acid homology). The leucine zipper in P5CS in plants may function to allow close association even between the originally separate enzymes, γ-glutamyl kinase and γ-glutamyl phosphate reductase, as in prokaryotes[2]. These evidences strongly suggest that *BgP5CS* should be a bifunctioanal enzyme which catalyzes the first two steps in proline biosynthesis from glutamate.

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References

1) M. POPP: *Progress in Botany*, **56**, 416–429 (1995).
2) C.A. HU, A.J. DELAUNEY and D.P.S. VERMA: *Proc. Natl. Acad. Sci. USA*, **89**, 9354–9358 (1992).
3) P.B.K. KISHOR, Z. HONG, G. MIAO, C.A. HU and D. VERMA: *Plant Physiol.*, **108**, 1387–1394 (1995).
4) A. SAVOUÉ, S. JAOUA, X. HUA, W. ARDILES and M. MONTAGU: *FEBS letters*, **372**, 13–19 (1995).
5) A.P. STINES, D.J. NAYLOR, P.B. HØJ and R.V. HEESWIJCK: *Plant Physiol.*, **120**, 923–931 (1999).
6) T. FUJITA, A. MAGGIO, M. GARCIA-RIOS, R.A. BRESSAN and

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*2 Laboratory of Gene Expression.
*3 Present address: Kaneka Sun Spice Co. Ltd., Osaka.
*4 Present address: Hitec Corp., Osaka.

L.N. Csonka: *Plant Physiol.*, **118**, 661–674 (1998).
7) Y. Igarashi, Y. Yoshiba, Y. Sanada, S.K. Yamaguchi, K. Wada and K. Shinozaki: *Plant Mol. Biol.*, **33**, 857–865 (1997).
8) E. Walton, E. Podivinsky, R. Wu, P. Reynolds and L. Young: *Physiolgia Plantarum*, **102**, 171–178 (1998).
9) I. Ginzberg, H. Stein, Y. Kapulnik, L. Szabados, N. Strizhov, J. Schell, C. Koncz and A. Zilberstein: *Plant Mol. Biol.*, **32**, 755–764 (1998).
10) F. Sanger, S. Nicklen and A.R. Coulson: *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467 (1977).
11) A. Moriya and F. Sakai: *Wood Research*, No. **85**, 62–65 (1998).

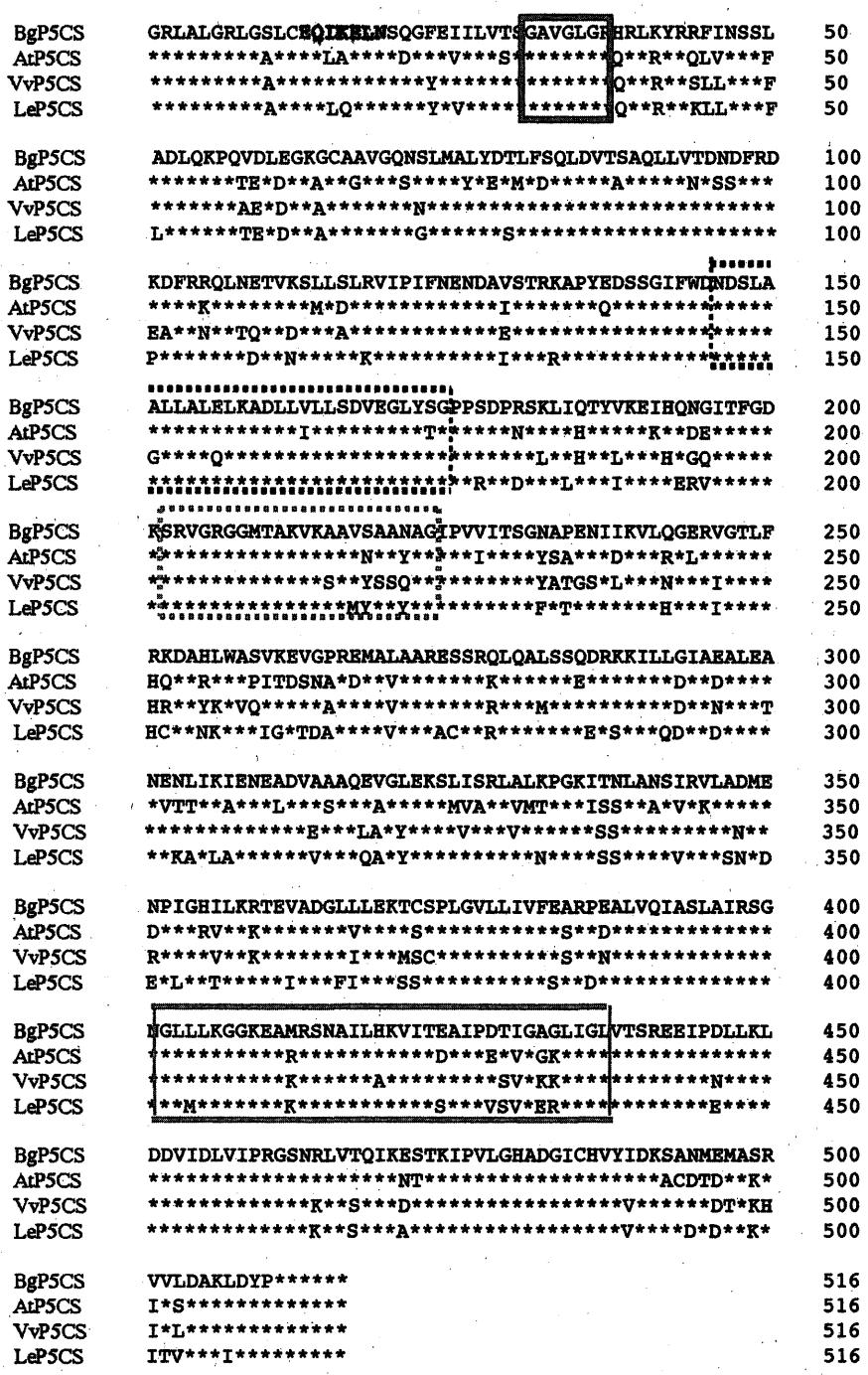

Fig. 1. Comparisons of the predicted BgP5CS amino acid sequence of *B. gymnorrhiza* with other plant P5CS. The sequence of BgP5CS is aligned with AtP5CS predicted from *Arabidopsis* genomic Gene[4], with VvP5CS from grape cDNA clone[5], and with LeP5CS from tomato cDNA clone[6]. Asterisks are identical with the amino acid residue of *B. gymnorrhiza* BgP5CS. Boxes indicated a putative ATP-binding domain with thick solid line, a putative NADPH-binding domain with light solid line, a luecine zipper with thick dotted line, and a putative γ-glutamyl kinase motif with light dotted line.