Structural Analysis of Δ1-Pyrroline-5-carboxylate Synthetase Gene from *Bruguiera gymnorrhiza*¹

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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 stress⁵. 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 Δ1-pyrroline-5-carboxylate synthetase (P5CS) which catalyzes the first two steps of proline biosynthesis⁶,³. 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 powder. Genomic DNA was extracted from the pulverized tissue with CTAB (Cetyltrimethyl 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 equivalent introns of P5CS gene from the other mangrove species, *B. sexangula* reported previously⁴,¹³. The deduced amino acid sequence of *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 y-glutamyl phosphate reductase, as in prokaryotes². These evidences strongly suggested that *BgP5CS* should be a bifunctional enzyme which catalyzes the first two steps in proline biosynthesis from glutamate.

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References

6) T. Fujita, A. Maggio, M. Garcia-Rios, R.A. Bressan and ¹ A part of this work was presented at the annual meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in Fukuoka, April 1998.
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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 [1], with VvP5CS from grape cDNA clone [2], and with LeP5CS from tomato cDNA clone [3]. 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 leucine zipper with thick dotted line, and a putative γ-glutamyl kinase motif with light dotted line.