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Molecular Cloning of Peroxidase Genomic DNAs from Sweetpotato

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Objectives

Expression of some peroxidase (POD) genes shows very distinctive pattern in cell cultures of sweetpotato (*Ipomoea batatas*). In the previous studies, it was known that these POD genes are active not only in cell cultures but also in stress-stimulated tissues of intact plant (1). Some of them showed salt- and hydrogen peroxide-stimulated gene expression as well as abscisic acid and methyl jasmonate responsiveness. Taken together these results, we can postulate that promoters of these POD genes share common stress-responsive *cis*-element(s). To isolate upstream region of POD genes from sweetpotato, PCR-assisted chromosomal walking (PACW) method was introduced by using gene-specific primers based on the cloned cDNA sequences.

Materials and Methods

1. Plant materials: sweetpotato (*Ipomoea batata* (L.) Lam. cv. White Star)
2. Methods
   - Promoter cloning by chromosome walking
   - Computer-aided alignment of genomic DNA sequences

Results and Discussion

Six of sweetpotato POD genomic DNAs were isolated by chromosomal walking. On the basis of primary structure of encoding proteins, we divided the isolated genes into two groups. One group including *gSWPA5*, *gSWPB1* and *gSWPB3* has three introns in their genomes. Interestingly, the protein structure of SWPA5 is very similar to that of *arabidopsis* ATPA2 that shows secretory pattern into the culture medium in cell cultures. We, therefore, can postulate SWPA5 is the homologous one of *arabidopsis* ATP. Other group of POD genes, *gSWPA3*, *gSWPA6* and *gSWPN1*, posses two introns. To search the 'consensus' sequence, computer-aided analysis was performed. Using MEME (Multiple Em for Motif Elicitation) program, we found the consensus, $CAA^{G}/_{C}CCCTTC^{T}/_{G}T^{C}/_{G}TTCA$, located in the region of 5´UTR. To investigate the function of this novel cis-element, promoter analysis with transient expression assay will be done. Further extensive analyses of these promoters will provide us more useful information of POD gene expression mechanism against stresses.

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Reference

Park SY et al. (2003) Molecular cloning and characterization of six peroxidase cDNAs from cell cultures of sweet potato and differential expressions in response to stress. *Molecular Genetics and Genomics* (*in press*).

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