

### 3 Isolation of Cytosolic Ascorbate Peroxidase Gene and Its Expression in Strawberry

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An cytosolic ascorbate peroxidase of strawberry (ApxSC) is an enzyme relating with fruit ripening. Our previous studies showed that the mRNA is strongly expressed in fruit and weakly in leaf, root, and petiole, but not in seed. Also, during ripening, the expression of the mRNA increased to the maximum level at the turning stage. Recombinant ApxSC protein was expressed in *E.coli* and was purified through chromatographies, which showed that *ApxSC* is functional gene. We isolated a genomic DNA harboring a *ApxSC* from a strawberry genomic library. Restriction mapping and sequence analyses showed that the *ApxSC* genomic DNA contains 10 exons and 9 introns, which is similar to the structure of pea *ApxI*. A primer extension analysis suggested that the transcription of *ApxSC* gene was started at three start sites with different degrees. The promoter region of *ApxSC* gene contains a sequence or structure distinct from other reported plant ascorbate peroxidase genes, though with several known functional elements such as a TATA box. Since strawberry has the polyploidic genome, we further isolated fifty-three cDNAs encoding ApxSC from a strawberry fruit cDNA library. These clones were categorized (i) into nine homologous (95 to 99 %) gene groups on the basis of their nucleotide sequences and (ii) into four groups of similar (>98%) polypeptides on the basis of their deduced amino acid sequences. Sequence variation among the gene groups was dispersed throughout the gene, while differences among the polypeptide groups were observed only at three amino acid positions (9, 63, and 233). These results imply that the *ApxSC* genes show co-dominant expression resulting from multiple alleles. This hypothesis is supported by genomic blots and primer extension analyses.

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Keywords: Ascorbate Peroxidase, Multiple Alleles, Strawberry