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SUMMARY OF DOCTORAL THESIS

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Title: Characterization of allene oxide synthases of potato and soybean

Metabolism of polyunsaturated fatty acids by peroxidation in plants is called as octadedanoide pathway, which provides various physiological mediators. The peroxidation is catalyzed by lipoxygenases (LOXs), which produce fatty acid hydroperoxides usually in a regio- and stereo-specific manner. The fatty acid hydroperoxides can be metabolized further by several kinds of enzymes. Among the enzymes, allene oxide synthases (AOSs) are the most major, because they can contribute to the synthesis of jasmonic acid, a genuine plant hormone involved in senescenece promotion, responses to wounding and drought, and defenses against insects and pathogens. Although a lot of research has been performed to evaluate AOSs in various plants, still some major commercially important farm products have not been fully examined. In this thesis, AOSs in two major farm products, potato and soybean, were investigated.

Potato (*Solanum tuberosum*) plants are rich in 9-lipoxygenase that converts linoleic acid and α-linolenic acid to 9*S*-hydroperoxy-10*E*,12*Z*-octadecadienoic acid (9-HPOD) and 9*S*-hydroperoxy-10*E*,12*Z*,15*Z*-octadecatrienoic acid (9-HPOT), respectively. The allene oxide synthase (AOS) involved in 9-HPOD/9-HPOT metabolism in potato, however, has not been characterized in detail. We cloned a cDNA encoding a novel AOS from potato sprouts by reverse transcriptase-PCR based on a partial sequence in the EST database. This AOS was successfully expressed in the yeast, *Pichia pastoris*, and purified using Ni-NTA resin. The recombinant enzyme metabolized 9-HPOD, 9-HPOT, 13-HPOD and 13-HPOT with reaction efficiency of 2.5 x 10^7, 1.0 x 10^7, 2.5 x 10^6, and 7.6 x 10^6 $M^{-1}s^{-1}$, respectively. The α-ketol formed from 9-HPOD was mainly composed of the 9*R*-enatimomer (90%). Besides sprouts, mRNA of this AOS was detected in buds, flowers and stems, but not in leaves, tubers and roots of mature plants, suggesting the tissue-specific function of this enzyme.

A plant allene oxide synthase (AOS) reacting with 13*S*-hydroperoxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid (13-HPOT), a lipoxygenase product of α-linolenic acid, provides an allene oxide which functions as an intermediate for jasmonic acid (JA) synthesis, making AOS a key enzyme regulating the JA level in plants. Although AOSs in various plants have been investigated, there is only limited information about AOSs in soybean (*Glycine max*). In this research, we cloned and characterized two soybean *AOS*s, *GmAOS1* and *GmAOS2*, sharing 95% homology in the predicted amino acid sequences. GmAOS1 and GmAOS2 comprised of 564 and 559 amino acids, respectively, with predicted N-terminal chloroplast-targeting signal peptides. Both AOSs expressed in *E. coli* were selective for 13*S*-hydroperoxides of α-linolenic and linoleic acids, suggesting the potential of GmAOS1 and GmAOS2 in contributing to JA synthesis. *GmAOS1* and *GmAOS2* were expressed in leaves, stems, and roots, suggesting their broad distribution in soybean plants.