

P 86

Enhanced Production of Protopanaxdiol (ginsenoside) in Roots of *Panax ginseng* Overexpressing Squalene Synthase Gene

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Objectives

Panax ginseng is an important medicinal plants and pharmacologically active component has been accepted as triterpenoid (ginsenoside). Squalene synthase gene represents a putative branch point capable of diverting carbon flow to the biosynthesis of non-saponifiable sterols and saponifiable triterpens. The squalene synthase gene of *Panax ginseng* could play an important role in triterpene (ginsenoside) production. Here we reported the alteration of ginsenoside contents of *Panax ginseng* by introducing squalene synthase gene.

Materials and Methods

1. Plant materials: *Panax ginseng*
2. Cloning of the squalene synthase gene from *Panax ginseng*: Screened from EST cDNA library.
3. Genetic transformation: *Agrobacterium tumefaciens* harboring ginseng squalene synthase gene
4. Analysis of transgenic plantlets: PCR, Southern, RT-PCR and Northern analysis.
5. Ginsenoside and squalene analysis: HPLC and GC.

Results and Discussion

1. Cloning of the squalene synthase gene: A full cDNA of SQ gene was isolated by EST. The nucleotide of SQ gene was 1476 bp and the putative ORF encodes a polypeptide of 415 amino acid residues. A deduced amino acid sequence showed similarity with *Arabidopsis thaliana* (75.78%) and *Nicotiana tabacum* (81.45%). A southern analysis indicates that at least 4 copies of squalene synthase genes exist in genome of *Panax ginseng*.
2. Genetic transformation: Genetic transformation protocol was followed by the our previous report (Choi et al. 2003).
3. Analysis of transgenic plantlets: Transgenic plants was confirmed by PCR, and GFP reaction, and Southern analysis. RT-PCR revealed that expression of SQ gene was enhanced in transgenic ginseng.
4. Bioassay for ginsenoside: Adventitious roots were obtained from transgenic and non-transgenic ginseng plants. Ginsenoside analysis of in vitro induced roots revealed that about 25% of ginsenoside of total one increased in transgenic ginseng roots, particularly notable increase only in protopanaxadiol (about double in ginsenoside Rb1). The preferential increase of protopanaxadiol group in transgenic *Panax ginseng* overexpressing SQ gene was similar to the result of Methyl jasmonic acid treatment.