

## 원자로부터 베타 아미린 합성 유전자 분리

작물과학원 인삼약초연구소  
 김옥태\*, 방경환, 신유수, 김영창, 현동윤, 차선우

Molecular Cloning of a gene encoding  $\beta$ -amyrin Synthase from *Polygala tenuifolia*

Ginseng and Medicinal Plant Research Institute, NICS, RDA  
 Ok-Tae Kim\*, Kyong-Hwan Bang, Young-Chang Kim, Yu-Su Shin,  
 Dong-Yun Hyun, Seon-Woo Cha

**Objectives**

*Polygala tenuifolia*, a member of the Polygalaceae family, has been for the treatment of a tonic, sedative, expectorant and anti-inflammation agent and has been cultivated throughout East Asia, including Korea. The roots of *P. tenuifolia* is a well-known traditional medicine, and various xanthenes, saponins, phenones and oligosaccharide esters have been isolated from this plant. Onjisaponins in *P. tenuifolia* shares with these compounds a common biosynthetic intermediate,  $\beta$ -amyrin, which is synthesized by the OSC  $\beta$ -amyrin synthase.  $\beta$ -Amyrin synthase, which to our knowledge is the first triterpene synthase to be discovered in *P. tenuifolia*, is directly or indirectly connected with onjisaponin production. Therefore, we decided to isolate the gene encoding oxidosqualene cyclase from *P. tenuifolia* as a first step towards improving saponin yield.

**Materials and Methods**

- Plant materials

Seeds of *Polygala tenuifolia* were obtained from our institute (Eumseong, Chungbuk).

- Cloning of a full-length PtbAS cDNA

Full-length cDNA sequence of  $\beta$ -amyrin were obtained by RACE using degenerate primer. 5'-RACE and 3'-RACE was performed using two nested primers matching the adapter sequences and two nested gene-specific primers in two rounds of PCR amplifications.

**Results and Discussion**

To isolate a full-length cDNA of PtbAS as the first step, the resulting 0.5 kb amplification product was cloned; two clones were sequenced and were found to be identical to each other. The full-length cDNA was obtained by 5- and 3-RACE using specific primers and was named *PtbAS* (*Polygala tenuifolia*  $\beta$ -amyrin synthase). The *PtbAS* open reading frame (ORF) consists of 2,289 bp nucleotides encoding a 763-

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\*Corresponding author: (TEL)+82-43-871-5535, (E-mail): kimot@rda.go.kr

amino acid protein of around 87.4 kDa (GenBank accession no. EF107623). The PtbAS amino acid sequence revealed 87%, 80%, and 79% identity with the  $\beta$ -amyrin synthases of *Glycyrrhiza glabra* (AB037203), *Betula platyphylla* (AB055512), and *Panax ginseng* (AB009030), respectively. Phylogenetic tree analysis of Figure 1 shows that PtbAS is closely related to  $\beta$ -amyrin synthases of other plants. However, this gene has not been shown to synthesize  $\beta$ -amyrin in the yeast mutant *erg7* in this paper. Therefore, additional experiments will be performed to clarify the function of PtbAS gene.

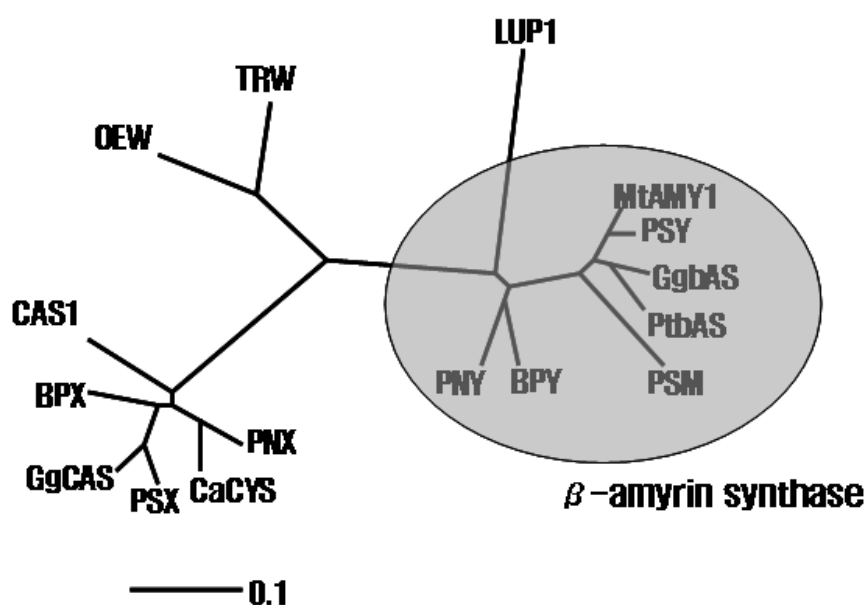


Fig. 1. Phylogenetic tree constructed from the deduced amino-acid sequences of PtbAS from *P. tenuifolia* and OSCs from other plants.