

고려인삼으로부터 Squalene Synthase 유전자의 Cloning 및 형질전환체의 특성

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ABSTRACT

Introduce of gene connected with disease and transformation system of ginseng, Squalene synthase(PSS) gene cloned from and disease resistant gene were carried out for expression and transformation of plant using *Agrobacterium*. PSS of 35S-35S-AMV-PSS-Tnos, has been constructed which were mobilized into *Agrobacterium tumefaciens* strain MP 90 disarmed Ti-plasmid. PSS gene were introduced into the binary vector pRD 400. The transgenic ginseng plants were propagated using repetitive secondary embryogenesis and introduced NPTII and PSS genes of the transgenic ginseng were successfully identified by the PCR and survival test on the medium.

연구목적

고려인삼(*Panax ginseng* C.A. Meyer)의 사포닌 생합성 관련 유전자중 triterpenonid 합성에 관여된 효소인 Squalene synthase gene(PSS)을 cloning 및 식물형질전환용 운반체에 재조합 후 인삼 부정근을 이용하여 유전자를 발현시킴으로써 dammarene type의 사포닌 생합성을 증대시키고자 한다.

재료 및 방법

인삼의 EST로부터 인삼의 Full length cDNA library로부터 clone을 선발하여 사포닌 합성 관련 PSS의 Sequencing(ABI377, PerkinElmer)을 통해 확인된 유전자의 염기서열 분석 및 확인하였다.

PSS의 ORF(Open reading frame)를 PCR로 증폭하여 식물형질전환용 pRD400 binary vector를 사용하였다. 인삼 발현용으로 cassette vector는 524-XbaI을 사용하였다.

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결과 및 고찰

PSS는유전자의 염기서열 분석에서 일본에서 분리된 *Panax ginseng* (Japan-1)과 100% 상동성을 보였다. 본 유전자를 식물형질전환용 binary vector에 재조합하여 다시 인삼에 형질전환시킴으로서 PSS유전자가 인삼에서 고발현되도록 하였다. 인삼 자엽에서 cloning한 유전자를 발현시킨 것을 PCR을 통하여 형질전환체임을 확인하였다. 또한 형질전환된 인삼으로부터 유도된 부정근에서 total ginsenoside가 wild type보다 높게 나타났다. 이것은 인삼 사포닌 생합성과정에서 FPP(C15)에서 Squalene(C30)으로 진행되는 과정에서 형질전환된 유전자가 증가하여 Tri-terpenoids phytosterols saponins의 증가에 기여할 가능성을 시사한다.

<i>P. ginseng</i>	1	MGSLGAILKHPEDFYPLLLKLEFAARHAEKQIPPEPHUWFCYSLHLKVSRSFGLVIQQLGPCLRDAV
<i>P. ginseng (Japan-1)</i>	1	MGSLGAILKHPEDFYPLLLKLEFAARHAEKQIPPEPHUWFCYSLHLKVSRSFGLVIQQLGPCLRDAV
<i>G. max</i>	1	mgslgailkhpddfyplllkklmaarhaekqippephwfcysmlhkvsrsfalviqqlgiclrnav
<i>L. corniculatus</i>	1	mgslgavrhpdldfyplllkklmaarhaekqippephwfcysmlhkvsrsfalviqqlatcllrnav
<i>G. glabra</i>	1	mgslgavrhpdldfyplllkklmaarhaekqippephwfcysmlhkvsrsfalviqqlatcllrnav
<i>N. tabacum</i>	1	mgslgailkhpddfyplllkklmaarhaekqippephwfcysmlhkvsrsfalviqqlpvelrnav
<i>A. thaliana</i>	1	mgslgavrhpdldfyplllkklmaarhaekqippephwfcysmlhkvsrsfalviqqlatcllrnav
<i>P. ginseng</i>	121	EFHHVSAFLELGGSCYOEALIEDITWRMGAGMAKFIKKEVETINDYDEYCHYVAGLVGLGSLKLFHA
<i>P. ginseng (Japan-1)</i>	121	EFHHVSAFLELGGSCYOEALIEDITWRMGAGMAKFIKKEVETINDYDEYCHYVAGLVGLGSLKLFHA
<i>G. max</i>	121	efhhvscalfelcknygcaieditwrmgagmakfikkevetiddedyechyvaglvglgsklfha
<i>L. corniculatus</i>	121	efhhvscalfelcknygcaieditwrmgagmakfikkevetiddedyechyvaglvglgsklfha
<i>G. glabra</i>	121	efhhvscalfelcknygcaieditwrmgagmakfikkevetiddedyechyvaglvglgsklfha
<i>N. tabacum</i>	121	efhhvscalfelcknygcaieditwrmgagmakfikkevetiddedyechyvaglvglgsklfha
<i>A. thaliana</i>	121	efhhvscalfelcknygcaieditwrmgagmakfikkevetiddedyechyvaglvglgsklfha
<i>P. ginseng</i>	241	EDLKYEENSRAKAVQCLNDNVTDALVHAEEDCLKYHSDLRGPAIFRFAIPQIMAIQTALGCFNMTOV
<i>P. ginseng (Japan-1)</i>	241	EDLKYEENSRAKAVQCLNDNVTDALVHAEEDCLKYHSDLRGPAIFRFAIPQIMAIQTALGCFNMTOV
<i>G. max</i>	241	edlkyeensrkavqclndnmvtnalvhaeedclkymsdlrddsfrrfcaipqimaigtalgcynniev
<i>L. corniculatus</i>	241	edlkyeensrkavqclndnmvtnalvhaeedclkymsdlrddsfrrfcaipqimaigtalgcynniev
<i>G. glabra</i>	241	edlkyeensrkavqclndnmvtnalvhaeedclkymsdlrddsfrrfcaipqimaigtalgcynniev
<i>N. tabacum</i>	241	edlkyeensrkavqclndnmvtnalvhaeedclkymsdlrddsfrrfcaipqimaigtalgcynniev
<i>A. thaliana</i>	241	kledlkyeentkavqclndnmvtnalvhieedclkymsdlrddsfrrfcaipqimaigtalgcynniev
<i>P. ginseng</i>	361	EAIQKTCRESGTLNKRKSYIRKENGYSGLLAILVILVLSLIRAYLSAKRQDN* 416
<i>P. ginseng (Japan-1)</i>	361	EAIQKTCRESGTLNKRKSYIRKENGYSGLLAILVILVLSLIRAYLSAKRQDN* 416
<i>G. max</i>	361	eaiqktcrdesglnkrksyirskengysglailvvlvllsrlraylsanhhns* 414
<i>L. corniculatus</i>	361	eaiqktcrdesglnkrksyirskengysglailvvlvllsrlraylsanhhns* 414
<i>G. glabra</i>	361	eaiqktcrdesglnkrksyirskengysglailvvlvllsrlraylsakrqn* 414
<i>N. tabacum</i>	361	eailkterdesglnkrksyirskengysglailvvlvllsrlraylsanhhns* 412
<i>A. thaliana</i>	361	rleavqklcrdegvlqnrksyivndkqpnsvfllmvvlllaivraylran* 411

Fig. 1. Comparison of the amino acid residues among PSS isolated in other species.

Table 1. List of PSS registered in other plants.

Species	Gene	Amino acid residue	Nucleotide identity(%)	Tissue type	Lineage
<i>Panax ginseng</i>	PSS	415		Leaf	Plant
<i>Panax ginseng</i> (Japan-1)		415	100		Plant
<i>Glycine max</i>		413	87		Plant
<i>Lotus corniculatus</i>	LjSqS	413	85	Root nodule	Plant

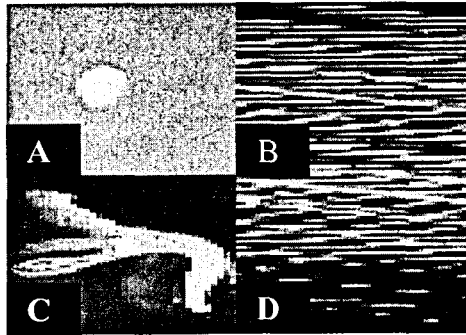


Fig. 2. Acquisition of ginseng transgenic ginseng growth on the medium. A : Nodular tissues formed on the surface of ginseng cotyledons harboring PSS, B : Cotyledon explants of transgenic secondary somatic embryos on the 100µg/ml Kanamycin, C : Transgenic plants with well developed roots.

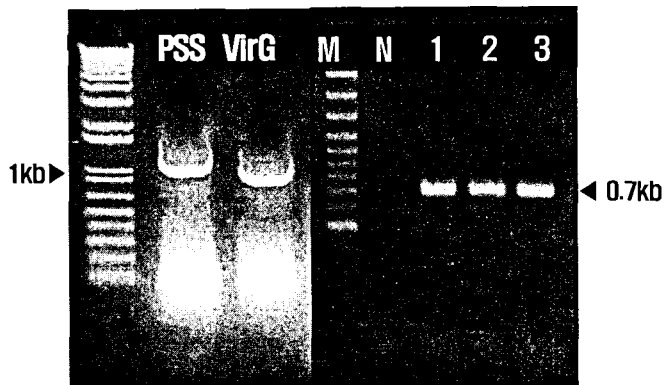


Fig. 3. PCR products of PSS and NPT gene from transgenic ginseng plant.

참고문헌

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