J. Ginseng Res.
Vol. 32, No. 2, 114-119 (2008)

Isolation and Characterization of Terpene Synthase Gene from *Panax ginseng*

Yu-Jin Kim*, Ah-Rom Ham*, Ju-Sun Shim*, Jung-Hye Lee*, Dae-Young Jung*, Jun-Gyo In, Bum-Soo Lee** and Deok-Chun Yang*#**

**Korean Ginseng Center for Most Valuable Products & Ginseng Genetic Resource Bank, Kyung Hee University, Suwon 449-701, Korea*

***Biopia Co., Ltd., Yongin 449-598, Korea*

(Received March 24, 2008; Accepted May 6, 2008)

Abstract : Terpene synthase plays a key role in biosynthesis of triterpene saponins (ginsenosides) and is intermediate in the biosynthesis of a number of secondary metabolites. A terpene synthase (PgTPS) cDNA was isolated and characterized from the root of *Panax ginseng* C.A. Meyer. The deduced amino acid sequence of *PgTPS* showed a similarity with *A. deliciosa* (AAX16121) 61%, *V. vinifera* (AAS66357) 61%, *L. hirsutum* (AAG41891) 55%, *M. truncatula* (AAV36464) 52%. And the segment of a terpene synthase gene was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR). We studied expression of terpene synthase under stressful conditions like chilling, salt, UV, and heavy metal stress treatment. Expression of *PgTPS* was increased gradually after exposure to stresses such as chilling, salt, and UV illumination. But its transcription seems to be reduced by cadmium and copper treatment.

Key words : *Panax ginseng*, terpene synthase, abiotic stress, cDNA, RT-PCR.

INTRODUCTION

Plants produce a vast and diverse arrays of low-molecular weight organic compounds, the overwhelming majority of which are secondary metabolites with nonessential, yet important functions such as defense[1,2]. Terpenes are useful for making defense compounds in many plants against herbivores and environmental stresses[3-5].

Terpene synthase (TPS) comprises of vast family of terpenes. TPS is very important enzyme, which is useful for making defense compounds in many plants against herbivores and environmental stress[6]. And terpene synthase gene plays a key role in biosynthesis of secondary metabolites including triterpene saponins (ginsenosides). Roots of *P. ginseng*, one of the most famous and widely used medicinal plants, contain at 25 different triterpene saponins[7]. Especially both tetracyclic dammarane- and pentacyclic oleanane-type triterpene saponins are produced in *P. ginseng* roots and they are referred to ginsenosides[8]. And secondary metabolites include various kinds of terpenes, such as mono, sesqui, di and triterpenes[9]. In *P. ginseng*, β-amyrin synthase (bAS) and cycloartenol synthase (CAS) belong to oxidosqualene cyclase (OSC) family that situates at the branching point for triterpene and sterol biosynthesis[5].

Ginsenosides have been shown to have pharmacological effects, including immune system modulation, anti-stress activities, and anti-hyperglycemic activities, anti-inflammatory, anti-oxidant and anti- cancer effects[10,11]. Therefore, over-accumulation of ginsenosides in transgenic *P. ginseng* by metabolic engineering can provide better quality of medicine. In this study, we report cloning of terpene synthase genes from *Panax ginseng* and provide detailed analyses on the expression profile of the genes in the defense response to abiotic stresses.

MATERIALS AND METHODS

1. Plant materials

Four-year old *Panax ginseng* plants grown at field were used for cDNA library construction. This material and cDNA were provided by Ginseng Genetic Resource Bank.

2. RNA purification and cDNA library construction

Total RNA was isolated from *p. ginseng* by an aqueous phenol extraction procedure[12]. A commercial cDNA syn-

#To whom correspondence should be addressed.
(Tel) +82-31-201-2688; (Fax) +82-31-202-2687
(E-mail) dcyang@khu.ac.kr

thesis kit was used to construct a library according to the manufacture's instruction manual (Clontech, PT3000-1, USA). Fractions containing cDNA greater than 500bp were recovered and this library was amplified to yield a final titer of 2×10^9 pfu ml^{-1}. Individual colonies were propagated and saved at -80°C until further use.

3. Nucleotide sequencing and sequence analysis

The pTriplEx phagemids were excised from the Uni-ZAP XR library and used as templates for sequence analysis. The 5' ends of randomly selected cDNA inserts were sequenced by an automatic DNA sequencer (ABI prism 3700). Nucleotide and amino acid sequence analyses were performed using DNASIS program (Hitachi). Comparison of sequences to DNA and protein databases at NCBI was performed using the blast algorithm[13]. The functional classification of EST clone was based on the results of a comparison to the non-redundant protein database of GenBank using the blastx algorithm. EST clone was annotated manually following the Munich Information Center for Protein Sequences (MIPS) role categorization[14].

We used ClustalX with default gap penalties to perform multiple alignment of glutaredoxins isolated in ginseng and previously registered in other species[15]. Based on this alignment, a phylogenetic tree was constructed according to the neighbor-joining method, using the MEGA3 programs[16]. Bootstrap analysis with 1,000 replicates was also conducted in order to obtain confidence levels for the branches[17].

4. Application of abiotic stresses

To investigate the response of the *PgTPS* gene to various stresses, we used the *P. ginseng* plantlets. *Panax ginseng* C. A. Meyer cv. "Hwang-Sook Jong" seeds (Ginseng Genetic Resource Bank, Korea) were culture on MS[18] basal medium 10 mg/L giberrellic acid, 3% (w/v) sucrose and 0.7% plant agar under controlled conditions of 25/18°C and a 16-h photoperiod from white fluorescent lamps. Healthy, 3-week-old plantlets were used for the treatments and nucleic acid extractions.

For chemical stress or plant hormone treatments, the plantlets were placed for various periods in MS media containing indicated concentrations of chemicals; 100 mM NaCl, 500 μM $CuSO_4$ and 500 μM $CdSO_4$. Chilling stress was applied by exposing the plantlets to a temperature of 4°C, and for the UV treatment, the plantlets were irradiated under UV lamps at 1.35 μE $m^{-2}s^{-1}$ for 1, 4, 8, 24, 48, or 72 h, repectively. In all cases, stress treatments were carried out on the MS media and 10 plantlets were treated with each stress. Control plants were held in a growth room at 25°C under a 16-h photoperiod. The stressed plant materials from all completed treatments were immediately frozen in liquid nitrogen and stored at -70°C until required.

5. Semi-quantitative RT-PCR Analysis

Total RNA was extracted from seedlings of *P. ginseng* using RNeasy mini kit (Qiagen, Valencia, CA, USA). For RT-PCR, 200 ng of total RNA was used as a template for reverse transcription using oligo$(dT)_{15}$ primer (0.2 mM) (INTRON Biotechnology, Inc., South Korea) for 10 min at 65°C. Then reaction mixture was incubated with AMV Reverse Transcriptase (10 U/μl) (INTRON Biotechnology, Inc., South Korea) for 60 min at 42°C. The reaction was inactivated by heating the mixture at 70°C for 5 min. The PCR reaction was then performed using a 1 μl aliquot of the first strand cDNA in a final volume of 20 μl containing 5 pmol of specific primers for coding region of *PgTPS* gene (forward, 5'- GAA GGA TGT GCG AGG AAT GT -3'; reverse, 5'- CTT TGT CGG AAT GAC GGA CT -3'). As a control, the primers specific to *P. ginseng* actin gene were used (forward, 5'- CGT GAT CTT ACA GAT AGC TTG ATG A -3'; reverse, 5'- AGA GAA GCT AAG ATT GAT CCT CC -3'). PCR was carried out using 2X Taq Premix (SolGent Co., South Korea) in a thermal cycler programmed as follows: an initial denaturation for 5 min at 96°C, 36 amplification cycles [20 s at 95°C (denaturation), 45 s at 57°C (annealing), and 1 min at 72°C (polymerization)], followed by a final elongation for 10 min at 72°C. Actin gene was PCR-amplified in the same PCR conditions as *PgTPS* gene with the same amplification cycles (36 cycles) and was used as an internal control to normalize each sample for variations in the amounts of RNA used. Seven ul of the reaction mixture was analyzed on a 1% (W/V) agarose gel in 1X TAE buffer and then photographed for the expression analysis.

RESULTS

1. Cloning and analysis of a PgTPS cDNA

From our expressed sequence tags (EST) analysis of a cDNA library, which was prepared from the root of *P. ginseng*, a cDNA clone encoding a terpene synthase gene was identified. We named this gene *PgTPS* (*P. ginseng* terpene synthase); its nucleotide sequence is presented in Fig. 1. The *PgTPS* cDNA was 1883 nucleotides long and had the putative open reading frame of 1707 bp. This ORF encodes a terpene synthase protein of 568 amino

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CTCCTCTTCAATATTCCTTTTCTATATATTATTTGAACATGGATGTGAATATTCTTGCTT 60
                                    M  D  V  N  I  L  A

CATCTGGTTCTTTTTCAGCCAAAAATAATGCGGTTGTGCCGTCCACAATTGTTCGACGCA 120
S  S  G  S  F  S  A  K  N  N  A  V  V  P  S  T  I  V  R  R

CAGCCAATTTCCATCCCAGCGTTTGGGGAGACCATTTTCTCGCATACACTAGCTCCGATC 180
T  A  N  F  H  P  S  V  W  G  D  H  F  L  A  Y  T  S  S  D

TCCTGAGAGCCGATGATGATACAGAGGGCAAATGTCAACTACTAAAAGAGGAAGTGAAGA 240
L  L  R  A  D  D  D  T  E  G  K  C  Q  L  L  K  E  E  V  K

AGATGCTATTGGCAGCTACCGATAAGCCACAAAAACTACTCAACTTAATTTCTGATATCC 300
K  M  L  L  A  A  T  D  K  P  Q  K  L  L  N  L  I  S  D  I

AACTATTAGGTGTGGCTTACCATTTTGAAGCTGAGATTGATGCGGCATTACAACACGTGA 360
Q  L  L  G  V  A  Y  H  F  E  A  E  I  D  A  A  L  Q  H  V

ATGCCACCTTTCATGAATATGTTGCTAGCAAAAAAGACCATGACCTTCACTTTGTATCAC 420
N  A  T  F  H  E  Y  V  A  S  K  K  D  H  D  L  H  F  V  S

TTTGTTTTCGATTACTTAGGCAAGATGGACATCATGTCTCTTCTGATGTGTTCTGCAAAT 480
L  C  F  R  L  L  R  Q  D  G  H  H  V  S  S  D  V  F  C  K

TCAAGGACAATGATGGGAAATTCAAGGAGTGCTTGACGAAGGATGTGCGAGGAATGTTAA 540
F  K  D  N  D  G  K  F  K  E  C  L  T  K  D  V  R  G  M  L

GCTTGTTTGAAGCAACACATCTTAGGGTTCACGGAGAAGATATACTTGAAGAAGCACTTG 600
S  L  F  E  A  T  H  L  R  V  H  G  E  D  I  L  E  E  A  L

CATTCACCACCTCTCAACTTCACCATCTCAAATCTCACTTACAAAATCATCCTCTTGCGG 660
A  F  T  T  S  Q  L  H  H  L  K  S  H  L  Q  N  H  P  L  A

CCCATGTTACTCATGCACTTAAGATGCCCATCCGCAAGGGTTTAACAAGGCTGGAAGCCA 720
A  H  V  T  H  A  L  K  M  P  I  R  K  G  L  T  R  L  E  A

GGCGTTACATATCCATTTATCATGAACATGATTCACATGATAAAGTGCTCTTAGAATTTG 780
R  R  Y  I  S  I  Y  H  E  H  D  S  H  D  K  V  L  L  E  F

CAAAATTAGACTTTAACAAATTGCAAAAAGTTCATCAAGGAGAGCTAAGCTATATTACCA 840
A  K  L  D  F  N  K  L  Q  K  V  H  Q  G  E  L  S  Y  I  T

GGTGGTGGAAAGATTTAAATTTTGCAGAGAAACTGTCTTTTGCAAGAGATAGAATGGTGG 900
R  W  W  K  D  L  N  F  A  E  K  L  S  F  A  R  D  R  M  V

AGTGCTACTTTTGGACATTAGGGGTTTATTTTGAGCCACAATACCTCCTGGCCCGAAGAT 960
E  C  Y  F  W  T  L  G  V  Y  F  E  P  Q  Y  L  L  A  R  R

TAATGACAAAGACAATTTCAATGTTATCGATTATTGATGACATATATGACGCTTTTGGTA 1020
L  M  T  K  T  I  S  M  L  S  I  I  D  D  I  Y  D  A  F  G

CTTTCGAAGAACTTGTGATCTTCACGGATGCAATTGAAAGGTGGGATATTAGTGCATTAG 1080
T  F  E  E  L  V  I  F  T  D  A  I  E  R  W  D  I  S  A  L

ATCAGCTACCAGTATACATGAGACTTTGTTATCAAGCTCTCTTAGATGTCTATACTGAAA 1140
D  Q  L  P  V  Y  M  R  L  C  Y  Q  A  L  L  D  V  Y  T  E

TGGAGGAAGAAATGGCCAGGGCAGGAAGACCATTATACCGTGTTCACTACGCAAAATATG 1200
M  E  E  E  M  A  R  A  G  R  P  L  Y  R  V  H  Y  A  K  Y

AGCTCAAAAGATTAGTTAAAGCATACTTTGAAGAAGCTAAATGGCTTCACAAAGGCTACA 1260
E  L  K  R  L  V  K  A  Y  F  E  E  A  K  W  L  H  K  G  Y

TTCCAACAATGGAGGAGCATATGGAGGTGGCACTCGTATCCGCAGGTTATATGATGATAT 1320
I  P  T  M  E  E  H  M  E  V  A  L  V  S  A  G  Y  M  M  I

CAACCACTTCCTTTGTCGGAATGACGGACTTGGCAACCAAGGAGGCCTTCGAATGGGTAT 1380
S  T  T  S  F  V  G  M  T  D  L  A  T  K  E  A  F  E  W  V

CTAATGATCCTTTAATTGTTAGGGCTTCGTCCGTGATTAACCGACTCACTGACGACATTG 1440
S  N  D  P  L  I  V  R  A  S  S  V  I  N  R  L  T  D  D  I

TGGGGCATGAGATAGAGCAGGAGAGAGGACATGTAGCCTCAGGGGTTGAGTGCTACATGA 1500
V  G  H  E  I  E  Q  E  R  G  H  V  A  S  G  V  E  C  Y  M

AACAACATGGTGTTCCAAAGGAAGATGTTTATGCCGAATTTCAGAAACAAATTGTAAATG 1560
K  Q  H  G  V  P  K  E  D  V  Y  A  E  F  Q  K  Q  I  V  N

CTTGGAAGGATATGAACCAGGAATGCCTTCGTCCCACGGCGGTTGCAATGCCTCTTCTTA 1620
A  W  K  D  M  N  Q  E  C  L  R  P  T  A  V  A  M  P  L  L

CTAGAGTTTTCAATCTGGCACGCGTCATAAATCTTCTGTACGATGATTTTGATGGCTACT 1680
T  R  V  F  N  L  A  R  V  I  N  L  L  Y  D  D  F  D  G  Y

CAAATTCGAAAACTAGGACCAAAGATTTGATAACCTCCGTGCTCATAGATCCTGTTCCCA 1740
S  N  S  K  T  R  T  K  D  L  I  T  S  V  L  I  D  P  V  P

TATAAGGATAGATGAAAGGCAGTTGGTTTGTTCATCATGCTACTTGGGGTTAAATGTTAA 1800
I  *  G  *  M  K  G  S  W  F  V  H  H  A  T  W  G  *  M  L

ATAAAGTGTTATGATCATTATAGTAGTAAGTTTATTAATGGATTGTTAAACACCACCACT 1860
N  K  V  L  *  S  L  *  *  *  V  Y  *  W  I  V  K  H  H  H

TTAAAAAAAAGAAAAAAAAAAAA 1883
F  K  K  K  K  K  K  K
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Fig. 1. Nucleotide and deduced amino acid sequence of a terpene synthase cDNA isolated from root of Panax ginseng. The deduced amino acid sequence is shown in single letter code below the nucleotide sequence. Numbers to the right refer to nucleotides.

acids, beginning at the initiation codon ATG (Position 38) and ending at the stop codon TAA (Position 1745) of the cDNA.

2. Homology Analysis

BLASTX database searches with terpene synthase sequence various other plant species as the top best matches. The deduced amino acid sequence of PgTPS was compared to other related gene products registered previously. The deduced amino acid of terpene synthase showed the highest similarity (61%) with the germacrene-D synthase from *A. deliciosa* and the terpene synthase from *V. vinifera*. Only 61% of similarity with other plants suggests that this *P. ginseng* gene is not characterized very

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P.ginseng       EATHLRVHGEDILEEALAFTTSQLHHLKSHLQNH--PLAAHVTHAL-KMPIRKGLTRLEA 227
O.basilicum     EASNYGMEGEDILDKALEISTSHLEPLASRSR--------RINEAL-EMPISKTLVRLGA 210
L.hirsutum      EASYLRVRDEEILEEALAFTTTHLKSIVSTMSNNNNSLKVEVSEAL-TQPIRMTLPRMEA 203
L.esculentum    EASHLRVRNEEILEEALTFTTTHLESIVSNLSNNNNSLKVEVGEAL-TQPIRMTLPRMGA 211
M.truncatula    EATHMRIHGEEILDDALSFTSLHLEMMTTQLSPS---VATKINHSL-KRPLFKNLPRLVA 222
A.deliciosa     EATCLRIHGEDILDEALAFTITQLRSALPNLSTP---FKEQIIHAL-NQPIHKGLTRLNA 224
V.vinifera      EATHFRVHGEDILDEALAFTTTHLQSATKHSSNP---LAEQVVHAL-KQPIRKGLPRLEA 217
C.sinensis      EAAYMAVRGEHILDEAIAFTTTHLKSLVAQDHVTP-KLAEQINHAL-YRPLRKTLPRLEA 206
C.junos         EAGHLAIRGEGILDEAIAFTRTHLQSMVSQDVCPN-NLAEQINHTL-DCPLRRALPRVET 216
S.tuberosum     EASHVRTHGEDILEEALVFSTAHLESAAPHLESP---LSKQVTHAL-EQSLHKSIPRVET 211
C.annuum        EASHVRSHGEDILEDALAFSTTHLESATPHLEYP---LKEQVRHAL-EQSLHKGIPRIEI 218
G.arboreum      EASYMRVHGEDILDEAISFTTAQLTLALPTLHHP---LSEQVGHAL-KQSIRRGLPRVEA 215
A.annua         EATYLRVQGEGVLDDALVFTRTCLEKIAKDLVHTNPTLSTYIQEAL-KQPLHKRLTRLEA 208
C.sativus       EASHMRGHGEALLEEALEFTTTHLKAYIHLYSNINPNFASEVSNAL-KLPIRKCVPRVKA 227
P.cablin        EATHLRVHGEDVLDNAFDFTRNYLESVYATLNDP---TAKQVHNALNEFSFRRGLPRVEA 211
                **     ..* :*:.*: ::   *                 : .:*   .:   : *:   56

P.ginseng       RRYIS-IYH--EHDSHDKVLLEFAKLDFNKLQKVHQGELSYITRWWKDLNFAEKLSFARD 284
O.basilicum     RKFIS-IYE--EDESRDEDLLKFAKLDFNILQKIHQEELTHIARWWKELDLGNKLPFARD 267
L.hirsutum      RRYIS-IYE--NNDAHNHLLLKFAKLDFNMLQKLHQRELSDLTRWWKDLDFANKYPYARD 260
M.truncatula    RHYIS-IYE--NNDAHNHLLLKFAKLDFNLLQKLHQRELCEITRWWKDLNFAKTLPFARD 281
A.deliciosa     RSHIL-FFE--QNDCHSKDLLNFAKLDFNLLQKLHQRELCEITRWWKDLNFAKTLPFARD 281
V.vinifera      RHYFS-VYQ--ADDSHNKALLKLAKLDFNLLQKLHQKELSDISAWWKDLDFAHKLPFARD 274
C.sinensis      RYFMS-MINSTSDHLCNKTLLNFAKLDFNILLELHKEELNELTKWWKDLDFTTKLPYARD 265
C.junos         RFFLS-VYP--RDDKHDKTLLKFSKLDFNLVQRIHQKELSAITRWWKDLDFTTKLPYARD 273
S.tuberosum     RYFIS-IYE--EEEFKNDVLLRFAKLDYNLLQMLHKHELSEVSRWWKDLDFVTTLPYARD 268
C.annuum        QFFISSVYD--KQAIKNDVLLRFAKLDYNMLQMLHKQELAEVSRWWKDLNFVNTLPYARD 276
G.arboreum      RNFIS-IYQ--DLESHNKSLLQFAKIDFNLLQLLHRKELSEICRWWKDLDFTRKLPFARD 272
A.annua         LRYIP-MYE--QQASHNESLLKLAKLGFNLLQSLHRKELSEVSRWWKGLDVPNNLPYARD 265
C.sativus       REYFE-IYQ--QQPSHNETLLTFSKLDFNILQKLHQKEIAEICRWWKDLNVSTNFPFARD 284
P.cablin        RKYIS-IYE--QYASHHKGLLKLAKLDFNLVQALHRRELSEDSRWWKTLQVPTKLSFVRD 268
                  .:                ** ::*:.:* :   *: *:     *** *:.  . .:.*: 78

P.ginseng       RMVECYFWTLGVYFEPQYLLARRLMTKTISMLSIIDDIYDAFGTFEELVIFTDAIERWDI 344
O.basilicum     RVVECYFWILGVYFEPQYNIARRFMTKVIAMTSIIDDIYDVHGTLEELQRFTDAIRSWDI 327
L.hirsutum      RLVECYFWILGVYFEPKYSRARKMMTKVLKMTSIIDDTFDAYATFDELEPFNDAIQRWDA 320
L.esculentum    RLVECYFWILGVYFEPKYSRARKMMTKVLNLTSIIDDTFDAYATFDELVTFNDAIQRWDA 328
M.truncatula    RIVEAYFWILGVYFEPQYSFGRRIMTKVISLASVIDDIYDVYGTIEELQLFTQAIERWDI 339
A.deliciosa     RMVECYFWILGVYFEPQYLLARRMLTKVIAMISIIDDIYDVYGTLEELVLFTDAIERWEI 341
V.vinifera      RVVECYFWILGVYFEPQFFFARRILTKVIAMTSIIDDIYDVYGTLEELELFTEAVERWDI 334
C.sinensis      RLVELYFWDLGTYFEPQYAFGRKIMTQLNYILSIIDDTYDAYGTLEELSLFTEAVQRWNI 325
C.junos         RIVELYFWIVGTYFEPKYTLARKIMTKTIYTASIIDDTFDAYGFFEELKLFAEAVQRWDI 333
S.tuberosum     RAVECYFWTMGVYAEPQYSQARVILAKTIAMISIVDDTFDAYGIVKELEVYTDAIQRWDI 328
C.annuum        RVVECYFWALGVYYEPQYSQARVMLVKTIAMISIVDDTYDAYGTVDELAIYTDVIQRWDI 336
G.arboreum      RVVEGYFWIMGVYFEPQYSLGRKMLTKVIAMASIVDDTYDSYATYDELIPYTNAIERWDI 332
A.annua         RMVECYFWALGVYFEPKYSQARIFLAKVISLATVLDDTYDAYGTYEELKIFTEAIQRWSI 325
C.sativus       RIVECYFWILSIYFEPYFKFGRKILTKVIAMTSIMDDIYDAYGTFEELQLFTLAIKRWDM 344
P.cablin        RLVESYFWASGSYFEPNYSVARMILAKGLAVLSLMDDVYDAYGTFEELQMFTDAIERWDA 328
                * ** ***  . * ** :  .* ::.:     :::** :* ..  .**  :  .:. *.  104
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Fig. 2. Multiple alignment of the deduced amino acid sequences of *PgTPS* from other plant species; *A. deliciosa* (AAX16121), *V. vinifera* (AAS66357), *L. hirsutum* (AAG41891), *L. esculentum* (AAG41890), *M. truncatula* (AAV36464), *C. sativus* (AAU05952), *C. sinensis* (AAQ04608), *O. basilicum* (AAV63786), *C. junos* (AAG01339). Alignment of sequences was performed using Clustal X. Amino acid positions are shown on the right. Identical amino acid residues in all sequences are indicated by asterisks. Semicolons indicate residues that are mostly conserved (identity in at least four out of seven amino acids). Gaps that were introduced for optimal alignment are marked with dashes.

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P.ginseng       SALDQLPVYMRLCYQALLDVYTEMEEEMARAGRPLYRVHYAKYELKRLVKAYFEEAKWLH 404
O.basilicum     RAIDELPPYMRLCYEALLGMYAEMENEMVKQNQ-SYRIEYARQEMIKLVTTYMEEAKWCY 386
L.hirsutum      NAIDSIPPYMRPAYQAFLDIYSEMEQVLSKKGK-LDRVYYAKNEMKKLVRAYFKETQWLN 379
L.esculentum    NAIDSIQPYMRPAYQALLDIYSEMEQVLSKEGK-LDRVYYAKNEMKKLVRAYFKETQWLN 387
M.truncatula    SCMDFLPQYMKFCYKAVLDVYEEMEQEMVKEGR-AFCVFYAKNEMKRLVQAYFTEAKWFS 398
A.deliciosa     SALDQLPEYMKLCYQALLDVYSMIDEEMAKQGR-SYCVDYAKSSMKILVRAYFEEAKWFH 400
V.vinifera      SAIDQLPEYMRVCYQALLYVYSEIEEEMAKEGR-SYRLYYAKEAMKNQVRAYYEEAKWLQ 393
C.sinensis      EAVDMLPEYMKLIYRTLLDAFNEIEEDMAKQGR-SHCVRYAKEENQKVIGAYSVQAKWFS 384
C.junos         GAMDILPEYMKVLYKALLDTFNEIEQDLAKEGR-SSCLPYGKEKMQELVQMYFVQAKWFS 392
S.tuberosum     SQIDRLPEYMKVSFKALLDLYEDYEKELSKDGR-SDVVHYAKERMKEIVRNYFVEAKWFI 387
C.annuum        KEIDSLPDYMKISYKALLDLYKDYEKEMSRDGR-SHVVYYAKERLKELVKSYNIEAKWFI 395
G.arboreum      KCMNQLPNYMKISYKALLDVYEEMEQLLANQGR-QYRVEYAKKAMIRLVQAYLLEAKWTH 391
A.annua         TCIDMLPEYLKLLYQGVLDIYIEMEEIMGKEGK-AHHLSYAKESMKEFIRSYMMEAKWAN 384
C.sativus       SMVNLLPQYMKVHYTTLLDLFEEMDKGIVNDGI-SYRSCFGKEAMKRQAESYFKEAEWLN 403
P.cablin        SCLDKLPDYMKIVYKALLDVFEEVDEELIKLGA-PYRAYYGKEAMKYAARAYMEEAQWRE 387
                  :: :  *:: : .* :  :: : . .      :.:       * :::*  123

P.ginseng       LTDDIVGHEIEQERGHVASGVECYMKQHGVPKEDVYAEFQKQIVNAWKDMNQEC-LRPTA 521
O.basilicum     LMDDVVGHGIEQK----ISSVDCYMKENGCSKMEAVGEFSKRVKKAWKNLNEEW-VEPRA 500
L.hirsutum      AMNDIVGHEVEQERGHVASLIECYMKDYGASKQEAYAKFKKDVTNAWKDINKEF-FRPTE 497
L.esculentum    AMNDIVGHEDEQERGHVASLIECYMKDYGASKQETYIKFLKEVTNAWKDINKQF-FRPTE 505
M.truncatula    LMDDIVSNEFEQERGHGASAIECYMNEHGVSREDAVNELSRQVTNAWKDTNEEL-LDPTE 515
A.deliciosa     LKDDIVGHKFEQKRGHVASAVECYSKQHGTTEEEAIIELDKQVTHSWKDINAEC-LCPIK 517
V.vinifera      LMDDMVFHKFEQKRGHVASAVECYMKQHGASEQETPNEFPQPVREAWKDINEEC-LIPTA 510
C.sinensis      LMDDMQGHEFEQKRGHVASAIECYTKQHGVSKEEAIKMFEEEVANAWKDINEELMMKPTV 502
C.junos         LRNDIVSHQFEQKRGHIASGVECYIKQYGVSAEEVVTVFTEEVENAWKDMNEEF-LKPTA 509
S.tuberosum     VVDDIATYEVEKGRGQIATGIECYMRDYDVSTEVAMEKFQEMAEIAWKDVNEGI-LRPTP 503
C.annuum        VIDDIATYEVEKNRGQLSTGIECYMRDYSVSTKEAMAKFQEMGESGWKDINEGM-LRPTP 511
G.arboreum      FMDDIAEHKFNHRREDDCSAIECYMKQYGVTAQEAYNEFNKHIESSWKDVNEEF-LKPTE 508
A.annua         LMDDIHSQKEEKERIHVASSVESYMKQYDVTEEHVLKVFNKKIEDAWKDITRES-LVRKD 501
C.sativus       LMDDVVSYKFEKEREHIVSAVECYMSNHGRSEDETCAELLKQVEDAWKTINECC-LHPMN 520
P.cablin        LVNDITGHEFEKKREHVRTAVECYMEEHKVGKQEVVSEFYNQMESAWKDINEGF-LRPVE 505
                  :*:     ::      : ::.* :       .  : .   .**  .   .     147

P.ginseng       VAMPLLTRVFNLARVINLLYDDF-DGYSNSKTRTKDLITSVLIDPVPI- 568
O.basilicum     ASMVILVRVVNLARVINLLYVGE-DSYGNSSVKTKELIKGVLVHPIK-- 546
L.hirsutum      VPMFVLERVLNLTRAAEPLYKEK-DAYTNAKGKLKNMINSILIESVKI- 544
L.esculentum    VPMFVLERVLNLTRVADTLYKEK-DTYTNAKGKLKNMINSILIESVKI- 552
M.truncatula    VPKPLLIRVLNLSRVIDVLYKDG-DCYTNSQGSTKNDIISILLNPCPV- 562
A.deliciosa     VPMPLLARVLNLARVLYVIYQDE-DGYTHPGTKVENFVTSVLIDSMPIN 565
V.vinifera      VPMPILMRVLNLARVIDVIYKNE-DGYTHFGAVLKDFVTSMLIDPVPI- 557
C.sinensis      VARPLLGTILNLARAIDFIYKED-DGYTHS-YLIKDQIASVLGDHVPF- 548
C.junos         FPVALIERPFNIARVIEFLNKKG-DWYTHS-HAIKDQIAAVLRDPVTI- 555
S.tuberosum     VSTEILTRILNLARIIDVTYKHNQDGYTHPEKVLKPHIIALLVDSIEI- 551
C.annuum        IPMEFLSRILNLARLVDVTYKHNEDGYTHPEKVIKPHIIAMVVDSFKI- 559
G.arboreum      MPTPVLCRSLNLARVMDVLYREG-DGYTHVGKAAKGGITSLLIDPIQI- 555
A.annua         IPMPLMMRVINLAQVMDVLYKHK-DGFTNVGEELKDHIKSLLVHPIPI- 548
C.sativus       VPMPFLICLLNLTRVMALLYSHE-DGYTNSKGRTKLLIQSLLIDPLHL- 567
P.cablin        FPIPLLYLILNSVRTLEVIYKEG-DSYTHVGPAMQNIIKQLYLHPVPY- 552
                 .  .:   .* :          * : :    :  :  :  .      156
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Fig. 2. Continued.

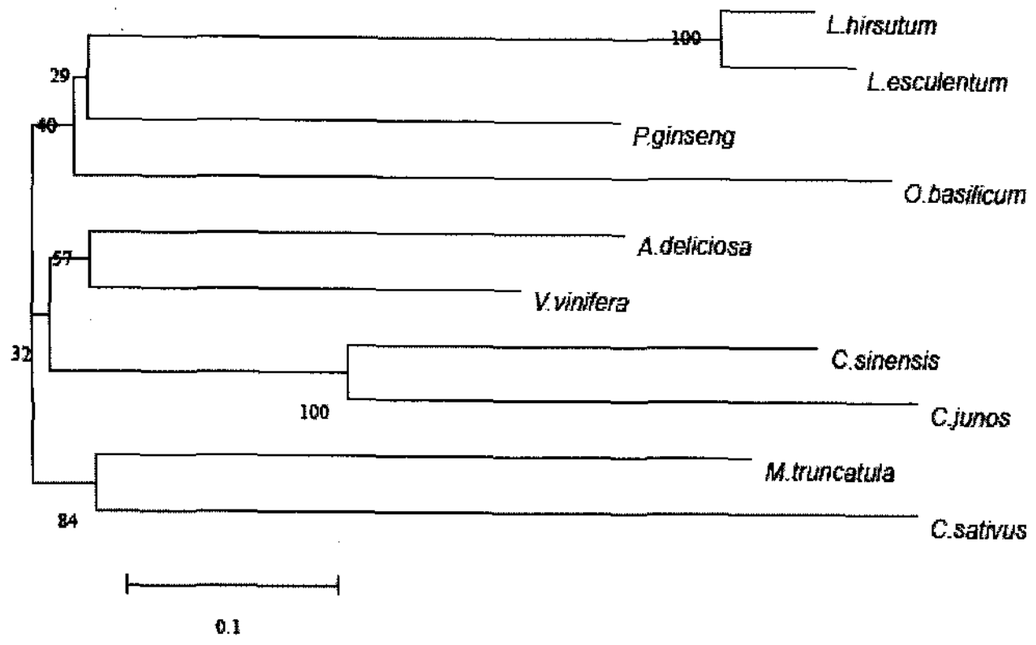

Fig. 3. Phylogenetic relationship of terpene synthase proteins from *P. ginseng* and other plants. Phylogenetic analysis is based on the deduced amino acid sequences of terpene synthase genes from various plant species. Neighbor-joining method was used and the branch lengths are proportional to divergence, with the scale of 0.1 representing 10% changes.

well in other organisms. An alignment of terpene synthase sequence available from NCBI and GenBank libraries was performed using the Clustal method. The result identified several regions of homology. The multiple alignments revealed regions of high homology as illustrated in (Fig. 2). A phylogenetic analysis of 9 different plants has been carried out using the Clustal method (Fig. 3).

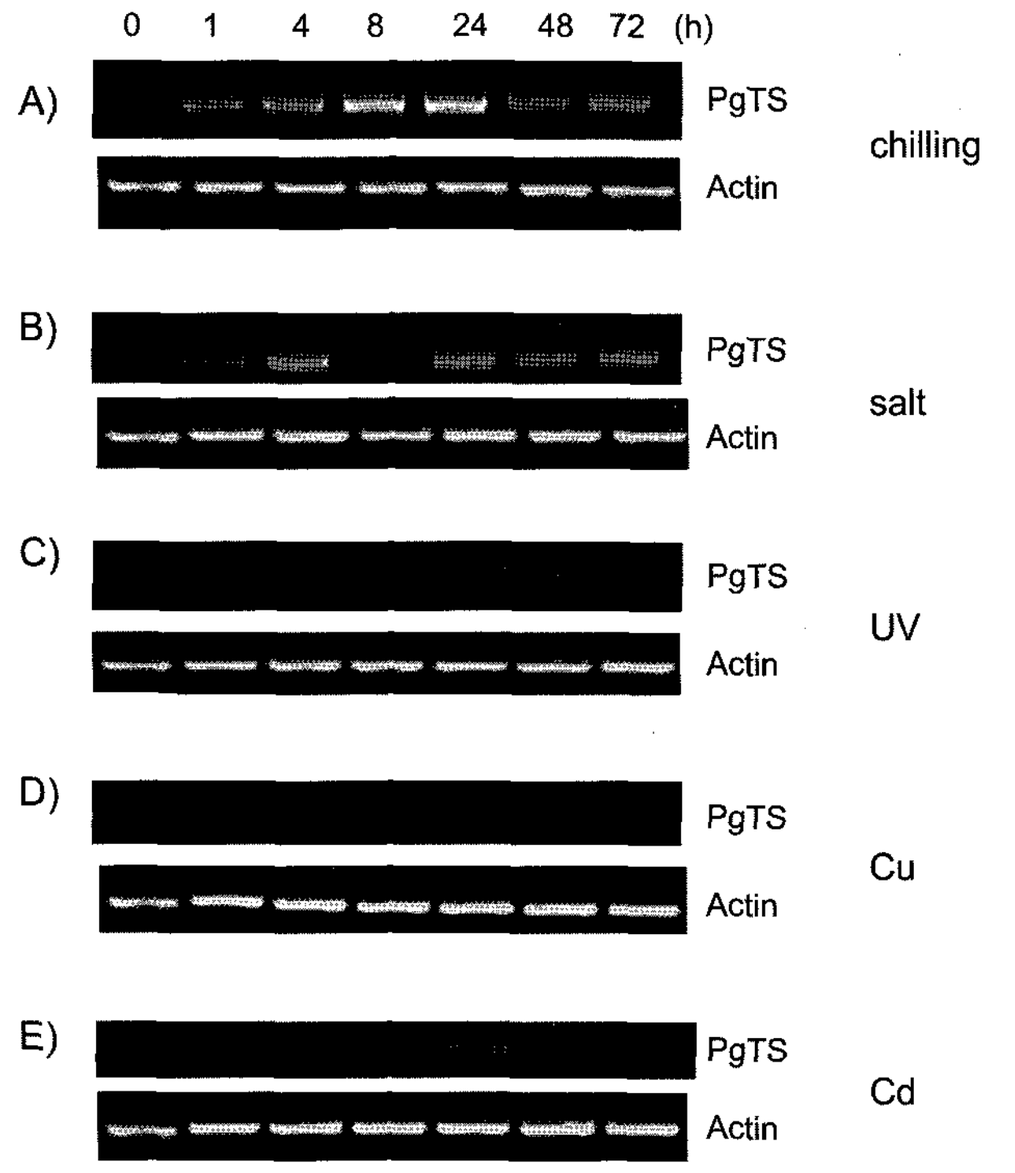

Fig. 4. RT-PCR analysis of the expression of the *PgTPS* genes in seedlings of *Panax ginseng* at various time points (h) post-treatment with various stresses. A, chilling; B, 100 mM NaCl; C, UV light; D, 500 uM CuSO4; E, 500 uM CdSO4. Actin was used as an internal control.

3. Differential expression of terpene synthase under abiotic stress

The expression patterns of the *PgTPS* gene after exposure to various abiotic stresses, such as chilling, salt, heavy metal, and UV were investigated by RT-PCR. When ginseng plantlets exposed to chilling or salt stresses, the *PgTPS* expression profiles were similar. Under chilling stress, *PgTPS* mRNA was gradually accumulated at corresponding time points and peaked at 24 h. After 48 h of chilling stress treatment, *PgTPS* expression was decreased, but the expression level was still higher, and then increased again at 72 h (Fig. 4A). A salinity stress (100 mM NaCl) also caused a rapid induction immediately, *PgTPS* gene transcription level was the highest at 4 h post-treatment (Fig. 4B), transcription was reduced at 8 h treatment, and then it was restored from 24 h. During UV exposure, the transcriptional expression of *PgTPS* was strongly expressed at 1 h, and then reduced gradually,

however it was higher than normal. At 1 day after treatment under UV exposure, it was strongly transcripted once again (Fig. 4C). The *PgTPS* gene expression seemed to be inhibited similarly by heavy metal stresses, such as Cu or Cd. A copper stress (500 uM $CuSO_4$) treatment caused induction immediately at 1 h and then reduction at 4 h. After that, *PgTPS* recovered to normal transcript level at 8 h; however, its expression was dramatically decreased after 1 day after treatment (Fig. 4D). With 500 μM Cadmium treatment, although *PgTPS* transcript was induced at first, it was gradually decreased to 8 h, shown lower than control. Later, *PgTPS* was transcripted strongly at 24 h and inhibited once again, as Cu treatment (Fig. 4E).

DISCUSSION

We report here the functional characterization of cDNA clone, terpene synthase gene from *Panax ginseng*, originally obtained from cDNA libraries. Terpene synthase gene encoded a polypeptide 568 amino acid residues with 49~61% identities to the terpene synthase gene sequences from other plant species, respectively. The highest similarity is just 61% with other plants so this gene from *Panax ginseng* is a study of unexplored value. This terpene synthase gene has not been known that is involved in process of *Panax ginseng* exactly. TPS catalyze the formation of the most abundant and structurally diverse group of natural metabolites in plants. The divergent evolution of TPSs, their ability to form multiple products, and their differential expression that is related with development or stress, have known to drive the complexity and plasticity in terpene production[6].

To investigate the expression of a *PgTPS* gene related with abiotic stresses, such as chilling, UV exposure, salt and heavy metals, we performed quantitative RT-PCR analysis using the plantlet of *P. ginseng*. In our study, the expression of *PgTPS* gene was highly expressed in 24h after treatment by chilling, salt, UV stresses. That is, defense substances is the result of increased gene expression within the abiotic stress affected plantlets of *Panax ginseng*. The increased levels of TPS transcripts were accompanied by major changes in terpene accumulation, a response against defense. In rice, biosynthesis of terpene is increased and TPS is induced by elicitor and UV treatment[6]. Plants generally produce secondary metabolites as a defense mechanism against environmental stresses. Secondary metabolites are low-molecular weight organic compounds, and do not seem to be necessary for growth. Nevertheless, many of these natural products have important roles in plant defense and allelopathy[6]. Terpene metabolites are involved in several ecological and physiological functions on the basis of the differential expression profiles of TPS genes observed in response to biotic and abiotic environmental factors[19,20].

TPSs capable of synthesizing sesquiterpenes and monoterpenes have been reported in several plants[21-24]. In case of *Panax ginseng*, triterpene saponins were increased by sodium chloride treatment or UV irradiation, even if it inhibited root growth factors[25,26], suggesting that *PgTPS* transcription is related saponin synthesis and is able to regulated by abiotic stresses.

It is the first time study of various environmental stresses about terpene synthase gene of *Panax ginseng*. However, the possible role for TPS in development or biotic stress remains elusive. Therefore, we will continuously study further to find relations between *PgTPS* and biotic stress or development and then produce the transformant by re-introduction of *PgTPS* into *P. ginseng*. These approaches will improve our understanding of the role of TPS and terpenes in plant-environment interactions. Interestingly, if this gene is concerned about ginsenosides biosynthesis, over-accumulation of ginsenosides in *P. ginseng* by specific stress treatment or over-expression of this gene is anticipated to provide better quality of medicine.

ACKNOWLEDGEMENTS

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (No. R01-2006-000-11178-0).

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