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Cloning and Characterization of Soybean IFS (Isoflavone Synthase) Genes from Korean Cultivar, Sinpaldalkong

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Two genes, *SinIFS1* and *SinIFS2* from Korean soybean cultivar, Sinpaldalkong known as one of isoflavone-rich cultivars, were cloned with PCR and degenerate primers. The sequences of two genes were analyzed with previously reported IFS genes of leguminous plants and their expression pattern in various environmental conditions was surveyed. The genomic clone of *SinIFS1* contained 1,828bp nucleotides and encoded a polypeptide of 521 amino acids, and 1912bp nucleotides and a polypeptide of 521 amino acids for *SinIFS2*. Both genes included several conserved motifs, oxygen binding and activation (A/G-G-X-E/D-T-T/S), ERR triad (E...R....R), and heme binding (F-X-X-G-X-R-X-C-X-G) domain, which are typical in any member of cytochrome P450 superfamily. Very high sequence homology (>98%) was observed in the comparison with other IFSs of legumes. In the northern blot analysis to check the expression and increase of *SinIFS1* to various environmental conditions (low temperature, light, dark, UV, and fungal elicitor), the most significant induction, more than 6 times of transcript level compared to the dark treatment as a control, was observed from the fungal elicitor treatment. The next up-regulated expression was from UV treatment (4×), low temperature and light conditions.

Key words – IFS, PCR, degenerate primer, cytochrome P450

The remarkable attention of large research groups has become focused on the synthesis of isoflavone and its related compounds in plant. The most interesting feature of these secondary metabolite stems from the fact that isoflavones and related compounds can act as estrogens in human, even though they were often the product of defense related response against pathogen attack and stressful conditions, such as low temperature. However, the anti-cancer activity of the isoflavonoids is not applicable widely to human health due to their taxonomically limited presence in the Leguminosae and poor dietary habit of westerners with these plant species [5,9].

Isoflavones are produced through a branch of the phenylpropanoid pathway of secondary metabolism [3]. After production of flavanone catalyzed by chalcone isomerase, 2-hydroxylation coupled to aryl migraton of the B-ring of a flavanone yields the corresponding isoflavone (genistein or daidzein) mediated by 2-hydroxyisoflavanone synthase (2-HIS or IFS) [11]. The gene for aryl migration enzyme (IFS) has been cloned from soybean, where these phytoestrogen accumulated in seeds as well as isoflavonoid-derived phytoalexin (glyceollin) in pathogen on infected tissues [2]. With the successful founding of the gene for IFS, researchers could initiate the potential engineering of isoflavone production in wide variety of plant normally lacking IFS, but containing its substrate. Fortunately, many plant species produce IFS substrate, flavanone, as a intermediate for tannins and anthocyanins [8].

Domestically, the important physiological function of isoflavone was acknowledged in breeding field and analytical research to seek high isoflavone content was carried [7]. The result demonstrated that individual genetic background as well as environmental condition, such as low temperature, affected the amount of isoflavone accumulation in soybean. In this study, we cloned two genes, *SinIFS1* and *SinIFS2* from Korean soybean cultivar, Sinpaldalkong, known as one of isoflavone-rich cultivars, and demonstrated their sequence homology to pre-existed IFSs and expression on various conditions.

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Materials and Methods

Plant materials and genomic DNA extraction

The seeds of Sinpaldalkong was sown to plastic pot

containing vermiculite and grown under 12hrs light/8hrs dark cycle at room temperature. Twenty days old seedlings were harvested, immediately frozen in liquid nitrogen and stored at -70℃ until used for DNA isolation. Soybean genomic DNA was prepared as described previously [6].

SinIFS1 and SinIFS2 amplification by PCR

Genomic fragment containing *IFS1* was produced by PCR with primers:5′-TGCTGGAACTTGCACTTGGT-3′ and 5′-GTATATGATGGG TACCTTAATTAAGAAAGGAG-3′. A DNA fragment containing *IFS2* was produced with primers:5′-AAAATTAGCCTCACAAAAGCAAAG-3′ and 5′-GCAAACGAAGACAAATGGGAGATGATA-3′ [2]. These PCR fragments were cloned into the pCR2.1 vector (Invitrogen, USA) and sequenced. Homology search was initially carried out through BLAST Network Service (National Center for Biotechnology Information, USA) and nucleotide and deduced amino acid sequences were analysed using PC/GENE program (Intelligenetics, Switzerland)

Northern analysis

For northern blot, plant materials (20 day-old seedlings) were exposed to various conditions; low temperature (5℃), light, dark, UV light, and yeast extract (10%) for 6hrs. The detailed expression patterns at low temperature were investigated according to the different treatment time from 1hr to 6 hrs. Total RNA was purified by Chomczynski's method [1]. About 20 μg of total RNA was electrophoresed on 1% agarose-formaldehyde gel, and transferred to Hybond-N^+ membrane by capillary blotting. The membranes were hybridized with the ^{32}P-labeled probe at 65℃ for 20 hrs. The probe was produced using Ladderman™ Labeling Kit (Takara, Japan). Membranes were washed twice with 0.2× SSC, 0.1% SDS under high stringency condition at 42℃ for 20min and at 65℃, for 30min/3 replicates. The membranes were exposed to X-ray film.

Results and Discussions

Two genes, *SinIFS1* and *SinIFS2* were cloned from the Korean soybean cultivar Sinpaldalkong with two different sets of primer originally designed to amplify cytochrome P450. The PCR reaction generated a single band amplification at the size of about 1.8kb, and subsequently subcloned and sequenced (Fig. 1, 2). The genomic clone of *SinIFS1* contained 1,828bp nucleotides and encoded a

```
ggggatcccacgatgttgctggaacttgcacttggtttgtttgtgttagctttgtttctg
            M  L  L  E  L  A  L  G  L  F  V  L  A  L  F  L
cacttgcgtcccacaccaagtgcaaaatcaaaagcacttcgccacctcccaaaccctcca
 H  L  R  P  T  P  S  A  K  S  K  A  L  R  H  L  P  N  P  P
agcccaaagcctcgtcttcccttcattggccaccttcacctcttaaaagataaacttctc
 S  P  K  P  R  L  P  F  I  G  H  L  H  L  L  K  D  K  L  L
cactatgcactcatcgatctctccaaaaagcatggcccctattctctctctccttcggc
 H  Y  A  L  I  D  L  S  K  K  H  G  P  L  F  S  L  S  F  G
tccatgccaaccgtcgttgcctccacccctgagttgttcaagctcttcctccaaacccac
 S  M  P  T  V  V  A  S  T  P  E  L  F  K  L  F  L  Q  T  H
gaggcaacttccttcaacacaaggttccaaacctctgccataagacgcctcacttacgac
 E  A  T  S  F  N  T  R  F  Q  T  S  A  I  R  R  L  T  Y  D
aactctgtggccatggttccattcggaccttactggaagttcgtgaggaagctcatcatg
 N  S  V  A  M  V  P  F  G  P  Y  W  K  F  V  R  K  L  I  M
aacgaccttctcaacgccaccaccgtcaacaagctcaggcctttgaggacccaacagatc
 N  D  L  L  N  A  T  T  V  N  K  L  R  P  L  R  T  Q  Q  I
cgcaagttccttagggttatggcccaaagcgcagaggcccagaagccccttgacgtcacc
 R  K  F  L  R  V  M  A  Q  S  A  E  A  Q  K  P  L  D  V  T
gaggagcttctcaaatggaccaacagcaccatctccatgatgatgctcggcgaggctgag
 E  E  L  L  K  W  T  N  S  T  I  S  M  M  M  L  G  E  A  E
gagatcagagacatcgctcgcgaggttcttaagatcttcggcgaatacagcctcactgac
 E  I  R  D  I  A  R  E  V  L  K  I  F  G  E  Y  S  L  T  D
ttcatctggcctttgaagtatctcaaggttggaaagtatgagaagaggattgatgacatc
 F  I  W  P  L  K  Y  L  K  V  G  K  Y  E  K  R  I  D  D  I
ttgaacaagttcgaccctgtcgttgaaagggtcatcaagaagcgccgtgagatcgtcaga
 L  N  K  F  D  P  V  V  E  R  V  I  K  K  R  R  E  I  V  R
aggagaaagaacggagaagttgttgagggcgaggccagcggcgtcttcctcgacactttg
 R  R  K  N  G  E  V  V  E  G  E  A  S  G  V  F  L  D  T  L
cttgaattcgctgaggacgagaccatggagatcaaaattaccaaggagcaaatcaagggc
 L  E  F  A  E  D  E  T  M  E  I  K  I  T  K  E  Q  I  K  G
cttgttgtcgtaagtttccttcttctctcctactttattactttctttcattcatcatat
 L  V  V▲
gtattggcattaaatagtatactatatgagaaaatatgttacgcactcacggtgtaaaga

tatgtggtgtttttttaaaaagagatacagaagttgcttttatgcatgtatgttaacgta

tatttactcaagtggaaactaattaattctcaattttgggtatgtaggacttttttctct
                                    ▲D  F  F  S

gcagggacagattccactgcggtggcaacagagtgggcattggcagagctcatcaacaat
 A  G  T  D  S  T  A  V  A  T  E  W  A  L  A  E  L  I  N  N
cccagggtgttgcaaaaggctcgtgaggaggtctacagtgttgtgggcaaagatagactc
 P  R  V  L  Q  K  A  R  E  E  V  Y  S  V  V  G  K  D  R  L
gttgacgaagttgacactcaaaaccttccttacattagggccattgtgaaggagacattc
 V  D  E  V  D  T  Q  N  L  P  Y  I  R  A  I  V  K  E  T  F
cgaatgcacccaccactcccagtggtcaaaagaaagtgcacagaagagtgtgagattaat
 R  M  H  P  P  L  P  V  V  K  R  K  C  T  E  E  C  E  I  N
gggtatgtgatcccagagggagcattggttcttttcaatgtttggcaagtaggaagggac
 G  Y  V  I  P  E  G  A  L  V  L  F  N  V  W  Q  V  G  R  D
cccaaatactgggacagaccatcagaattccgtcccgagaggttcttagaaactggtgct
 P  K  Y  W  D  R  P  S  E  F  R  P  E  R  F  L  E  T  G  A
gaaggggaagcagggcctcttgatcttaggggccagcatttccaactcctctcatttggg
 E  G  E  A  G  P  L  D  L  R  G  Q  H  F  Q  L  L  S  F  G
tctgggaggagaatgtgccctggtgtcaatttggctacttcaggaatggcaacacttctt
 S  G  R  R  M  C  P  G  V  N  L  A  T  S  G  M  A  T  L  L
gcatctcttatccaatgctttgacctgcaagtgctgggccctcaaggacaaatattgaaa
 A  S  L  I  Q  C  F  D  L  Q  V  L  G  P  Q  G  Q  I  L  K
ggtgatgatgccaaagttagcatggaagagagggctggcctcacagttccaagggcacat
 G  D  D  A  K  V  S  M  E  E  R  A  G  L  T  V  P  R  A  H
agtctcgtttgtgttccacttgcaaggatcggcgttgcatctaaactcctttcttaatta
 S  L  V  C  V  P  L  A  R  I  G  V  A  S  K  L  L  S  -
aggtaatcatcatatacggtaccggg
```

Fig. 1. Nucleotide and deduced amino acid sequences of the *SinIFS1* gene. Underlines represented three main conserved motifs of P450, oxygen binding and activation, ERR triad, and heme binding in order. Intron sequences are shown in italics between arrow head.

```
gggggatccaaaattagcctcacaaaagcaaagatcaaacaaaccaaggacgagaacacg

atgttgcttgaacttgcacttggtttattggttttggctctgtttctgcacttgcgtccc
 M  L  L  E  L  A  L  G  L  L  V  L  A  L  F  L  H  L  R  P
acacccactgcaaaatcaaaagcacttcgccatctcccaaacccaccaagcccaaagcct
 T  P  T  A  K  S  K  A  L  R  H  L  P  N  P  P  S  P  K  P
cgtcttcccttcataggacaccttcatctcttaaaagacaaacttctccactacgcactc
 R  L  P  F  I  G  H  L  H  L  L  K  D  K  L  L  H  Y  A  L
atcgacctctccaaaaaacatggtcccttattctctctctactttggctccatgccaacc
 I  D  L  S  K  K  H  G  P  L  F  S  L  Y  F  G  S  M  P  T
gttgttgcctccacaccagaattgttcaagctcttcctccaaacgcacgaggcaacttcc
 V  V  A  S  T  P  E  L  F  K  L  F  L  Q  T  H  E  A  T  S
ttcaacacaaggttccaaacctcagccataagacgcctcacctatgatagctcagccgcc
 F  N  T  R  F  Q  T  S  A  I  R  R  L  T  Y  D  S  S  A  A
atggttcccttcggaccttactggaagttcgtgaggaagctcatcatgaacgaccttctc
 M  V  P  F  G  P  Y  W  K  F  V  R  K  L  I  M  N  D  L  L
aacgccaccactgtaaacaagttgaggcctttgaggacccaacagatccgcaagttcctt
 N  A  T  T  V  N  K  L  R  P  L  R  T  Q  Q  I  R  K  F  L
agggttatggcccaaggcgcagaggcacagaagccccttgacttgaccgaggagcttctg
 R  V  M  A  Q  G  A  E  A  Q  K  P  L  D  L  T  E  E  L  L
aaatggaccaacagcaccatctccatgatgatgctcggcgaggctgaggagatcagagac
 K  W  T  N  S  T  I  S  M  M  M  L  G  E  A  E  E  I  R  D
atcgctcgcgaggttcccaagatctttggcgaatacagcctcactgacttcatctggcca
 I  A  R  E  V  P  K  I  F  G  E  Y  S  L  T  D  F  I  W  P
ttgaagcatctcaaggttggaaagtatgagaagaggatcgacgacatcttgaacaagttc
 L  K  H  L  K  V  G  K  Y  E  K  R  I  D  D  I  L  N  K  F
gaccctgtcgttgaaagggtcatcaagaagcgccgtgagatcgtgaggaggagaaagaac
 D  P  V  V  E  R  V  I  K  K  R  R  E  I  V  R  R  R  K  N
ggagaggttgttgagggtgaggtcagcggggttttccttgacactttgcttgaattcgct
 G  E  V  V  E  G  E  V  S  G  V  F  L  D  T  L  L  E  F  A
gaggatgagaccatggagatcaaaatcaccaaggaccacatcgagggtcttgttgtcgtg
 E  D  E  T  M  E  I  K  I  T  K  D  H  I  E  G  L  V  V  ▲
agtttcctgcttcattcattgatcgaaatatgcagtattttgttaacaagagatcgagaa

ttgacatttatatattcatgtggtggcaattaattaacggtacgcattcttaatcgatat

tgtgtatgtgcaggactttttctcggcaggaacagactccacagcggtggcaacagagtgg
          ▲D  F  F  S  A  G  T  D  S  T  A  V  A  T  E  W
gcattggcagaactcatcaacaatcctaaggtgttggaaaaggcccgtgaggaggtctac
 A  L  A  E  L  I  N  N  P  K  V  L  E  K  A  R  E  E  V  Y
agtgttgcgggaaaggacagacttgtggacgaagttgacactcaaaaccttccttacatt
 S  V  A  G  K  D  R  L  V  D  E  V  D  T  Q  N  L  P  Y  I
agagcaatcgtgaaggagacattccgcatgcacccgccactcccagtggtcaaaagaaag
 R  A  I  V  K  E  T  F  R  M  H  P  P  L  P  V  V  K  R  K
tgcacagaagagtgtgagattaatggatatgtgatcccagagggagcattgattctcttc
 C  T  E  E  C  E  I  N  G  Y  V  I  P  E  G  A  L  I  L  F
aatgtatggcaagtaggaagagaccccaaatactgggacagaccatcggagttccgtcct
 N  V  W  Q  V  G  R  D  P  K  Y  W  D  R  P  S  E  F  R  P
gagaggttcctagagacaggggctgaaggggaagcagggcctcttgatcttaggggacaa
 E  R  F  L  E  T  G  A  E  G  E  A  G  P  L  D  L  R  G  Q
cattttcaacttctcccatttgggtctgggaggagaatgtgcctggagtcaatctggct
 H  F  Q  L  L  P  F  G  S  G  R  R  M  C  P  G  V  N  L  A
acttcgggaatggcaacacttcttgcatctcttattcagtgcttcgacttgcaagtgctg
 T  S  G  M  A  T  L  L  A  S  L  I  Q  C  F  D  L  Q  V  L
ggtccacaaggacagatattgaagggtggtgacgccaaagttagcatggaagagagagcc
 G  P  Q  G  Q  I  L  K  G  G  D  A  K  V  S  M  E  E  R  A
ggcctcactgttccaagggcacatagtcttgtctgtgttccacttgcaaggatcggcgtt
 G  L  T  V  P  R  A  H  S  L  V  C  V  P  L  A  R  I  G  V
gcatctaaactccttttcttaattaagatcatcatcatatataatatttacttttgtgtgt
 A  S  K  L  L  S  -
tgataatcatcatttcaataaggtctcgttcatctacttttttatgaagtatataagccct
tccatgcacattgtatcatctcccatttgtcttcgtttgcggtaccggg
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Fig. 2. Nucleotide and deduced amino acid sequences of the *SinIFS2* gene. Underlines represented three main conserved motifs of P450, oxygen binding and activation, ERR triad, and heme binding in order. Intron sequences are shown in italics between arrow head.

polypeptide of 521 amino acids. This reveals their distinctive feature in length, considering that most P450s contain approximately 500 amino acid. The *SinIFS1* included 219bp of single intron, indicating that it belongs to A-type P450, and 46bp of 5′ and 3′ UTR (Fig. 1). *SinIFS2* contained a total of 1912bp nucleotides and encoded a polypeptide of 521 amino acid. It has a intron with a size of 136bp, relatively smaller than that of *SinIFS1* (Fig. 2). Both introns were flanked by typical border sequences, GT and AG, and their locations were at the same position of 897bp from start codon, though the size of intron were different as mentioned above (Fig. 1, 2). Soybean IFS gene also belongs to a member of cytochrome P450, heme-thiolate monooxygenase that utilize a flavoprotein system to transfer electron from NADPH and/or NADH to a substrate [10], and can be recognized by several conserved motifs. The three main characteristic domains of P450, oxygen binding and activation (A/G-G-X-E/D-T-T/S), ERR triad (E...R....R), and heme binding (F-X-X-G-X-R-X-C-X-G) were found in *SinIFS1* and *SinIFS2*, respectively. Especially, the heme-binding domain near the C-terminus of the gene contains a conserved Cys residue, as expected, that is known to be served as the axial ligand to the heme. Both genes contained proline-rich region between hydrophobic domain at N-terminal and catalytic domain. The proline-rich region is known to act as a type of hinge region [4].

The deduced amino acid sequences of two genes, *SinIFS1* and *SinIFS2*, were aligned with homologous sequences from other legumes, soybean (*Glycin max*; GenBank accession number AF195798), mung bean (*Vigna radiata*; AF195806), and red clover (*Trifolium pratense*; AF195810) (Fig. 3, 4). As expected, the predicted protein sequences of *SinIFS1* and *SinIFS2* showed high similarity more than 98% amino acid compared with those proteins from other legumes. In the alignment with other *IFS1s*, *SinIFS1* showed only 7 amino acid sequences difference to soybean and mung bean *IFS1s* (99%) and 9 amino acid sequences to red clover (98%) (Fig. 3). This result is in well accodance with the previous report [2] where more than 95% amino acid similarity was found among *IFS1s* in legume plants. In a case of *SinIFS2*, much higher sequence homology was observed (Fig. 4). The alignment of *SinIFS2* showed 4 amino acid difference to *IFS2s* of mung bean (99%) and soybean (99%), respectively, and 5 amino acid to red clover *IFS2* (99%). At this point, we don't have enough data or information to explain clearly about this high sequence similarity among

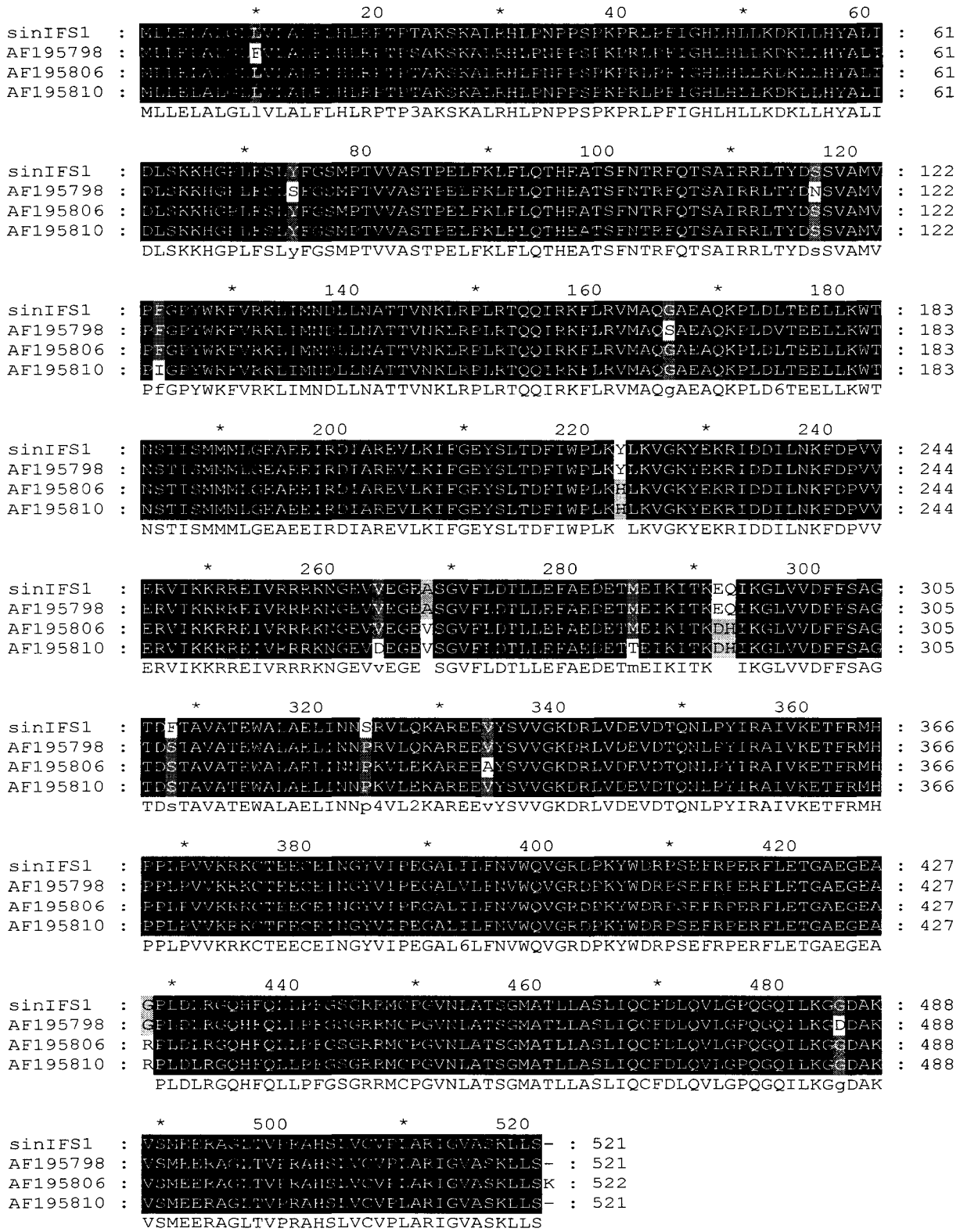

Fig. 3. Alignment of the deduced SinIFS1 protein sequence with homologous sequences of *Glycine max* isoflavone synthase 1 (ifs1) gene (GenBank accession number: AF195798), *Vigna radiata* isoflavone synthase 1 (ifs1) (GenBank accession number: AF195806) and *Trifolium pratense* isoflavone synthase 1 (ifs1) (GenBank accession number: AF195810). Blank shade represented identical amino acid residues.

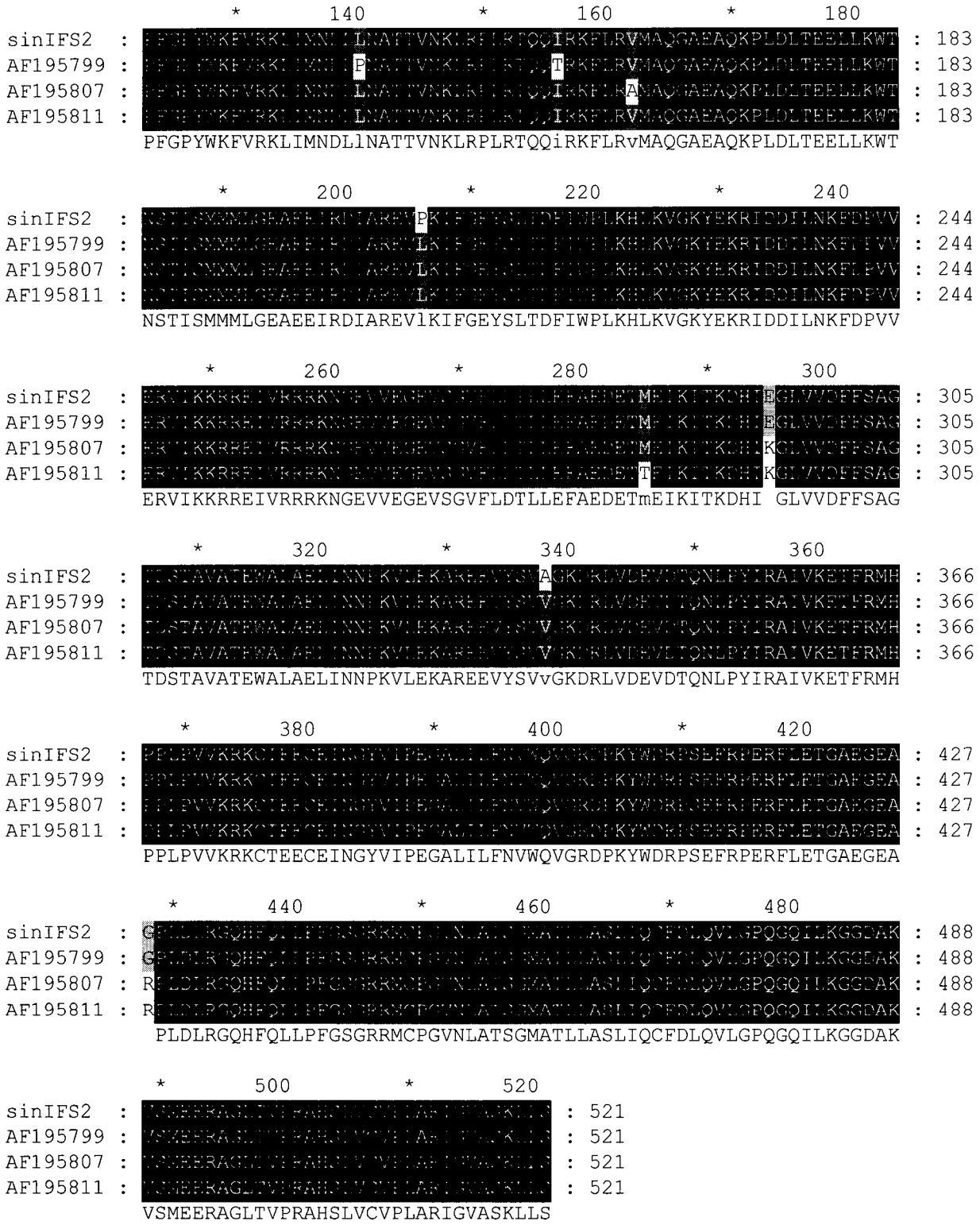

Fig. 4. Alignment of the deduced SinIFS2 protein sequence with homologous sequence of *Glycine max* isoflavone synthase 2 (ifs2) gene (GenBank accession number: AF195799), *Vigna radiata* isoflavone synthase 2 (ifs2) (GenBank accession number: AF195807) and *Trifolium pratense* isoflavone synthase 2 (ifs2) (GenBank accession number: AF195811). Blank shade represented identical amino acid residues.

IFSs. However, considering the presence of this gene mainly in leguminous plants, not widely spread to other plants in terms of evolution, the high sequence similarity might result from temporally far-near origin of the *IFS*. It is intriguing that *IFS* belongs to the super family of P450 genes and P450 genes are one of the most abundant and widely spread gene families in the plant kingdom. More detailed study on gene characterization should be addressed to obtain closer look on its origin and narrow range of presence in plant species.

The expression and induction of *SinIFS1* to various environmental conditions were investigated by northern blot analysis. Five different conditions, low temperature, light, dark, UV, and fungal elicitor (yeast extract) were given to 20 day-old seedlings of soybean to see the induction of transcripts. The most significant induction in gene expression, more than 6 times of transcript level compared to the dark treatment as a control, was observed from the fungal elicitor treatment (Fig. 5). The next up-regulated expression was from UV treatment (4×), and low temperature and light conditions. Considering the fact that isoflavanoids comprise a large group of secondary metabolites involved in plant-pathogen interaction, its induction

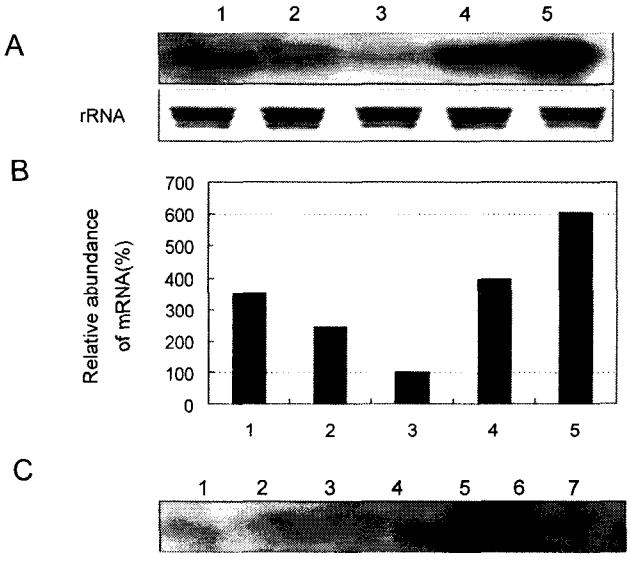

Fig. 5. Induced gene expression profiles of *SinIFS1* by various environmental conditions. (A) Northern blot analysis of *SinIFS1* from leaf treated with low temperature (lane 1), light (lane 2), dark (lane 3), UV (lane 4), and fungal elicitor (lane 5). (B) The histogram of the relative abundance of mRNA in percentage. (C) Northern blot analysis of *SinIFS1* on the various treatment of low temperature; control (lane 1), 1-6 hour exposure (lane 2-7).

and response to fungal elicitor and harmful environmental conditions seem to be feasible. In the detailed northern analysis of low temperature, detectable amount of transcripts was visualized 5hrs after low temperature treatment (Fig. 5, lane 6). The expression level was similar to the result of low temperature treatment as shown in the figure 5 (B).

In this study, we cloned two genes, *SinIFS1* and *SinIFS2*, and represented their sequence homology and expression profile on various environmental conditions. Further study on gene expression, e.g., spatial manner of gene expression and induction patterns, seems to be required to understand this complicate gene more evidently.

Acknowledgement

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References

1. Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162**, 156-159.
2. Chung W. S., O. Yu, S. C. Lau, D. P. O'Keefe, J. Odell, G. Fader and B McGonigle. 2000. Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes. *Nat. Biotechnol.* **18**, 208-212.
3. Dixon, R. A. and N. L. Pavia. 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell* **7**, 1085-1097.
4. Hasemann, C. A., R. G. Kurumbail, S. S. Boddupalli, J. A. Peterson, and J. Deisenhofer. 1995. Structure and function of cytochrome P450: A comparative analysis of three crystal structures. *Structure*. **3**, 41-62.
5. Hollman, P. C. and M. B. Katan. 1998. Bioavailability and health effects of dietary flavanols in man. *Arch. Toxicol.* (suppl) **20**, 237-248.
6. Kim C. S., C. H. Lee., J. S. Shin, Y. S. Chung, and N. I. Hyung 1997. A simple and rapid method for isolation of high quality genomic DNA from fruit trees and conifers using PVP. *Nucleic Acid Research.* **25**(5), 1085-1086.
7. Kim S. R., H. D. Hong, and S. S. Kim. 1999. Some properties and contents of isoflavone in soybean and soybean foods. *Korea Soybean Digest.* **16**(2), 35-46.
8. Padmavati, M. and A. R. Reddy. 1999. Flavanoid biosynthetic pathway and cereal defense response: an emerging trend in crop biotechnology. *Plant Biochem. Biotechnol.* **8**, 15-20.
9. Rice-Evans, C. A., N. J. Miller and G. Paganga. 1997. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **2**, 152-159.
10. Schuler M. A. 1996. Plant cytochrome P450 monooxygenases. *Crit. Rev. in Plant Sci.* **15**, 235-284.

11. Steel, C. L., M. Gijzen, D. Outob and R. A. Dixon. 1999. Molecular characterization of the enzyme catalyzing the aryl migration reaction of isoflavonoid biosynthesis in soybean. *Arch. Biochem. Biophys.* **367**, 146-150.

초록 : 신팔달콩 유래 IFS (isoflavone synthase)유전자 클로닝 및 기능 규명

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이소플라본의 함량이 매우 높은 것으로 알려진 국내 콩품종 신팔달로부터 2개의 유전자 *IFS1 (SinIFS1)*과 *IFS2 (SinIFS2)*가 클로닝되었다. 유전자의 염기서열을 밝힌 후, 기존에 알려진 콩과의 다른 *IFS* 유전자들과 유전자 염기서열의 유사성을 비교분석하였다. 유전자 *SinIFS1*은 전체 1,828bp의 nucleotide와 521개의 아미노산으로 이루어져 있었고 *SinIFS2*의 경우, 1912bp의 nucleotide와 521의 아미노산으로 이루어져 있었다. 두 유전자 모두 cytochrome P450 superfamily의 일원이었고, 상응하는 conserve된 motif들을 가지고 있었다. 콩과의 다른 식물에서 클로닝된 IFS들과의 염기서열비교에서는 매우 높은 염기서열 유사성(98% 이상)이 관측되었다. 유전자의 발현과 유발에 관한 노던분석 실험 결과, 무처리구로 사용한 암처리보다 모두 유발된 유전자의 발현을 나타났는데, 특히 곰팡이 elicitor 처리구의 경우, 무처리보다 6배 이상의 유전자 유발을 보였다. 그 다음으로는 자외선 처리가 높은 유전자 발현 유발효과를 나타내었고, 그 다음으로 저온과 명처리순으로 유발효과를 나타내었다.