

[Back to article](#)

Table S1. Primer sequences used for quantitative-real time polymerase chain reaction (PCR) expression analysis and the cDNA cloning of isoflavone synthase IFS1 and IFS2 genes

Gene	Primer	Sequence
IFS1-qRT	F	AGAATTCCGTCGGAGAGGTT
	R	TGCCATTCTGAAGTAGCCAA
IFS2-qRT	F	AATGTGCCCTGGAGTCAATCTG
	R	GGCGTCACCAACCCTCAATAT
Actin 2/7-qRT	F	ACTTGCCCATCAGGAAGCTC
	R	TGTTCACCAACCTCTGCCAAG
IFS1	F	CACCATGTTGCTGAACTTGCACTTGGT
	R	TTAAGAAAGGAGTTAGATGCAACGCC
IFS2	F	CACCATGTTGCTGAACTTGCACTTGGT
	R	TTAAGAAAGGAGTTAGATGCAACGCC

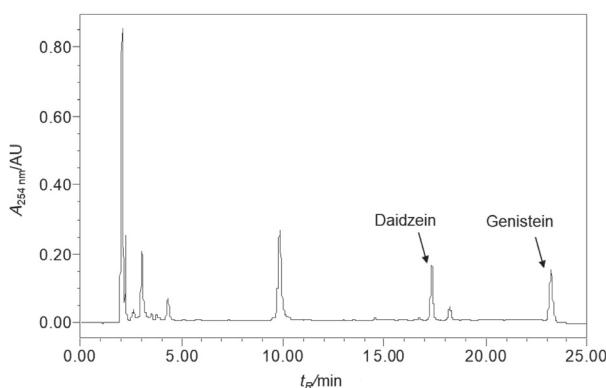
[Back to article](#)

Fig. S1. A representative chromatogram of soybean cultivar (PK1042) for isoflavone estimation. Peaks corresponding to major isoflavones, *i.e.* daidzein and genistein, are labelled

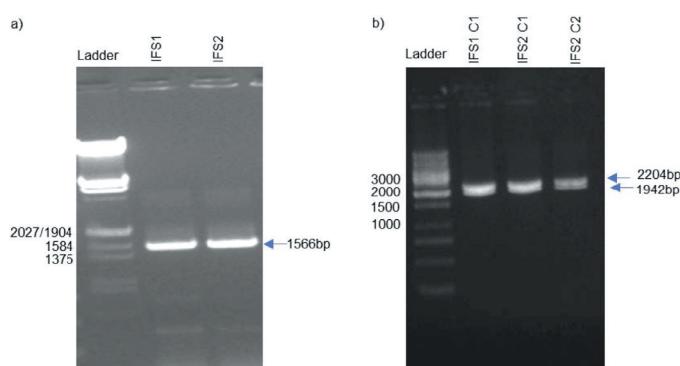
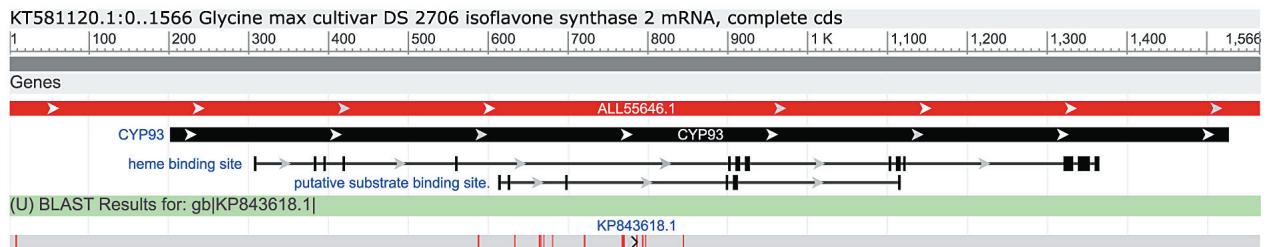
[Back to article](#)

Fig. S2. Polymerase chain reaction (PCR) amplification of isoflavone synthase isomers: a) IFS1 and IFS2 transcripts, b) Pvull restriction products (2204 and 1942 bp) of IFS1 and IFS2 cloned into the pEN-TR-D-TOPO vector

[Back to article](#)

a)

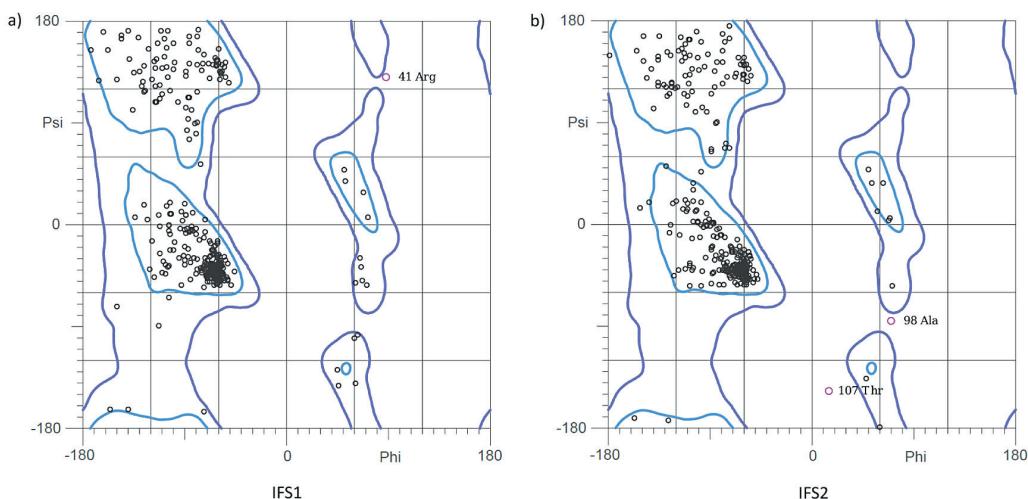


b)

Fig. S3. Nucleotide sequence alignment of IFS1 and IFS2 coding sequences showing 99.04 % identity: a) graphical summary of the alignment, and b) sequence alignment with IFS1 as query sequence and IFS2 as subject sequence

[Back to article](#)

Query 1	MLLELALGLFVLALFLHRLRPTSAKS	KALRHLNPSPSKPRLPFIGHLHLLKD	KLLHYAL	60
Sbjct 1	MLLELALGLFVLALFLHRLRPTSAKS	KALRHLNPSPSKPRLPFIGHLHLLKD	KLLHYAL	60
Query 61	IDLSKKHGPLFSLSFGSMP	TVA	STPELFKLFLQTHEATSFNTRFQTS	AIRRLTYDNSVA
Sbjct 61	IDLSKKHGPLFSLSFGSMP	TVA	STPELFKLFLQTHEATSFNTRFQTS	AIRRLTYDNSVA
Query 121	MVPFGPYWKFVRKLI	MNDLLATT	VNKLRPLRTQQIRKF	LRVMAQS
Sbjct 121	MVPFGPYWKFVRKLI	MNDLLATT	VNKLRPLRTQQIRKF	LRVMAQS
Query 181	KWTNSTISMMMLGEAEE	EIRDIAREV	LKIFGEYSLTDFI	PLKYLKV
Sbjct 181	KWTNSTISMMMLGEAEE	EIRDIAREV	LKIFGEYSLTDFI	PLKYLKV
Query 241	DPVVERVIKKRREIVR	RKNGEV	ASGVFLDTL	LEFAE
Sbjct 241	YPVVERVIKKRREIVR	RKNGEV	ASGVFLDTL	LEFAE
Query 301	FFSAGTD	STA	VEWALAEI	NNPRVLQKAREEV
Sbjct 301	FFSAGTD	STA	VEWALAEI	NNPRVLQKAREEV
Query 361	ETFRMHPLPVKRKCTEE	CINGYV	IP	EGALVLFN
Sbjct 361	ETFRMHPLPVKRKCTEE	CINGYV	IP	EGALVLFN
Query 421	TGAEGEA	GPLDLRGQHFQ	LLPF	PGSGRRMCPGVNLATSGM
Sbjct 421	TGAEGEA	GPLDLRGQHFQ	LLPF	PGSGRRMCPGVNLATSGM
Query 481	ILKGDDAKVSMEERAGL	TVPRAHSLVC	VPLARIGVASKL	LLS
Sbjct 481	ILKGDDAKVSMEERAGL	TVPRAHSLVC	VPLARIGVASKL	LLS

Fig. S4. Sequence alignment of isoflavone synthase IFS1 and IFS2 proteins showing 98.27 % identity[Back to article](#)**Fig. S5.** Ramachandran plot generated on the RoseTTAFold (25) for isoflavone synthase: a) IFS1 and b) IFS2 3D models

[Back to article](#)

a)

Your Input:

IFS1	Isoflavone synthase; Isoflavone synthase 1; Uncharacterized protein; Belongs to the cytochrome P450 family (521 aa)
------	--

Predicted Functional Partners:

			Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
HIDH	2-hydroxyisoflavanone dehydratase; Dehydratase that mediates the biosynthesis of isoflavonoids. Can use both 4'-...			●	●						0.970
GLYMA13G24210.1	Uncharacterized protein; Belongs to the class I-like SAM-binding methyltransferase superfamily. Cation-Independen...		●		●						0.942
F3H	Flavanone 3-hydroxylase; Belongs to the iron/ascorbate-dependent oxidoreductase family			●	●						0.939
F3H2	Flavanone 3-hydroxylase; Uncharacterized protein ; Belongs to the iron/ascorbate-dependent oxidoreductase family			●	●						0.939
GLYMA01G37120.1	Uncharacterized protein; Belongs to the iron/ascorbate-dependent oxidoreductase family			●	●						0.907
CYP71D9	Cytochrome P450 71D9		●		●	●	●				0.907
CHI	Chalcone–flavonone isomerase 1A; Catalyzes the intramolecular cyclization of bicyclic chalcones into tricyclic (S)-f...			●	●	●					0.889
DFR2	Dihydroflavonol-4-reductase 2; Uncharacterized protein				●	●	●				0.859
GLYMA02G18380.1	Uncharacterized protein				●	●	●				0.830
LOC548087	Dihydroflavonol-4-reductase DFR1				●	●					0.818

b)

Your Input:

IFS2	2-hydroxyisoflavanone synthase; Involved in isoflavanoid biosynthesis. Converts liquiritigenin to 2-hydroxyisoflavanone which spontaneously dehydrates to daidzein (521 aa)
------	---

Predicted Functional Partners:

			Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
HIDH	2-hydroxyisoflavanone dehydratase; Dehydratase that mediates the biosynthesis of isoflavonoids. Can use both 4'-...		●	●	●						0.984
CHI	Chalcone–flavonone isomerase 1A; Catalyzes the intramolecular cyclization of bicyclic chalcones into tricyclic (S)-f...		●	●	●						0.935
GLYMA13G24210.1	Uncharacterized protein; Belongs to the class I-like SAM-binding methyltransferase superfamily. Cation-Independen...			●	●						0.926
F3H	Flavanone 3-hydroxylase; Belongs to the iron/ascorbate-dependent oxidoreductase family			●	●						0.907
F3H2	Flavanone 3-hydroxylase; Uncharacterized protein ; Belongs to the iron/ascorbate-dependent oxidoreductase family			●	●						0.907
CYP71D9	Cytochrome P450 71D9			●	●	●					0.906
GLYMA02G18380.1	Uncharacterized protein				●	●	●				0.877
DFR2	Dihydroflavonol-4-reductase 2; Uncharacterized protein				●	●	●				0.861
GLYMA01G37120.1	Uncharacterized protein; Belongs to the iron/ascorbate-dependent oxidoreductase family			●	●						0.853
chs8	Uncharacterized protein; Belongs to the chalcone/stilbene synthases family			●		●					0.846

Fig. S6. STRING scores for isoflavone synthase isomers: a) IFS1 and b) IFS2 with different proteins