

Supplemental Data

Disrupting Flavone Synthase II Alters Lignin and Improves Biomass Digestibility

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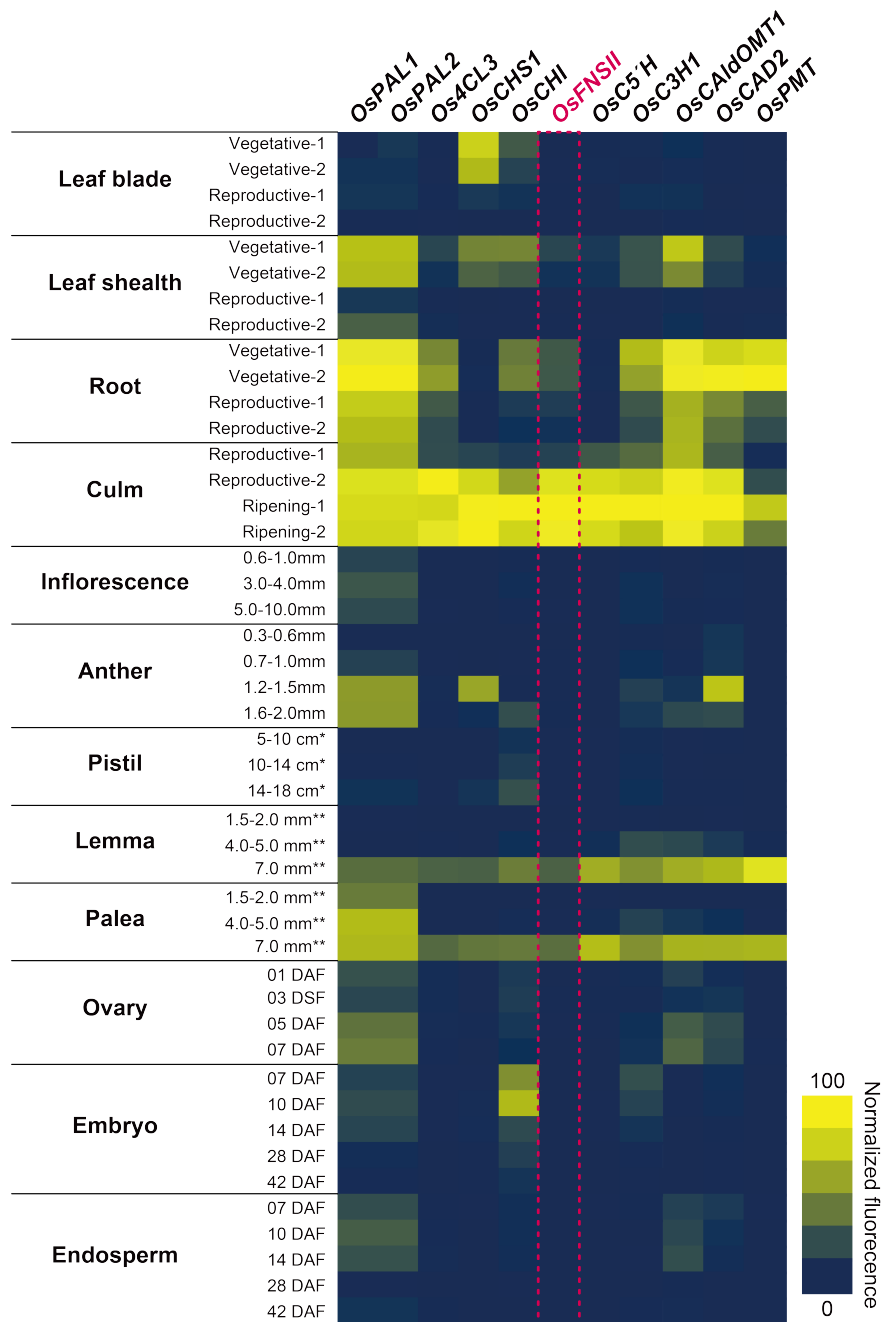
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List of Materials

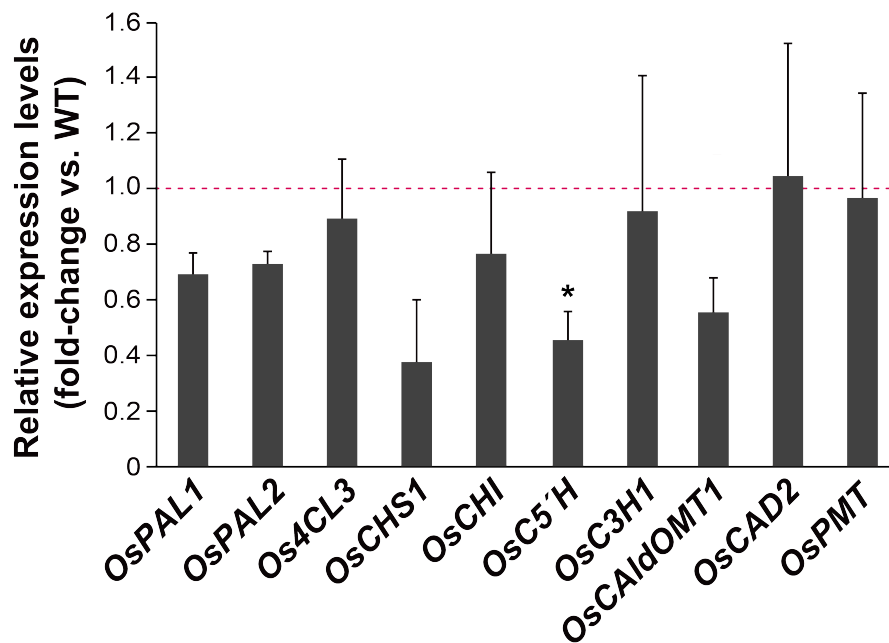
- Supplemental Figure S1.** Gene expression data of flavonoid and monolignol biosynthetic genes in wild-type rice plants.
- Supplemental Figure S2.** Relative expression levels of flavonoid and monolignol biosynthetic genes in *fnsII* mutant culms.
- Supplemental Figure S3.** Thioacidolysis yield per thioglycolic lignin content in wild-type and *fnsII* mutant rice tissues.
- Supplemental Figure S4.** Cell wall-bound *p*-coumarate content per thioglycolic lignin content in wild-type and *fnsII* mutant rice tissues.
- Supplemental Figure S5.** Sugar composition in wild-type and *fnsII* mutant rice tissues.
- Supplemental Figure S6.** HSQC NMR spectra of whole culm cell walls from wild-type and *fnsII* mutant rice.
- Supplemental Figure S7.** Phylogenetic tree of FNSII proteins.
- Supplemental Table S1.** Primers used in this study.



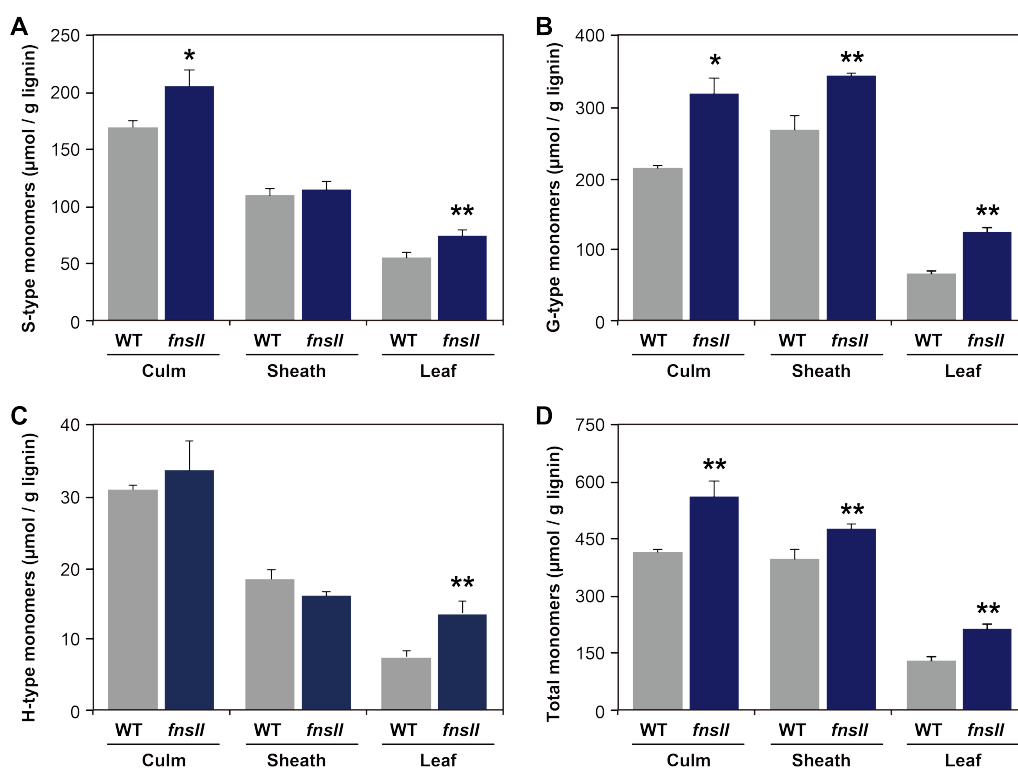
Supplemental Figure S1. *In silico* gene expression data of major flavonoid and monolignol biosynthetic genes [*OsPAL1* (AK102817), *OsPAL2* (NM_001054), *Os4CL3* (AK120964), *OsCHS1* (AB00801), *OsCHI* (AK061390), *OsFNSII* (*CYP93G1*; AK105745), *OsC5'H* (AK070442), *OsC3H1* (AK099695), *OsCALdOMT1* (AK064768), *OsCAD2* (AK105011), and *OsPMT* (AK60689)]. Microarray-based expression data were retrieved from Rice Expression Profile Database (Sato et al., 2012). Vegetative-1, -2: vegetative stages at day and night time; Reproductive-1, -2: reproductive stages at the day and night time; Ripening-1, -2: ripening stages at day and night time; *: panicle size; **: floret size; DAF: days after flowering.

Reference

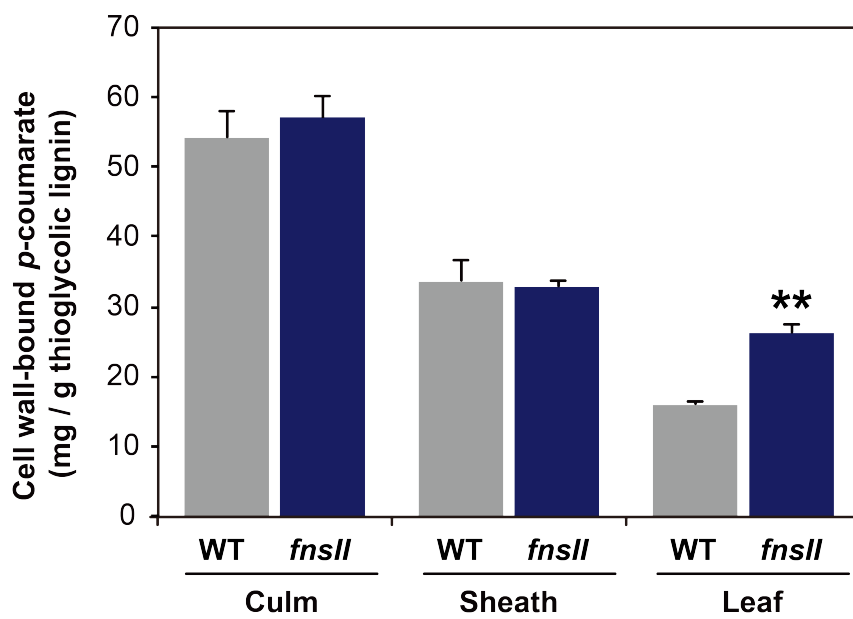
Sato Y, Takehisa H, Kamatsuki K, Minami H, Namiki N, Ikawa H, Ohyanagi H, Sugimoto K, Antonio BA, Nagamura Y (2012) RiceXPro version 3.0: expanding the informatics resource for rice transcriptome. *Nucleic Acids Res.* **41**: D1206–D1213



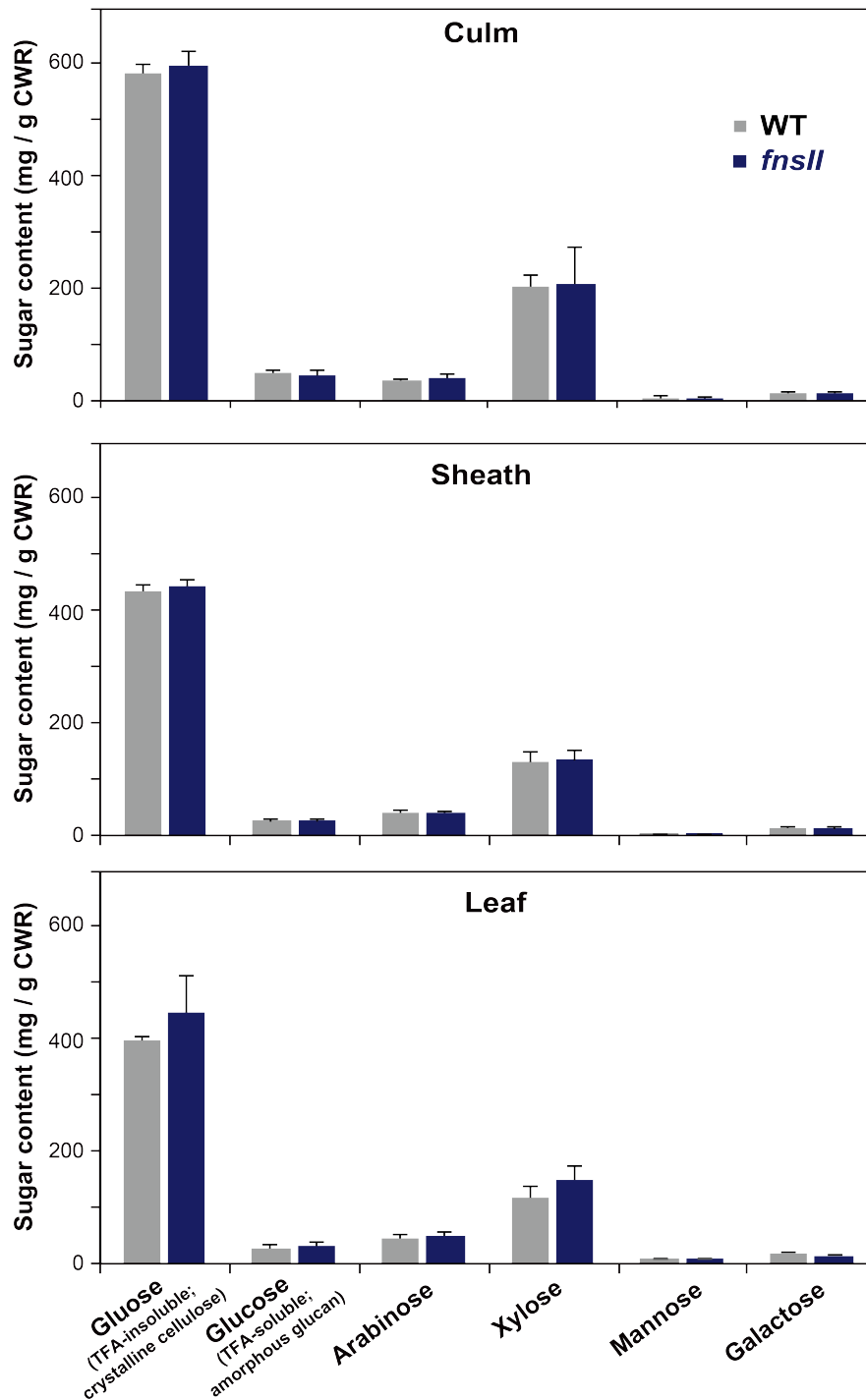
Supplemental Figure S2. Relative expression levels of major flavonoid and monolignol biosynthetic genes in *fnsII* mutant culms compared with wild-type plants. A ubiquitin gene (*OsUBQ5*; AK061988) was used as an internal control. For gene abbreviations, see the footnote of Supplemental Fig. S1. Values are means \pm SD ($n = 5$) and asterisks indicate significant differences between wild-type and *fnsII* plants (Student's *t*-test, *: $P < 0.05$).



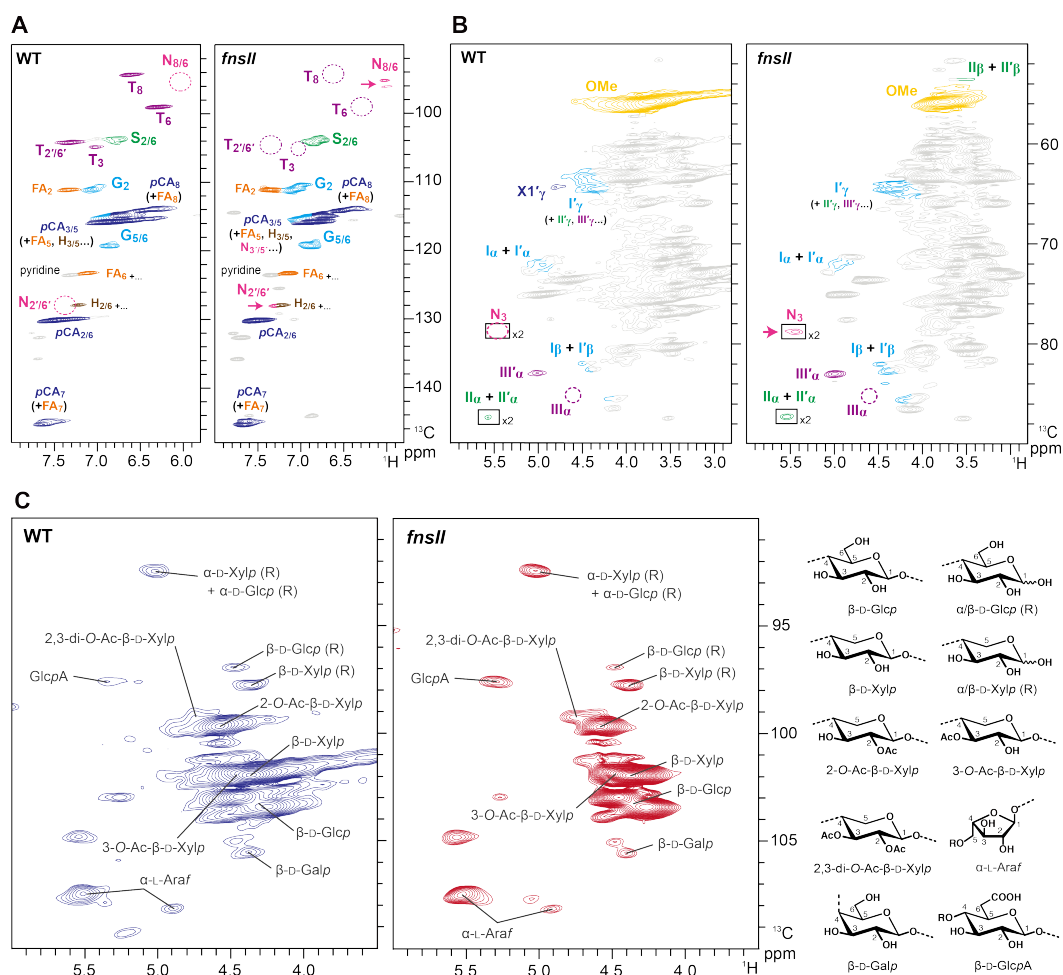
Supplemental Figure S3. Thioacidolysis monomer yield in culm, sheath, and leaf tissues from wild-type (WT) and *FNSII*-knockout mutant (*fnsII*) rice plants. The yields of S-type (A), G-type (B), H-type (C), and total trithioethylpropane monomers (D) are expressed per lignin content based on thioglycolic acid lignin assay. Values are means \pm SD ($n = 3$) and asterisks indicate significant differences between WT and *fnsII* plants (Student's *t*-test, *: $P < 0.05$; **: $P < 0.01$).



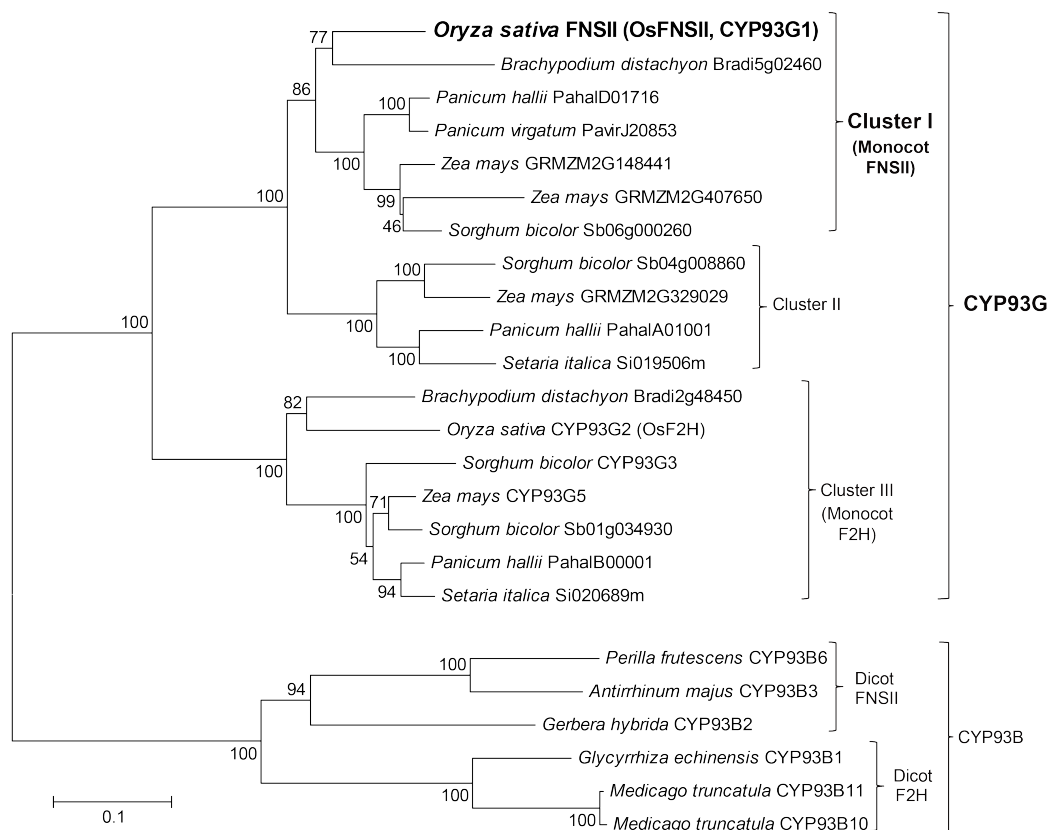
Supplemental Figure S4. Cell wall-bound *p*-coumarate content per thioglycolic lignin content in culm, sheath, and leaf tissues from wild-type (WT) and *FNSII*-knockout mutant (*fnsII*) rice plants. Values are means \pm SD ($n = 3$) and asterisks indicate significant differences between WT and *fnsII* mutant plants (Student's *t*-test, **: $P < 0.01$).



Supplemental Figure S5. Sugar composition analysis of culm, sheath, and leaf cell walls from wild-type (WT) and *FNSII*-knockout mutant (*fnsII*) rice plants. Values are means \pm SD ($n = 3$). No statistically significant differences between WT and *fnsII* rice tissues were observed (Student's *t*-test, $P < 0.05$).



Supplemental Figure S6. Aromatic (**A**), aliphatic (**B**), and sugar anomeric (**C**) sub-regions of short range ^1H - ^{13}C correlation (HSQC) NMR spectra of whole culm cell walls of wild-type (WT) and *FNSII*-knockout mutant (*fnsII*) rice plants. For structure abbreviations, also see Figure 5 and Figure 6 in the main article.



Supplemental Figure S7. Phylogenetic analysis of CYP93 proteins. The unrooted phylogenetic tree was constructed by neighbor-joining method. Bootstrapping with 1,000 replications was performed and scale bar represents 0.1 amino acid substitution per site. The CYP93G monocot proteins were found in three clusters. **Cluster I** contains the rice FNSII (OsFNSII, CYP93G1) and other putative FNSII proteins in monocots (71–74% identity to OsFNSII). **Cluster II** consists of uncharacterized monocot proteins that show 63–66% identity to OsFNSII. No rice protein falls in this clade. **Cluster III** consists of the previously characterized monocot flavanone 2-hydroxylases (F2Hs) in rice (OsF2H, CYP93G2) (Du et al., 2010a), sorghum (CYP93G3, Sb02g000220) (Du et al., 2010b), and maize (CYP93G5, GRMZM2G162783) (Morohashi et al., 2012), and their close homologs. Dicot FNSIIs and F2Hs are classified in CYP93B subfamily and form independent clades distinct from the monocot FNSIIs and F2Hs. These proteins were named with their gene identifiers or accession numbers: AF156976 (*Gerbera hybrid* CYP93B2), AB028151 (*Anitrrhinum majus* CYP93B3), AB045592 (*Perilla frutescens* CYP93B6), AB001380 (*Glycyrrhiza echinensis* CYP93B1), DQ354373 (*Medicago truncatula* CYP93B10), and AC146789 (*Medicago truncatula* CYP93B11).

References

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- Du Y, Chu H, Wang M, Chu IK, Lo C (2010b) Identification of flavone phytoalexins and a pathogen-inducible flavone synthase II gene (SbFNSII) in sorghum. *J. Exp. Bot.* **61**: 983–994.
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Supplemental Table S1. Primers used in this study.

Purpose	Primer names	Sequence (from 5' to 3')	Target gene (accession no.)
Genotyping	CL2323-LP	TTGGCTCTTCTTTTCCCAAC	<i>OsFNSII</i> (AK105745)
	CL2324-RP	AGATCCAGATCCGAGGGTTC	
	CL1204-BP	CCACAGTTTTTCGCGATCCAGACTG	
qRT-PCR	OsPAL1-F	AGGAGCTCGGCTGCGTATT	<i>OsPAL1</i> (AK102817)
	OsPAL1-R	ATGCCGAGGAACACCTTGTT	
	OsPAL2-F	TGGCTCGGCCACAGAT	<i>OsPAL2</i> (NM_001054)
	OsPAL2-R	GTTGTCGTTGACGGAGTTGATC	
	Os4CL3-F	GGACAGGGTTACGGTATGACTGA	<i>Os4CL3</i> (AK120964)
	Os4CL3-R	TTAACCTTGAAGGGCTCCTTGGCA	
	OsCHS1-F	ACCCGGACTACTACTTCAGGAT	<i>OsCHS1</i> (AB00801)
	OsCHS1-R	TCTTCCTGATCTGCGACTTGT	
	OsCHI-F	CGCAGGTTTGTGGTGGTTGC	<i>OsCHI</i> (AK061390)
	OsCHI-R	CGGATCGATCAAACCAGCAAG	
	CYP75B4-F	ACACGGAAATCAAGGCCCTT	<i>OsC5'H</i> (AK070442)
	CYP75B4-R	TCGAATCAGCTCTGCCATTGT	
OsC3H1-F	TTGGGCTTCTATGGGACATGA	<i>OsC3H</i> (AK099695)	
OsC3H1-R	CCATTGCCCACTCGACTGAT		
OsCAldOMT1-F	CCTCGCCCTCATGAACCA	<i>OsCAldOMT1</i> (AK064768)	
OsCAldOMT1-R	CGTCCAGGACTGCGTCCTTA		
OsCAD2-F	TGTGTGAGACTCTGACGACTTGTC	<i>OsCAD2</i> (AK105011)	
OsCAD2-R	CATATATTGCGAGGCCGAATTT		
OsPMT-F	GTCATACCCTTCGCGTACTACC	<i>OsPMT</i> (AK60689)	
OsPMT-R	GGCCTTGATCTCCTCCTTGAA		
OsUBQ5-F	ACCACTTCGACCGCCACTACT	<i>OsUBQ5</i> (AK061988)	
OsUBQ5-R	ACGCCTAAGCCTGCTGGTT		