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Differential expression of *GS5* regulates grain size in rice

Chunjue Xu, Yu Liu, Yibo Li, Xiaodong Xu, Caiguo Xu, Xianghua Li, Jinghua Xiao and Qifa Zhang[1]

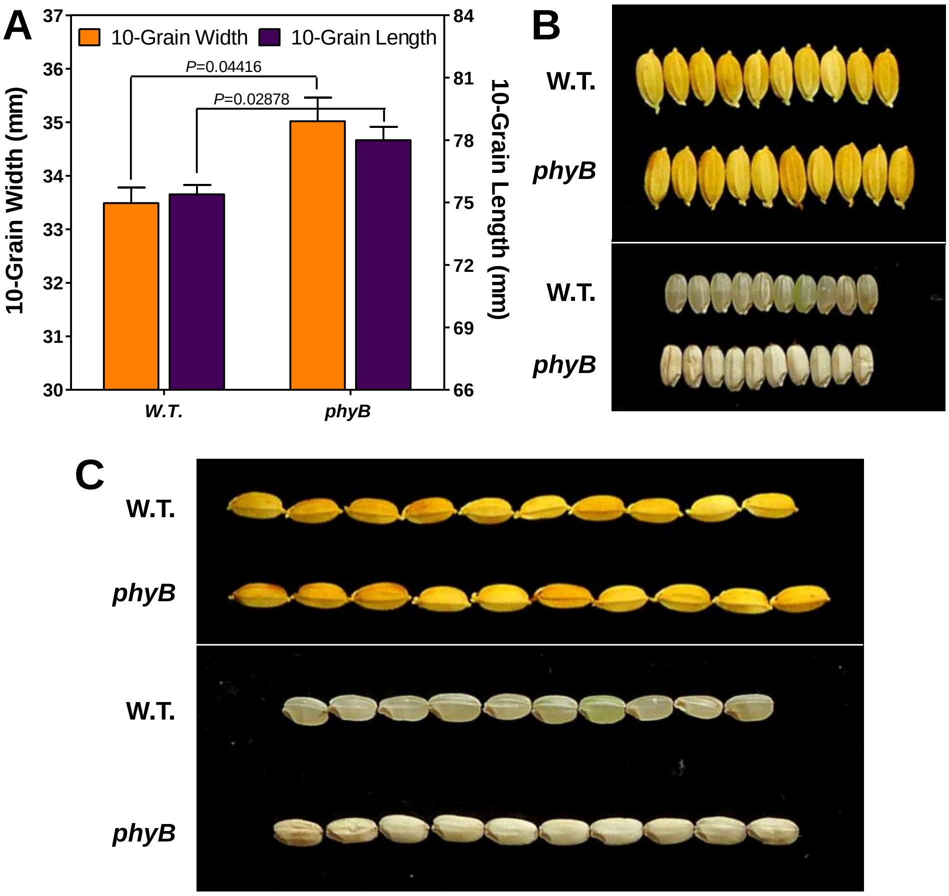


Figure S1. Grain size and chalkiness of *phyB* mutant. (A) Significant difference in grain size between *phyB* mutant and wild type (W.T.). All data are presented as mean ± SE (n≥3). *P* values were calculated using Student's *t*-test. (B) Grain width and chalkiness of *phyB* mutant and wild type. (C) Grain length and chalkiness of *phyB* mutant and wild type.

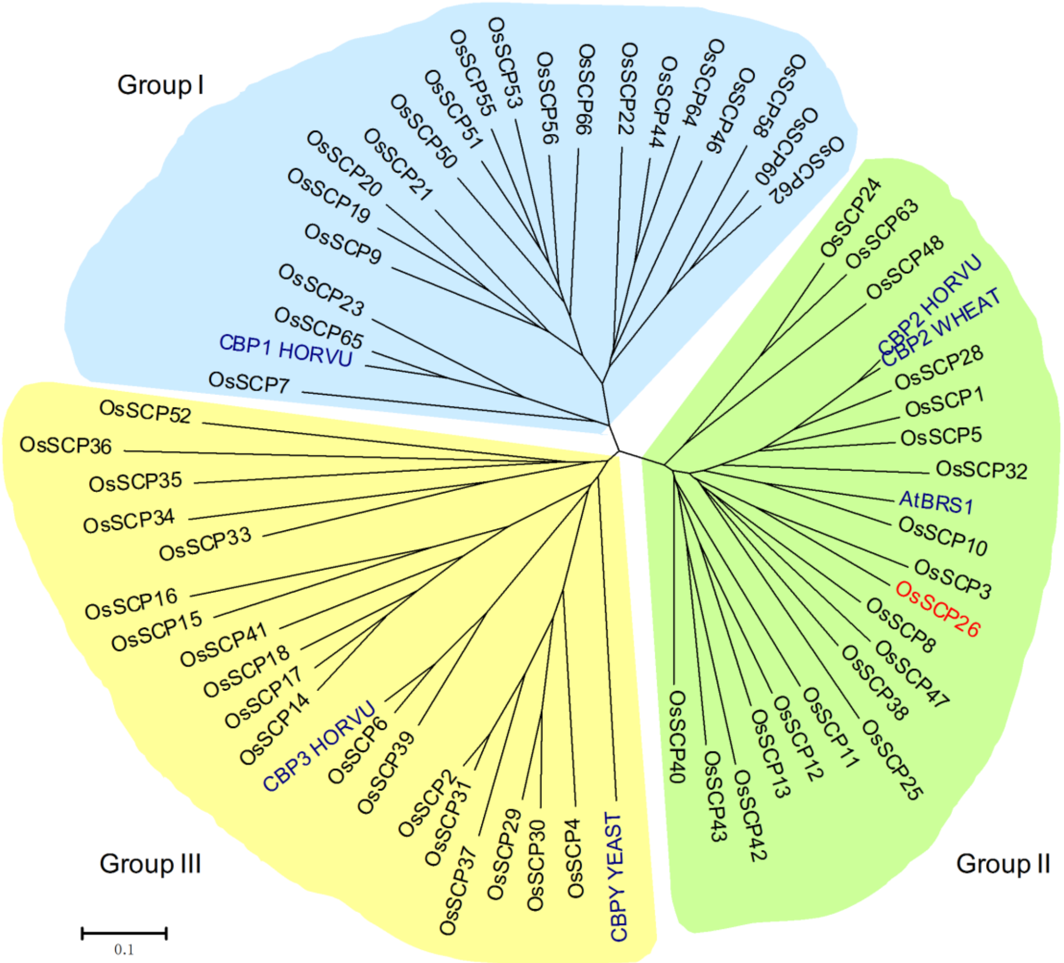


Figure S2. Phylogenetic tree of members of the serine carboxypeptidase-like protein family. Full-length protein sequences of OsSCPLs were downloaded from http://rice.plantbiology.msu.edu/ca/gene_fams/27_76.shtml and aligned with ClustalX and calculated with MEGA 3.1. *CBP1 HORVU* (NCBI accession NO. P07519), *CBP2 HORVU* (NCBI accession NO. P08818), *CBP3 HORVU* (NCBI accession NO. P21529) and *CBP2 WHEAT* (NCBI accession NO. P08819) were used to indicate the three groups of SCPL. *GS5* (*OsSCP26*) and *AtBRS1* (NCBI accession NO. NP_194790) belonging to SCPL group II; *CBPY YEAST* (NCBI accession NO. P00729) belongs to SCPL group III.

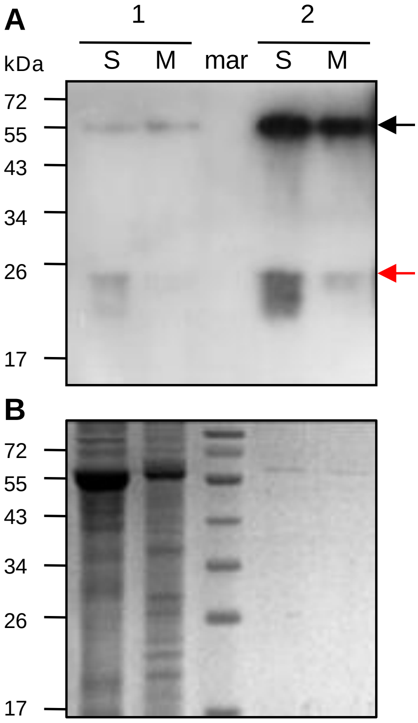


Figure S3. Assay of GS5-FLAG protein in *P_{Ubi}::GS5-FLAG* transgenic positive plants. (A) Western blot assay of GS5-FLAG transgenic plants using anti-FLAG antibody showing the cleavage of GS5-FLAG in *P_{Ubi}::GS5-FLAG* transgenic positive plants into two chains (A and B). 1, Total protein extracted from transgenic positive plants; 2, Eluted proteins after anti-FLAG M2 agarose affinity purification; mar, Marker; S, Soluble protein sample; M, solubilizd membrane protein sample; black arrow, full-length GS5-FLAG protein; red arrow, the C-terminal of GS5, B chain-FLAG peptide. (B) Coomassie Blue-stained protein samples of (A).

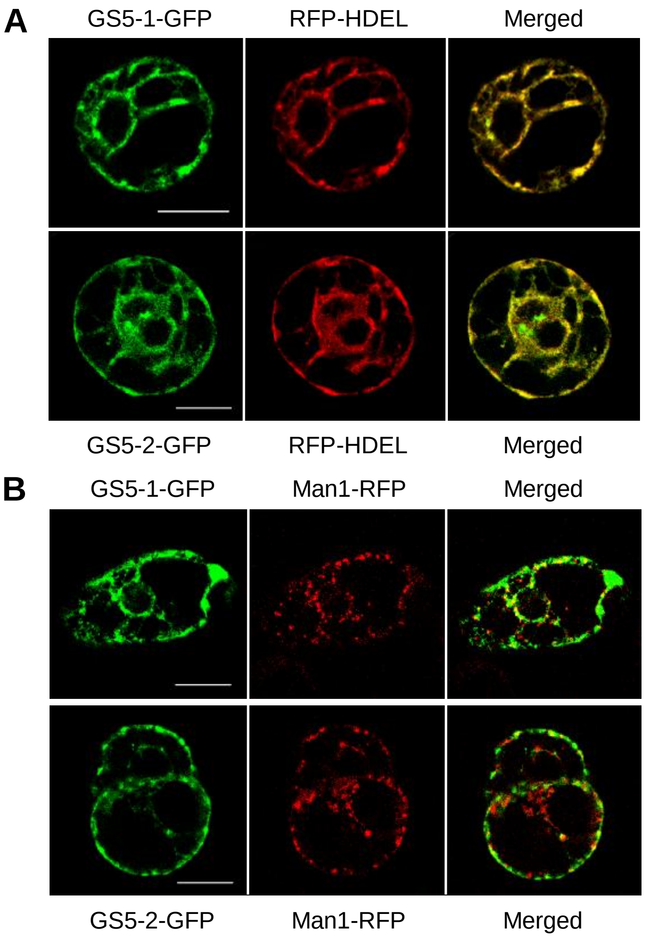
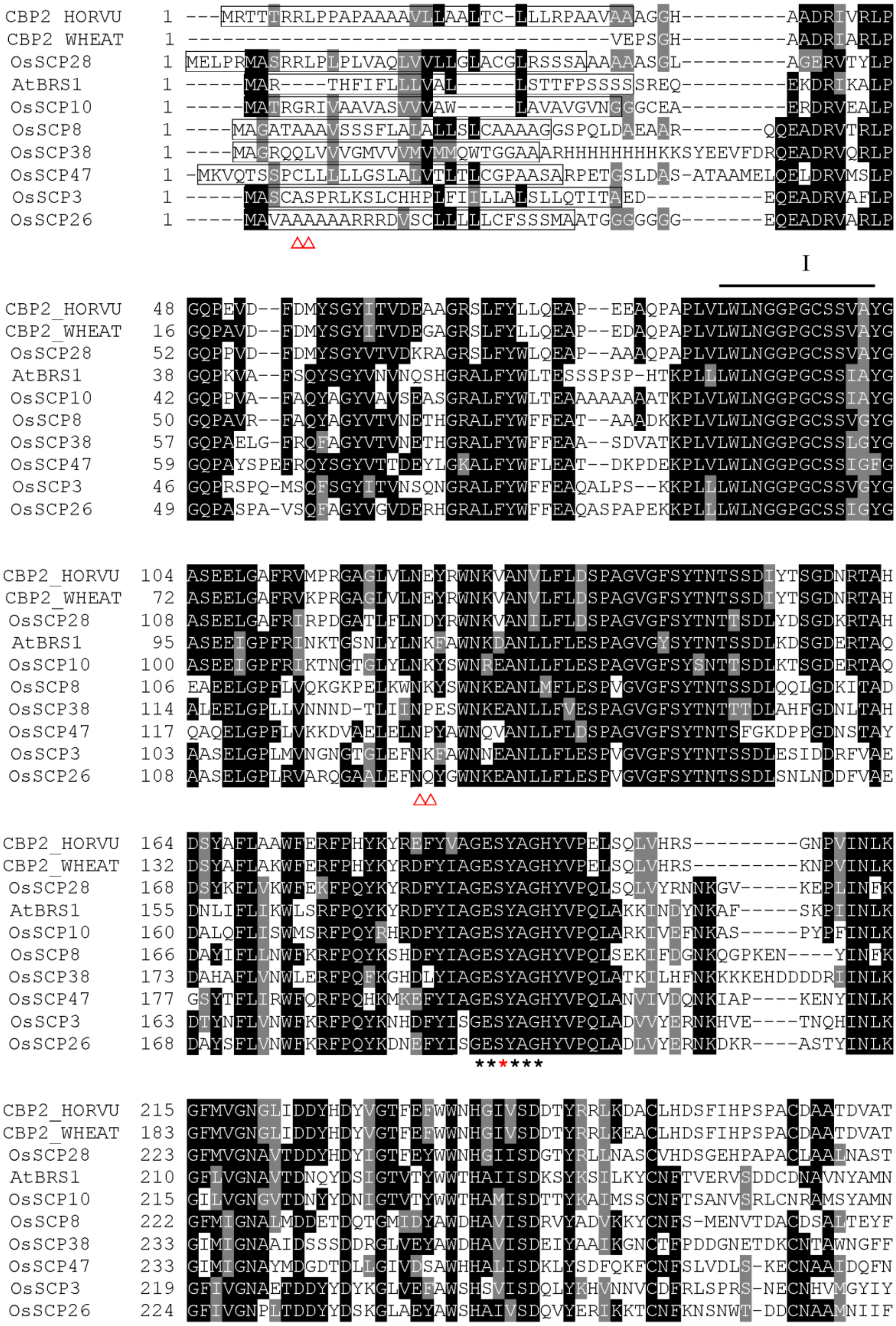


Figure S4. Co-localization assay of GS5 with endoplasmic reticulum marker inside the cell. (A) Co-expression of GS5-GFP and endoplasmic reticulum marker RFP-HDEL in tobacco BY-2 protoplasts showing co-localization of the two proteins. (B) Co-expression of GS5-GFP and *cis*-Golgi marker Man1-RFP in tobacco BY-2 protoplasts showing distinct localization of the two proteins. The experiments were repeated three times, yielding similar results, and representative images are shown. Scales bars = 20 μm.



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CBP2 HORVU     1 ---MRTTTRRLPPAPAAAAVILAALTC-LLLRPAAVAAAGGH---------AADRIVRLP
CBP2 WHEAT     1 ------------------------------------VEPSGH---------AADRIARLP
OsSCP28        1 MELPRMASRRLPLPLVAQLVVLLGLACGLRSSSAAAAAASGL---------AGERVTYLP
AtBRS1         1 -----MAR----THFIFLLLVAL-----LSTTFPSSSSSREQ---------EKDRIKALP
OsSCP10        1 -----MATRGRIVAAVASVVVAW-----LAVAVGVNGGGCEA---------ERDRVEALP
OsSCP8         1 ----MAGATAAAVSSSFLALALLSLCAAAAGGSPQLDAEAAR-------QQEADRVTRLP
OsSCP38        1 ----MAGRQQLVVVGMVVVMVMMQWTGGAAARHHHHHHHHKKSYEEVFDRQEADRVQRLP
OsSCP47        1 -MKVQTSSPCLLLLLGSLALVTLTLCGPAASARPETGSLDAS-ATAAMELQELDRVMSLP
OsSCP3         1 -----MASCASPRLKSLCHHPLFIILLALSLLQTITAED----------EQEADRVAFLP
OsSCP26        1 -----MAVAAAAAARRRDVSCLLLLLCFSSSMAATGGGGGGG-------EQEADRVARLP
                          ΔΔ

                                                                     I
CBP2_HORVU    48 GQPEVD--FDMYSGYITVDEAAGRSLFYLLQEAP--EEAQPAPLVLWLNGGPGCSSVAYG
CBP2_WHEAT    16 GQPAVD--FDMYSGYITVDEGAGRSLFYLLQEAP--EDAQPAPLVLWLNGGPGCSSVAYG
OsSCP28       52 GQPPVD--FDMYSGYVTVDKRAGRSLFYWLQEAP--AAAQPAPLVLWLNGGPGCSSVAYG
AtBRS1        38 GQPKVA--FSQYSGYVNVNQSHGRALFYWLTESSSPSP-HTKPLILWLNGGPGCSSIAYG
OsSCP10       42 GQPPVA--FAQYAGYVAVSEASGRALFYWLTEAAAAAAAATKPLVLWLNGGPGCSSIAYG
OsSCP8        50 GQPAVR--FAQYAGYVTVNETHGRALFYWFFEAT--AAADKKPLVLWLNGGPGCSSVGYG
OsSCP38       57 GQPAELG-FRQFAGYVTVNETHGRALFYWFFEAA--SDVATKPLVLWLNGGPGCSSLGYG
OsSCP47       59 GQPAYSPEFRQYSGYVTTDEYLGKALFYWFLEAT--DKPDEKPLVLWLNGGPGCSSIGFG
OsSCP3        46 GQPRSPQ-MSQFSGYITVNSQNGRALFYWFFEAQALPS--KKPLLLWLNGGPGCSSVGYG
OsSCP26       49 GQPASPA-VSQFAGYVGVDERHGRALFYWFFEAQASPAPEKKPLLLWLNGGPGCSSIGYG

CBP2_HORVU   104 ASEELGAFRVMPRGAGLVLNEYRWNKVANVLFLDSPAGVGFSYTNTSSDIYTSGDNRTAH
CBP2_WHEAT    72 ASEELGAFRVKPRGAGLVLNEYRWNKVANVLFLDSPAGVGFSYTNTSSDIYTSGDNRTAH
OsSCP28      108 ASEELGAFRIRPDGATLFLNDYRWNKVANILFLDSPAGVGFSYTNTTSDLYDSGDKRTAH
AtBRS1        95 ASEEIGPFRINKTGSNLYLNKFAWNKDANLLFLESPAGVGYSYTNTSSDLKDSGDERTAQ
OsSCP10      100 ASEEIGPFRIKTNGTGLYLNKYSWNREANLLFLESPAGVGFSYSNTTSDLKTSGDERTAQ
OsSCP8       106 EAEELGPFLVQKGKPELKWNKYSWNKEANLMFLESPVGVGFSYTNTSSDLQQLGDKITAD
OsSCP38      114 ALEELGPLLVNNND-TLIINPESWNKEANLLFVESPAGVGFSYTNTTTDLAHFGDNLTAH
OsSCP47      117 QAQELGPFLVKKDVAELELNPYAWNQVANLLFLDSPAGVGFSYTNTSFGKDPPGDNSTAY
OsSCP3       103 AASELGPLMVNGNGTGLEFNKFAWNNEANLLFLESPVGVGFSYTNTSSDLESIDDRFVAE
OsSCP26      108 AASELGPLRVARQGAALEFNQYGWNKEANLLFLESPVGVGFSYTNTSSDLSNLNDDFVAE
                                    ΔΔ

CBP2_HORVU   164 DSYAFLAAWFERFPHYKYREFYVAGESYAGHYVPELSQLVHRS---------GNPVINLK
CBP2_WHEAT   132 DSYAFLAKWFERFPHYKYRDFYIAGESYAGHYVPELSQLVHRS---------KNPVINLK
OsSCP28      168 DSYKFLVKWFEKFPQYKYRDFYIAGESYAGHYVPQLSQLVYRNNKGV-----KEPLINFK
AtBRS1       155 DNLIFLIKWLSRFPQYKYRDFYIAGESYAGHYVPQLAKKINDYNKAF-----SKPIINLK
OsSCP10      160 DALQFLISWMSRFPQYRHRDFYIAGESYAGHYVPQLARKIVEFNKAS-----PYPFINLK
OsSCP8       166 DAYIFLLNWFKRFPQYKSHDFYIAGESYAGHYVPQLSEKIFDGNKQGPKEN----YINFK
OsSCP38      173 DAHAFLVNWLERFPQFKGHDLYIAGESYAGHYVPQLATKILHFNKKKKEHDDDDRIINLK
OsSCP47      177 GSYTFLIRWFQRFPQHKMKEFYIAGESYAGHYVPQLANVIVDQNKIAP----KENYINLK
OsSCP3       163 DTYNFLVNWFKRFPQYKNHDFYISGESYAGHYVPQLADVVYERNKHVE----TNQHINLK
OsSCP26      168 DAYSFLVNWFKRFPQYKDNEFYISGESYAGHYVPQLADLVYERNKDKR----ASTYINLK
                                         ******

CBP2_HORVU   215 GFMVGNGLIDDYHDYVGTFEFWWNHGIVSDDTYRRLKDACLHDSFIHPSPACDAATDVAT
CBP2_WHEAT   183 GFMVGNGLIDDYHDYVGTFEFWWNHGIVSDDTYRRLKEACLHDSFIHPSPACDAATDVAT
OsSCP28      223 GFMVGNAVTDDYHDYIGTFEYWWNHGIISDGTYRLLNASCVHDSGEHPAPACLAALNAST
AtBRS1       210 GFLVGNAVTDNQYDSIGTVTYWWTHAIISDKSYKSILKYCNFTVERVSDDCDNAVNYAMN
OsSCP10      215 GILVGNGVTDNYYDNIGTVTYWWTHAMISDTTYKAIMSSCNFTSANVSRLCNRAMSYAMN
OsSCP8       222 GFMIGNALMDDETDQTGMIDYAWDHAVISDRVYADVKKYCNFS-MENVTDACDSALTEYF
OsSCP38      233 GIMIGNAAIDSSSDDRGLVEYAWDHAVISDEIYAAIKGNCTFPDDGNETDKCNTAWNGFF
OsSCP47      233 GIMIGNAYMDGDTDLLGIVDSAWHHALISDKLYSDFQKFCNFSLVDLS-KECNAAIDQFN
OsSCP3       219 GFIVGNAETDDYYDYKGLVEFAWSHSVISDQLYKHVNNVCDFRLSPRS-NECNHVMGYIY
OsSCP26      224 GFIVGNPLTDDYYDSKGLAEYAWSHAIVSDQVYERIKKTCNFKNSNWT-DDCNAAMNIIF
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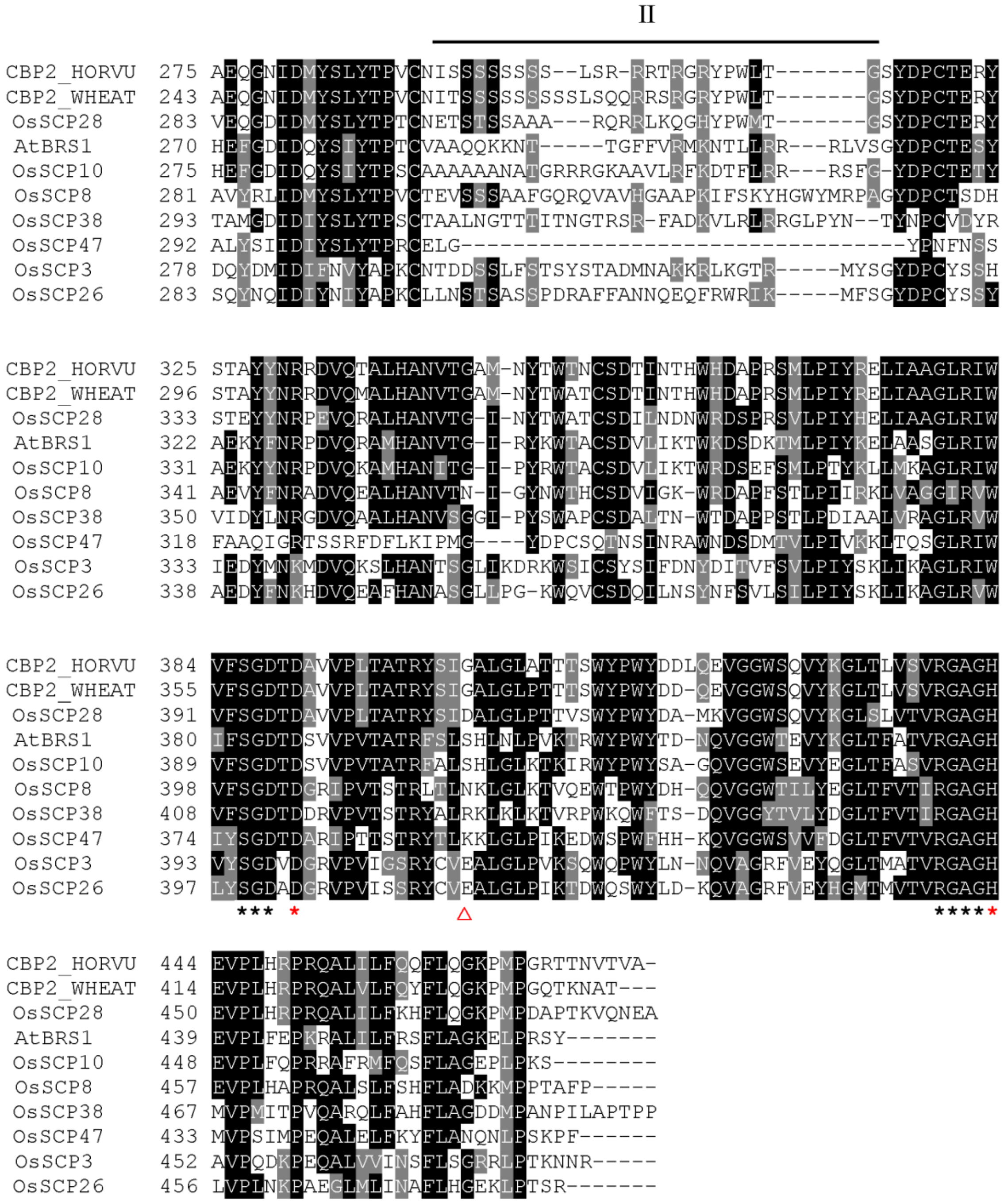

Figure S5. Amino acid alignment of group II serine carboxypeptidase-like proteins. The boxed amino acids at the N terminus represent the putative signal peptide. I and II indicate the putative substrate binding region and linker peptide respectively. The A-chain is between signal peptide and linker peptide, and B-chain is downstream of the linker peptide. The red triangles indicate the different residues between GS5-1 and GS5-2 (OsSCP26). The red asterisks indicate the Ser-Asp-His catalytic triad of SCPL, and the black asterisks mark the residues essential for catalysis.

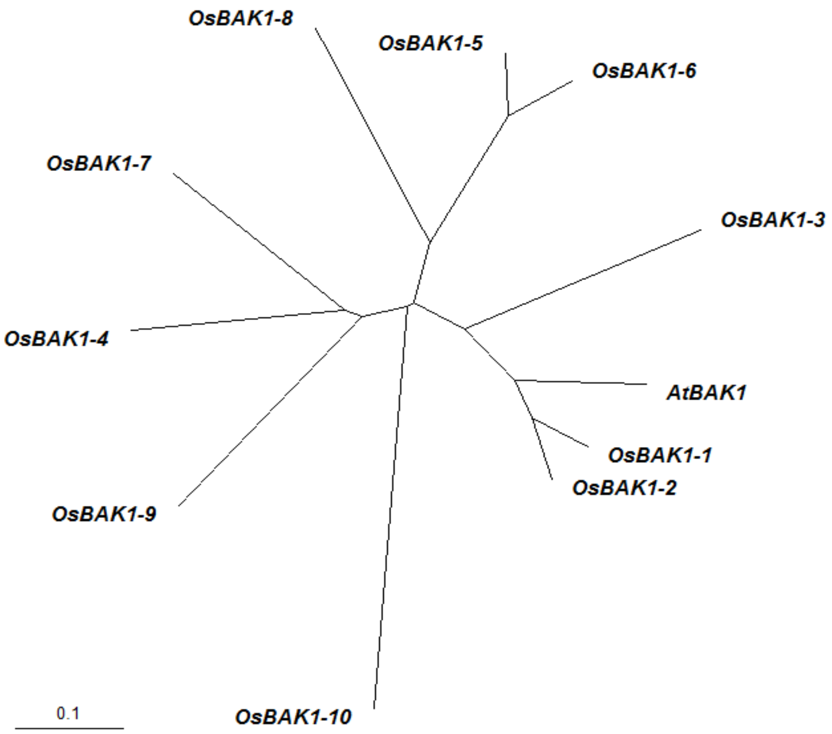

Figure S6. Phylogenetic tree of the rice BAK1 homologues. Full-length protein sequences of the rice BAK1 homologues were obtained by BLAST using the AtBAK1 (NCBI accession NO. NM_119497) sequence from http://rice.plantbiology.msu.edu/index.shtml, aligned with ClustalX and calculated with MEGA 3.1. OsBAK1-1, LOC_Os08g07760; OsBAK1-2, LOC_Os04g38480; OsBAK1-3, LOC_Os06g12120; OsBAK1-4, LOC_Os01g07630; OsBAK1-5, LOC_Os02g18320; OsBAK1-6, LOC_Os03g49620; OsBAK1-7, LOC_Os06g16330; OsBAK1-8, LOC_Os11g39370; OsBAK1-9, LOC_Os02g14120; and OsBAK1-10, LOC_Os03g16010.

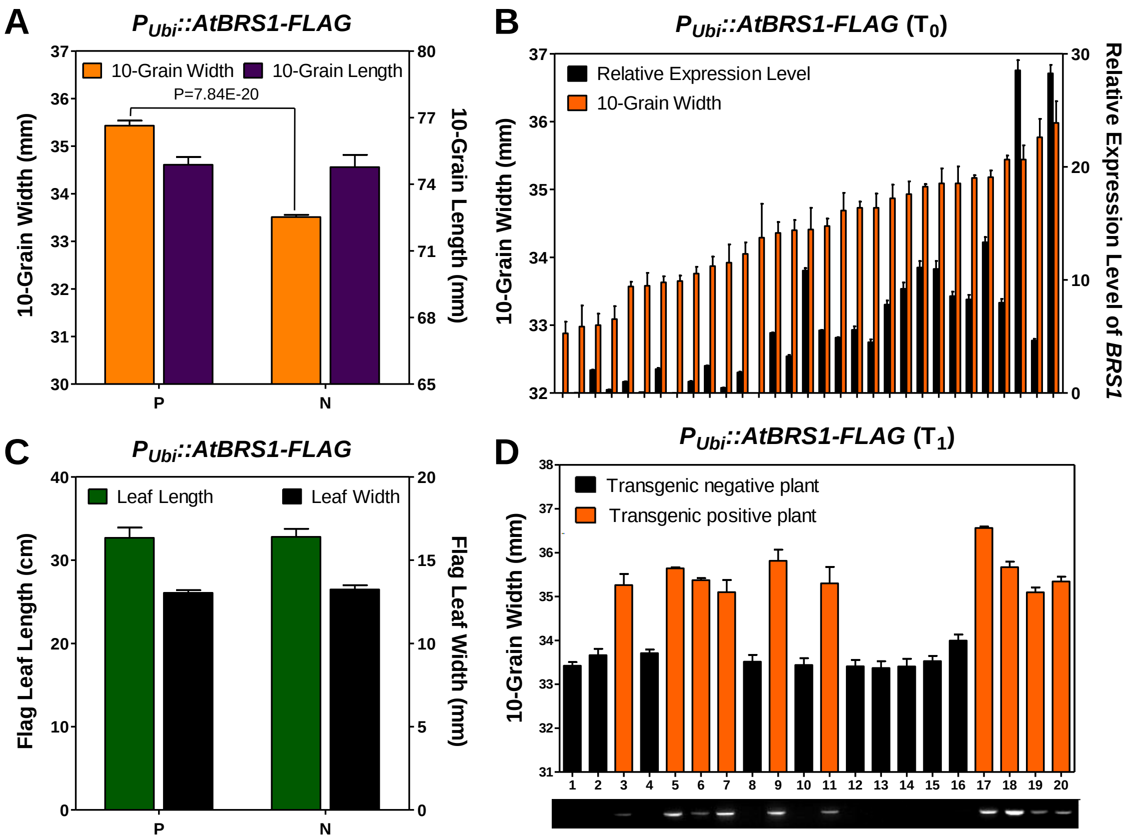

Figure S7. Grain size and leaf size of P_{Ubi}:: *AtBRS1-FLAG* transgenic plants. (A) Grain width and grain length of T1 transgenic rice plants overexpressing *AtBRS1*. P, transgenic positive plants; N, transgenic negative plants. (B) Co-segregation of grain width with expression level of *AtBRS1* in T0 transgenic rice plants. (C) Flag leaf width and length of T1 transgenic rice plants overexpressing *AtBRS1*. P, transgenic positive plants; N, transgenic negative plants. (D) Co-segregation of grain width with *AtBRS1* genotype in T1 transgenic rice plants. All data are presented as mean ± SE (n≥3). *P* values were calculated using Student's *t*-test.

Table S1. Polymorphisms and *cis* -acting elements in the 2-kb promoter of *GS5* .

Position	**SNPs/InDels**		**Sequence**	***Cis* -acting elements**	**Organism**	**Function**
	GS5-1	***GS5-2***				
–1961	/	/	CAANNNNATC	circadian	*Lycopersicon esculentum*	Circadian control
–1723	/	/	ATTAAT	Box4	*Petroselinum crispum*	Light responsiveness
–1656	/	/	AATCTAATCT	ATCT-motif	*Arabidopsis thaliana*	Light responsiveness
–1630	/	/	CAACTG	MBS	*Arabidopsis thaliana*	MYB binding site, drought-inducibility
				E-box	*Arabidopsis thaliana*	Brassinosteroid responsiveness
–1620	/	/	GGAGATG	GAG-motif	*Hordeum vulgare*	part of a light responsive element
–1595	C	T	/	/	/	/
–1539	/	/	CATATG	E-box	*Arabidopsis thaliana*	Brassinosteroid responsiveness
–1518	/	/	CACGTG	ABRE	*Arabidopsis thaliana*	Abscisic acid responsiveness
				G-box	*Arabidopsis thaliana*	Light responsiveness
				E-box	*Arabidopsis thaliana*	Brassinosteroid responsiveness
–1514	/	/	TGACG	TGACG-motif	*Hordeum vulgare*	MeJA-responsiveness
–1489	/	/	CCGTCC	A-box	*Petroselinum crispum*	*cis* -acting regulatory element
				CCGTCC-box	*Arabidopsis thaliana*	Meristem specific activation
–1476	-	A	/	/	/	/
–1466	T	A	/	/	/	/
–1464	T	G	/	/	/	/
–1463	A	G	/	/	/	/
–1391	G	A	/	/	/	/
–1376	/	/	ATTAAT	Box4	*Petroselinum crispum*	Light responsiveness
–1345	/	/	ATTTTCTTCA	TC-rich repeats	*Nicotiana tabacum*	Defense and stress responsiveness
–1314	/	/	CGGTCA	MBS	*Zea mays*	MYB Binding Site
–1303	A	G	/	/	/	/
–1281	T	-	/	/	/	/
–1242	/	/	TGACG	TGACG-motif	*Hordeum vulgare*	MeJA-responsiveness
–1223	/	/	CACTTG	E-box	*Arabidopsis thaliana*	Brassinosteroid responsiveness
–1218	/	/	GTCAT	Skn-1-motif	*Oryza sativa*	Endosperm expression
–1204	/	/	CCG/ACCC	Spl	*Zea mays*	Light responsive element
–1164	/	/	CACTTG	E-box	*Arabidopsis thaliana*	Brassinosteroid responsiveness
–1159	/	/	GTCAT	Skn-1-motif	*Oryza sativa*	Endosperm expression
–1142	/	/	CATTTG	E-box	*Arabidopsis thaliana*	Brassinosteroid responsiveness
–1109	C	T	/	/	/	/
–1096	/	/	CAATTG	E-box	*Arabidopsis thaliana*	Brassinosteroid responsiveness
–1088	/	/	AAACAGA	GARE-motif	*Brassica oleracea*	Gibberellin-responsive element
–1076	A	G	/	/	/	/
–1032	A	T	/	/	/	/
–1015	G	T	/	/	/	/

Continuing Table S1

–1013	/	/	CAGATG	E-box	*Arabidopsis thaliana*	Brassinosteroid responsiveness
–895		G	GATAGGA	GATA-motif	*Arabidopsis thaliana*	part of a light responsive element
	A		GATAAGATT	I-box	*Arabidopsis thaliana*	part of a light responsive element
–879	/	/	TTTCAAA	BoxI	*Pisum sativum*	Light responsive element
–847	T	-	/	/	/	/
–825		T	TCTTAC	TCT-motif	*Arabidopsis thaliana*	part of a light responsive element
	C		/	/	/	/
–788		C	CCG/ACCC	Spl	*Zea mays*	Light responsive element
	A		/	/	/	/
–756	/	/	ATTAAT	Box4	*Petroselinum crispum*	Light responsiveness
–710	/	/	GTCAT	Skn-1-motif	*Oryza sativa*	Endosperm expression
–671		G	TGACG	TGACG-motif	*Hordeum vulgare*	MeJA-responsiveness
	A		/	/	/	/
–409	/	/	AAACAGA	GARE-motif	*Brassica oleracea*	Gibberellin-responsive element
–375	/	/	TAACTG	MBS	*Arabidopsis thaliana*	MYB binding site, drought-inducibility
–371	/	/	TGAGTCA	GCN4-motif	*Oryza sativa*	Endosperm expression
–368	/	/	GTCAT	Skn-1-motif	*Oryza sativa*	Endosperm expression
–326	-	C	/	/	/	/
–325	-	A	/	/	/	/
–324	-	A	/	/	/	/
–323	-	A	/	/	/	/
–225	/	/	ATTTTCTTCA	TC-rich repeats	*Nicotiana tabacum*	Defense and stress responsiveness
–100	/	/	TTTCTTCTCT	5'UTR Py-rich stretch	*Lycopersicon esculentum*	Conferring high transcription levels

-, single nucleotide deletion; /, not detected.

Table S2. Primers used in this work.

Name	Sequence
Expression analysis	
GS5qF	AGTGGACTGCTTCCAGGGAAG
GS5qR	CACGCAGTACCGAGAACTGA
Act1F	TGCTATGTACGTCGCCATCCAG
Act1R	AATGAGTAACCACGCTCCGTCA
Promoter constructs	
PAF	AAGCTTTCGTATGGAATTACCTAGCTATGCA
PBF	AAGCTTTTGACGAGAGGCAAGGACCCCA
PCF	AAGCTTGATGGGAGAGATAGATAGACTC
PDF	AAGCTTTGCAACCCGGATCTGGACAGA
PDR	GGATCCTGCTAACCCGTGGTGCCGAGCTCTAGC
–1109mutF	ACACAGGATTTTGACAGGAATAC
–1109mutR	GTATTCCTGTCAAAATCCTGTGT
–1032mutF	TGATTGGACCACTGAAAAAAC
–1032mutR	GTTTTTTCAGTGGTCCAATCA
–825mutF	CTCCAAAATTCTTACATTTTTTCT
–825mutR	AGAAAAAATGTAAGAATTTTGGAG
B_GAmutF	ACAATTGTAACCAAGAGGATTTGTA
B_GAmutR	TACAAATCCTCTTGGTTACAATTGT
C_LIGHTmutF	CTCCAAAATCCTTACATTTTTTC
C_LIGHTmutR	GAAAAAATGTAAGGATTTTGGAG
cDNA constructs	
GS5cDNAF	GGTACCTAGCTAGAGCTCGGCACCACGGGTTAGCAA
GS5cDNAR	GGATCCTCTGCTTGTCGGAAGCTTCTCACCATGAAG
OsBAK1-7cDNAF	GGTACCTACCTGAGCTCAGCAATGGA
OsBAK1-7cDNAR	GTTAACTCTGGGGCCGGAGAGCTC
OsMSBP1cDNAF	CTCGAGATGGCGGCGGCGGTGGCGGA
OsMSBP1cDNAR	GGATCCACTTTCTTTGGCGCCTTCATCAGGTGCA
GS5AchainF	GAATTCGGGATCCATATGGAGCAGGAGGCTGACAGGGT
GS5AchainR	CTGCAGCTCGAGGCGGCCGCTTAGAAGGCACTTTGGGGCA
GS5BchainF	GAATTCGGGATCCATATGTTTTCAGGCTATGATCCATGC
GS5BchainR	CTGCAGCTCGAGGCGGCCGCTTCTGCTTGTCGGAAGCTT
OsBAK1-4LRRF	ACTAGTATGGAATTCAACTACGAAGTGGTGGCGCTGA
OsBAK1-4LRRR	CTCGAGGATATCATCTCCAGACTTGACACCACAGATC
OsBAK1-7LRRF	ACTAGTATGGAATTCAACAACGAAGTGCAAGCTCTGATTG
OsBAK1-7LRRR	GCGGCCGCGGATCCATCCTGCTCCCTGTTGGCGTC
OsBAK1-9LRRF	ACTAGTATGGAATTCAACTACGAAGTGGCGGCGCTGATG
OsBAK1-9LRRR	CTCGAGGGATCCGCAACCATGCATAATTGATGAATTGC
OsBAK1-4KDF	GGATCCCATATGAGGCGCAATCAGCAGATCTT
OsBAK1-4KDR	ACTAGTGAATTCCCTCGGTCCAGAGAGCTCCA
OsBAK1-7KDF	GGATCCCATATGCGCCGCAACCGGCAGATCCT
OsBAK1-7KDR	ACTAGTGAATTCTCTGGGGCCGGAGAGCTCGA
OsBAK1-9KDF	CATATGGAATTCTGCAGATGGCGCTTGCCTTT
OsBAK1-9KDR	ACTAGTCTCGAGTCTGGGACCAGATAGCTCAATTGG