

# Fig. S1. Signal distribution of the rice ASDM after background correction (a), normalization between slides (b) and a consistency test between microarrays (c).

**a**, **b** The data were processed with limma package in R (http://www.bioconductor.org/) using backgroundCorrect and normalizeBetweenArrays functions, respectively. plotDensities functions were used to analyze the distribution of probe intensities from 8 microarray data. Frequency densities before and after normalization were plotted against log2-based intensities. **c** Log2-based intensities of 2 leaf samples were compared. The linear model was y=0.998x + 0.018, r2 = 0.996.



seq_id (ASDM)	Leaf	Root	P1cm	S21DAP	CV
① Os04t0612500-01	33,864	309	1,348	5,214	1.6
② Os04t0612500-01_UE	7,667	135	325	1,045	1.6
③ Os04t0612500-02	41,814	273	2,979	5,436	1.6

SEQ_ID (RNA-Seq)	Leaf	Root	P1cm	Seed	CV
Os04t0612500-01_E004	2,274	13	44	144	1.8
Os04t0612500-02_E006	602	1	3	37	1.8



#### Fig. S2A. The analysis of a locus, Os04g0612500, which has two transcripts, Os04t0612500-01 and Os04t0612500-02 (proline-rich proteins).

**a** Design of feature probes in ASDM. Feature probes and primers for PCR were indicated as single arrows and thick arrows, respectively. UniqExonJ was indicated as arrow heads in Os04t0612500-01 in the junctions of two spliced exons. **b** Intensities in ASDM (left) and counts in RNA-Seq (right). Exons in RNA-Seq are designated as in Fig. 1B. **c** Analysis by RT-PCR and **d** quantitative real-time PCR. A locus, Os04g0612500, was highly expressed in the leaf. The rice Tubulin gene was used as a control. The expression of the lowest stage in each transcript was set to 1.



## b

seq_id (ASDM)	Leaf	Root	P1cm	S21DAP	CV	SEQ_ID (RNA-Seq)	Leaf	Root	P1cm	Seed	CV
1 Os10t0471100-01	238	182	32,324	1,839	1.8	Os10t0471100-01_E001	1	0	401	13	1.9
② Os10t0471100-01_UE	56	54	2,606	211	1.7	0.1010471100 00 5000	0	0	244	F	1.0
③ Os10t0471100-02	44	23	1,475	48	1.8	OS10t04/1100-02_E008	0	0	244	5	1.9



### Fig. S2B. Os10g0471100 was active in the P1cm stage.

Os10t0471100-01 and Os10t0471100-02 encoded a protein with high similarity to proteins involved in wax production and CER1, respectively. **a** Feature probes and primers for PCR as indicated above. **b** Intensities in ASDM (left) and counts in RNA-Seq (right). **c** Analysis by RT-PCR and **d** quantitative real-time PCR.



## b

seq_id (ASDM)	Leaf	Root	P1cm	S21DAP	CV	SEQ_ID (RNA-Seq)	Leaf
1 Os07t0673400-01	1,614	228	1,377	40,940	1.8	Os07t0673400-01 E001	16
② Os07t0673400-01_UE	419	172	369	3,138	1.4		-
③ Os07t0673400-02	309	191	176	788	0.8	Os0/t06/3400-02_E003	1

SEQ_ID (RNA-Seq)	Leaf	Root	P1cm	Seed	CV
Os07t0673400-01_E001	16	1	4	14	0.8
Os07t0673400-02_E003	1	0	1	10	1.6



### Fig. S2C. Os07g0673400 was active in the S21DAP stage.

Os07t0673400-01 and Os07t0673400-02 encoded a protein with high similarity to proteins involved in Rossmann-like alpha/beta/alpha sandwich fold domain containing protein and similar to universal stress protein, respectively. a Feature probes and primers for PCR as indicated above. b Intensities in ASDM (left) and counts in RNA-Seq (right). c Analysis by RT-PCR and d quantitative real-time PCR.





## Fig. S3. Data distribution of raw RNA-Seq. Dispersion of raw (a) and normalized counts (b) and a consistency test between 2 independent RNA-seq using leaf samples (c).

a, The data was processed with density function of R. b The initial perData set was loaded and normalized with DEXSeqDataSetFromHTSeq and estimateSizeFactors, respectively in DEXSeq package. -exon dispersion estimates (shown by black points), the fitted mean-dispersion values function (red line), and the shrunken values in blue. c Log2-based intensities of 2 leaf samples were compared. The linear model was y=0.943x + 0.404, r2 = 0.889.



**Fig. S4.** Data comparison between the intensities of ASDM and the counts of RNA-seq in a public database, TENOR (http://tenor.dna.affrc.go.jp). RNA-Seq data Soot\_Count\_10d and Root\_Coun\_10d were obtained from the TENOR database. The values are represented in x- and y-axes, respectively. The linear regression model gave slopes of 0.48 and 0.55 for the leaf (a) and root (b), respectively. Pearson correlation coefficients ranged 0.25-0.27.



**Fig. S5.** The distribution of CVs of 100 constitutively expressed genes in CREP (mean = 0.18, sd 0.02) and their corresponding genes in ASDM (mean = 0.11, sd 0.05) and RNA-Seq (mean = 0.34, sd 0.19) datasets. The gene list was obtained from reported in Wang et al. (2010) and the values in the ASDM and RNA-Seq are retrieved in table S8 in this report.

### Whole loci by ASDM

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GO:0032502\_developmental process GO:0032501\_multicellular organismal process GO:0065007\_biological regulation GO:0050896\_response to stimulus GO:0044699\_single-organism process GO:0002376\_immune system process GO:0051704\_multi-organism process GO:0044848\_biological phase GO:000003\_reproduction GO:0022414\_reproductive process GO:0071840\_cellular component organization or biogenesis GO:0070271\_protein complex biogenesis GO:0019953\_sexual reproduction GO:0019882\_antigen processing and presentation GO:0040011\_locomotion GO:0032504\_multicellular organism reproduction GO:0040007\_growth GO:0006488\_dolichol-linked oligosaccharide\_ GO:0034969\_histone arginine methylation GO:0008168\_methyltransferase activity GO:0023052\_signaling GO:0009987\_cellular process GO:0008152\_metabolic process GO:0051179\_localization GO:0022613\_ribonucleoprotein complex biogenesis

## Single ts loci





GO:0051179 localization GO:0008152 metabolic process GO:0032501 multicellular organismal process GO:0032502 developmental process GO:0050896 response to stimulus GO:0044699 single-organism process GO:0065007 biological regulation GO:0071840\_cellular component organization or biogenesis GO:0009987 cellular process GO:0009657 plastid organization GO:0022414 reproductive process GO:000003 reproduction GO:0044848 biological phase GO:0051704\_multi-organism process GO:0002376 immune system process GO:0061024 membrane organization GO:0071554\_cell wall organization or biogenesis GO:0001906 cell killing GO:0023052\_signaling GO:0031163\_metallo-sulfur cluster assembly GO:0040007 growth GO:0006665 sphingolipid metabolic process GO:0080165 callose deposition in phloem sieve plate GO:0002252 immune effector process GO:0009453\_energy taxis GO:0022622\_root system development GO:0031647 regulation of protein stability GO:0044419 interspecies interaction between organisms GO:0010229 inflorescence development GO:0009615\_response to virus GO:0002683\_negative regulation of immune system process

GO:0043901\_negative regulation of multi-organism process

### Alternatively spliced ts loci

CA CAN CANE AND

GO:0032501 multicellular organismal process GO:0065007 biological regulation GO:0032502 developmental process GO:0051179 localization GO:0071840 cellular component organization or biogenesis GO:0044699 single-organism process GO:0050896 response to stimulus GO:0051704 multi-organism process GO:0002376 immune system process GO:0009987 cellular process GO:0008152 metabolic process GO:0009608 response to symbiont GO:0002504\_antigen processing and GO:000003 reproduction GO:0070555\_response to interleukin-1 GO:0007610 behavior GO:0044848\_biological phase GO:0022610\_biological adhesion GO:0040011\_locomotion GO:0023052 signaling GO:0022414\_reproductive process GO:0040007 growth

#### Whole loci by RNA-Seq

CN CN CHICHT



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GO:0008152\_metabolic process GO:0051704\_multi-organism process GO:0002376\_immune system process GO:0050896\_response to stimulus GO:0044699\_single-organism process GO:0065007\_biological regulation GO:0051179\_localization GO:0051179\_localization GO:0071840\_cellular component organization or biog enesis GO:0022613\_ribonucleoprotein complex biogenesis GO:0009987\_cellular process

GO:0023052\_signaling

GO:0010228\_vegetative to reproductive phase transit ion of meristem GO:0097354\_prenylation

GO:0006914\_autophagy

GO:0070085\_glycosylation

GO:0032501\_multicellular organismal process

GO:0032502\_developmental process

GO:0040007\_growth

GO:0009880\_embryonic pattern specification GO:0048508\_embryonic meristem development Fig. S6. GO analysis of loci of whole genes (a) and the genes with single loci (b, left) or alternatively spliced transcripts (b, right) loci between CVI, CVII, CVIII-i and CVIII-ii based on ASDM. GO analysis of loci of whole genes by RNA-Seq is presented in (c). As the expression of the genes in CVIII-iii is very low, the group is disregard. GO terms that are enriched in CVI and CVII are GO:0032502 developmental process, GO:0032501 multicellular organismal process. GO:0065007 biological regulation. GO:0050896 response to stimulus. GO:0044699 single-organism process. GO:0009987 cellular process and GO:0008152 metabolic process are also enriched in CVIII-i, GO terms that are enriched in CVII are GO:0044848 biological phase. GO:0000003 reproduction. GO:0022414 reproductive process. GO terms that are enriched in CVIII-i are GO:0019953 sexual reproduction, GO:0019882 antigen processing and presentation, GO:0040011 locomotion, GO:0032504 multicellular organism reproduction, GO:0040007 growth. GO analysis of loci of single transcript loci (b, left) and alternatively spliced transcript loci (b, right) between CVI, CVII, CVIII-i and CVIII-ii. Next we tested GO enrichments of CV groups are affected by the loci with single or alternative spliced transcripts. The number of loci with single and alternative spliced transcripts are 16.999 and 5844, respectively. The number of loci with single transcript (16.999) are 4.172. 4,607, 3,154, 5,056 according to the groups of CVI, CVII, CVIII-I, and CVIII-ii, respectively. For these genes, GO enrichments are tested. GO:0009657 plastid organization is highly enriched by single transcript in CVI. GO terms that are moderately enriched by single transcript in CVI are GO:0051704 multi-organism process, GO:0002376 immune system process, GO:0061024 membrane organization, GO:0071554 cell wall organization or biogenesis, GO:0001906 cell killing, GO:0023052 signaling, GO:0031163 metallo-sulfur cluster assembly, GO:0040007 growth. GO terms that are moderately enriched by single transcript in CVII are GO:0009987 cellular process, GO:0000003 reproduction, GO:0044848 biological phase, GO:0080165 callose deposition in phoem sieve plate, GO:0002252 immune effector process. GO terms that are enriched by genes with single transcript in CVI and CVII are GO:0032501 multicellular organismal process, GO:0032502 developmental process, GO:0050896 response to stimulus, GO:0044699 single-organism process, GO:0065007 biological regulation. GO terms that are moderately enriched by single transcript in CVIII-i are GO:0009453 energy taxis, GO:0022622 root system development, GO:0031647 regulation of protein stability, GO:0044419 interspecies interaction between organisms, GO:0010229 inflorescence development, GO:0009615 response to virus, GO:0002683 negative regulation of immune system process, GO:0043901 negative regulation of multi-organism process. The number of loci with alternatively spliced transcripts (5844) are 690, 1276, 1632, 2246 according to the groups of CVI, CVII, CVIII-i, and CVIII-ii, respectively. In general the GO enrichement by the loci with alternative spliced transcripts are in agreement with those of the loci with single transcript. A exception is GO:0040007 growth. The term is highly enriched in this group while that of loci with single transcript is enriched a little bit. For these genes, GO enrichments are tested. GO terms are enrinched by CVI, CVII, CVIII-i are GO:0032501 multicellular organismal process, GO:0065007 biological regulation, GO:0032502 developmental process. GO:0051179 localization, GO:0071840 cellular component organization or biogenesis, GO:0044699 single-organism process, GO:0050896 response to stimulus, GO:0051704 multi-organism process, GO:0002376 immune system process, GO:0009987 cellular process, GO:0008152 metabolic process. GO:0040011 locomotion, GO:0044848 biological phase, GO:0007610 behavior are moderately enriched in CVI, CVII, CVIII-I, respectively. c GO analysis of loci of whole genes between CVI, CVII, CVIII-i and CVIII-ii based on RNA-Seq dataset. In the analysis, 47 GO terms are enriched by RNA-Seq while 25 terms are by ASDM. Generally speaking, many terms are overlapped in both analyses such as GO:0032501 multicellular organismal process, GO:0065007 biological regulation, GO:0050896 response to stimulus, and GO:0009987 cellular process. Among 20 highly enriched processes by RNA-Seq 14 terms are found in ASDM. GO terms that are enriched in CVI and CVII are GO:0008152 metabolic process, GO:0050896 response to stimulus, GO:0044699 single-organism process, GO:0065007 biological regulation. GO:0071840 cellular component organization or biogenesis, GO:0022613 ribonucleoprotein complex biogenesis, GO:0009987 cellular process are also enriched in CVIII-i. GO:0010228 vegetative to reproductive phase transition of meristem GO:0097354 prenylation GO:0006914 autophagy GO:0070085 glycosylation are also enriched in CVIII-ii.



**Fig. S7.** Distribution of the highest and the lowest expression among alternatively spliced transcripts of the CVI: CVI\_Leaf (f=323, mean=3.3, 9.9-fold, sd=1.9), CVI\_Root (f=110, mean=2.9, 7.4- fold, sd=1.7), CVI\_P1cm (f=78, mean=3.1, 8.5- fold, sd=1.8), and S21DAP (f=88, mean=3.4, 10.8- fold, sd=2.2).



**Fig. S8.** Distribution of the highest and the lowest expression among alternatively spliced transcripts of the CVIII: CVIII (f=3382, mean=2.7, 6.3- fold, sd=1.6), CVIIIi (f=1488, mean=3.4, 10.5- fold, sd=1.7), CVIIIii (f=1244, mean=2.1, 4.3- fold, sd=1.1) and CVIIIiii (f=379, mean=0.9, 1.9- fold, sd=0.6)<sup>3</sup>



Fig. S9. Types of alternative splicing which affect the expression. Types are denoted as in Table S8

Campbell et al. classified the type of alternative splicing into 9 groups base on the results of the Program to Assemble Spliced Alignments (PASA, Campbell et al., 2006); Alternate Acceptor (AA), Alternate Donor (AD), Alternate Terminal Exon (ATE), Retained Exon and Skipped Exon (RE/SE), Initiation within an Intron (IWI), Termination within an Intron (TWI), and Spliced Intron and Retained Intron (RI/SI). PASA detected 5,028 alternative splicing from 2,438 loci.