New Phytologist Supporting Information

A (conditional) role for labdane-related diterpenoid natural products in rice stomatal closure

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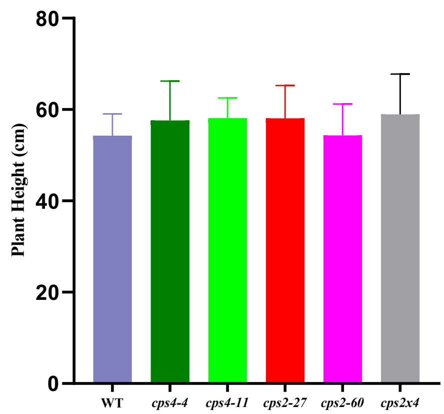
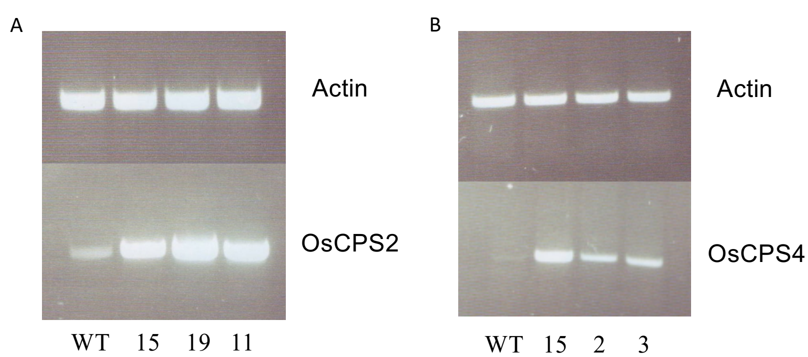
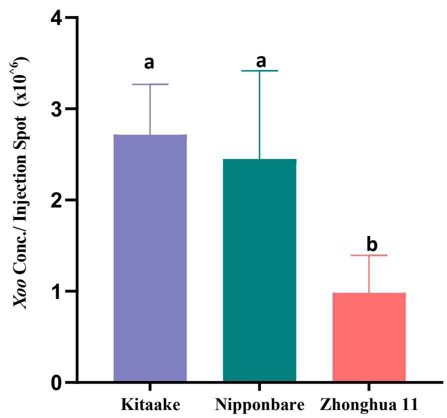


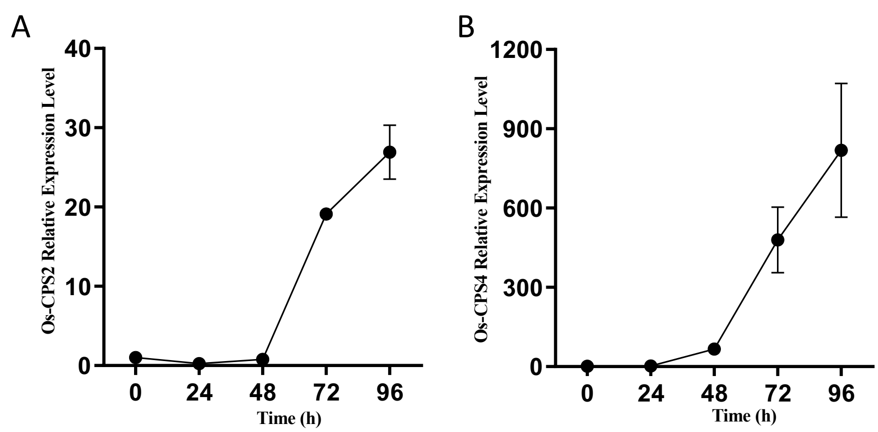
Supplemental Figure S1. Rice cultivar Kitaake *cps* mutants exhibit normal growth. Histogram indicating plant height of wild-type (WT), *cps2*, *cps4* and *cps2x4* plants grown in parallel, 10 weeks after germination (n = 12, error bars indicate standard deviation). Three independent experiments were performed with similar results.



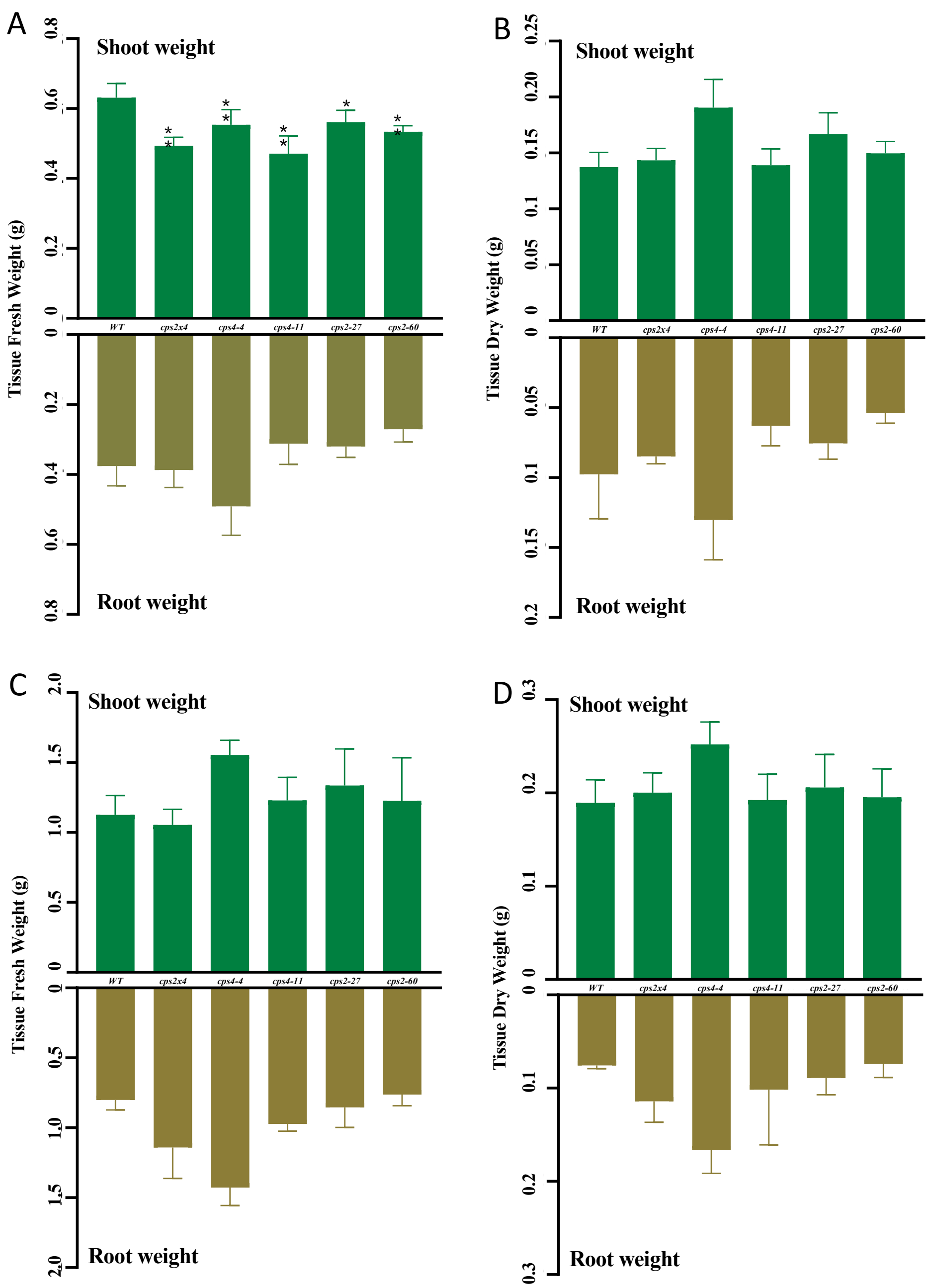
Supplemental Figure S2. Selection of rice cultivar Kitaake *CPS* over-expression (OE) lines. Image of semi-quantitative RT-PCR of leaves from WT and OE (numbered) three-week old seedlings for A) *OsCPS2* and B) *OsCPS4*. Actin was used as the control gene.



Supplemental Figure S3. Variation between rice cultivars in susceptibility to *Xanthomonas oryzae* pathovar *oryzae* (*Xoo*). Histograms indicating *Xoo* colony forming units found in infected leaves (n = 9, error bars indicate standard deviation). Different letters indicate significant difference calculated by Fisher's LSD test (p<0.01). Three independent experiments were performed with similar results.

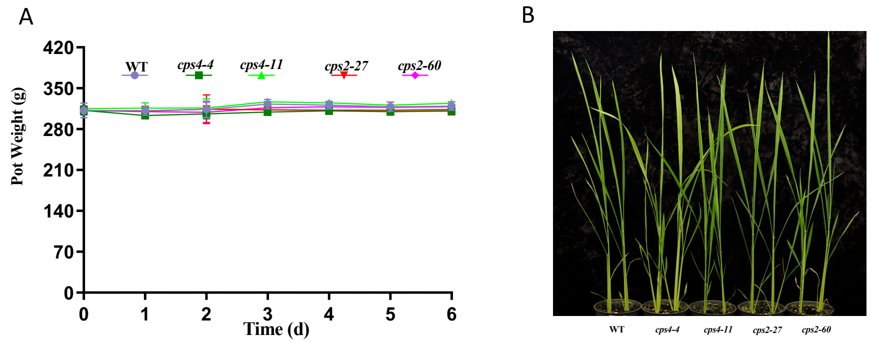


Supplemental Figure S4. Induction of rice *CPSs* by *Xanthomonas oryzae* pathovar *oryzae* (*Xoo*) infection in rice cultivar Kitaake. Graphs indicating relative levels of mRNA measured by qRT-PCR from leaves at various time after inoculation for A) *OsCPS2* and B) *OsCPS4* (n = 3, error bars indicate standard deviation). Two independent experiments were performed with similar results.

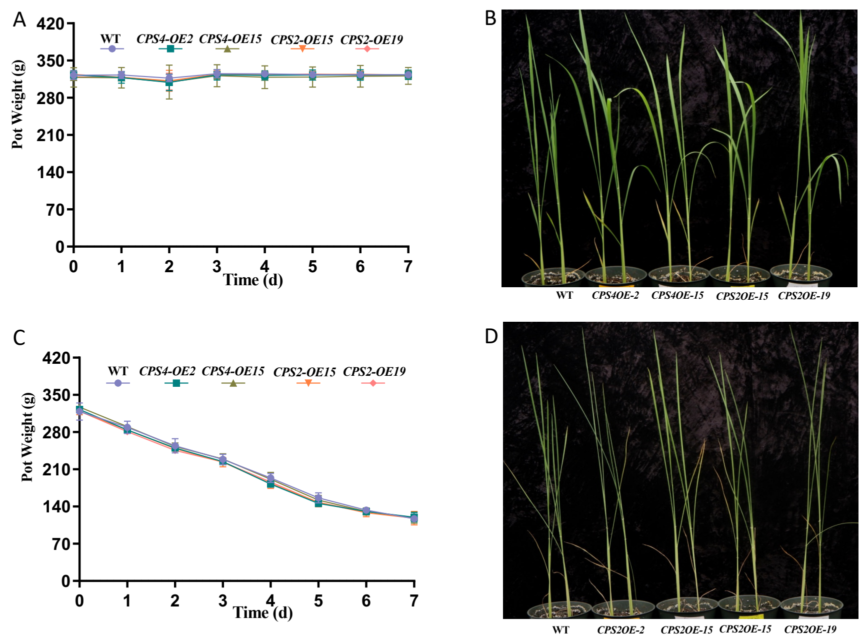


Supplemental Figure S5. Rice cultivar Kitaake *cps* mutants do not affect biomass. (A and B) Control plant (A) fresh and (B) dry weights of shoots (top) and roots (bottom), as indicated. (C and D) Plants following drought treatment (withholding water for six days) (C) fresh and (D) dry weights of shoots (top) and roots (bottom), as indicated. Data is averaged from six plants (error bars show standard deviation). While significant differences are observed for shoots of the *cps* mutants relative to wild-type (WT) in (C), this reflects their reduced water content (*P < 0.05, **P < 0.01, calculated using one-way ANOVA followed by Dunnett's multiple comparisons tests to compare the different lines against the wildtype).

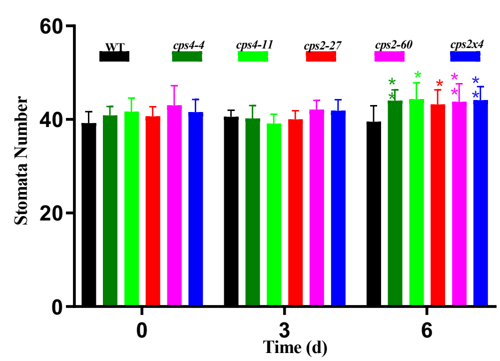
Supplemental Figure S6. Equivalent water loss for rice cultivar Kitaake *cps* mutants under well-watered conditions. (A) Representative image of control plants from wild-type (WT), *cps2* and *cps4* lines with continued watering. (B) Analysis of water loss during drought stress treatment from pots with WT, *cps2* or *cps4* plants (n = 5; error bars show standard deviation).

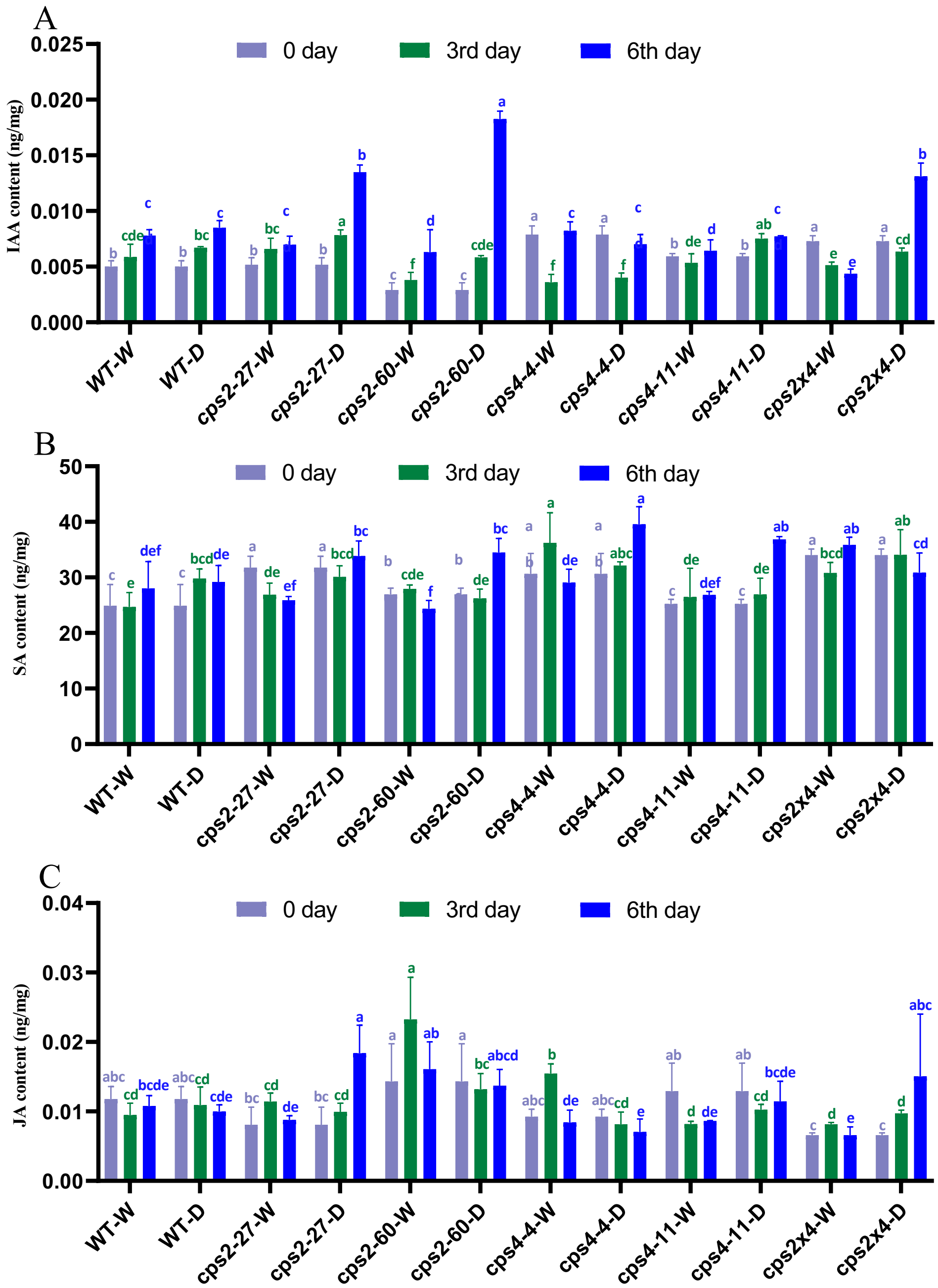


Supplemental Figure S7. Rice *CPS* over-expression (OE) does not affect drought resistance. (A – D) Analysis of wild-type (WT), *CPS2-OE* and *CPS4-OE* lines assayed together with graphs indicating pot weights over time (A) when watered, or (C) following withholding of water, along with representative pictures of plants after each treatment (B and D). For both graphs, n = 5 and error bars indicate standard deviation. Three independent experiments were performed with similar results.

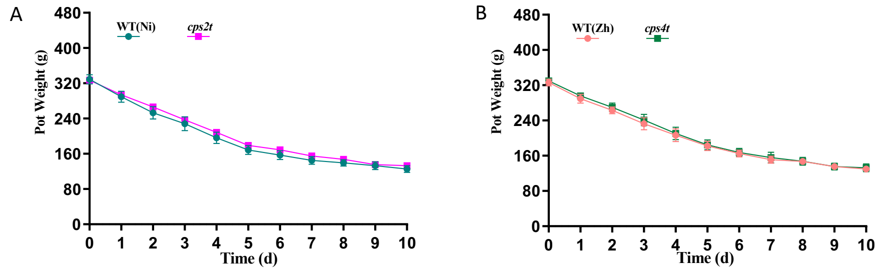


Supplemental Figure S8. Rice cultivar Kitaake *cps* mutants do not affect stomata density. Histogram indicating number of stomata in standard sized images of wild-type (WT)*, cps2*, *cps4* and *cps2x4* either before (0) or following withholding of water for 3 or 6 days (as indicated; n = 12, error bars show standard deviation; *P < 0.05 and **P < 0.01, calculated using one-way ANOVA test). Four independent experiments were performed with similar results.



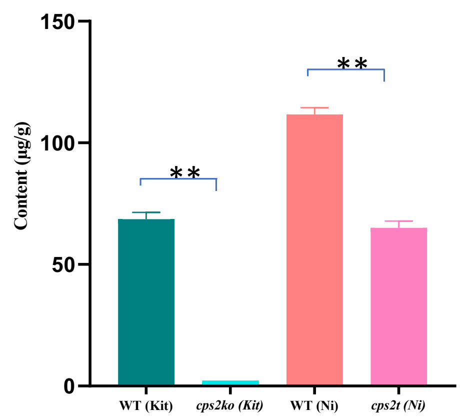


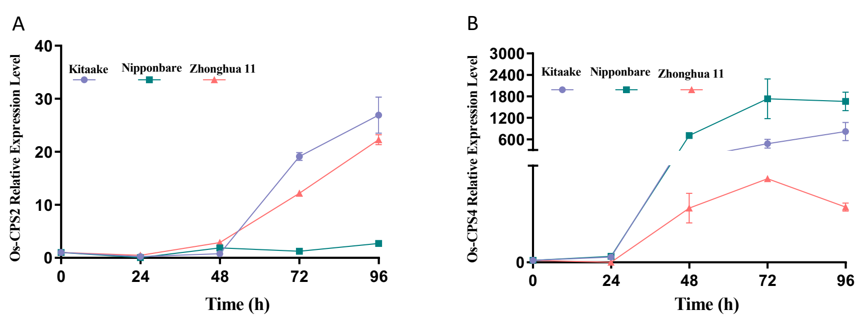
Supplemental Figure S9. Rice cultivar Kitaake *cps* mutants do not uniformly affect other phytohormone levels in response to drought stress treatment either before, three or six days after withholding water (n = 6, error bars indicate standard deviation). (A) Indole-acetic acid (IAA). (B) Salicylic acid (SA). (C) Jasmonic acid (JA). Significant differences within each time point are indicated by different letters of the same color (P<0.01; using two-sided Fisher's LSD).



Supplemental Figure S10. Rice cultivar (cv.) Nipponbare *cps2t* and cv. Zhonghua 11 *cps4t* mutants do not affect drought resistance. Graphs indicating pot weights over time following withholding of water for (A) wild-type cv. Nipponbare (Ni) and *cps2t*, and (B) wild-type cv. Zhonghua 11 (Zh) and *cps4t* (n = 5 and error bars indicate standard deviation). Three independent experiments were performed with similar results.

Supplemental Figure 11. Contrasting effect of rice cultivar (cv.) Kitaake (Kit) *cps2* and cv. Nipponbare (Ni) *cps2t* relative to their respective wild-type (WT) on phytocassanes level in root exudates (values reflect total peak area for all phytocassanes relative to that of the internal standard; n = 3, error bars show standard deviation; **P < 0.01, calculated using unpaired two tailed Student's *t*-test). Three independent experiments were performed with similar results.





Supplemental Figure S12. Induction of rice *CPSs* by *Xanthomonas oryzae* pathovar *oryzae* (*Xoo*) infection varies between cultivars. Graphs indicating relative levels of mRNA measured by qRT-PCR from leaves of cultivar (cv.) Kitaake (purple), cv. Nipponbare (green) or cv. Zhonghua 11 (red) at various time after inoculation for A) *OsCPS2* and B) *OsCPS4* (n = 10, error bars show standard deviation). Three independent experiments were performed with similar results.

Supplemental Table S1. Genotypes from CRISPR/Cas9 mutagenesis.

	1[st] target	2[nd] target
WT (*Kitaake*)	GTTTGGGCAGCCAGCATCGGCGG	GACGCCCAGCCATTGCTGCAAGG
cps4-4	GTTTGGGCAGCCAGCATTCGGCGG	GACGCCCAGCCATTGCTTGCAAGG
cps4-10	GTTTGGGCAGCCAGCATTCGGCGG	GACGCCCAGCCATTGC AAGG
WT (*Kitaake*)	GGGAATGGAAAACTTGGACTGGG	GCTTACGCTCTCAGCGAAACGGG
cps2-27	GGGAATGCTAGGCCGAACACTGGG	GCTTACGCTCTCAGCGATAACGGG
cps2-60	GGGAATGGAAAACTTGGAACTGGG	GCTTACGCTCTCAGCGATAACGGG
WT (*Kitaake*) (CPS4)	GTTTGGGCAGCCAGCATCGGCGG	GACGCCCAGCCATTGCTGCAAGG
cps2x4 (CPS4)	GTTTGGGCAGCCAGCATTCGGCGG	GACGCCCAGCCATTGC AAGG
WT (*Kitaake*)	GGGAATGGAAAACTTGGACTGGG	GCTTACGCTCTCAGCGAAACGGG
cps2x4 (CPS2)	GGGAATGGAAAACTTGGAACTGGG	GCTTACGCTCTCAGCGATAACGGG

indicates insertion; indicates deletion; indicates replacement

Supplemental Table S2. Primers used in this study.

gOs-CPS2-1 F	tgtt GGGAATGGAAAACTTGGACT
gOs-CPS2-1 R	aaac AGTCCAAGTT TTCCATTCCC
gOs-CPS2-2 F	gtgt GCTTACGCTCTCAGCGAAAC
gOs-CPS2-2 R	aaac GTTTCGCTGA GAGCGTAAGC
gOs-CPS4-1 F	tgtt GTTTGGGCAGCCAGCATCGG
gOs-CPS4-1 R	aaac CCGATGCTGG CTGCCCAAAC
gOs-CPS4-2 F	gtgt GACGCCCAGCCATTGCTGCA
gOs-CPS4-2 R	aaac TGCAGCAATG GCTGGGCGTC
Os-CPS2Seq F	ATATACTAACGAAATTGAAAAGGG
Os-CPS2Seq R	ACGGTTTTAAAGAGGAACAT
Os-CPS4Seq F	ATCATTCATCCCAATATCTATTG
Os-CPS4Seq R	AACGTTTAACTATTTATCTTA
OsCas9-F	GGGTAATGAACTCGCTCTGC
OsCas9-R	TGGCGTCAAGAACTTCCTTTG
U6P-F1	AAGAACGAACTAAGCCGGAC
Os-CPS2F	CGGGGTACCATGCAGATGCAGGTGCTCACC
Os-CPS2R	GCTCTAGACTAATTGACATCCTCGAACA
Os-CPS4F	CGGGGTACCATGCCGGTCTTCACTGCGTC
Os-CPS4R	GCTCTAGACTAAATCACATCTTGGAATATGAC
Os-CPS2-qRT-F	ACGAGGAGTGGATGATGGTC
Os-CPS2-qRT-R	GCTATGGAGCAAGGTGGTC
Os-CPS4-qRT-F	CCCAGGCGAGGTTGAGTAT
Os-CPS4-qRT-R	TGAGGTACAGGTCGTTGCAG
Os-CPS2-RT-F	CGAGGACGACGAGGAGTG
Os-CPS2-RT-R	GGACTCAGGTGCCTGTAGGC
Os-CPS4-RT-F	ATGCTAATAAGCAAATCACCG
Os-CPS4-RT-R	GGCATAGAGTGTAGCACGTCTC
OsActin-F	CTCAGCACATTCCAGCAGAT
OsActin-R	ACAGATAGGCCGGTTGAAAA