

New Phytologist Supporting Information

Dissecting the labdane-related diterpenoid biosynthetic gene clusters in rice reveals directional cross-cluster phytotoxicity

Riqing Li, Juan Zhang, Zhaohu Li, Reuben J. Peters and Bing Yang

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The following Supporting Information is available for this article:

Supporting Information Table S1 Primers for mutation detection in rice plants.

| Primer | Sequence (5'-3') | Usage |
|--------------|--------------------------|------------------------------------|
| F1 | TGCTTTTAGATCGATAAGAAC | Mutation detection of c2BGC |
| R1 | TGAGCAGGTTGCCGATGAGA | Mutation detection of c2BGC |
| F2 | ACCGCTGCATCCATAGCTCGC | Mutation detection of c2BGC |
| R2 | AGTTCCGGTTCCTATTCGAAC | Mutation detection of c2BGC |
| F3 | TTGAACCTAGCTAATGTGCTC | Mutation detection of c4BGC |
| R3 | TGCATGTTTCCAGATTCATCCTG | Mutation detection of c4BGC |
| F4 | GGCGGATTCTGCTTCGGCGAC | Mutation detection of c4BGC |
| R4 | GTGAACATATCCTGCACAATG | Mutation detection of c4BGC |
| gCYP76M7/8-F | GTGTGAGCTCCGACCCGAGTGGA | CRISPR/Cas9 construct of CYP76M7/8 |
| gCYP76M7/8-R | AAACTCCACTGCGGGTCGGAGCTC | CRISPR/Cas9 construct of CYP76M7/8 |
| CYP76M7-F | CTACGCCGCGGCAAACCCC | Mutation detection of CYP76M7 |
| CYP76M7-R | ACGAGGTTGAGGAGGCCAGT | Mutation detection of CYP76M7 |
| CYP76M8-F | TACTTGGTTCGGCGGCG | Mutation detection of CYP76M8 |
| CYP76M8-R | GGCTGACACCATGGCTCTT | Mutation detection of CYP76M8 |

Supporting Information Table S2 Segregation of T2 populations of rice mutants

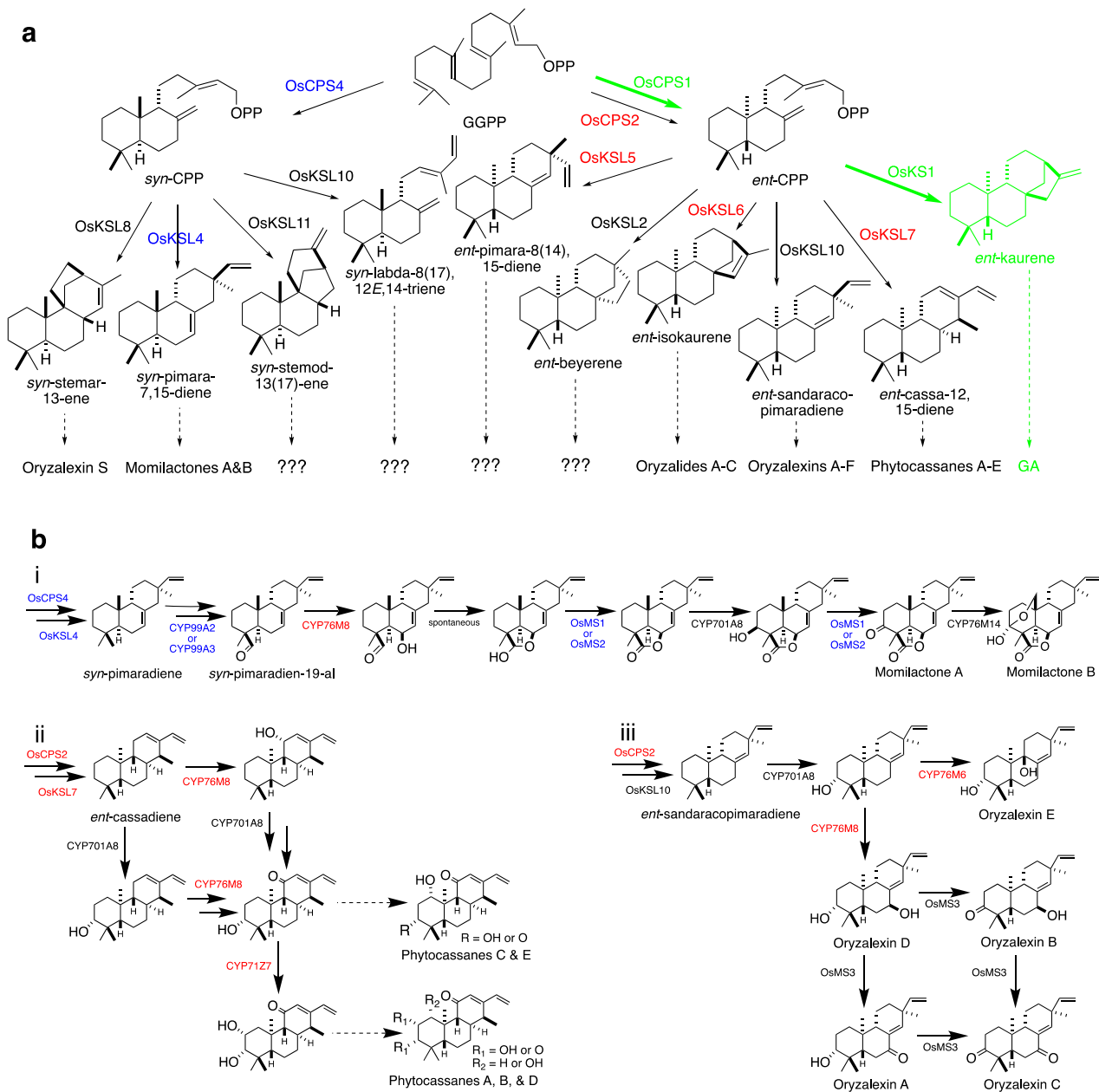
| Population | Total No. | Individual NO. of a particular genotype | | | χ^2 ^a |
|--------------------|-----------|---|--------------|------------|-----------------------|
| | | WT | Heterozygous | homozygous | |
| $\Delta 2bgc$ (T2) | 99 | 25 | 51 | 23 | 0.17 |
| $\Delta 4bgc$ (T2) | 107 | 27 | 54 | 26 | 0.03 |

^a $\chi^2_{0.05} (1:2:1) = 5.99$.

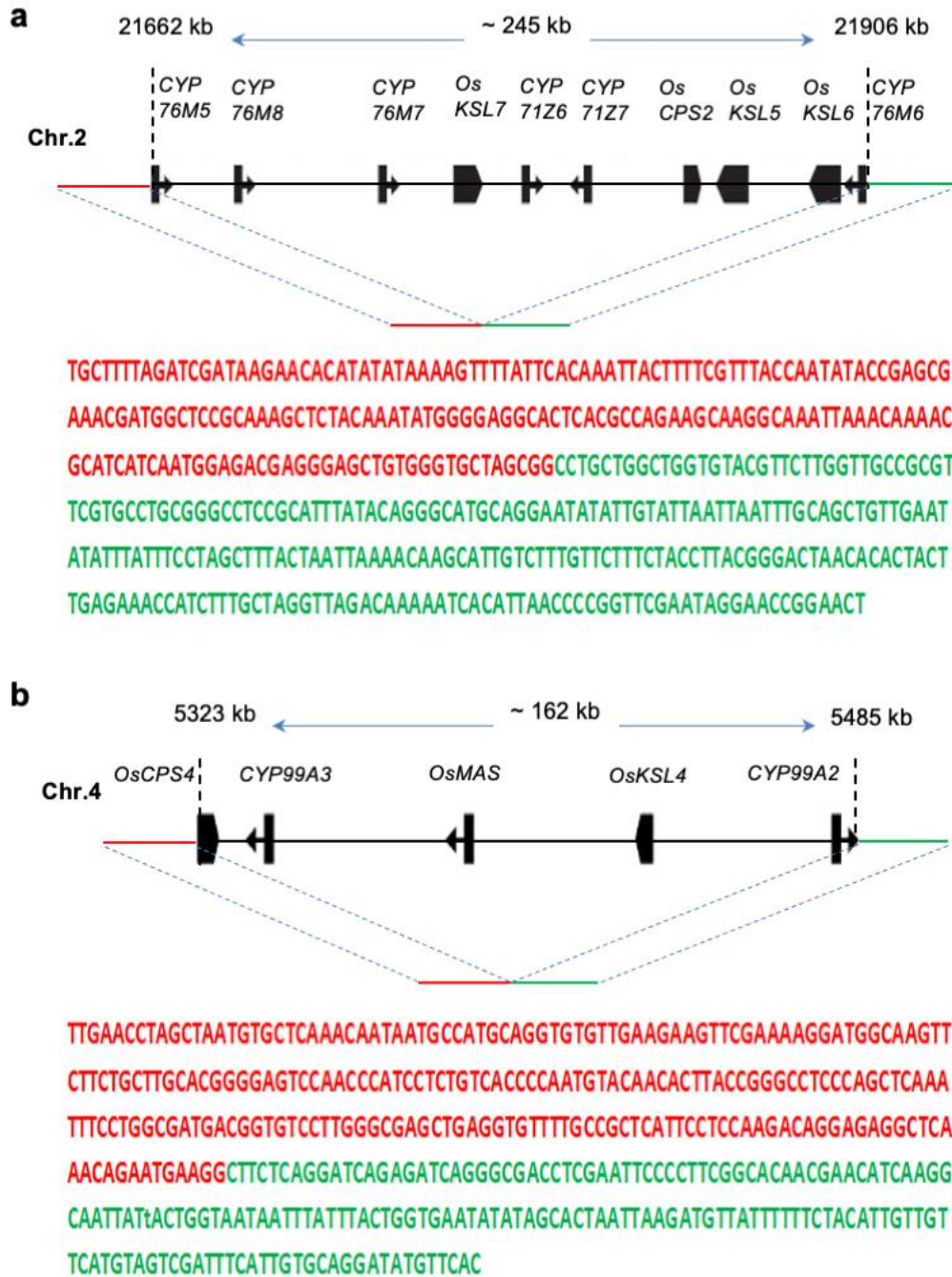
Supporting Information Table S3 Primers for RT-PCR

| Gene | Kitaake rice gene ID ^a | Forward primer (5'-3') | Reverse primer (5'-3') |
|----------------|--|-------------------------------|-------------------------------|
| <i>OsCPS4</i> | OsKitaake04g030200 | ggttcttgcccaggagaatg | gaccttgatgatgtggctat |
| <i>CYP99A3</i> | OsKitaake04g030300 | ccttagctcaacacacgtc | gtggtcttggtggctctgt |
| <i>OsMAS</i> | OsKitaake04g030500 | tgtcctgctcaacagcag | gttgggaagagtgaatacatgc |
| <i>OsKSL4</i> | OsKitaake04g030600 | gtggtcgtctccagaatgat | gacaccgtcttcttaacaac |
| <i>CYP99A2</i> | OsKitaake04g030800 | gccctggtgatacatttgg | gagccgtatatccatcagatt |
| <i>CYP76M5</i> | OsKitaake02g214700 | gtcgtgcctgtcatcgtcaa | cctggaggaagtgtatgaag |
| <i>CYP76M8</i> | OsKitaake02g214900 | agactgccaatgtccttgct | cctgtgtaggcttatacttgt |
| <i>CYP76M7</i> | OsKitaake02g215000 | cgtgtccgagaagttcaagt | gtgccactgggtcccgtat |
| <i>OsKSL7</i> | OsKitaake02g215400 | cacctgttctactaccagac | gtcatccacattctgattacc |
| <i>CYP71Z6</i> | OsKitaake02g215500 | gtgcgtaacatccgtcctaa | gtggtagcattacacactgg |
| <i>CYP71Z7</i> | OsKitaake02g215600 | cgtcctccaagacagcacat | gctacacaaataggccagtc |
| <i>OsCPS2</i> | OsKitaake02g215700 | agcagcagcaacgatgtcag | gagccatgctgtagacaca |
| <i>OsKSL5</i> | OsKitaake02g215800 | cgcaggaagctcttctggtt | tcacagcgttcccaaaccag |
| <i>OsKSL6</i> | OsKitaake02g216000 | tagagaagtgggacgacctat | gattcggcgatatgatccac |
| <i>CYP76M6</i> | OsKitaake02g216100 | caaccaagaacgtacaccag | ggatggtgaggtacaacaag |
| <i>PR1</i> | OsKitaake07g021300 | cgtcttcatcacctgcaa | tcagcgtacgatagtagta |
| <i>PBZ</i> | OsKitaake12g 174100 | ctcaagatgatcgaggac | tggacatttctgctgctc |
| <i>POX22.3</i> | OsKitaake07g273000 | acgacataaacgggccac | aggtgctaagccatggct |
| <i>Actin1</i> | OsKitaake03g316400 | ctcagcacattccagcagat | acagataggccggtgaaaa |

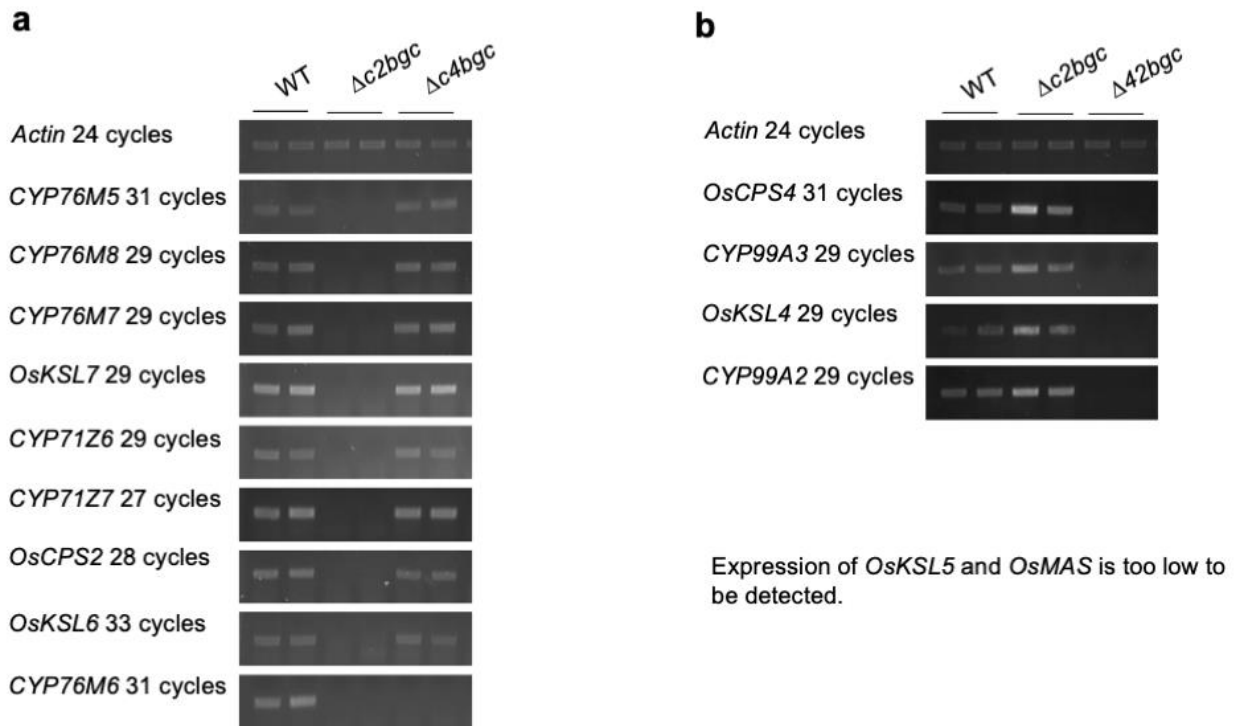
^a Kitaake gene IDs are from JGI (Joint Genome Institute) website, https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_OsativaKitaake_er



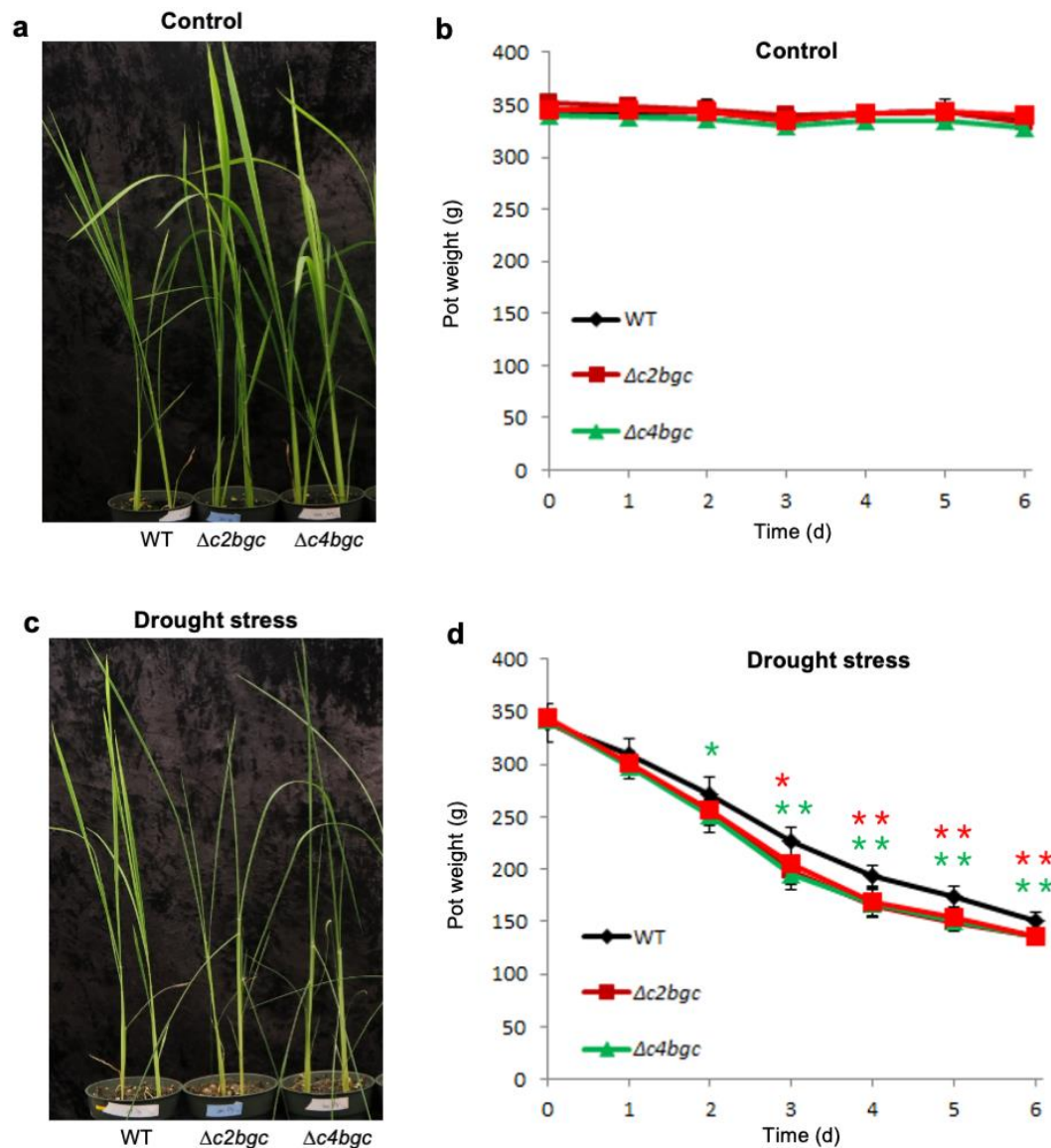
Supporting Information Figure S1. Rice LRD biosynthesis. Green indicates gibberellin biosynthesis, blue text the enzymes encoded in the c4BGC, and red text the enzymes encoded in the c2BGC. Dashed arrows indicate multiple reactions. **(a)** Map of known diterpene cyclases, with indicated LRDs where known (??? indicates unknown metabolic fates). **(b)** Downstream map of **(i)** momilactone, **(ii)** phytocassane and **(iii)** oryzalexin biosynthesis showing known relevant enzymes.



Supporting Information Figure S2. Genomic DNA sequence of $\Delta c2bgc$ and $\Delta c4bgc$ mutations in rice. (a,b) Top, schematic of large deletions one chromosome 2 (a) and 4 (b). Bottom, the flanking genomic DNA sequence of large deletions. Red and green color indicates genomic DNA sequences flanking the gene cluster deletions, respectively.



Supporting Information Figure S3. Relative expression level of enzymatic genes from c2GBC and c4GBC in rice plants. **(a)** Gel images of RT-PCR amplicons using gene-specific primers for nine genes in c2GBC on RNA transcripts from $\Delta c2bgc$ and $\Delta c4bgc$ plant leaves. **(b)** Gel images of RT-PCR amplicons using gene-specific primers for four genes in c4GBC on RNA transcripts from $\Delta c2bgc$ and $\Delta c4bgc$ plant leaves.



Supporting Information Figure S4. Effect of $\Delta c2bgc$ and $\Delta c4bgc$ mutants of rice on drought resistance. (a, c) Representative pictures of wild-type (WT), $\Delta c2bgc$ and $\Delta c4bgc$ mutant plants in normal condition (continued watering) (a) and analysis of water loss in normal condition (continued watering) (c). (b, d) Representative pictures of wild-type (WT), $\Delta c2bgc$ and $\Delta c4bgc$ mutant plants after drought stress treatment (withholding of water for 6 d) (b) and analysis of water loss during drought stress treatment (d) from pots with WT, $\Delta c2bgc$ and $\Delta c4bgc$ mutant plants (n = 6, error bars indicate SD). Asterisks represent significant differences derived from one-way ANOVA followed by Dunnett's multiple comparisons tests to compare the different lines against WT at each time point (*, $p < 0.05$ and **, $p < 0.01$; colors correlated to that for $\Delta c2bgc$ and $\Delta c4bgc$ mutant lines, respectively). Four independent experiments were performed with similar results. Error bars represent +/- SD.

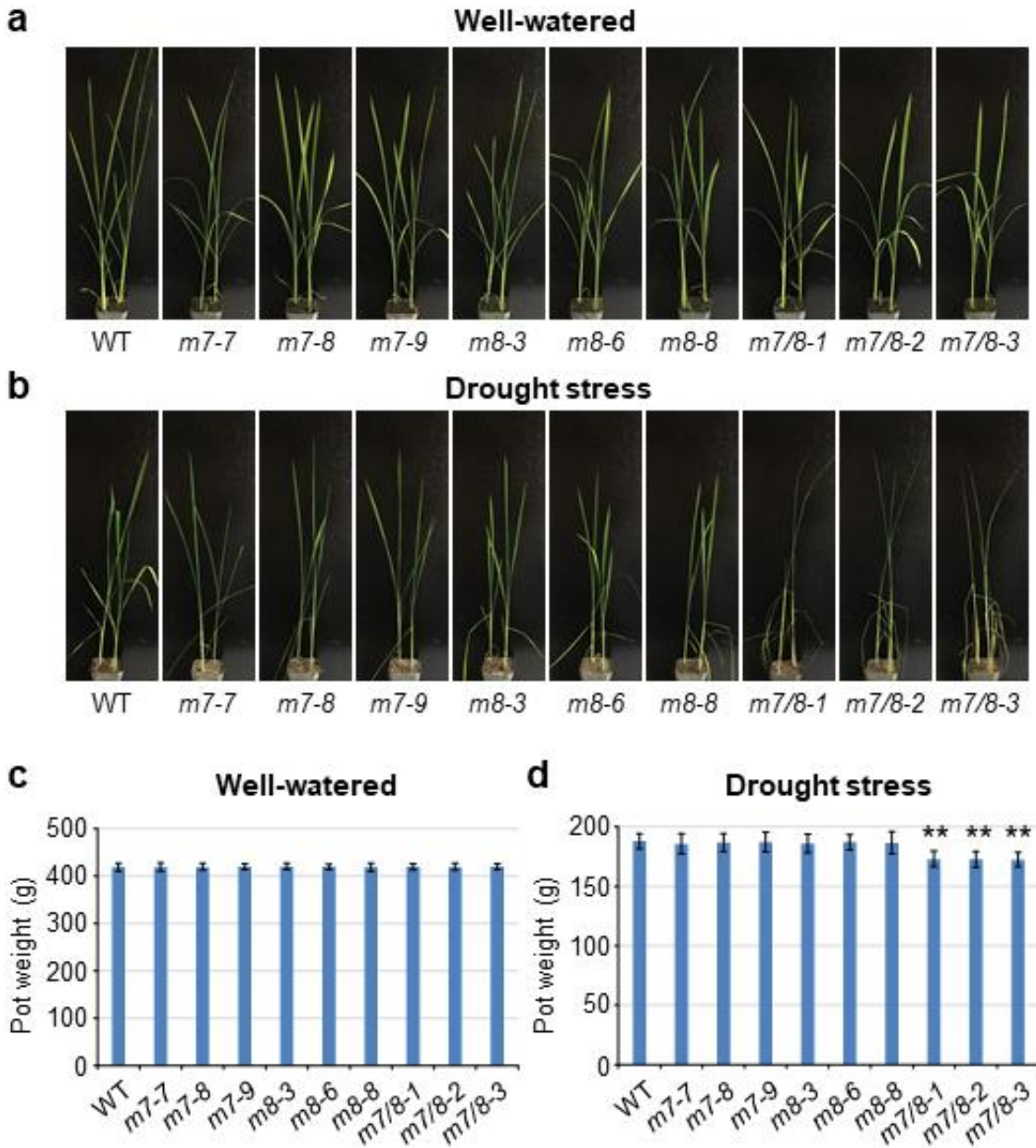
CYP76M7 mutation site

| | |
|---------------------|---|
| WT | ATCCCGAGCTCCGACCCGCAGTGGAAAGGCCCTGCGCGGGATCCAGGG |
| <i>cyp76m7/8</i> #1 | ATCCCGAGCTCCGACCCGCAG-GGAAGGCCCTGCGCGGGATCCAGGG |
| <i>cyp76m7/8</i> #2 | ATCCCGAGCTCCGACCCGCAGT T GGAAGGCCCTGCGCGGGATCCAGGG |
| <i>cyp76m7/8</i> #3 | ATCCCGAGCTCCGACCCGCAGT T GGAAGGCCCTGCGCGGGATCCAGGG |

CYP76M8 mutation site

| | |
|---------------------|---|
| WT | CCCGAGCTCCGACCCGCAGTGGAAAGGCCCTGCGGGGGATCCACGCCTCG |
| <i>cyp76m7/8</i> #1 | CCCGAGCTCCGACC-----TGGAAGGCCCTGCGGGGGATCCACGCCTCG |
| <i>cyp76m7/8</i> #2 | CCCGAGCTCCGACCCGCAGT T GGAAGGCCCTGCGGGGGATCCACGCCTCG |
| <i>cyp76m7/8</i> #3 | CCCGAGCTCCGACCCGCAGT T GGAAGGCCCTGCGGGGGATCCACGCCTCG |

Supporting Information Figure S5. Mutations of *CYP76M7* and *CYP76M8* genes in *cyp76m7/8* double mutant lines. The *CYP76M7* and *CYP76M8* genes were mutated simultaneously using CRISPR/Cas9 approach in rice. "-" and letters in red color represent deletion and insertion mutations, respectively.



Supporting Information Figure S6. Effect of *cyp76m7*, *cyp76m8* and *cyp76m7/8* mutants on drought resistance in rice plants. **(a, c)** Representative pictures of wild-type (WT), *cyp76m7*, *cyp76m8* and *cyp76m7/8* mutant plants under normal condition (continued watering) (a), and analysis of water loss under normal condition (continued watering) (c) (n = 5, error bars indicate SD). **(b, d)** Representative pictures of wild-type (WT), *cyp76m7*, *cyp76m8* and *cyp76m7/8* mutant plants after drought stress treatment (withholding of water for 7 d) (b), and analysis of water loss after drought stress treatment (d) (n = 5, error bars indicate SD). Asterisks represent significant differences derived from one-way ANOVA followed by Dunnett's multiple comparisons tests to compare the different lines against WT (**, $p < 0.01$). Error bars represent +/- SD.