Supporting Information

Mutations in a sub-family of abscisic acid receptor genes promote rice growth and productivity

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SI Materials and Methods

Vector Construction and Plant Cultivation. A multi-gene editing system for rice *PYLs* was constructed. In this system, Cas9 was directed by the maize Ubiquitin promoter, and four sgRNA expression cassettes, which were directed by OsU6-1, OsU3-1, OsU6-2 and OsU3-2 promoters respectively (see *SI Appendix*, Fig. S8 for the promoter sequences), were arranged in tandem. For target recognition (see *SI Appendix*, Table S1 for the target sequences), 20-nt guide oligo-nucleotides were synthesized with appropriate adaptors for seamless ligation with the four promoters. The tandem sgRNA expression cassettes were first constructed in the PUC19 intermediate vector, and then sub-cloned into PCAMBIA1300 backbone with the Cas9 expression cassette. Two separate multiplex CRISPR/Cas9 vectors were constructed for rice *PYL* gene editing. To mutate the *PYLs* individually, thirteen single-targeting vectors were constructed (see *SI Appendix*, Table S2 for the target sequences). The constructs were transformed into the calli of rice variety Nipponbare through *Agrobacterium*-mediated transformation.
The transgenic plants were grown in paddy fields. The seeds from the transgenic plants were sowed in Lingshui County of Hainan Province (China) in late December and in Shanghai (China) in mid-June. For the phenotypic observation, at least 24 plants were cultivated for each line in the paddy field.

Shoot Length and Fresh Weight Measurements. After pre-soaking in water for one day and then germinating for two days at room temperature, the seeds of different lines were sowed at the same time in the paddy field with a density of 1.5 x 1.5 cm in late December in Hainan and in mid-June in Shanghai. After 20 days, the shoot length and fresh weight of the seedlings were measured. The results of these measurements were confirmed in two successive planting seasons with independent lines.

Heading Date Comparison. In two successive years, we recorded the date when all the plants of one line had just begun heading, and then the heading dates of the wild type and *pyl* mutants were compared.

Histological Analyses and Epidermal Cell Observation. For histological sectioning and epidermal cell observation, the materials were first fixed in FAA's solution. For leaf sectioning analyses, semi-thin (10–15 μm) sections were made using Leica CM 3050 cyrostat microtome. For the internode transverse section analyses, sections were made by hand-held slicing. For epidermal cell observations, fresh or fixed leaves were soaked in boiled water for 10 minutes, and then transferred to 95% ethanol and boiled

for about 1 hour until being completely faded. Then the materials were soaked in 85% lactic acid of 96 ^{o}C for 8 minutes. After cooling to room temperature, the epidermal layers were observed under a microscope. The transverse sections and epidermal cells were imaged with a Leica DM2500 microscope (Leica Microsystems).

Cell Cycle Comparison. After soaking in water for one day at 37 ^{o}C, the seeds of the wild type and *pyl1/4/6* were transferred to a greenhouse (28 ^{o}C, 80% humidity, and 12 h light/12 h dark) to germinate for two days. Then the tips of buds on the germinated seeds were collected. Nuclei were prepared from the collected materials as described previously (1). The isolated nuclei were analyzed with BD FACS AriaIII flow cytometer. No fewer than 10,000 nuclei were collected for each treatment.

Seed Dormancy Analyses. Pre-harvest sprouting was investigated in the paddy field just after the finishing of seed filling, and in every line, the sprouting data from three plants were obtained (all of the seeds from a plant were investigated). The pre-harvest sprouting analyses were finished in one day.
For ABA treatment, the seeds from *pyl1/2/3/4/5/6/12*, *pyl7/8/9/10/11/13*, and the wild type were harvested on the same day, and then dried in 42 ^{o}C in a dry oven for seven days. After storing at 20 ^{o}C in a dry cabinet for two weeks, the seeds were used for ABA treatment. 100 de-husked seeds of each line were placed on filter papers soaked with PBS buffer (pH 7.0) containing ABA and 300 mg/L carbenicillin. Then the seeds were placed in 30 ^{o}C to culture for five days, and the germination rate was determined every day. This assay was repeated three times.
Stomatal Movement and Water Loss Assays. For stomatal movement assay, the fourth leaves from 20-day-old seedlings were sampled at 10 a.m.in the greenhouse (26 ^{o}C, 80% humidity, and 12 h light/12 h dark) and then floated on stomata opening buffer (l mM MES-NaOH, 20 mM KCl, pH6.0) containing 0 and 30 μM ABA for 1 and 3 hours. After the treatment, the samples were frozen in liquid nitrogen immediately, and then dried in -80 ^{o}C vacuum freeze drier (Labogene ScanVac Maxi Vac beta) for 5 hours. Then the stomata were investigated with a Hitachi TM3000 scanning electron microscope. At least 200 stomata were examined in each treatment of each line, and the assays were repeated three times.
For water loss assays, detached flag leaf blades were placed in room temperature and weighed at indicated time points.

ABA Sensitivity Assays of Seedling Growth. The seedlings of two-leaf stage with similar size were transferred to 2/3 liquid MS media with 0 and 2.5 μM ABA and then cultured for five days in greenhouse (26 ^{o}C, 80% humidity, and 12 h light/12 h dark). Then the fresh weight and shoot length were measured.

Real-time RT-PCR. Total RNA was extracted individually from the roots, second internodes, flag leaves (leaf blades), young panicles (5–7 cm), auxiliary buds from the second internodes, seeds at 25 DAP, and germinated seeds (when the buds of the germinated seeds were just observed). Except for seeds, the other materials were sampled at the heading stage. Reverse transcription was performed using the SuperScript® III Reverse Transcriptase (Invitrogen, Cat. no. 18080-044). Real-time

RT-PCR analyses were performed using the Bio-Rad CFX96 real-time PCR instrument and EvaGreen (Biotium, Cat. no. 31000). The RT-PCR was conducted with gene-specific primers for *PYLs*, and Actin-F and Actin-R for *ACTIN* (see *SI Appendix*, Table S3 for the primer sequences).

Plot Field Test. Plants of the wild type and *pyl1/4/6* were grown in Shanghai and Hainan paddy fields under natural conditions. The planting density was 15 x 15 cm, with one plant one hill. 144 (12 x 12) plants were cultivated in every plot. The plot yield was determined when the seeds were harvested.

Accession Numbers. Sequence data from this article can be found in the GenBank data libraries under the following gene locus identifiers: *PYL1*, Os10g0573400; *PYL2*, Os06g0562200; *PYL3*, Os02g0226801; *PYL4*, Os01g0827800; *PYL5*, Os05g0473000; *PYL6*, Os03g0297600; *PYL7*, Os06g0526400; *PYL8*, Os06g0527800; *PYL9*, Os06g0528300; *PYL10*, Os02g0255500; *PYL11*, Os05g0213500; *PYL12*, Os02g0255300; and *PYL13*, Os06g0526466.

SI Reference

1. Hefner E, Huefner N, & Britt AB (2006) Tissue-specific regulation of cell-cycle responses to DNA damage in *Arabidopsis* seedlings. *DNA Repair* 5(1):102-110.

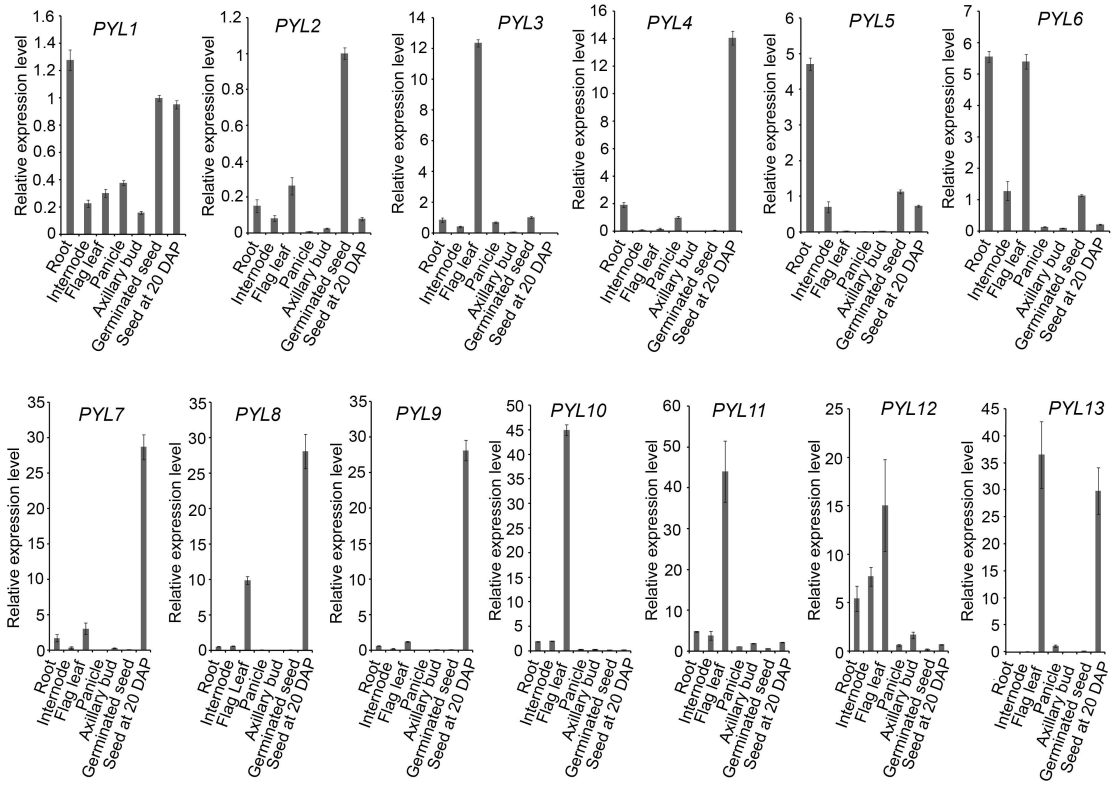

Fig. S1. Real-time RT-PCR analyses of rice *PYL* expression. Except *PYL13*, the expression of each gene was detected in all the tissues tested. *PYL13* expression was not detected in roots, internodes, and auxiliary buds. The error bar represents the SE of the mean values of three biological replicates.

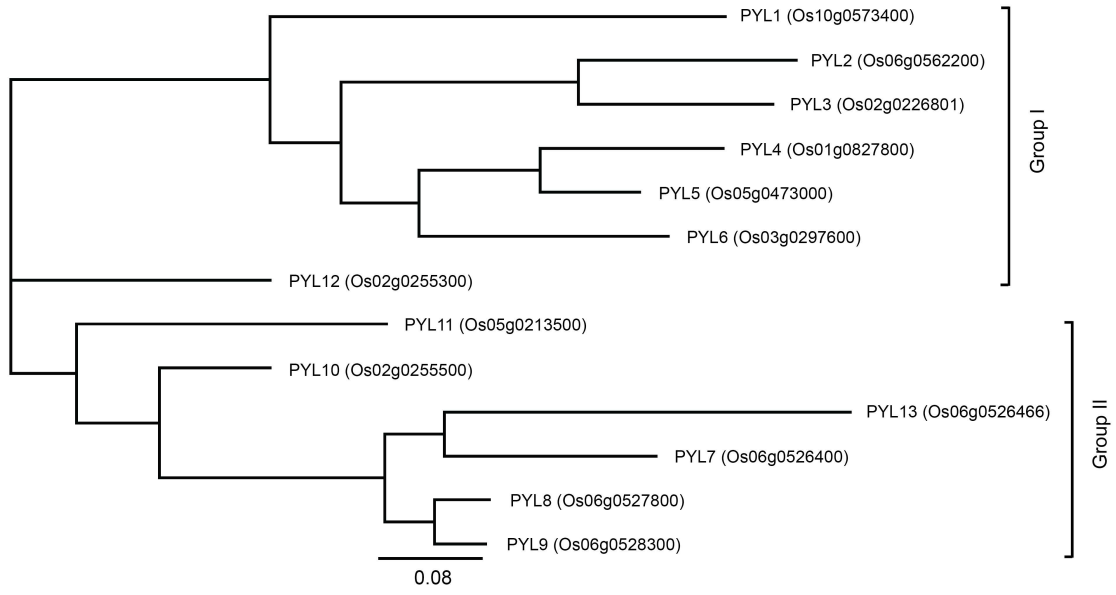

Fig. S2. Phylogenetic tree of rice PYLs.

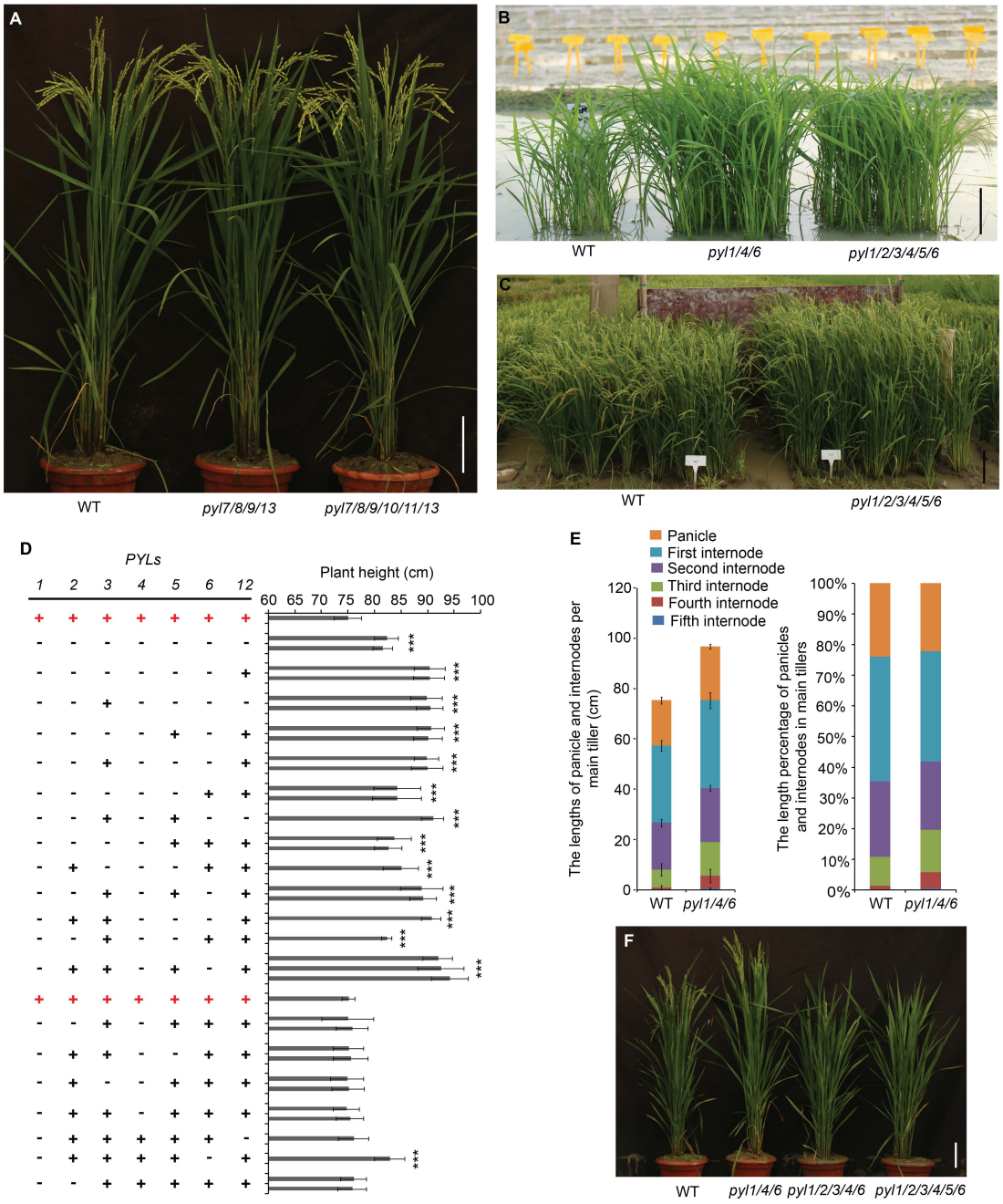

Fig. S3. Phenotypic analyses of rice *pyl* mutants. (***A***) Morphological features of wild-type, *pyl7/8/9/13*, and *pyl7/8/9/10/11/13* plants at the seed-filling stage. (*B*) Phenotypes of wild-type, *pyl1/4/6*, and *pyl1/2/3/4/5/6* seedlings. (*C*) Comparison of wild-type and *pyl1/2/3/4/5/6* plants at the seed-filling stage. (***D***) Plant heights of group I *pyl* mutants at the mature stage in Hainan Province. Every column represents an independent line. The "+" and "-" represent the wild-type and mutated genes, respectively. *P* values (versus the wild type) were calculated by the Student's *t*-test. ***, $P < 0.001$. (***E***) Diagram of the lengths of every internode and panicle in main tillers. The left panel shows the lengths of every internode and panicle; the right panel shows the length percentage of the internodes and panicles. (***F***) Comparison of the wild type, *pyl1/4/6*, *pyl1/2/3/4/6*, and *pyl1/2/3/4/5/6* at the heading stage. Scale bars, 10 cm. Data are presented as means ± SD. WT, the wild type.

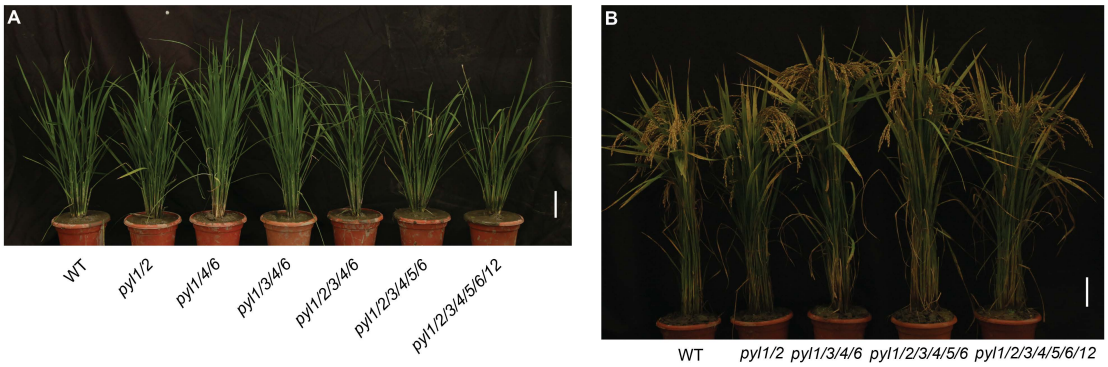

Fig. S4. The sensitivity of group I *pyl* mutants to the high temperature climate in 2016. (***A***) Phenotypic comparison of the wild type and group I mutants in the high temperature climate. The materials were 55-day-old plants. The plants had just experienced the high temperature climate during July in 2016 in Shanghai. (***B***) Mature stage phenotype of the group I mutants that had experienced the high temperature climate in the year 2016. Scale bars, 10 cm. WT, the wild type.

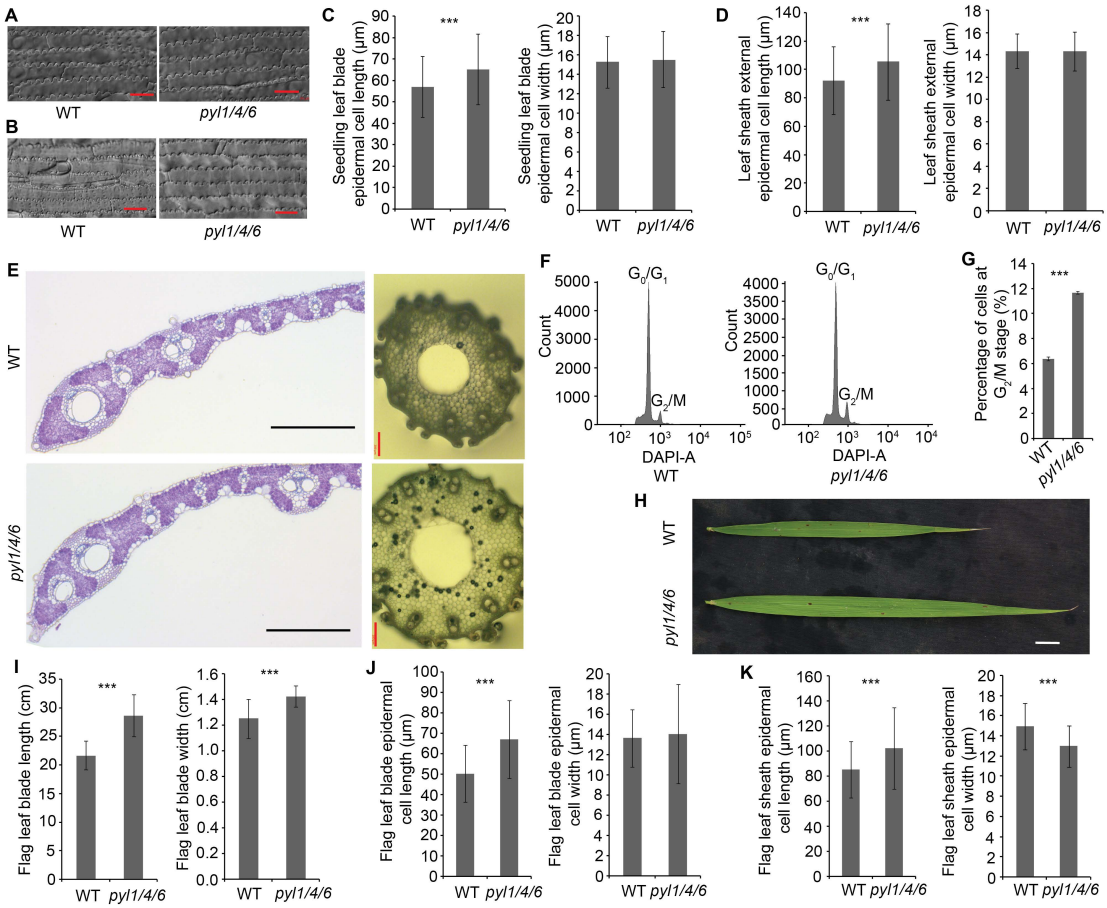

Fig. S5. Mutations in group I *PYLs* promote rice growth through increasing cell length and accelerating cell division. (***A***) The upper epidermal cells of wild-type and *pyl1/4/6* leaf blades of 21-day-old seedlings. Scale bars, 20 μm. **(*B*)** The external epidermal cells of wild-type and *pyl1/4/6* leaf sheaths of 21-day-old seedlings. Scale bars, 20 μm. (*C*) The upper epidermal cell lengths and widths of the leaf blades of 21-day-old seedlings. (*D*) The external epidermal cell lengths and widths of the leaf sheaths of 21-day-old seedlings. (*E*) Cross-sections of the seedling leaf blades and uppermost internodes. Scale bars, 200 μm. (*F*) Cell cycle analyses using flow cytometry. Count, nucleus number; DAPI-A, DNA content. (*G*) The percentage of cells at G_2/M phase. (*H*) The flag leaf blades of *pyl1/4/6* and the wild type. Scale bar, 2 cm. (*I*) The flag leaf blade lengths and widths of wild-type and *pyl1/4/6* main tillers. (*J*) The upper epidermal cell lengths and widths of wild-type and *pyl1/4/6* flag leaf blades. (*K*) The external epidermal cell lengths and widths of the flag leaf sheaths. WT, the wild type. Data are presented as means ± SD. *P* values were calculated by the Student's *t*-test. ***, $P < 0.001$.

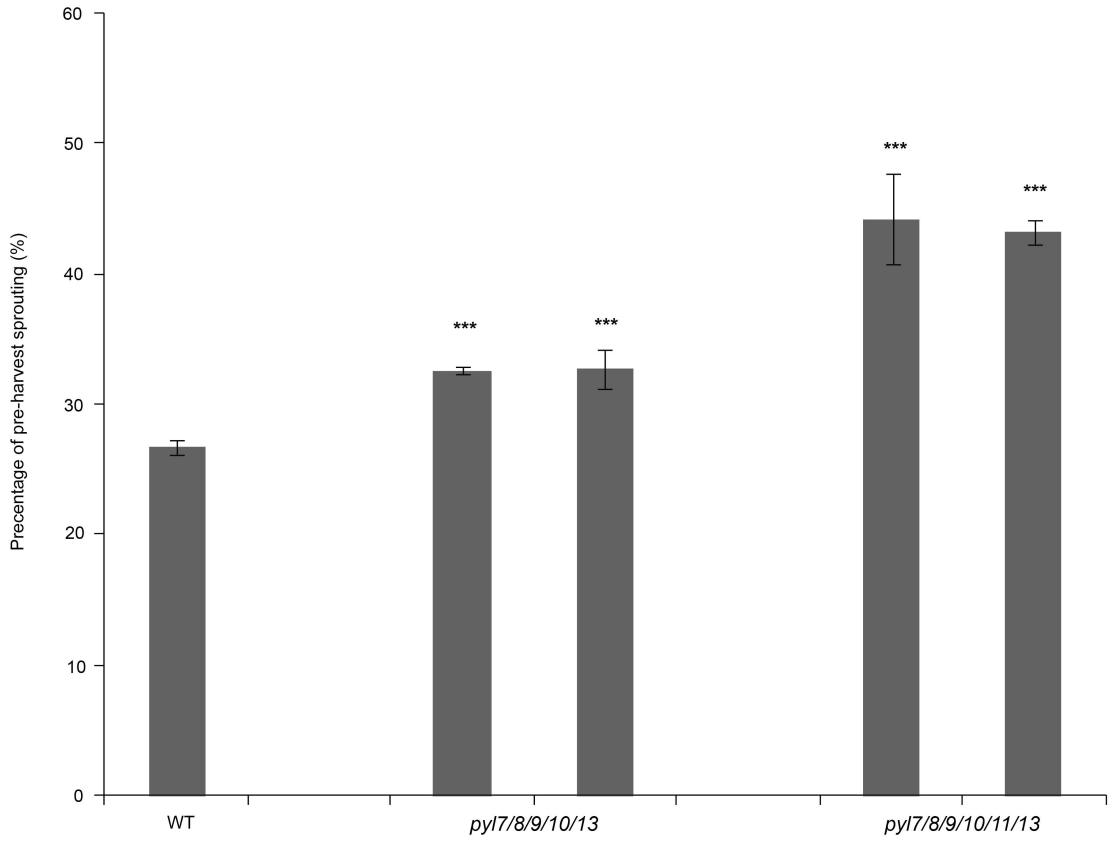

Fig. S6. Rates of seed germination on panicles when the seed harvesting was delayed by about 25 days. Data are presented as means ± SD. *P* values (versus the wild type) were calculated by the Student's *t*-test. ***, $P < 0.001$. WT, the wild type.

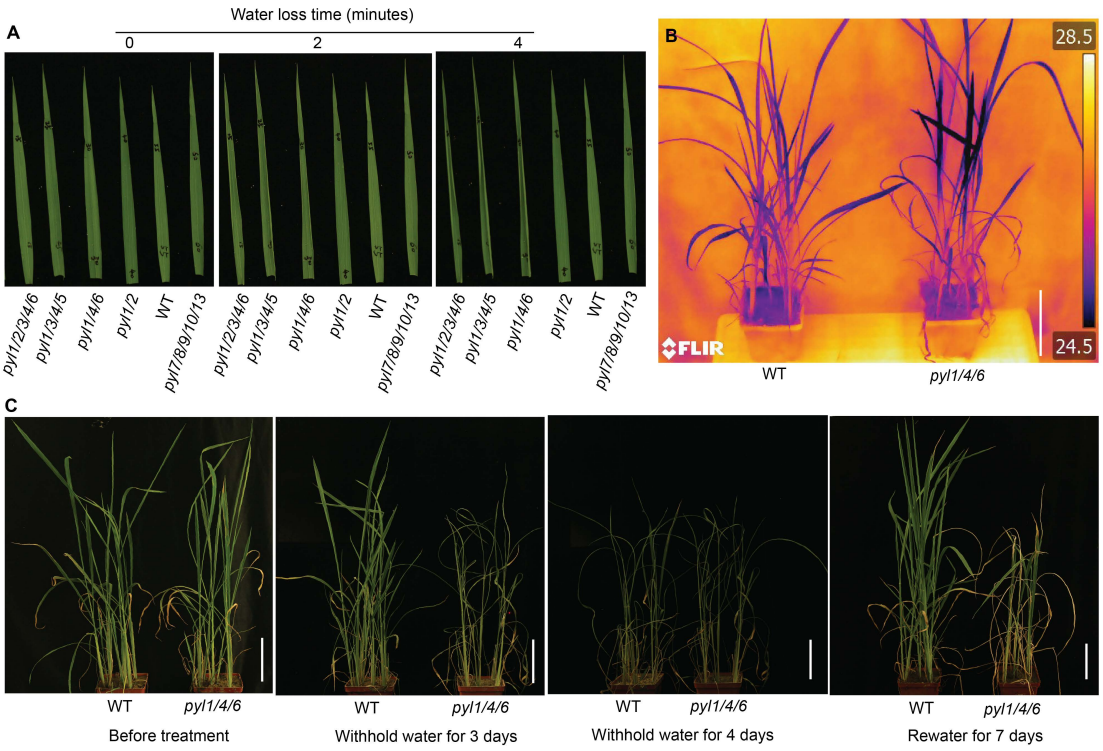

Fig. S7. Group I mutations led to severe defects in water loss control. (***A***) Comparison of transpirational water loss from detached flag leaves of wild-type and *pyl* plants. (***B***) Infra-red images of the wild type and *pyl1/4/6***.** The right color scale bar indicates leaf surface temperature. (***C***) Drought stress assays of wild-type and *pyl1/4/6* plants. Forty-day-old plants were used for the drought stress assays. Scale bars, 10 cm. WT, the wild type.

A

5'-AAGGAATCTTTAAACATACGAACAGATCACTTAAAGTTCTTCTGAAGCAACTTAAAGTTATCAGGCATGCATGGATCTTGGAGGAATCAGATGTGCA
GTCAGGGACCATAGCACAAGACAGGCGTCTTCTACTGGTGCTACCAGCAAATGCTGGAAGCCGGGAACACTGGGTACGTTGGAAACCACGTGATG
TGAAGAAGTAAGATAAACTGTAGGAGAAAAGCATTTCGTAGTGGGCCATGAAGCCTTTCAGGACATGTATTGCAGTATGGGCCGGCCCATTACGCAA
TTGGACGACAACAAAGACTAGTATTAGTACCACCTCGGCTATCCACATAGATCAAAGCTGATTTAAAAGAGTTGTGCAGATGATCCGTGGC-3'

B

5'-GGATCATGAACCAACGGCCTGGCTGTATTTGGTGGTTGTGTAGGGAGATGGGGAGAAGAAAAGCCCGATTCTCTTCGCTGTGATGGGCTGGAT
GCATGCGGGGGAGCGGGAGGCCCAAGTACGTGCACGGTGAGCGGCCCACAGGGCGAGTGTGAGCGCGAGAGGCGGGAGGAACAGTTTAGTA
CCACATTGCCCAGCTAACTCGAACGCGACCAACTTATAAACCCGCGCGCTGTCGCT-3'

C

5'-TGCCACGGATCATCTGCACAACTCTTTTAAACCAGCTTTGATCTATGTGGATAGCCGAGGTGGTACTAATACTAGTCTTTGTTGTCGTCCAATTGC
GTAATGGGCCGGCCCATACTGCAATACATGTCCTGAAAGGCTTCATGGCCCACTACGAAATGCTTTTCTCCTACAGTTTATCTTACTCCACATCACG
TGGTTTCCAACGTACCCAGTGTTCCCGGCTTCCAGCATTTGCTGGTAGCACCAGTAGAAGACGCCTGTCTTGTGCTATGGTCCCTGACTGCACAT
CTGATTCCTCCAAGATCCATGCATGCCTGATAACTTTAAGTTGCTTCAGAAGAACTTTAAGTGATCTGTTCGTATGTTTAAAGATCCCTT-3'

D

5'-TTTTTTCCTGTAGTTTTCCCACAACCATTTTTTACCATCCGAATGATAGGATAGGAAAAATATCCAAGTGAACAGTATTCCTATAAAATTCCCGTAAAA
AGCCTGCAATCCGAATGAGCCCTGAAGTCTGAACTAGCCGGTCAACTATACAGGCTATCGAGATGCCATACACGAGACGGTAGTAGGAACTAGGAA
GACGATGGTTGATTCGTCAGGCGAAATCGTCGTCCTGCAGTCGCATCTATGGGCCTGGACGGAATAGGGGAAAAAATTGGCCGGATAGGAGGGAA
AGGCCCAGGTGCTTACGTGCGAGGTAGGCCTGGGCTCTCAGCGCTTCGATTCGTTGGCACCGGGGTAGGATGCAATAGAGAGCAACGTTTAGTAC
CACCTCGCTTAGCTAAACTGGACTGCCTTATATGCGCGGGTGCTGGCTTGGC-3'

Fig. S8. The promoter sequences of sgRNA-expressing cassettes. (***A***) OsU3-1 promoter sequence. (***B***) OsU6-1 promoter sequence. (***C***) OsU3-2 promoter sequence. (***D***) OsU6-2 promoter sequence.

Table S1. The target sites for the multiplex *PYL* gene editing

Target sites	Target genes
TCAGCTTCCGCGTCGTCGGCGG	*PYL2*
	PYL3
	PYL5
	PYL6
GCGCACCACCGCTACGCCGTGGG	*PYL1*
GGCGGCGGTAAGGCGTGCCCGG	*PYL4*
GGATCATTGGAGGTGACCATAGG	*PYL12*
AGCGGCAGGGAAGTTGCCAATGG	*PYL8*
	PYL9
CCGGGAGGAGGAGATGGAGTAC	*PYL7*
	PYL13
GGTGGCGGCTGGCGGACGAGAGG	*PYL10*
GAGGAGGAGGTTGGAGGGTCGGG	*PYL11*

Table S2. The target sites for the single *PYL* gene editing

Target sites	Target genes
GCGCACCACCGCTACGCCGTGGG	*PYL1*
CCATCGTGCGCAGCTTCGGCAAC	*PYL2*
GCGGGTTCGCCAACCCGCAGCGG	*PYL3*
GTGCTCAGCTTCCGGATCGTCGG	*PYL4*
GTGTGAGATAAGAGCGTGGTGGG	*PYL5*
CCCTGGCATCCCGCACCAGCACC	*PYL6*
CCGCCACGAAATCGGTAGCAACC	*PYL7*
CCGGCAGTTCCACCGCCACGAGC	*PYL8*
CCATTATAACAGAATTACTCATC	*PYL9*
GGTGGCGGCTGGCGGACGAGAGG	*PYL10*
GAGGAGGAGGTTGGAGGGTCGGG	*PYL11*
GGATCATTGGAGGTGACCATAGG	*PYL12*
CCACTTGGAGGTCATCGATGGCC	*PYL13*

Table S3. Primers for RT-PCR

Name	Sequence (5’ to 3’)
QPYL1-F	TACCGATCCGTCACCACCGTC
QPYL1-R	GACTTGAGCTTCTGGAGGTTG
QPYL2-F	CGACGACCGCCACATCATCAG
QPYL2-R	TGGTGTCCTCCGCCGTGTTC
QPYL3-F	TCCGTCACCGAGTTCTCCTCC
QPYL3-R	TGATCAATCACTAATGGTGGTTG
QPYL4-F	GTGGTCGAGTCGTACGTGGTG
QPYL4-R	CCGTTGGCGCGCGGCGCTTC
QPYL5-F	GTCGTGGAGTCCTACGTGGTG
QPYL5-R	TCAGGTCAGGTCAGGCAGGAG
QPYL6-F	GACACCATCGTCAAGTGCAAC
QPYL6-R	GCGCCGAAGAAACACACATCC
QPYL7-F	CCACCAATAAACTTGACACGGAGG
QPYL7-R	CTTTTGTGAGAAAGTGTGTAATGCG
QPYL8-F	GTTCGTCGCCAAGCATATCAAG
QPYL8-R	GAACATTGACCTCCCTAACAC
QPYL9-F	TTCCAGCCACAAGGAGCACTG
QPYL9-R	TTCGACCACCAGTGTTCCAAG
QPYL10-F	ACATACTCAGTGTCAGGTTCG
QPYL10-R	GAAGTAGCATGTCTCATCCTTG
QPYL11-F	CGACGACGAGCACATCCTCAG
QPYL11-R	AGTAGCATGTCTCGTCCTTTG
QPYL12-F	CGGTGTCAGGATCATTGGAGG
QPYL12-R	GATGAGGAACTCGACGAAGTG
QPYL13-F	GATTGAGTCATTTGTGGTGGAC
QPYL13-R	AAGAAGGAATGTGAGATCAAGG
Actin-F	CTGACAGGATGAGCAAGGAG
Actin-R	GGCAATCCACATCTGCTGGA

Table S4. Effect of *pyl* mutations on ABA-induced closure of stomata

		WT		*pyl1/2/3/4/5/6/12*		*pyl7/8/9/10/11/13*	
		cc	po + co	cc	po + co	cc	po + co
Before ABA treatment		26.7 ±1.8	73.3 ±1.8	0	100	25.8 ±2.5	74.2 ±2.5
After treatment for one hour	0 μM ABA	12.8 ±0.8	87.2 ±0.8	0.2 ±0.3	99.8 ±0.3	10 ±0.5	90 ±0.5
	30μM ABA	33.5 ±1.3	66.5 ±1.3	4.7 ±1.3	95.3 ±1.3	28.7 ±1.0	71.3 ±1.0
After treatment for three hours	0 μM ABA	7.0 ±1.5	93.0 ±1.5	0	100	5.3 ±1.6	95.8 ±2.1
	30μM ABA	60.2 ±3.1	39.8 ±3.1	8.0 ±0.5	92.0 ±0.5	47.8 ±1.0	52.2 ±1.0

(cc) = % of stomata completely closed; (po + co) = % of stomata partially or completely open; at least 200 stomata were counted in each treatment of each line, and the assays were repeated three times.

Dataset S1. The list of rice *pyl* mutants.