Mutations in a subfamily of abscisic acid receptor genes promote rice growth and productivity

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Abscisic acid (ABA) is a key phytohormone that controls plant growth and stress responses. It is sensed by the pyrabactin resistance 1 (PYR1)/PYR1-like (PYL)/regulatory components of the ABA receptor (RCAR) family of proteins. Here, we utilized CRISPR/Cas9 technology to edit group I (*PYL1–PYL6* and *PYL12*) and group II (*PYL7–PYL11* and *PYL13*) *PYL* genes in rice. Characterization of the combinatorial mutants suggested that genes in group I have more important roles in stomatal movement, seed dormancy, and growth regulation than those in group II. Among all of the single *pyl* mutants, only *pyl1* and *pyl12* exhibited significant defects in seed dormancy. Interestingly, high-order group I mutants, but not any group II mutants, displayed enhanced growth. Among group I mutants, *pyl1/4/6* exhibited the best growth and improved grain productivity in natural paddy field conditions, while maintaining nearly normal seed dormancy. Our results suggest that a subfamily of rice *PYLs* has evolved to have particularly important roles in regulating plant growth and reveal a genetic strategy to improve rice productivity.

hormone | signaling | stress | crop | yield

Increasing the productivity of food and fiber has been a continual effort of human cultures since the advent of agriculture nearly 10,000 y ago. Abscisic acid (ABA) is a crucial stress hormone that enhances plant adaptation to abiotic and biotic stresses, partly by controlling aspects of plant growth and development, including productivity (1–4). Abiotic stresses, especially water deficit, induce ABA accumulation, which triggers rapid biochemical and physiological responses that enhance stress adaptation. Although ABA may promote root growth in response to drought stress, it generally has inhibitory roles in affecting overall plant growth (1–9). ABA is perceived by the soluble pyrabactin resistance 1 (PYR1)/PYR1-like (PYL)/regulatory components of the ABA receptor (RCAR) family of proteins (9–12). The binding of ABA to PYLs triggers conformational changes that enable interactions between the PYLs and clade A type 2C protein phosphatases (PP2Cs). This leads to the inhibition of the PP2Cs, thus releasing the sucrose nonfermenting 1-related protein kinase 2s (SnRK2s) from inhibition by the PP2Cs (9–19). Activated SnRK2s then phosphorylate, among other proteins, ABA-responsive element-binding factors and components of the machinery regulating stomatal aperture (20–24). These components include the slow anion channel SLAC1 and the NADPH oxidase AtrbohF (21–24). The phosphorylation ultimately results in activation of many responses that contribute to stress adaptation.

In *Arabidopsis*, the *PYL* family consists of 14 genes (11). High-order mutants, from several types of triple mutants to the *pyr1pyl1pyl2pyl4pyl5pyl8* sextuple mutant, have been generated (11, 17, 25). The high-order mutants exhibit reduced vegetative growth and strong ABA-insensitive phenotypes (25). Although extensive gene redundancy exists in the *PYL* family, specific functions and characteristics have been attributed to several *Arabidopsis PYLs*. For example, the *Arabidopsis PYL8* plays particularly important roles in regulating root growth (26–28). Thirteen *PYLs* have been predicted in the rice genome (29, 30). These PYLs have different inhibitory activities on PP2Cs in in vitro assays (29, 30). Overexpression of rice *PYL3*, *PYL5*, *PYL9*, or *PYL11* improved drought stress resistance and led to hypersensitivity to ABA for inhibition of seed germination and seedling growth (29, 31, 32). Among the rice PYLs, PYL12 is unique because it is unable to bind ABA but shows potent constitutive inhibition on the activities of PP2Cs in vitro (30).

To date, no high-order *pyl* mutants have been reported in rice, and the function of rice *PYLs* remains to be identified. Here, we utilized CRISPR/Cas9 technology to systematically mutate the *PYL* genes. Through analyses of the *pyl* mutants, we found that combinatorial mutations of group I genes (*PYL1–PYL6* and *PYL12*) promote rice growth, whereas mutations of group II genes (*PYL7–PYL11* and *PYL13*) have no significant effects on growth. Among group I mutants, *pyl1/4/6* exhibited the most robust growth and improved grain productivity, while maintaining near-normal seed dormancy and other agronomic traits. These results show functional differentiation of rice *PYLs* and provide a genetic strategy to improve rice productivity.

Results

Multigene Knockouts of Rice *PYLs*. Our real-time RT-PCR analyses showed a differential expression pattern for each of the 13 rice *PYLs* in various tissues (*SI Appendix*, Fig. S1), suggesting functional differences between rice *PYLs*. We constructed two CRISPR/Cas9 vectors to edit the 13 genes (Fig. 1*A* and specific target sites on each *PYL* gene are shown in *SI Appendix*, Table S1). Each vector targets a group of neighboring genes on the phylogenetic tree (Fig. 1*A* and *SI Appendix*, Fig. S2). One vector (vector I) targets group I genes, including *PYL1*, *PYL2*, *PYL3*, *PYL4*, *PYL5*, *PYL6*, and *PYL12*, and another (vector II) targets group II genes, including *PYL7*, *PYL8*, *PYL9*, *PYL10*, *PYL11*, and *PYL13* (Fig. 1*A*). We also constructed individual vectors to edit each *PYL* independently (target sequences are shown in *SI Appendix*, Table S2).

Significance

Climate change is challenging plant agriculture and our ability to manage food security. Crop growth and yield are controlled by several phytohormones and their overlapping signal networks. We report here an unexpected aspect of the abscisic acid (ABA) signal network that directly impacts rice productivity. Simultaneously mutating the genes encoding the ABA receptors pyrabactin resistance 1-like 1 (PYL1), PYL4, and PYL6 causes improved growth and increased grain yield in rice. Our work thus reveals an important role of these ABA receptors in growth control and a genetic strategy to improve rice yield.

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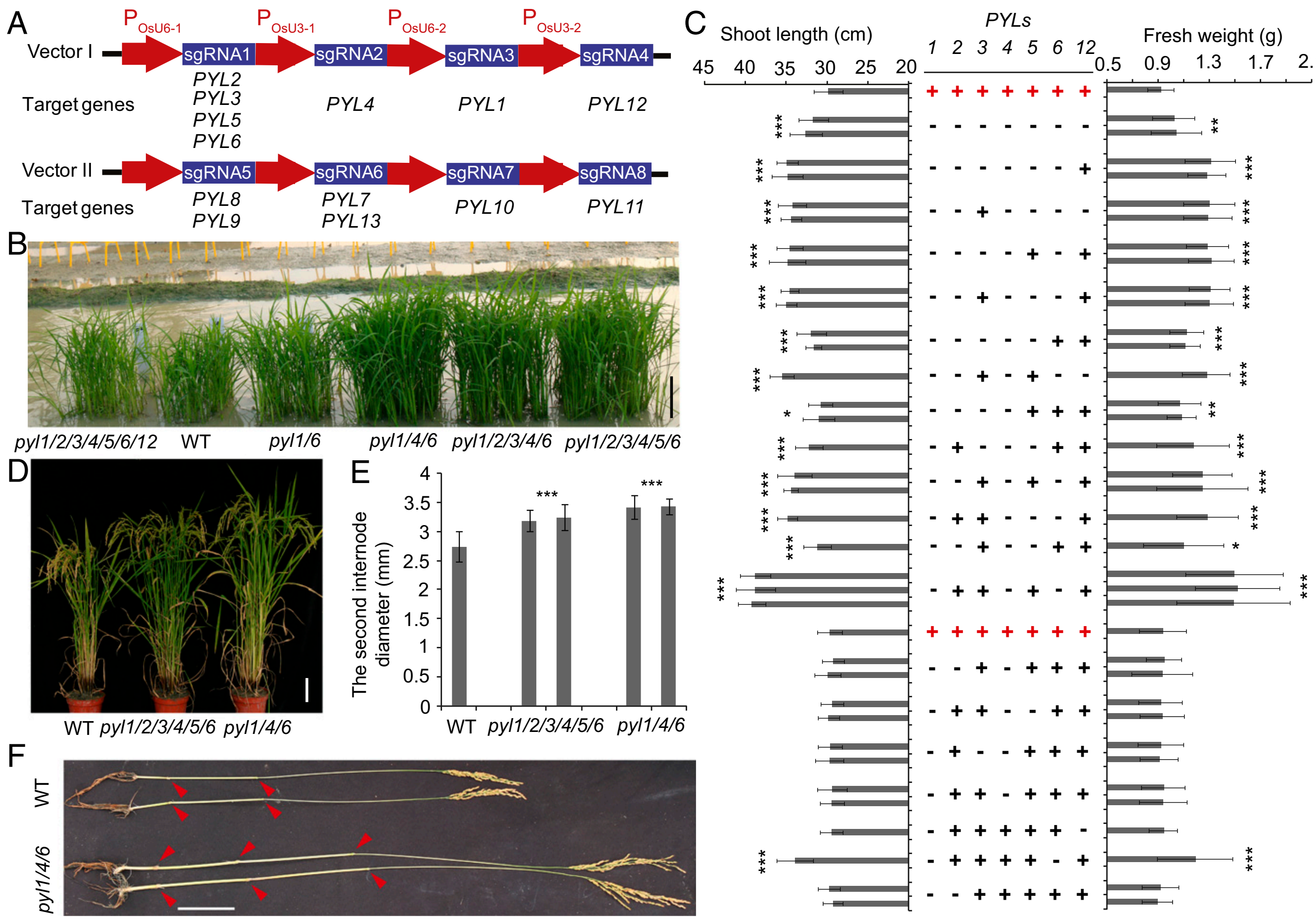

Fig. 1. Group I *pyl* mutations promote rice growth. (*A*) Strategy of vector construction for editing rice *PYL* genes. (*B*) Comparison of wild-type, *pyl1/6*, *pyl1/4/6*, *pyl1/2/3/4/6*, *pyl1/2/3/4/5/6*, and *pyl1/2/3/4/5/6/12* seedlings. (*C*) Shoot lengths and fresh weights of 21-d-old group I *pyl* seedlings. Every column represents an independent line. The "+" and "−" represent the wild-type and mutated genes, respectively. (*D*) Morphological features of wild-type, *pyl1/2/3/4/5/6*, and *pyl1/4/6* plants at the mature stage. (*E*) Diameters of wild-type, *pyl1/2/3/4/5/6*, and *pyl1/4/6* second internodes. (*F*) Comparison of the panicles and internodes of the wild type and *pyl1/4/6*. The arrowheads indicate the stem nodes. (Scale bars, 10 cm.) WT, wild type. Data are presented as means ± SD. *P* values (versus the wild type) were calculated by the Student's *t* test: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

Through *Agrobacterium*-mediated transformation of the rice variety Nipponbare, seedlings of the first (T0) generation with specific mutations in *PYLs* were obtained. In the T2 generation, 153 lines (A1–A153) of homozygous mutants for group I genes and 84 lines (B6–B89) of homozygous mutants for group II genes were identified (Dataset S1; A1–A108 and B6–B51 were identified from the multiplex gene-edited lines, and A109–A153 and B52–B89 were obtained from the single gene-edited lines). Since *pyl7/8/9/10/11/13* mutants were not found among the multiplex gene-edited lines, we crossed *pyl7/8/9/10/13* lines with *pyl11* and identified five homozygous *pyl7/8/9/10/11/13* lines from the segregating second (F2) population (Dataset S1; B1–B5). In subsequent analyses, we focused on plants with frameshift mutations since they are most likely knockout mutants. To ascertain the genotype and heritability of every line, we sequenced the target genes to confirm the genotype in the following generations and selected CRISPR/Cas9-free plants for seed harvesting.

High-Order Group I, but Not Group II, Mutants Exhibit Improved Growth Compared with the Wild Type. Phenotypic analyses of the mutants were made in the paddy fields of Shanghai and Lingshui County of Hainan Island under natural conditions. We did not observe significant morphological differences between the wild type and any single mutant of the 13 *PYLs*. During the entire rice life cycle, high-order group II mutants also appeared to have similar morphological phenotypes to the wild type (*SI Appendix*, Fig. S3*A*). Interestingly, we found that many group I mutants had more robust growth than the wild type (Fig. 1 *B–D* and *SI Appendix*, Fig. S3 *B–D*). During the seedling stage (one- to five-leaf stage) in both Shanghai and Hainan, among *pyl1/6* to *pyl1/2/3/4/5/6/12*, most mutants had a larger stature than the wild type (Fig. 1 *B* and *C* and *SI Appendix*, Fig. S3*B*). Among the mutants, *pyl1/4/6* had the most robust growth, indicating important roles for *PYL1*, *PYL4*, and *PYL6* in inhibiting rice growth (Fig. 1 *B* and *C*). All lines of *pyl1/4/6* seedlings had larger fresh weight and longer shoot length than the wild type and appeared larger than the other mutants overall (Fig. 1*C*). Among the double (*pyl1/6*, *pyl1/4*, *pyl1/2*, and *pyl1/12*) and triple (*pyl1/4/6*, *pyl1/2/4*, *pyl1/4/5*, and *pyl1/3/4*) mutants, only *pyl1/6* and *pyl1/4/6* seedlings were larger than the wild type (Fig. 1*C*). The other high-order group I mutants were all larger than the wild type. From the triple to sextuple group I mutants, all of the seedlings that contained a mutation in *PYL6* had longer shoot length and larger fresh weight than those without the *pyl6* mutation (Fig. 1*C*), indicating that *PYL6* plays a crucial role in controlling rice plant growth.

In Hainan, after the seedling stage, high-order group I mutants continued to exhibit more robust growth than the wild type. However, in Shanghai in the year 2016, phenotypic differences from those in Hainan appeared. In Shanghai in 2016, from mid-July, a high-temperature climate (about 41 °C at midday) appeared and lasted for more than 2 wk. Passing through this high-temperature

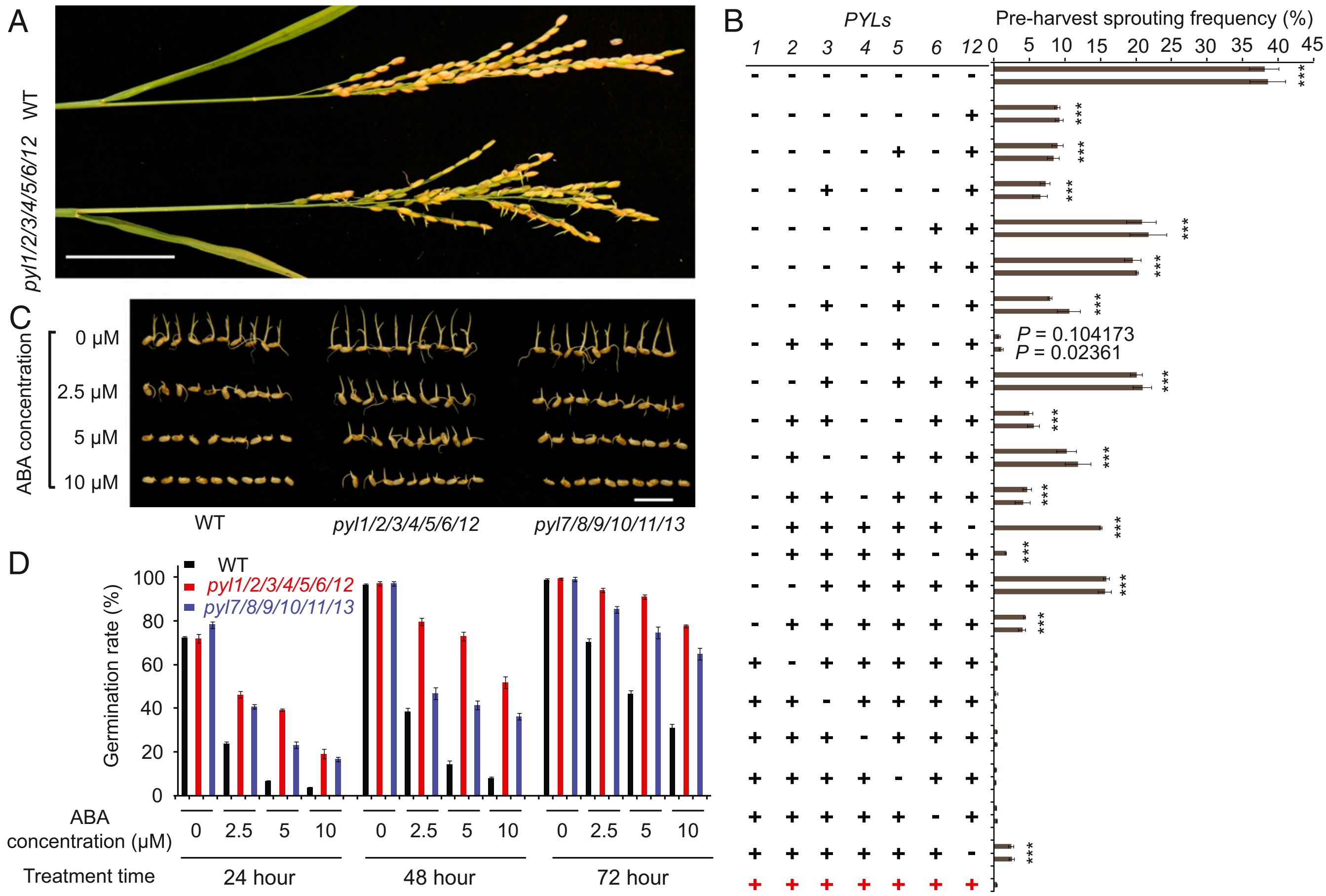

Fig. 2. PHS and seed dormancy of the *pyl* mutants. (*A*) PHS of *pyl1/2/3/4/5/6/12* mutant. (Scale bar, 5 cm.) (*B*) PHS frequencies in group I mutants. Every column represents an independent line. In every line, all of the seeds of three plants were counted. The "+" and "−" represent the wild-type and mutated genes, respectively. *P* values (versus the wild type) were calculated by the Student's *t* test: ****P* < 0.001. (*C*) Germinating seeds of the wild type, *pyl1/2/3/4/5/6/12*, and *pyl7/8/9/10/11/13* treated with ABA for 4 d. (Scale bar, 2 cm.) (*D*) Effect of ABA on the seed germination rates of the wild type, *pyl1/2/3/4/5/6/12*, and *pyl7/8/9/10/11/13*. In every treatment, 100 seeds were used. The treatment was repeated three times. WT, wild type. Data are presented as means ± SD.

period, higher order group I mutant plants (~30–60 d post-germination) suffered more severely retarded growth (*SI Appendix*, Fig. S4*A*), suggesting hypersensitivity of the higher order mutants to the high-temperature climate. During and after this high-temperature period, the *pyl1/4/6* lines were still larger than the wild type (*SI Appendix*, Fig. S4*A*).

After the heading stage, most high-order group I mutant plants in both Shanghai and Hainan were larger than the wild type (Fig. 1*D* and *SI Appendix*, Fig. S3 *C* and *D*). We measured the plant height at this stage on Hainan Island, and the data revealed a generally similar growth pattern to that during the seedling stage (*SI Appendix*, Fig. S3*D*). From *pyl1/6* to *pyl1/2/3/4/5/6/12*, most mutant lines were taller than the wild type (Fig. 1*D* and *SI Appendix*, Fig. S3*D*). Among the high-order group I mutant lines, the *pyl1/4/6* lines were often the tallest (Fig. 1*D* and *SI Appendix*, Fig. S3*D*). In Shanghai in 2016, despite the high-temperature episode, the group I mutants regained their faster growth phenotype after the heading stage (*SI Appendix*, Fig. S4*B*). Except for *pyl1/2/3/4/5/6/12* mutants, all of the plants in Shanghai showed a similar phenotype to those in Hainan at the mature stage (*SI Appendix*, Fig. S4*B*). The *pyl1/2/3/4/5/6/12* plants in Shanghai showed similar plant height to the wild type at the mature stage (*SI Appendix*, Fig. S4*B*), whereas they were slightly taller than the wild type in Hainan (*SI Appendix*, Fig. S3*D*). At the mature stage, we also measured the internode diameters of wild-type, *pyl1/2/3/4/5/6*, and *pyl1/4/6* plants. The *pyl1/2/3/4/5/6* and *pyl1/4/6* mutants had larger internode diameters than the wild type (Fig. 1 *E* and *F*). Compared with the wild type, the length of every internode in *pyl1/4/6* was also increased, especially the lower internodes (Fig. 1*F* and *SI Appendix*, Fig. S3*E*).

To further characterize the increased growth in group I mutants, we compared the cell size of *pyl1/4/6* and the wild type at the seedling stage. The results showed that the epidermal cells of *pyl1/4/6* leaf blades and sheaths were longer than those of the wild type (*SI Appendix*, Fig. S5 *A*–*D*). However, no significant differences ($P > 0.1$) in the cell width between *pyl1/4/6* and the wild type were observed (*SI Appendix*, Fig. S5 *A*–*D*). At the seed-filling stage, the *pyl1/4/6* flag leaf blades were longer and wider than the wild type (*SI Appendix*, Fig. S5 *H* and *I*). Consistent with this phenotype, the epidermal cells of the flag leaf blades in *pyl1/4/6* were longer than the wild type, whereas the epidermal cell widths of the flag leaf blades showed no significant differences between *pyl1/4/6* and the wild type (*SI Appendix*, Fig. S5*J*). The epidermal cells of flag leaf sheaths in *pyl1/4/6* were longer but narrower than those of the wild type (*SI Appendix*, Fig. S5*K*). Histological analyses of cross-sections of leaf blades and internodes revealed no obvious differences in the sizes of the cells between *pyl1/4/6* and the wild type (*SI Appendix*, Fig. S5*E*). Using flow cytometry, we examined DAPI-stained nuclei isolated from the bud tips of germinated seeds (germinated for 2 d). The results showed that *pyl1/4/6* had a higher percentage of cells in G_2/M phase than the wild type (*SI Appendix*, Fig. S5 *F* and *G*), indicating a faster cell cycle in *pyl1/4/6*. Our data suggest that

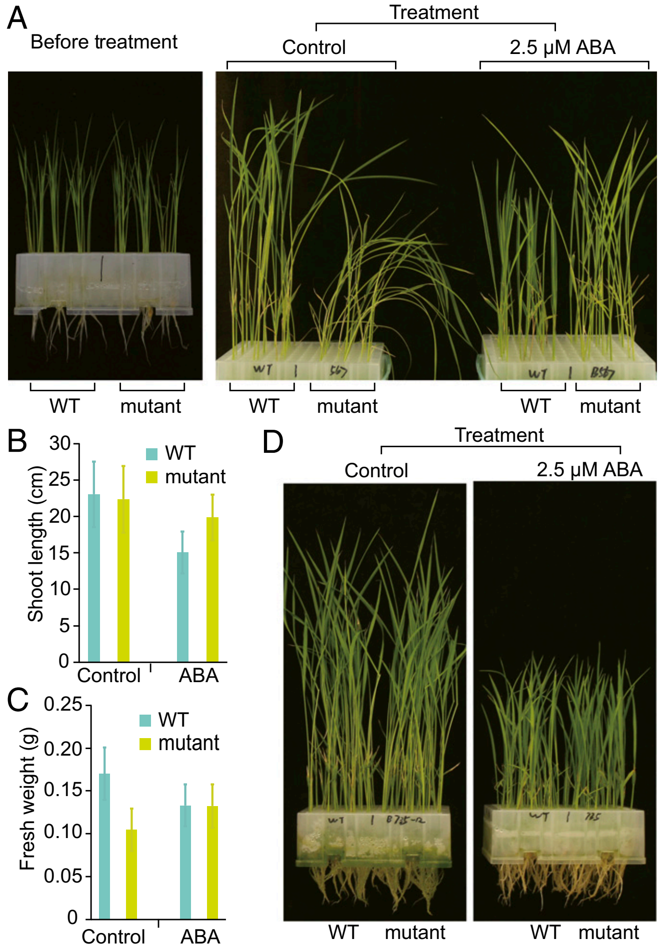

Fig. 3. Sensitivity of wild-type and *pyl* seedlings to ABA. (*A*) Wild-type and *pyl1/2/3/4/5/6/12* (mutant) seedlings grown for 5 d in 2/3 Murashige and Skoog (MS) liquid medium without (control) or with 2.5 μM ABA. (*B*) Shoot lengths of the wild-type and *pyl1/2/3/4/5/6/12* (mutant) seedlings treated with 0 (control) and 2.5 μM ABA for 5 d. Twenty-four seedlings of each material in the control and ABA treatments were measured. (*C*) Fresh weights of the wild-type and *pyl1/2/3/4/5/6/12* (mutant) seedlings treated with 0 (control) and 2.5 μM ABA for 5 d. Twenty-four seedlings of each material in the control and ABA treatments were measured. (*D*) Wild-type and *pyl7/8/9/10/11/13* (mutant) seedlings grown for 5 d in 2/3 MS liquid medium without (control) or with 2.5 μM ABA. WT, wild type. Data are presented as means ± SD.

mutations in group I *PYLs* promote rice plant growth through accelerating cell division and increasing cell elongation.

Group I, but Not Group II, Mutations Delay Heading Dates. Heading date is an important trait for adapting crops to different cultivating regions and cropping seasons. In many of the group I mutants, a substantial delay in heading date was observed (*SI Appendix*, Fig. S3*F*). Compared with the wild type, the heading date of *pyl1/2/3/4/5/6* and *pyl1/2/3/4/5/6/12* was delayed by about 9 d, *pyl1/2/3/4/6* by about 7 d, *pyl1/2/3/4* by about 5 d, and *pyl1/4/6* by about 1 d. No differences in heading dates were observed between the wild type and other group I triple, double, and single mutants. The group II mutants had heading dates similar to the wild type.

Group I Mutations Result in Seed Dormancy Defects and Preharvest Sprouting. Seeds sometimes fail to enter a period of strong dormancy and may germinate while still attached to the parent plants. This condition is known as preharvest sprouting (PHS). PHS is usually associated with reduced levels of ABA or ABA signaling in the maturing seeds. Among the high-order group I, but not group II, mutants, significant PHS was often observed, especially in Shanghai (Fig. 2*A*). In Shanghai, during the harvest time of Nipponbare (late September and early October), there was often very abundant rainfall that can promote PHS. Therefore, we investigated the PHS of the *pyl* mutants in Shanghai in the year 2016. The highest frequency of PHS was observed in *pyl1/2/3/4/5/6/12* (Fig. 2*B*). Among the single mutants, only *pyl1* and *pyl12* had notably higher frequencies of PHS than the wild type (Fig. 2*B*), indicating that *PYL1* and *PYL12* play critical roles in establishing seed dormancy. Consistent with the critical role of *PYL12* in seed dormancy, the frequency of PHS in *pyl1/2/3/4/5/6/12* was much higher than that in *pyl1/2/3/4/5/6* (Fig. 2*B*). The important role of *PYL12* in seed dormancy is interesting, since PYL12 cannot bind to ABA in in vitro assays (30). Interestingly, we found that the frequency of PHS was lower in *pyl1/6* than in *pyl1*, and lower in *pyl1/4/6* than in *pyl1* and *pyl1/4* (Fig. 2*B*). Thus, there appears to be an antagonistic function between *PYL6* and the other group I *PYL* genes in establishing seed dormancy. Among the triple mutants (*pyl1/4/6*, *pyl1/2/4*, *pyl1/4/5*, and *pyl1/3/4*), *pyl1/4/6* had the lowest frequency of PHS (Fig. 2*B*). In fact, among the high-order mutants of group I, *pyl1/4/6* had the lowest PHS frequency, which was nearly comparable to the wild type (*P* values of 0.104173 and 0.02361 for two independent lines) (Fig. 2*B*). In other planting seasons, we did not observe obvious PHS in *pyl1/4/6*. The most robust growth and lowest PHS frequency observed in *pyl1/4/6* indicated that this combination of mutations may be used to

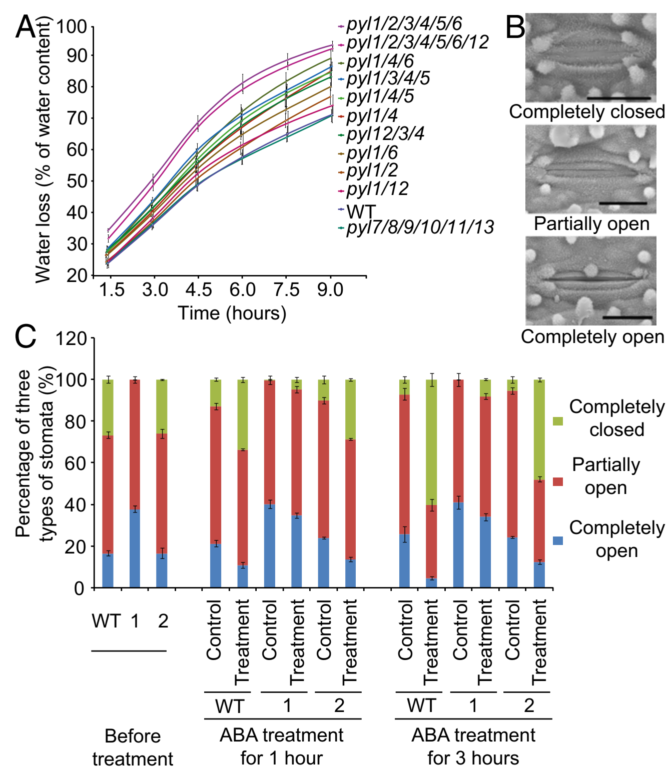

Fig. 4. Stomatal movement in the wild type and *pyl* mutants. (*A*) Cumulative transpirational water loss from the detached flag leaves of the wild type and *pyl* mutants. Five flag leaf blades of each mutant were used for this assay. (*B*) Scanning electron microscopy images of three levels of stomatal opening. (Scale bars, 10 μm.) Stomata with a similar or wider opening than the stoma as pictured in the *Bottom* panel were considered completely open. (*C*) Effect of *pyl* mutations on ABA-induced closure of stomata. 1, *pyl1/2/3/4/5/6/12*; 2, *pyl7/8/9/10/11/13*; Control, treatment in stomata opening buffer without ABA; WT, wild type. Data are presented as means ± SD.

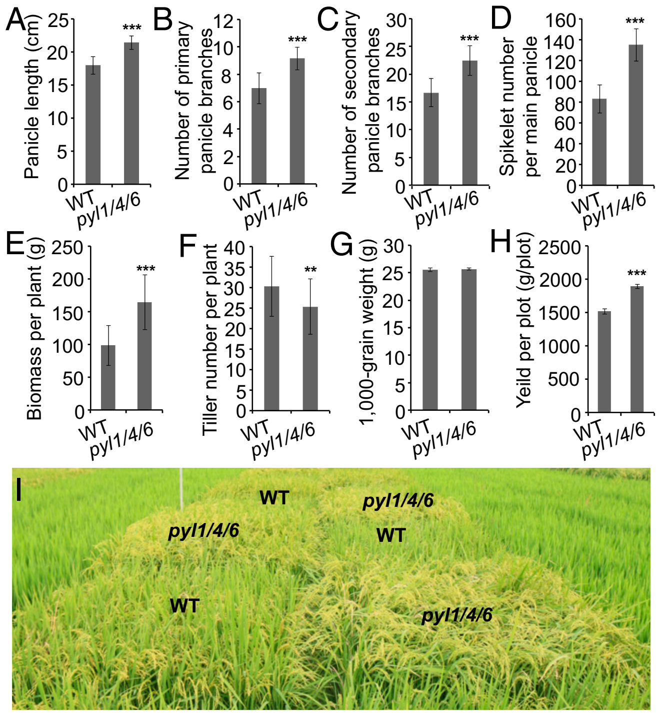

Fig. 5. Agronomic characteristics and yield test of the wild type and *pyl1/4/6*. (*A*) Main panicle lengths of the wild type and *pyl1/4/6*. Numbers of primary (*B*) and secondary (*C*) branches per main panicle are shown. (*D*) Spikelet numbers per main panicle of the wild type and *pyl1/4/6*. (*E*) Biomasses of the wild type and *pyl1/4/6* at the mature stage. (*F*) Tiller numbers of the wild type and *pyl1/4/6*. (*G*) One thousand-grain weights of the wild type and *pyl1/4/6*. (*H*) Grain yields per plot of the wild type and *pyl1/4/6* in Shanghai in the year 2016. The planting density was 15 × 15 cm, with one plant one hill. One hundred forty-four (12 × 12) plants were cultivated in every plot. (*I*) Plot yield test in Shanghai in the year 2016. WT, wild type. Data are presented as means ± SD. *P* values (versus the wild type) were calculated with the Student's *t* test: ****P* < 0.001; ***P* < 0.01.

generally improve rice productivity. No obvious PHS was observed in group II mutants at the normal harvest time. However, we did find higher frequencies of seed germination on panicles in *pyl7/8/9/10/13* and *pyl7/8/9/10/11/13* than in the wild type when the seed harvesting was delayed by about 25 d in the year 2016 in Shanghai (*SI Appendix*, Fig. S6). Thus, there appears to be some defects in seed dormancy in these group II mutants.

The observed PHS implies that differences in the sensitivity of seed germination to ABA may exist between the *pyl* mutants and the wild type. Therefore, we treated the germinating seeds of the wild type, *pyl1/2/3/4/5/6/12*, and *pyl7/8/9/10/11/13* with 2.5, 5, and 10 μM ABA for 4 d, and examined the germination status each day. These assays revealed the highest germination rate in *pyl1/2/3/4/5/6/12* under the ABA treatment conditions (Fig. 2 *C* and *D*). Notably higher germination rates in *pyl7/8/9/10/11/13* compared with the wild type were also observed during the treatments with each ABA concentration (Fig. 2*D*). These results indicate that both group I and group II *PYLs* control seed dormancy but that group I genes have more important functions in seed dormancy than group II genes.

The Growth of Group I, but Not Group II, Mutants Is Less Sensitive to ABA than the Wild Type. To investigate the sensitivity to ABA at the postgermination stage, 8- to 9-d-old seedlings with similar sizes were treated with 2.5 μM ABA. Five days after the treatment, compared with the untreated control, the shoot length of the wild type was reduced by about 35%, whereas the ABA treatment reduced the shoot length of *pyl1/2/3/4/5/6/12* by only 11%, indicating less sensitivity of *pyl1/2/3/4/5/6/12* seedlings to ABA (Fig. 3 *A* and *B*). The ABA treatment reduced the fresh weight of the wild type by 22%, whereas the fresh weight of *pyl1/2/3/4/5/6/12* increased by 27% after the treatment (Fig. 3 *A* and *C*). This suggests that a basal level of ABA signaling promotes plant growth. In fact, without ABA treatment, *pyl1/2/3/4/5/6/12* seedlings were not robust when grown in the greenhouse (26 °C, 80% humidity, and 12 h light/12 h dark), and it was even difficult for them to stand erect (Fig. 3*A*). ABA treatment made *pyl1/2/3/4/5/6/12* stronger physically and more robust (Fig. 3 *A* and *C*). No obvious differences in ABA response were observed between wild-type and *pyl7/8/9/10/11/13* seedlings (Fig. 3*D*).

Group I *PYL* Knockouts Lead to Severe Defects in Stomatal Movement and Water Loss Control. Given that ABA controls transpirational water loss by inducing stomatal closure, we investigated the water loss rate of detached flag leaves. Gravimetric measurement of water loss from detached leaves indicates that high-order group I, but not group II, mutants lose water more quickly than the wild type (Fig. 4*A* and *SI Appendix*, Fig. S7*A*). Among all of the *pyl* mutants tested, *pyl1/2/3/4/5/6* and *pyl1/2/3/4/5/6/12* lost water most quickly (Fig. 4*A*). Infrared imaging revealed a lower leaf surface temperature in *pyl1/4/6* than in the wild type in the greenhouse (26 °C, 80% humidity, and 12 h light/12 h dark) (*SI Appendix*, Fig. S7*B*), consistent with more rapid transpiration in group I mutants. Water withholding assays showed that *pyl1/4/6* mutant plants were more sensitive to drought than the wild type (*SI Appendix*, Fig. S7*C*). Next, we examined whether ABA induction of stomatal closure was affected in the *pyl* mutants. Before treatment with 30 μM ABA, ∼27% and ∼26% of the stomata in the wild type and *pyl7/8/9/10/11/13* were closed, respectively, whereas no stomata of *pyl1/2/3/4/5/6/12* leaves were closed (Fig. 4 *B* and *C* and *SI Appendix*, Table S4). After treatment in 30 μM ABA for 1 h, stomata of the wild type and *pyl7/8/9/10/11/13* began to close, reaching around 60% and 48% completely closed after 3 h, respectively (Fig. 4*C* and *SI Appendix*, Table S4). Only 8% of the stomata of *pyl1/2/3/4/5/6/12* were closed completely after the ABA treatment for 3 h (Fig. 4*C* and *SI Appendix*, Table S4). These results clearly show that ABA treatment induces much less stomatal closure in *pyl1/2/3/4/5/6/12* than in the wild type and *pyl7/8/9/10/11/13*, suggesting that the group I *PYLs* have a more important function in stomatal movement than group II *PYLs*.

Improving Productivity with *pyl1/4/6*. The above results revealed that *pyl1/4/6* lines exhibited the most robust growth among the *pyl* mutants, while maintaining nearly normal seed dormancy and heading date, suggesting that this mutant has important potential for use in agriculture. So, we compared several agronomic characteristics of *pyl1/4/6* with the wild type. The seed density of the panicles in *pyl1/4/6* was not obviously different from that in the wild type, but the panicles of *pyl1/4/6* were longer than the wild type (Figs. 1*F* and 5*A*). There were more primary and secondary branches in the panicles of *pyl1/4/6* (Fig. 5 *B* and *C*), and the spikelet number per main panicle was also increased in *pyl1/4/6* (Fig. 5*D*). Compared with the wild type, the biomass of *pyl1/4/6* at the mature stage was significantly increased (Fig. 5*E*), even though the tiller number was decreased (Fig. 5*F*). The 1,000-grain weight of *pyl1/4/6* plants showed no significant differences from the wild type (Fig. 5*G*; *P* > 0.2).

The productivity of *pyl1/4/6* in paddy field conditions was examined using plot yield tests. Our test in Shanghai in 2016 showed that compared with the wild type, *pyl1/4/6* had ∼25% higher grain yield (Fig. 5 *H* and *I*). We repeated the test in Hainan Island. Here, we observed an increase of about 31% in grain production (a yield per plot of 1,727.1 ± 34.9 g for *pyl1/4/6* versus 1,317.5 ± 25.7 g of yield per plot for the wild type, 15 × 15-cm plant density, 144 plants per plot). These results indicate that *pyl1/4/6* has significantly improved yields under natural paddy field conditions.

Discussion

The growth-repressing function of ABA has been thought to be a trade-off for improving stress adaptation (3, 33, 34). Since the plant growth and stress adaptation processes are contrary to each other in many ways, it is important to establish a balance between them that is appropriate for the environment. Therefore, ABA levels and signaling should have significant effects on both growth and adaptation. Genetic adjustment of the balance by manipulating ABA levels and/or signaling may generate useful crop varieties to improve productivity in specific environments.

The PYLs are currently the largest plant hormone receptor family known (35). In *Arabidopsis*, 14 *PYL* members have been identified, and redundant as well as differential functions of the genes have been documented (11, 26–28, 35, 36). In rice, 13 *PYL* members were predicted (29, 30). Our results indicate differential and redundant functions for different members as well. The functional differentiation and redundancy provide the possibility to adjust the balance between growth and stress resistance to improve crop productivity through editing certain *PYL*s. In rice, we found that among the *pyl* mutants, *pyl1/4/6* showed the best growth, while maintaining nearly normal seed dormancy. During the heat wave of the 2016 summer in the paddy field in Shanghai, although the growth of quintuple to septuple *pyl* mutants was increasingly retarded, the *pyl1/4/6* mutants still grew better than the wild type. This suggests that the *pyl1/4/6* lines may be more tolerant to the hot weather than the quintuple, sextuple, and septuple group I mutants. These results indicate that the balance between growth and stress adaptation is altered in high-order group I *pyl* mutants, and that the new balance in *pyl1/4/6* plants favors more growth, while stress adaptation is less compromised than in higher order group I *pyl* mutants.

Previous studies showed that *Arabidopsis* high-order *pyl* mutants exhibited retarded growth, and that this growth defect can be ameliorated by increasing the humidity of the growth environment (25, 37). Still, improved growth (compared with the wild type) was not observed in *Arabidopsis pyl* mutants (25). Although, overall, rice *PYLs* have similar functions to *Arabidopsis PYLs* in promoting seed dormancy and stomatal closure, they seem to differ in their impacts on plant growth. It is likely that under the paddy field growth conditions, some of the rice *PYLs* have been selected to have a particularly important role in restraining plant growth. In the future, it will be of interest to determine how PYL1, PYL4, and PYL6 (particularly PYL6) are linked to growth regulation in rice. In the paddy field, where water is not limiting, the higher transpiration of *pyl1/4/6* may contribute to faster growth and higher yield by facilitating rapid CO_2 absorption, while avoiding the negative effects of excessive water loss. Plant breeders have long suggested that the improvement of crop performance in certain climates may be achieved by reducing responses to ABA or endogenous ABA level (34). The *pyl1/4/6* rice mutants may represent an effective approach to achieve this historically coveted result.

Materials and Methods

Details are provided in *SI Appendix, SI Materials and Methods*, including vector construction and plant cultivation, shoot length and fresh weight measurements, heading date comparison, histological analyses and epidermal cell observations, cell cycle comparison, seed dormancy analyses, stomatal movement and water loss assays, ABA sensitivity assays of seedling growth, real-time RT-PCR, plot field test, and accession numbers.

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