The versatile functions of OsALDH2B1 provide a genic basis for growth–defense trade-offs in rice

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In plants, enhanced defense often compromises growth and development, which is regarded as trade-offs between growth and defense. Here we identified a gene, *OsALDH2B1*, that functions as a master regulator of the growth–defense trade-off in rice. OsALDH2B1 has its primary function as an aldehyde dehydrogenase and a moonlight function as a transcriptional regulator. Loss of function of *OsALDH2B1* greatly enhanced resistance to broad-spectrum pathogens, including fungal blast, bacterial leaf blight, and leaf streak, but caused severe phenotypic changes such as male sterility and reduced plant size, grain size, and number. We showed that its primary function as a mitochondrial aldehyde dehydrogenase conditions male fertility. Its moonlight function of transcriptional regulation, featuring both repressing and activating activities, regulates a diverse range of biological processes involving brassinolide, G protein, jasmonic acid, and salicylic acid signaling pathways. Such regulations cause large impacts on the morphology and immunity of rice plants. The versatile functions of OsALDH2B1 provide an example of the genic basis of growth–defense trade-offs in plants.

Oryza sativa | disease resistance | growth | development | male sterility

Growth, development, reproduction, and defense are basic processes for all organisms. Although defense is essential for an organism to survive, frequently, however, enhanced defense is achieved at the cost of other processes. In plants, for example, enhanced defense is often accompanied by a "syndrome" of compromised morphology such as growth retardation, male sterility, reduced plant size, etc. In other words, such compromised morphology often results in immunity activation as observed in transgenic rice overexpressing *OsWRKY45* and *Ideal Plant Architecture1* or knockdown of miR156 and knockout of *OsDOF11*, which is often referred to as trade-offs between growth and defense (1–5). Such a trade-off has posed great difficulty for crop genetic improvement in the effort to keep the balance between defense and productivity. In the last few decades tremendous progress has been made in the understanding of the genes, pathways, and regulatory networks underlying defense, growth, and reproduction, respectively (1, 3–14). However, much less is known on how these processes are coordinated or whether there is a master regulator that may modulate these processes.

The protein moonlight function, a second function in addition to the presumed regular one, has been proposed as an adaptive mechanism to yield a novel regulator meeting the needs of organismal complexity (15, 16). Such moonlighting proteins have been found in a wide range of organisms (17), and about two-thirds of them are enzymes, which play diverse biological roles (18, 19). A second function of enzymes as transcription regulators or factors has been found in multiple organisms (20–26). Moonlighting proteins playing roles in disease development and as new therapeutic targets for disease treatments have been extensively studied in mammals (27, 28). However, little is known about whether moonlighting proteins play any roles in plant disease response or whether such a function may impact plant growth and development.

Here we identified a gene, *OsALDH2B1*, in rice with a primary function as an aldehyde dehydrogenase and a moonlight function as a regulator at multiple tiers of regulatory activities. The activities of this gene integrate multiple processes and pathways governing defense, growth, and reproduction. The results unraveled the mechanistic connections of the various processes underlying the growth–defense trade-off in this special case of rice.

Results

Mutation in *OsALDH2B1* Impeded Rice Growth but Increased Defense. We identified a T-DNA (Ti plasmid deoxyribonucleic acid) insertion mutant, RMD_05Z11EQ94, in which the T-DNA was inserted in the first intron of the *OsALDH2B1* gene (*SI Appendix*, Fig. S1*A*). The transcript of *OsALDH2B1* was severely reduced in the mutant relative to the wild type (WT) (*SI Appendix*, Fig. S1*B*). We designated the mutant *osaldh2b1*.

The *osaldh2b1* mutant exhibited a defect phenotype in growth and development, including plant height, tiller number, lamina joint angle, panicle morphology, grain number, and seed setting (Fig. 1 *A* and *B* and *SI Appendix*, Fig. S1 *E–J*). Compared with WT, *osaldh2b1* showed increased tiller number (188.9%) and lamina joint angle (55.8%) and decreased plant height (−20.4%), panicle length (−18.6%), and grain number per panicle (−29.1%). The mutant was completely male sterile, leading to zero seed setting. Furthermore, seeds produced by the *osaldh2b1* plant pollinated with WT pollen were substantially smaller than seeds of WT (Fig. 1*C* and *SI Appendix*, Fig. S1*K*).

To investigate whether such a compromised phenotype is associated with increased immunity response as reported in previous studies, we inoculated *osaldh2b1* with the bacterial pathogens *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv.

Significance

Crops' defense activation often causes growth inhibition and yield reduction, which is referred to as trade-offs between growth and defense. In this study, we identified a gene, *OsALDH2B1*, that functions as a master regulator of the growth–defense trade-off in rice. The findings provide an example for the genic basis of growth–defense trade-offs in plants and may also have important implication for crop genetic improvement by exploring and modulating these components to achieve a balance between high yield and disease resistance.

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The authors declare no competing interest.

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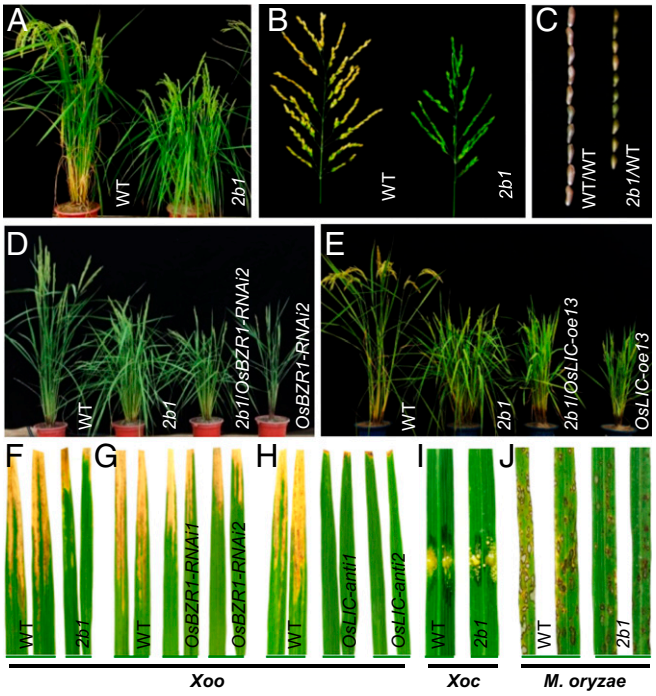

Fig. 1. Phenotypic effects of *OsALDH2B1*, *OsBZR1*, and *OsLIC*. Plants (*A*), panicles (*B*), and seeds (*C*) of WT and the *osaldh2b1* (*2b1*) mutant. (*D*) Plants of WT, *osaldh2b1*, *OsBZR1-RNAi2*, and *osaldh2b1/OsBZR1-RNAi2* double mutants segregated from F_2. (*E*) Plants of WT, *osaldh2b1*, *OsLIC-oe13*, and *osaldh2b1/OsLIC-oe13* double mutants segregated from F_2. (*F–H*) Increased resistance of the *osaldh2b1* mutant (*F*), *OsBZR1-RNAi* lines (*G*), and *OsLIC-anti* lines (*H*) to *Xoo* shown with leaves at 14 d after inoculation with *Xoo* strain PXO341. (*I*) Increased resistance of *osaldh2b1* mutant to *Xoc* shown with leaves at 7 d after inoculation with *Xoc* strain RH3. (*J*) Increased resistance of *osaldh2b1* mutant to *M. oryzea* shown with leaves at 7 d after inoculation with *M. oryzea* isolate *N2-2*.

oryzicola (*Xoc*) and the fungal pathogen *Magnaporthe oryzae* (*M. oryzae*). *Xoo* and *Xoc* cause rice bacterial leaf blight and bacterial leaf streak, respectively, and *M. oryzae* causes blast. All three diseases are highly devastating and cause heavy yield losses worldwide. The results showed that *osaldh2b1* enhanced resistance to all of the pathogen strains we tested. The lesion lengths of *osaldh2b1* plants resulting from inoculation of *Xoo* and *Xoc* were 66.5% and 43.1% shorter than those of WT (Fig. 1 *F* and *I* and *SI Appendix*, Fig. S1 *L* and *M*), and the disease index of *M. oryzae* was reduced by 37.1% relative to WT (Fig. 1*J* and *SI Appendix*, Fig. S1*N*). The amounts of bacterial growth of *Xoo* and *Xoc* and fungal biomass of *M. oryzae* were smaller, corroborating the observation of enhanced disease resistance of *osaldh2b1* (*SI Appendix*, Fig. S1 *L–N*).

The *osaldh2b1* complementary plants (*OsALDH2B1-C*) complemented the mutant phenotype (*SI Appendix*, Fig. S1*Q*), suggesting that *OsALDH2B1* was the gene responsible for the *osaldh2b1* mutant phenotype. Putting these findings together, these results indicated that mutation in *OsALDH2B1* hindered rice growth and development but enhanced disease resistance.

***osaldh2b1* Mutant Constitutively Activated Brassinolide- and Jasmonic Acid–Related Signaling Pathways.** The enlarged leaf angle in *osaldh2b1* is a typical phenotype caused by overdose of brassinolide (BR) or activated BR signaling in rice (29). As BR biosynthesis is negatively feedback regulated by BR signaling (30), we measured the expression levels of BR biosynthetic genes, *D4*, *D11*, and *BRD1*, in WT and the *osaldh2b1* mutant and found that these genes were down-regulated in the *osaldh2b1* mutant (*SI Appendix*, Fig. S1*B*). In rice, the lamina joint angle is an indicator highly sensitive to BR and has been used as a BR-sensitive marker (31, 32). Lamina joint angle was increased by eBL (24-epibrassinolide, an active form of BR) treatment both in WT and the *osaldh2b1* mutant and was induced to a much larger degree in the *osaldh2b1* mutant (*SI Appendix*, Fig. S1*P*). Thus, in terms of lamina joint angle, the *osaldh2b1* mutant was hypersensitive to BR. Collectively, these results indicated that the BR signaling was highly activated in the *osaldh2b1* mutant.

OsBZR1 positively regulates rice BR signaling, while OsLIC is a negative regulator of rice BR signaling; *OsBZR1*-RNAi plants and *OsLIC-oe* lines displayed a similar BR-deficient phenotype with reduced lamina joint angle and plant height (33). To genetically determine the position of *OsALDH2B1* in the BR signaling pathway, we crossed the *osaldh2b1* mutant with the *OsBZR1-RNAi2* plants and *OsLIC-oe* plants. In the F_2 population, the *osaldh2b1/OsBZR1-RNAi2* plants showed a phenotype similar to that of the *osaldh2b1* mutant, although they accumulated an amount of *OsBZR1* transcripts similar to that of *OsBZR1-RNAi2* plants (Fig. 1*D* and *SI Appendix*, Fig. S2*D*), suggesting that *OsALDH2B1* functioned genetically downstream of *OsBZR1*; *osaldh2b1/OsLIC-oe13* plants showed a phenotype similar to that of *OsLIC-oe13* plants, except that *osaldh2b1/OsLIC-oe13* plants were highly sterile and produced no seeds, similar to *osaldh2b1* (Fig. 1*E* and *SI Appendix*, Fig. S3*G*). Thus, *OsALDH2B1* functioned genetically upstream of *OsLIC* in the BR-related signaling pathway. Taken together, these results suggested that *OsALDH2B1* was a negative regulator of BR signaling and functioned genetically downstream of *OsBZR1* and upstream of *OsLIC* in the BR-related signaling pathway. However, the male fertility mediated by *OsALDH2B1* might not be regulated by the BR-related signaling pathway.

Immune response in plants usually occurs with the activation of defense-related genes, such as *AOS2*, involved in jasmonic acid (JA) biosynthesis; *JAZ8*, involved in the JA-dependent signaling pathway; and *PR1a*, which is related to the salicylic acid (SA)- and JA-dependent signaling pathway (34). The expression levels of these three genes were significantly up-regulated in *osaldh2b1* compared with WT (*SI Appendix*, Fig. S1*C*). Since SA and JA are two major defense signaling molecules, we quantified SA and JA contents, and the results showed that the *osaldh2b1* mutant accumulated a much higher level of JA and a slightly but significantly higher level of SA (*SI Appendix*, Fig. S1*O*). These results demonstrated that the broad-spectrum resistance of the *osaldh2b1* mutant to the pathogens was associated with activation of both JA- and SA-related signaling pathways.

OsALDH2B1 Functions as an Aldehyde Dehydrogenase in Both Nuclei and Mitochondria. The DNA sequence of *OsALDH2B1* was predicted to encode an aldehyde dehydrogenase (ALDH) with two putative active sites (Glu^{316} and Cys^{350}) and putatively localize in mitochondrion (*SI Appendix*, Fig. S4*A*) (35). In rice protoplasts transiently expressing assays, the OsALDH2B1-GFP protein was detected in both the nucleus and mitochondria (*SI Appendix*, Figs. S4 *B* and *C* and S6*A*).

It was previously shown that OsALDH2B1 expressed in bacteria had ALDH activity (36). To detect the ALDH activity of mitochondrial and nuclear OsALDH2B1 in vivo, the OsALDH2B1-HA protein was isolated with anti-HA (anti-hemagglutinin) antibody from total, nuclear, and mitochondrial fractions from *OsALDH2B1-C* plants expressing the OsALDH2B1-HA protein and subjected to ALDH activity assay by monitoring the reduction of NAD^+ to NADH with a fluorescence spectrophotometer at 440 nm. The absorbance value increased with the reaction time (Fig. 2*A*), suggesting that both nuclear and mitochondrial OsALDH2B1 had ALDH activity. *OsALDH2B1* is highly similar to the maize gene *ZmALDH2B1* (also known as *rf2a*), which is a functional mitochondrial ALDH regulating male sterility in maize (37). Thus,

it is highly likely that the male sterility of *osaldh2b1* is due to the deficiency of the enzyme activity of OsALDH2B1 in mitochondria.

Previous results showed that Pro^{323} is required for the ALDH activity of ZmALDH2B1 (38). To investigate whether Glu^{316} and Pro^{322} (corresponding to the Pro^{323} in ZmALDH2B1) in OsALDH2B1 are required for its ALDH activity, we mutated Glu^{316} to Arg (referred to as OsALDH2B1^{m1}), Pro^{322} to Ser (referred to as OsALDH2B1^{m2}), and both (Glu^{316} to Arg and Pro^{322} to Ser, referred to as OsALDH2B1^{2m}). To analyze the ALDH activity of these OsALDH2B1 variants in vivo, we transiently expressed the proteins that were fused with GFP (*SI Appendix*, Fig. S6*A*). We did not detect ALDH activity in any of these variants (Fig. 2*B*). Thus, both Glu^{316} and Pro^{322} are critical for its ALDH activity.

OsALDH2B1 Had the Characteristics of a Transcription Factor. The moonlight function of several enzymes has been identified as transcription regulators or factors (16, 26, 39, 40). Until now, no nuclear localization of ALDH has been found in plants. The nuclear localization of OsALDH2B1 suggests that it may function as a transcription factor or regulator. To investigate this possibility, we tested the transcriptional regulation activity of OsALDH2B1 using dual-LUC (luciferase) activity assays. We observed that the reporter LUC activity was repressed by OsALDH2B1 (*SI Appendix*, Fig. S6 *A* and *B*), indicating a transcriptional repression function of OsALDH2B1. We also analyzed the roles of Glu^{316} and Pro^{322} in OsALDH2B1 transcriptional repression activity. We found that mutation of Glu^{316} or Pro^{322} impaired the transcriptional repression activity of OsALDH2B1, and double mutations of Glu^{316} and Pro^{322} did not further reduce its transcriptional repression activity (Fig. 2*C* and *SI Appendix*, Fig. S6*A*). This suggests that ALDH activity is associated with the transcriptional repression of OsALDH2B1.

We then checked the DNA binding activity of OsALDH2B1 and the potential DNA sequence specificity using systematic evolution of ligands by exponential enrichment assays (41). The analysis showed that 46 and 44% of the selected and amplified sequences contained core sequences GCCGCCGCC/G and TTTTTTTTT, which might be OsALDH2B1 binding sequences (ABSs) (Fig. 2*D*). To test this inference, we conducted an electrophoretic mobility shift assay (EMSA). The results showed that the OsALDH2B1 protein bound to ABS1 (GCCGCCGCC/G) and ABS2 (TTTTTTTTT), while OsALDH2B1 did not bind to a random sequence (Fig. 2*E*). We also found that neither mutation of Glu^{316} nor mutation of Pro^{322} could reduce the DNA binding activity of OsALDH2B1 in an EMSA (Fig. 2*E*), suggesting that mutations of Glu^{316} and Pro^{322} singly or in combination did not affect the DNA binding activity of OsALDH2B1. Thus, mutations of Glu^{316} and Pro^{322} reduced the transcriptional repression activity of OsALDH2B1 without affecting the DNA binding activity. Taken together, these data indicated that OsALDH2B1 may function as a transcription factor.

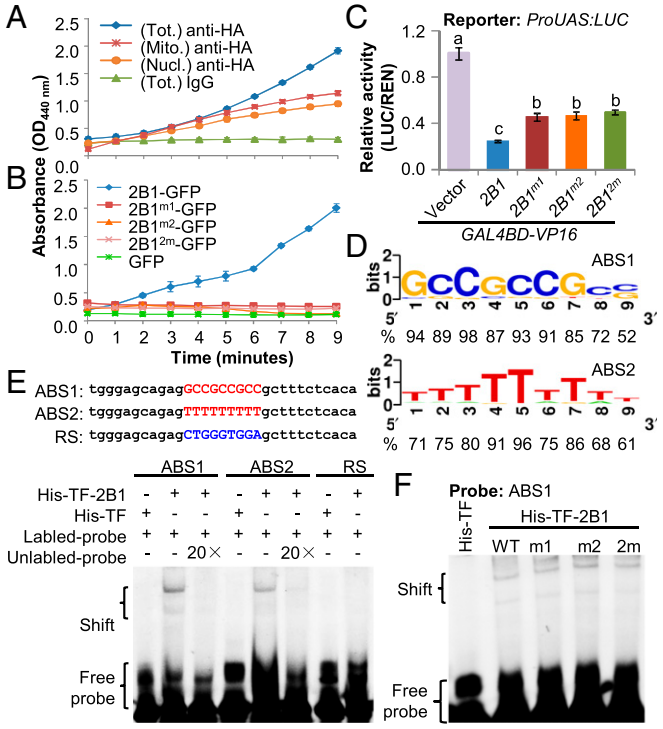

Fig. 2. Activity assays of the OsALDH2B1 protein. Data represent mean ± SEM (n = 3). 2B1, OsALDH2B1. (*A*) Aldehyde dehydrogenase activity of OsALDH2B1 in total homogenate (Tot.), mitochondria (Mito.), and nuclear (Nucl.) fractions. Protein isolated from total (Tot.) homogenate with IgG (immunoglobulin G) was used as a negative control. (*B*) Aldehyde dehydrogenase activity assay of OsALDH2B1 mutant proteins. Protein isolated from the total homogenate from rice protoplast transiently expressing GFP with anti-GFP antibody was used as a negative control. (*C*) The role of Glu^{316th} and Pro^{322nd} in the transcriptional repression activity of OsALDH2B1. *OsALDH2B1* variants were fused to GAL4DB-VP16 as effector constructs. Transient gene expression assays were performed in rice protoplast cells. Different letters above the bars indicate differences by a multiple range test at $P < 0.05$. (*D*) Putative OsALDH2B1 binding sequences identified using WebLogo. (*E*) DNA binding activity assay of OsALDH2B1 by EMSA. The red capital letters indicate the putative OsALDH2B1 binding sequences. The blue capital letters represent a random sequence (RS). (*F*) DNA binding activity of the Glu^{316th} and Pro^{322nd} mutant proteins of OsALDH2B1.

Differential Regulations of OsALDH2B1 on Expression of Target Genes. We searched for the targets of OsALDH2B1 in the rice genome by quarrying the two ABS sequences; three genes were found to contain two to four putative ABSs in the promoters (Fig. 3*A*). The first was *AOS2*, a defense gene that was up-regulated in the *osaldh2b1* mutant. The second was *OsLIC*, a negative regulator of rice BR signaling that we found to be genetically downstream of OsALDH2B1. Interestingly, *GS3*, a major QTL (quantitative trait locus) for grain size that encodes a Gγ protein (13, 42, 43), was also found to contain ABSs. We performed chromatin immunoprecipitation (ChIP)–qPCR assays on the binding of OsALDH2B1 to *AOS2*, *OsLIC*, and *GS3* promoters in vivo. OsALDH2B1 binding was enriched on positions A, C, D, E, and F in the *AOS2* promoter; H, I, and J in the *GS3* promoter; and K and L in the *OsLIC* promoter, which contained or was near the ABS (Fig. 3*C*), suggesting that OsALDH2B1 may bind to these promoters in vivo. EMSA showed that OsALDH2B1 could bind in vitro to the *AOS2* promoter at *P2* containing a sequence similar to ABS1, the *GS3* promoter at *P3* containing two ABS1s, and the *OsLIC* promoter at *P4* containing one ABS1 (*SI Appendix*, Fig. S5*A*). These results indicated that OsALDH2B1 bound to the promoters of the *AOS2*, *GS3*, and *OsLIC* genes both in vitro and in vivo.

We further analyzed OsALDH2B1 action on *AOS2*, *GS3*, and *OsLIC* expression in a transient expression assay with the LUC reporter driven by ∼2 kb putative promoter regions of *AOS2* (*ProAOS2:LUC*), *GS3* (*ProGS3:LUC*), and *OsLIC* (*ProOsLIC:LUC*) by cotransformation of the relevant constructs (*SI Appendix*, Fig. S6*A*). The LUC activity of *ProAOS2:LUC* and *ProGS3:LUC* was repressed by OsALDH2B1-GFP, while the LUC activity of *ProOsLIC:LUC* was enhanced by OsALDH2B1-GFP (Fig. 3*F*). Moreover, Glu^{316} and Pro^{322} were required for the full transcriptional regulation activity of OsALDH2B1 (Fig. 3*F*). Consistent

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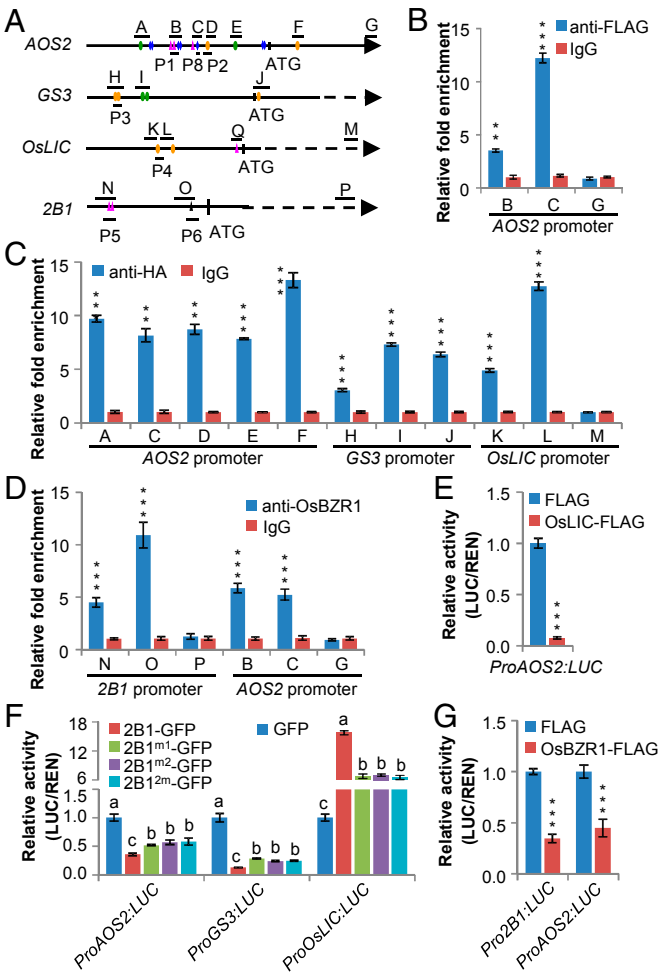

Fig. 3. In vivo DNA binding and transcriptional regulation activity assays. (*A*) Identifying binding sites in promoters by different transcription factors. The orange ellipses indicate OsALDH2B1 binding site ABS1, and the green ellipses indicate ABS2. The pink triangles indicate OsBZR1 binding site BRRE, and the black triangle indicates E-box. Blue diamonds indicate the OsLIC binding motif. P1, P5, and P6 indicate the probes used in EMSA bound by OsBZR1; P2, P3, and P4 indicate the probes used in EMSA bound by OsALDH2B, and P8 indicates the probe used in EMSA bound by OsLIC. A, C–F, and H–L indicate sequences tested in the ChIP–qPCR assay bound by OsALDH2B1; B and C indicate those bound by OsBZR1 and OsLIC, and N, O, and Q indicate those bound by OsBZR1. G, M, and P are negative controls. (*B*) Binding assay of OsLIC to the promoter of *AOS2* by ChIP–qPCR using *OsLIC-oe* plants. (*C*) Binding assays of OsALDH2B1 to the promoters of *AOS2*, *GS3*, and *OsLIC* by ChIP–qPCR with the *OsALDH2B1-C* plants using the anti-HA antibody. (*D*) Binding assays of OsBZR1 to the promoters of *AOS2* and *OsALDH2B1* by ChIP–qPCR with WT using anti-OsBZR1 antibodies. (*E*) Activity assay of OsLIC in regulating *AOS2* expression. (*F*) Activity assays of OsALDH2B1 in regulating target gene expression and the role of Glu^{316th} and Pro^{322nd} in the transcriptional regulation. (*G*) Activity assays of OsBZR1 in regulating *OsALDH2B1* and *AOS2* expression. OsBZR1-FLAG was used as an effector. In (*B*)–(*G*) data represent mean ± SEM (n = 3). $^{**}P < 0.01$ and $^{***}P < 0.001$, obtained with a one-tail Student's t test. Different letters above bars indicate differences by a multiple range test at $P < 0.05$. Transient gene expression assays were performed in rice protoplast cells.

with these transient assays, *osaldh2b1* plants accumulated more *AOS2* and *GS3* transcripts but fewer *OsLIC* transcripts (*SI Appendix*, Fig. S1 *B–D*). These results indicated that OsALDH2B1 differentially regulated expression of the target genes.

OsALDH2B1 had relatively high expression during the whole life cycle, while *GS3* and *AOS2* had relatively low expression (*SI Appendix*, Fig. S7*A*). *GS3* expression was significantly negatively correlated with that of *OsALDH2B1* (*SI Appendix*, Fig. S7*B*). Particularly, *GS3* had a relatively high expression in the young panicle, and the expression decreased gradually with panicle development (*SI Appendix*, Fig. S7*A*) (43), while *OsALDH2B1* had relatively low expression in the young panicle and the transcripts gradually increased with panicle development (*SI Appendix*, Fig. S7*A*). GS3 reduces grain length by competing with two other Gγ proteins, DEP1 and GGC2, both of which increase grain size, in forming the complex with the Gβ protein RGB1 in a G protein pathway (13). These results suggested that the grain size regulation of *OsALDH2B1* might be via the G protein pathway.

OsALDH2B1 Antagonized OsBZR1 Function. Since *OsLIC* expression is directly repressed by OsBZR1 (33) and activated by OsALDH2B1 (Fig. 3*F* and *SI Appendix*, Fig. S1*D*), we investigated the relationship between OsALDH2B1 and OsBZR1 in the regulation of *OsLIC* expression. We performed a transient expression assay in which we cotransformed a fixed amount (2 μg) of *OsBZR1-FLAG* (*SI Appendix*, Fig. S6*A*) with varying amounts (0.5 to 2 μg) of *OsALDH2B1-GFP* into rice protoplast cells using *ProOsLIC:LUC* as the reporter. The LUC activity increased gradually, in accordance with the amounts of *OsALDH2B1-GFP* (*SI Appendix*, Fig. S6*C*), indicating that OsALDH2B1 antagonized the repression of OsBZR1 on *OsLIC* expression. Conversely, we also transformed a fixed amount of *OsALDH2B1-GFP* with varying amounts of *OsBZR1-FLAG*. The LUC activity gradually decreased with the increased amounts of *OsBZR1-FLAG* (*SI Appendix*, Fig. S6*C*), indicating that OsBZR1 reduced the activation function of OsALDH2B1 on *OsLIC* expression. These results suggested that OsALDH2B1 and OsBZR1 antagonized each other to mutually reduce the activity of their counterparts in regulating *OsLIC* expression.

We investigated the possibility of physical interaction between OsALDH2B1 and OsBZR1 using a bimolecular fluorescence complementation assay. The yellow fluorescent protein (YFP) signals indicated that OsALDH2B1 interacted with OsBZR1 in the nucleus (*SI Appendix*, Fig. S8*A*). The interaction was further confirmed by LUC complementation activity and Co-IP (coimmunoprecipitation) assays (*SI Appendix*, Fig. S8 *B* and *C*), suggesting that OsALDH2B1 directly interacted with OsBZR1 in rice. We then analyzed the DNA binding activity of OsBZR1 and OsALDH2B1 in the presence of OsALDH2B1 and OsBZR1, respectively. The DNA binding activity of OsBZR1 was dramatically reduced in the presence of OsALDH2B1 (*SI Appendix*, Fig. S5*C*); in vivo assay showed that OsBZR1 displayed higher binding activity to the *OsLIC* promoter on position Q in *osaldh2b1* plants (*SI Appendix*, Fig. S6*D*), suggesting that OsALDH2B1 greatly inhibited the DNA binding activity of OsBZR1. Similarly, the DNA binding activity of OsALDH2B1 was gradually reduced with increased OsBZR1 (*SI Appendix*, Fig. S5*D*). Taken together, these results suggested that OsALDH2B1 and OsBZR1 may form a complex in inhibiting their DNA binding activity reciprocally.

***OsALDH2B1* and *AOS2* Were Direct Targets of OsBZR1.** To determine whether *OsBZR1* plays a role in rice disease resistance, we inoculated *OsBZR1-RNAi* plants with the bacterial pathogen *Xoo*. This assay showed that *OsBZR1-RNAi* plants developed shorter lesion length and grew fewer bacteria relative to WT (Fig. 1*G* and *SI Appendix*, Fig. S2*A*). Consistently, *OsBZR1-RNAi* plants accumulated a higher level of JA (*SI Appendix*, Fig. S2*B*). These results indicated that the increased disease resistance by suppressing *OsBZR1* expression was associated with activation of the JA-related signaling pathway.

OsBZR1 can directly bind to the promoter of a number of genes to regulate their expression via the BR response element (BRRE) and the E-box (enhancer-box) element (33, 44, 45).

OsALDH2B1 functioned genetically downstream of *OsBZR1* (Fig. 1*D*), and *OsBZR1* expression was negatively correlated with that of *OsALDH2B1* (*SI Appendix*, Fig. S7*B*); there are two BRREs and one E-box element in the *OsALDH2B1* promoter and three BRREs in the *AOS2* promoter (Fig. 3*A*). These prompted us to test whether OsBZR1 could bind to the promoters of *OsALDH2B1* and *AOS2*. We thus conducted EMSA and found that the His-TF-OsBZR1 protein could bind to *OsALDH2B1* and *AOS2* promoters in vitro (*SI Appendix*, Fig. S5*B*). We then confirmed the binding of OsBZR1 to *OsALDH2B1* and *AOS2* promoters using ChIP–qPCR, and the results revealed that OsBZR1 binding to the *OsALDH2B1* promoter was enriched on positions N and O containing BRRE and E-box in the *OsALDH2B1* promoter and on positions B and C containing BRRE in the *AOS2* promoter (Fig. 3*D*). We then conducted transient transcriptional regulation activity assay to check the effect of OsBZR1 on *OsALDH2B1* and *AOS2* expression with *ProOsALDH2B1:LUC* and *ProAOS2:LUC* as reporters (*SI Appendix*, Fig. S6*A*). We found that OsBZR1 transiently repressed the LUC activity (Fig. 3*G*), indicating that OsBZR1 could repress *OsALDH2B1* and *AOS2* expression. Consistent with the transient expression analyses, *OsBZR1-RNAi* plants accumulated more *OsALDH2B1* and *AOS2* transcripts relative to WT (*SI Appendix*, Fig. S2*C*). These data indicated that both *OsALDH2B1* and *AOS2* were targets of OsBZR1.

OsLIC Repressed *AOS2* Expression. OsLIC can directly bind to the promoter of a number of genes to regulate their expression via the "TCGC" element (33). The expression of *OsLIC* was negatively correlated with, but substantially higher than, those of *GS3* and *AOS2* (*SI Appendix*, Fig. S6), suggesting that OsLIC might repress *GS3* and *AOS2* expression. There are seven *OsLIC* binding elements in the *AOS2* promoter (Fig. 3*A*), indicating that OsLIC might bind to the promoter of *AOS2*. ChIP–qPCR assays showed that OsLIC binding was enriched on positions B and C containing the "TCGC" element in the *AOS2* promoter (Fig. 3*B*). The binding of OsLIC to the *AOS2* promoter was further confirmed in vitro by EMSA (*SI Appendix*, Fig. S5*E*). A transient transcriptional regulation assay using *ProAOS2:LUC* as a reporter also showed that OsLIC transiently repressed the LUC activity (Fig. 3*E*). Consistent with the transient expression analysis, *OsLIC-oe* plants accumulated fewer *AOS2* transcripts than WT, while the antisense transgenic plants of *OsLIC* (*OsLIC-anti*) accumulated more *AOS2* transcripts (*SI Appendix*, Fig. S3 *D* and *E*). These results indicated that OsLIC repressed *AOS2* expression.

To assess the function of *OsLIC* in rice resistance, we inoculated *OsLIC* transgenic plants with the bacterial pathogen *Xoo*. Overexpressing *OsLIC* could partially suppress the resistance of the *osaldh2b1* mutant (*SI Appendix*, Fig. S3 *F* and *G*), while *OsLIC-anti* plants significantly reduced lesion length and bacterial growth (Fig. 1*H* and *SI Appendix*, Fig. S3*B*). Consistently, *OsLIC-anti* plants accumulated a higher level of JA (*SI Appendix*, Fig. S3*D*), indicating that the increased disease resistance of *OsLIC-anti* plants was associated with activation of JA biosynthesis.

To investigate whether BR has any direct effect on rice defense, we treated WT seedlings with eBL and BRz (brassinazole, a BR biosynthesis inhibitor) and inoculated the treated seedlings with *Xoo*. Seedlings treated with eBL developed increased lesion length and bacterial growth compared to mock treatment, while the reverse was the case for plants treated with BRz (*SI Appendix*, Fig. S9*A*). *AOS2* expression was suppressed by eBL treatment (*SI Appendix*, Fig. S9*B*), which is consistent with a previous report (46), while BRz induced *AOS2* expression (*SI Appendix*, Fig. S9*B*). *JAZ8*, which is induced by pathogens and JA in the JA-dependent signaling pathway, showed an expression pattern similar to *AOS2* (*SI Appendix*, Fig. S9*B*). Accordingly, the JA level was reduced with eBL treatment but increased with BRz compared to mock treatment (*SI Appendix*, Fig. S9*C*), suggesting that BR negatively regulates rice defense via the JA pathway.

Discussion

We summarize the results of this work in Fig. 4 to illustrate how OsALDH2B1 works, allowing the possibility that OsALDH2B1 regulates different processes to be expanded in future studies.

OsALDH2B1 possesses both primary and moonlight functions (Fig. 4*A*). Its primary function as a mitochondrial enzyme regulates male fertility as observed in this study. Its moonlight function is transcriptional regulation in nuclear, featuring both repressing and activating activities at both transcription and posttranscription levels, which regulates a diverse range of biological processes and pathways (Fig. 4*B*).

Functioning as a transcription factor, OsALDH2B1 regulates the BR signaling pathway through both activation and repression and is also regulated by the BR pathway. OsALDH2B1 activates *OsLIC* expression. OsALDH2B1 is repressed by OsBZR1 at the transcriptional level; OsALDH2B1 and OsBZR1 reciprocally inhibit each other by forming a complex at the protein level. Mutation in *OsALDH2B1* reduces *OsLIC* expression and increases OsBZR1 activity, resulting in activation of the BR signaling pathway, involving genes both upstream and downstream. The net outcome indicates a negative role of OsALDH2B1 in BR signaling. OsALDH2B1, OsBZR1, and OsLIC have different roles in BR signaling, but they show similar negative roles in

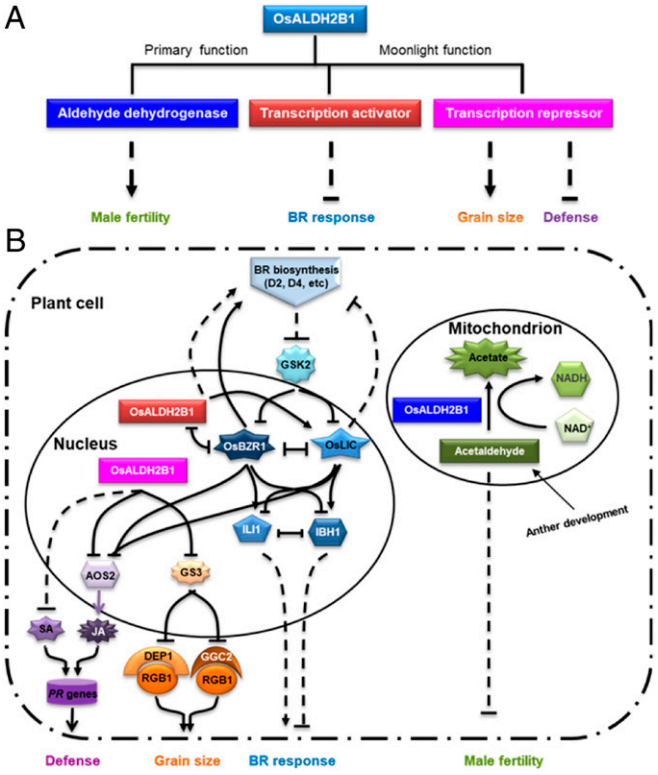

Fig. 4. A summary of the functions of OsALDH2B1 found in this study. The light blue OsALDH2B1 shows ALDH activity. The brown OsALDH2B1 shows transcription activation activity. The pink OsALDH2B1 shows transcription repression activity. Arrows and T lines indicate promoting and inhibiting effects, respectively. Solid and dotted lines indicate direct action and indirect actions, respectively. (*A*) The main functions of OsALDH2B1. (*B*) Pathways and processes regulated by OsALDH2B1. The G protein pathway, including DEP1, GGC2, and RGB1, was summarized by Sun et al. (13). The BR pathway, including D2, GSK2, ILI1, and IBH1, was summarized by Zhang et al. (29).

JA signaling. Simultaneously suppressing *OsBZR1* and *OsLIC* expression and knockout of *OsALDH2B1* activate both BR and JA signaling. The perception of BRs by membrane-localized coreceptors promotes OsBZR1 and OsLIC dephosphorylation and nucleus translocation (33, 47), which eventually activates the BR signaling and represses the JA signaling.

OsALDH2B1 regulates JA signaling pathway in two ways. It directly represses the expression of *AOS2* via the transcriptional repression activity and indirectly represses the expression of *AOS2* via the BR signaling pathway through OsBZR1 and OsLIC, both of which repress *AOS2* expression. Reducing the expression of *OsBZR1* and *OsLIC* increases *AOS2* transcripts, leading to JA accumulation. In addition, OsALDH2B1 also has an effect on SA via an as yet uncharacterized pathway. Together, these impact immune response and disease resistance.

The regulation of *OsALDH2B1* on *GS3* may have a fundamental effect on growth and development. A previous study suggested that GS3 was installed as a check to prevent grain size from becoming too large by expressing it only in the young panicle (13). The reduced expression of OsALDH2B1 only in the young panicle triggers GS3 to work as the check for grain size not to affect plant morphology; constitutive overexpression of *GS3* leads to a range of alterations in plant morphology such as increasing tiller number and reducing plant size, including grain size (43), which may largely account for the observed phenotype of the *osaldh2b1* mutant.

The multiple functions of *OsALDH2B1* are thus coordinated in two fundamental ways: the biochemical versatility and dynamic expression. In particular, the relatively low expression of *OsALDH2B1* in the young panicle only results in the relatively high expression of *GS3* to regulate grain size. Sufficient transcripts of *OsALDH2B1* in the stamen provide ALDH activity to detoxify acetaldehyde produced by ethanolic fermentation to produce fertile pollen. The dynamic expression of *OsALDH2B1* in nonreproductive organs keeps the balance of the biological processes, while alteration of its normal function causes an imbalance among defense, growth, development, and reproduction. These results may have implications for crop genetic improvement by modulating the OsALDH2B1 activities to balance defense and growth.

Materials and Methods

The rice variety ZH11 was used for transformation in most of this study. Rice plants were grown in the field for most of the studies and were grown in growth chambers for *Xoc* and *M. oryzae* inoculation. Details of experimental methods, including bioinformatic analysis, pathogen inoculation, subcellular localization, ALDH enzyme activity assay, systematic evolution of ligands by exponential enrichment, EMSA, transient expression assay in protoplasts, ChIP–qPCR, hormone treatment, and gene expression analysis, are given in *SI Appendix, SI Materials and Methods*.

Data Availability Statement. The vectors and genetic materials will be available upon request.

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1. D. L. Yang *et al.*, Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E1192–E1200 (2012).
2. S. Goto *et al.*, Development of disease-resistant rice by optimized expression of WRKY45. *Plant Biotechnol. J.* **13**, 753–765 (2015).
3. X. Li *et al.*, The systemic acquired resistance regulator OsNPR1 attenuates growth by repressing auxin signaling through promoting IAA-amido synthase expression. *Plant Physiol.* **172**, 546–558 (2016).
4. M. Liu *et al.*, Inducible overexpression of ideal Plant Architecture1 improves both yield and disease resistance in rice. *Nat. Plants* **5**, 389–400 (2019).
5. Y. Wu *et al.*, Rice transcription factor OsDOF11 modulates sugar transport by promoting expression of sucrose transporter and SWEET genes. *Mol. Plant* **11**, 833–845 (2018).
6. X. Huang *et al.*, Natural variation at the *DEP1* locus enhances grain yield in rice. *Nat. Genet.* **41**, 494–497 (2009).
7. Y. Jiao *et al.*, Regulation of *OsSPL14* by OsmiR156 defines ideal plant architecture in rice. *Nat. Genet.* **42**, 541–544 (2010).
8. Y. Deng *et al.*, Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. *Science* **355**, 962–965 (2017).
9. K. Hu *et al.*, Improvement of multiple agronomic traits by a disease resistance gene via cell wall reinforcement. *Nat. Plants* **3**, 17009 (2017).
10. H. Liu *et al.*, NBS-LRR protein pik-H4 interacts with OsBIHD1 to balance rice blast resistance and growth by coordinating ethylene-brassinosteroid pathway. *Front. Plant Sci.* **8**, 127 (2017).
11. G. Xu *et al.*, uORF-mediated translation allows engineered plant disease resistance without fitness costs. *Nature* **545**, 491–494 (2017).
12. D. De Vleesschauwer *et al.*, Target of rapamycin signaling orchestrates growth-defense trade-offs in plants. *New Phytol.* **217**, 305–319 (2018).
13. S. Sun *et al.*, A G-protein pathway determines grain size in rice. *Nat. Commun.* **9**, 851 (2018).
14. J. Wang *et al.*, A single transcription factor promotes both yield and immunity in rice. *Science* **361**, 1026–1028 (2018).
15. G. A. Wray *et al.*, The evolution of transcriptional regulation in eukaryotes. *Mol. Biol. Evol.* **20**, 1377–1419 (2003).
16. C. Feschotte, Transposable elements and the evolution of regulatory networks. *Nat. Rev. Genet.* **9**, 397–405 (2008).
17. C. J. Jeffery, Protein moonlighting: What is it, and why is it important? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **373**, 20160523 (2018).
18. C. J. Jeffery, An introduction to protein moonlighting. *Biochem. Soc. Trans.* **42**, 1679–1683 (2014).
19. M. Mani *et al.*, MoonProt: A database for proteins that are known to moonlight. *Nucleic Acids Res.* **43**, D277–D282 (2015).
20. P. Ostrovsky de Spicer, S. Maloy, PutA protein, a membrane-associated flavin dehydrogenase, acts as a redox-dependent transcriptional regulator. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4295–4298 (1993).
21. F. Moreno, P. Herrero, The hexokinase 2-dependent glucose signal transduction pathway of Saccharomyces cerevisiae. *FEMS Microbiol. Rev.* **26**, 83–90 (2002).
22. D. R. Edgell *et al.*, Intron-encoded homing endonuclease I-TevI also functions as a transcriptional autorepressor. *Nat. Struct. Mol. Biol.* **11**, 936–944 (2004).
23. N. G. Markova, A. Pinkas-Sarafova, M. Simon, A metabolic enzyme of the short-chain dehydrogenase/reductase superfamily may moonlight in the nucleus as a repressor of promoter activity. *J. Invest. Dermatol.* **126**, 2019–2031 (2006).
24. S. Hu *et al.*, Profiling the human protein-DNA interactome reveals ERK2 as a transcriptional repressor of interferon signaling. *Cell* **139**, 610–622 (2009).
25. K. Gunka *et al.*, Functional dissection of a trigger enzyme: Mutations of the bacillus subtilis glutamate dehydrogenase RocG that affect differentially its catalytic activity and regulatory properties. *J. Mol. Biol.* **400**, 815–827 (2010).
26. H. Zhang, Y. Zhao, D. X. Zhou, Rice NAD^+-dependent histone deacetylase OsSRT1 represses glycolysis and regulates the moonlighting function of GAPDH as a transcriptional activator of glycolytic genes. *Nucleic Acids Res.* **45**, 12241–12255 (2017).
27. M. Fairbank, P. St-Pierre, I. R. Nabi, The complex biology of autocrine motility factor/phosphoglucose isomerase (AMF/PGI) and its receptor, the gp78/AMFR E3 ubiquitin ligase. *Mol. Biosyst.* **5**, 793–801 (2009).
28. A. C. Mirando *et al.*, Aminoacyl-tRNA synthetase dependent angiogenesis revealed by a bioengineered macrolide inhibitor. *Sci. Rep.* **5**, 13160 (2015).
29. C. Zhang, M. Y. Bai, K. Chong, Brassinosteroid-mediated regulation of agronomic traits in rice. *Plant Cell Rep.* **33**, 683–696 (2014).
30. Z. Y. Wang *et al.*, Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. *Dev. Cell* **2**, 505–513 (2002).
31. K. Wada, S. Marumo, N. Ikekawa, M. Morisaki, K. Mori, Brassinolide and homobrassinolide promotion of lamina inclination of rice seedlings. *Plant Cell Physiol.* **22**, 323–325 (1981).
32. C. Yamamuro *et al.*, Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. *Plant Cell* **12**, 1591–1606 (2000).
33. C. Zhang *et al.*, Dynamics of brassinosteroid response modulated by negative regulator LIC in rice. *PLoS Genet.* **8**, e1002686 (2012).
34. Y. Ke, H. Liu, X. Li, J. Xiao, S. Wang, Rice OsPAD4 functions differently from Arabidopsis AtPAD4 in host-pathogen interactions. *Plant J.* **78**, 619–631 (2014).
35. S. O. Kotchoni *et al.*, Modeling-dependent protein characterization of the rice aldehyde dehydrogenase (ALDH) superfamily reveals distinct functional and structural features. *PLoS One* **5**, e11516 (2010).
36. H. Tsuji, N. Tsutsumi, T. Sasaki, A. Hirai, M. Nakazono, Organ-specific expressions and chromosomal locations of two mitochondrial aldehyde dehydrogenase genes from rice (*Oryza sativa* L.), *ALDH2a* and *ALDH2b*. *Gene* **305**, 195–204 (2003).
37. X. Cui, R. P. Wise, P. S. Schnable, The *rf2* nuclear restorer gene of male-sterile T-cytoplasm maize. *Science* **272**, 1334–1336 (1996).

38. F. Liu, X. Cui, H. T. Horner, H. Weiner, P. S. Schnable, Mitochondrial aldehyde dehydrogenase activity is required for male fertility in maize. *Plant Cell* **13**, 1063–1078 (2001).
39. H. Reinhold *et al*., β-amylase-like proteins function as transcription factors in *Arabidopsis*, controlling shoot growth and development. *Plant Cell* **23**, 1391–1403 (2011).
40. M. Xie *et al*., A 5-enolpyruvylshikimate 3-phosphate synthase functions as a transcriptional repressor in *Populus*. *Plant Cell* **30**, 1645–1660 (2018).
41. P. Bouvet, Identification of nucleic acid high affinity binding sequences of proteins by SELEX. *Methods Mol. Biol.* **1334**, 333–343 (2015).
42. C. Fan *et al*., *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor. Appl. Genet.* **112**, 1164–1171 (2006).
43. H. Mao *et al*., Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 19579–19584 (2010).
44. L. Y. Zhang *et al*., Antagonistic HLH/bHLH transcription factors mediate brassinosteroid regulation of cell elongation and plant development in rice and *Arabidopsis*. *Plant Cell* **21**, 3767–3780 (2009).
45. H. Tong *et al*., DWARF AND LOW-TILLERING, a new member of the GRAS family, plays positive roles in brassinosteroid signaling in rice. *Plant J.* **58**, 803–816 (2009).
46. K. Nahar, T. Kyndt, B. Hause, M. Höfte, G. Gheysen, Brassinosteroids suppress rice defense against root-knot nematodes through antagonism with the jasmonate pathway. *Mol. Plant Microbe Interact.* **26**, 106–115 (2013).
47. M. Y. Bai *et al*., Functions of OsBZR1 and 14-3-3 proteins in brassinosteroid signaling in rice. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 13839–13844 (2007).