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OsSERK1 regulates rice development but not immunity to *Xanthomonas oryzae* pv. *oryzae* or *Magnaporthe oryzae*

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Research Article

Abstract Somatic embryogenesis receptor kinase (SERK) proteins play pivotal roles in regulation of plant development and immunity. The rice genome contains two SERK genes, *OsSerk1* and *OsSerk2*. We previously demonstrated that *OsSerk2* is required for rice *Xa21*-mediated resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and for normal development. Here we report the molecular characterization of *OsSerk1*. Overexpression of *OsSerk1* results in a semi-dwarf phenotype whereas silencing of *OsSerk1* results in a reduced angle of the lamina joint. *OsSerk1* is not required for rice resistance to *Xoo* or *Magnaporthe oryzae*. Overexpression of *OsSerk1* in *OsSerk2*-silenced lines complements phenotypes associated with brassinosteroid (BR) signaling defects, but not the disease resistance phenotype mediated by *Xa21*. In yeast, OsSERK1 interacts with itself forming homodimers, and also interacts with the kinase domains of OsSERK2 and BRI1, respectively. OsSERK1 is a functional protein kinase capable of autophosphorylation *in vitro*. We conclude that, whereas OsSERK2 regulates both rice development and immunity, OsSERK1 functions in rice development but not immunity to *Xoo* and *M. oryzae*.

Keywords: *Oryza sativa*; OsSERK1; somatic embryogenesis receptor kinase; *Xanthomonas oryzae* pv. *Oryzae*; *Magnaporthe oryzae*
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INTRODUCTION

Somatic embryogenesis receptor kinase (SERK) proteins were initially identified because of their roles in the transition from somatic cells to embryogenic cells (Schmidt et al. 1997; Li 2010). In *Arabidopsis thaliana*, five SERK protein members (AtSERKs) have been reported (Schmidt et al. 1997; Li 2010). SERK proteins typically include five extracellular leucine-rich repeats (LRRs), a proline-rich region, a single-pass transmembrane domain, and a cytoplasmic kinase domain transducing extracellular signals to intracellular processes via protein phosphorylation (Hecht et al. 2001; Li 2010).

Apart from the role in plant regeneration from somatic embryos, AtSERK genes are better known for their functions in regulating plant development and immunity (Chinchilla et al. 2009; Roux et al. 2011; Gou et al. 2012). AtSERK3 (At4g33430) was independently identified as a brassinosteroid insensitive 1 (BRI1)-associated kinase (BAK1) because of its role in mediating brassinosteroid (BR) signal transduction (Li et al. 2002; Nam and Li 2002). *Atserk3/bak1* mutants display certain degrees of *bri1* symptoms whereas overexpression of *AtSERK3/BAK1* complements *bri1–5* dwarf phenotype (Gou et al. 2012). AtSERK3/BAK1 was later found to be required for immunity triggered by pathogen-associated molecular patterns (PAMPs), such as bacterial flagellin and elongation factor Tu (EF-Tu) (Chinchilla et al. 2007; Heese et al. 2007; Roux et al. 2011; Schwessinger et al. 2011). AtSERK3/BAK1 physically associates with the *Arabidopsis* pattern recognition receptors (PRRs) flagellin sensitive 2 (FLS2) and EF-Tu receptor (EFR) (Chinchilla et al. 2007; Heese et al. 2007; Roux et al. 2011; Schwessinger and Ronald 2012). The AtSERK3/BAK1 and FLS2 ectodomains form heterodimeric complexes and both directly interact with flg22 (Sun et al. 2013a, 2013b). Similarly the ectodomains of AtSERK3/BAK1 and BRI1 interact with BL as part of a heterodimeric complex (Wang and Chory, 2006; Santiago et al. 2013; Sun et al. 2013a, 2013b). Thus, AtSERK3/BAK1 functions as a co-receptor for receptor kinases BRI1, FLS2, and EFR and plays a pivotal role in regulating both plant development and immunity (Chinchilla et al. 2007; Schwessinger and Ronald 2012; Santiago et al. 2013; Sun et al. 2013a, 2013b). Subsequent studies have identified redundant roles among AtSERK members. For example, AtSERK4 (At2g13790) functions similarly as AtSERK3/BAK1 (BKK1, BAK1-like 1). Both are required for perception of PAMPs and for BR signaling (He et al. 2007; Roux et al. 2011). The AtSERK1 (At1g71830)

ortholog in tomato is required for immune receptor Ve1-mediated resistance to race 1 of *Verticillium dahlia* (Fradin et al. 2011). Transfer of tomato Ve1 into *Arabidopsis* revealed that AtSERK1 is required in addition to AtSERK3/BAK1 for Ve1-mediated resistance (Fradin et al. 2011). Overexpression of *AtSERK1*, *AtSERK2*, or *AtSERK4/BKK1* suppressed the *bri1–5* phenotype (Gou et al. 2012). *AtSERK5* (At2g13790) from the *Arabidopsis* Columbia ecotype is nonfunctional. Recent studies of AtSERK members have revealed the molecular mechanisms underlying the contributions of AtSERKs to plant development and immunity (Gou et al. 2012; Schwessinger and Ronald 2012).

In contrast to the five SERK members in *Arabidopsis*, the rice genome contains only two genes encoding predicted SERK proteins (OsSERK1/Loc_Os08g07760 and OsSERK2/Loc_Os04g38480) (~76% identity to AtSERK proteins) (Singla et al. 2009; Chen et al. 2014). OsSERK1 and OsSERK2 are clustered in the same group as AtSERK1 and AtSERK2, but not with AtSERK3/BAK1 and AtSERK4/BKK1 (Chen et al. 2014). Because of the high degree of similarity of OsSERK1 an OsSERK2 with all the AtSERK proteins, it had been difficult to identify the rice equivalent of AtSERK3/BAK1. In fact, Li et al. (2009) hypothesized that OsSERK1 serves as OsBAK1, mainly based on the ability of OsSERK1 to restore the dwarf phenotype of the *Arabidopsis bri1–5* mutant. Downregulation experiments of *OsSerk2* (named *OsSERK1* in Hu et al. 2005) expression showed that *OsSerk2* was involved in embryogenic cell formation and in plant development; overexpression of *OsSerk2* increased rice resistance to the hemi-necrotrophic fungus *Magnaporthe oryzae*, the causal agent of the rice blast disease. Simultaneous silencing of *OsSerk1*, *OsSerk2*, and other OsSERK-like genes enhanced rice susceptibility to *M. orzyae* (Hu et al. 2005; Park et al. 2011). These experiments indicated the involvement of OsSERK2 in resistance to *M. oryzae*, but did not specifically address the role of OsSERK1. In *Arabidopsis*, *AtSERK* genes are mainly associated with plant immunity to biotrophic pathogens although they are also involved in regulation of host resistance to hemi-necrotrophic and necrotrophic pathogens (Kemmerling et al. 2007; Roux et al. 2011). Recently, we reported that downregulation of *OsSerk2* expression almost completely abolished immunity mediated by XA21 and XA26, two rice PRRs (Chen et al. 2014). Both XA21 and XA26 are phylogenetically closely related to *Arabidopsis* FLS2 and EFR and belong to the same LRR-RLK subfamily XII (Chen et al. 2014). OsSERK2 functions as a regulatory co-receptor kinase of XA21 and also regulates BR-mediated signaling. Thus, OsSERK2 possesses dual roles in rice development and in PRR-mediated immunity (Chen et al. 2014).

Compared with OsSERK2, OsSERK1 has slightly higher identity to AtSERK3/BAK1 (Chen et al. 2014). It is unknown if OsSERK1 contributes to rice immunity. In this study, we show that like OsSERK2, OsSERK1 functions as rice development, but unlike OsSERK2, OsSERK1 is not required for rice XA21-mediated immunity and does not contribute to resistance to *Xoo* and *M. oryzae* in the absence of *XA21*. We also found that specific silencing of *OsSerk1* results in reduction of the angle of the lamina joint, but not affect other agronomic traits, such as leaf length and width, plant height, and seed set.

RESULTS

Overexpression of *OsSerk1* results in a semi-dwarf phenotype

To investigate the function of *OsSerk1*, we isolated the full-length coding region of *OsSerk1* and created an overexpression construct UbiC1300-*OsSerk1* by using the maize *ubiquitin 1* promoter to drive *OsSerk1* expression. Using *Agrobacterium*-mediated transformation, we obtained 18 independent transgenic plants in the rice Kitaake genetic background (called Kit-*OsSerk1ox*) and 30 in the *Xa21*-Kitaake background (called *Xa21*kit-*OsSerk1ox*) (Figure S1). Nearly all T_0 transgenic plants displayed semi-dwarf phenotypes compared to the wild type Kitaake control (Figure S1). The only exceptions were transgenic plants that did not overexpress *OsSerk1*. Two Kit-*OsSerk1ox* (#14 and #17) and four *Xa21*kit-*OsSerk1ox* (#3, #4, #7 and #18) T_0 plants with high transcript levels of *OsSerk1* were self-pollinated and used to analyze the correlation between the semi-dwarf phenotype and the transgene *OsSerk1ox* (Figure 1A, B). All plants carrying the *OsSerk1ox* transgene displayed significantly shorter than those lacking the *OsSerk1ox* transgene (Figure 1C, D), suggesting that overexpression of *OsSerk1* leads to the semi-dwarf phenotype. The semi-dwarf phenotype of homozygous *OsSerk1ox* plants included reduction of each internode length and increase of the angle of the lamina joint, compared with the control *Xa21*-Kitaake but did not affect seed size (Figures 1E, S2). These results suggest that *OsSerk1* controls rice plant stature and the angle of the lamina joint.

Overexpression of *OsSerk1* does not affect rice resistance to *Xoo*

To test whether the overexpression of *OsSerk1* enhanced rice resistance to the biotrophic pathogen *Xoo*, we inoculated Kit-*OsSerk1ox* T_1 plants with *Xoo* strain PXO99 at two developmental stages (3 or 6 weeks old). All progeny plants with or without the transgene displayed similar disease lesion lengths as the Kitaake control at both developmental stages (Figure S3A, B). Because *Xa21* only shows partial resistance at the juvenile stage, we also inoculated *Xa21*kit-*OsSerk1ox* 3-week-old plants to assess if overexpression of *OsSerk1* could enhance *Xa21* resistance at the seedling stage (Song et al. 1995; Park et al. 2010). We found no clear differences in lesion lengths among the plants with and without *OsSerk1ox* and the *Xa21*-Kitaake control (Figure S3B).

We further confirmed these results using lines homozygous for *OsSerk1ox*. Two Kit-*OsSerk1ox* lines, #K1630 and #K1634, and Kitaake were inoculated at the 3 and 6 weeks old stages. We found that these plants displayed similar lesion lengths as the susceptible control Kitaake (Figure 2A, B). Similarly, the *Xa21*kit-*OsSerk1ox* lines homozygous for both *Xa21* and *OsSerk1ox*, #X1904 and #X1953, displayed similar lesion lengths as the *Xa21*-Kitaake control at the seedling stage (Figure 2B). We also inoculated the two *Xa21*kit-*OsSerk1ox* lines and the *Xa21*-Kitaake control with the *Xoo-4* strain, which is unable to activate the XA21-mediated immune response (Figure S4). As expected, the *Xa21*-Kitaake showed full susceptibility to *Xoo-4*. The *Xa21*kit-*OsSerk1ox* lines showed similar susceptibility to *Xoo-4* as *Xa21* Kitaake. Taken together, we conclude that overexpression of *OsSerk1* does not affect rice resistance to *Xoo*.

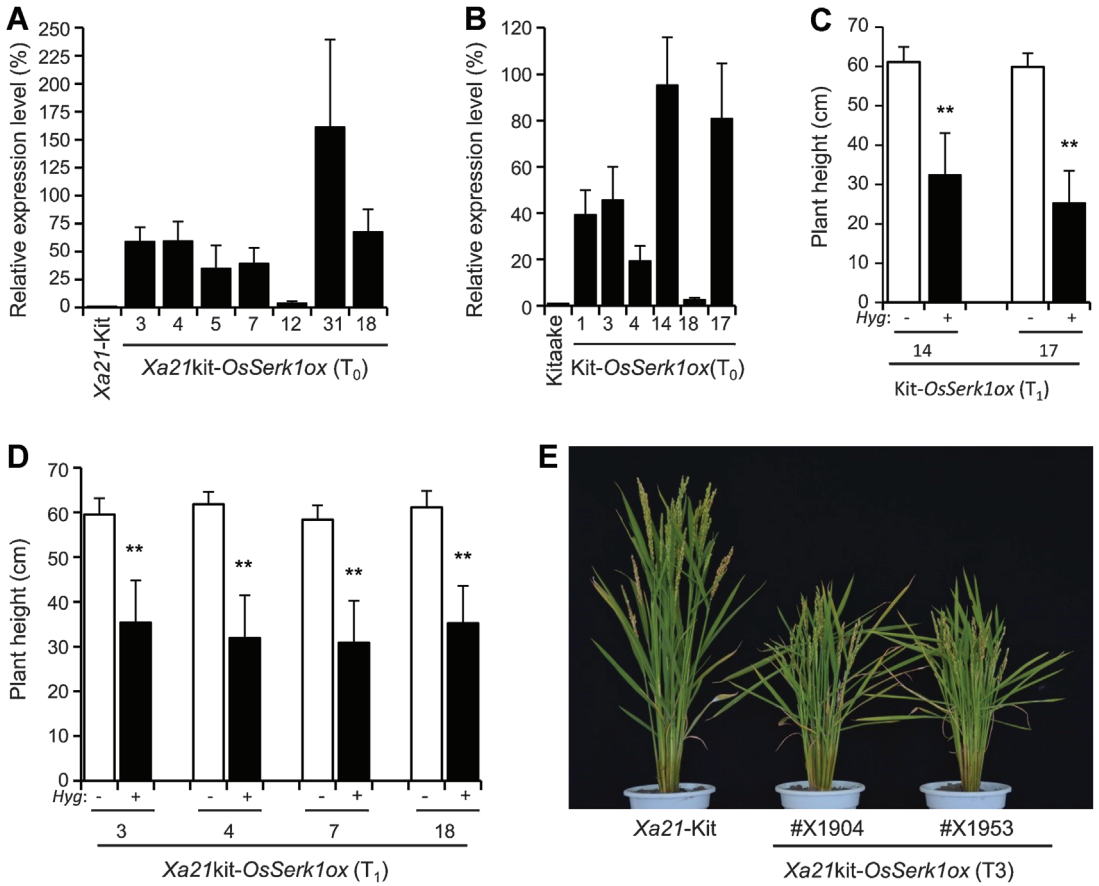

Figure 1. Identification of *OsSerk1* overexpression transgenic plants
(**A**) Transcript levels of *OsSerk1* among wild-type control *Xa21*-Kitaake (*Xa21*-Kit) and independent *Xa21*kit-*OsSerk1ox* transgenic plants revealed by real-time reverse transcription-polymerase chain reaction (RT-PCR). (**B**) Transcript levels of *OsSerk1* among the wild type Kitaake and independent Kit-*OsSerk1ox* T_0 transgenic plants revealed by real-time RT-PCR. (**C**) and (**D**) Plant height of the transgenic T_1 plants with or without the transgene *OsSerk1ox*. The primer pair (*Hyg*) specific to the *hygromycin phosphotransferase* gene was used to determine the plants with (represented by "+") or without (represented by "−") *OsSerk1ox*. Statistical significance comparison was conducted with ANOVA, where the mark "**" on the column indicates difference with $P \leqq 0.01$. (**E**) Stature of mature plants of wild-type *Xa21*-Kit, *Xa21*kit-*OsSerk1ox* #X1904 and #X1953. The #X1904 and #X1953 were the lines homozygous for *OsSerk1ox* that derived from *Xa21*kit-*OsSerk1ox* #3 and *Xa21*kit-*OsSerkox* #18 T_0 lines, respectively.

Silencing of *OsSerk1* mainly affects the angle of the lamina joint

To further clarify the function of *OsSerk1*, we generated an RNAi construct *pANDA-OsSerk1Ri* and transformed it into the *Xa21*-Kitaake genetic background through *Agrobacteria*-mediated transformation. We obtained five independent *Xa21*kit-*OsSerk1Ri* plants. Through real-time reverse transcription polymerase chain reaction (RT-PCR), we found that the *OsSerk1* transcript levels were significantly reduced in four of the five *Xa21*kit-*OsSerk1Ri* lines; while the *OsSerk2* and *Xa21* transcript levels in these lines showed no changes compared to the wild type *Xa21*-Kitaake used as the control (Figure 3A). This indicates that the four *Xa21*kit-*OsSerk1Ri* plants have specific downregulation of *OsSerk1* expression.

We found that all four *Xa21*kit-*OsSerk1Ri* lines displayed reduced angles of the lamina joint compared with the wild type *Xa21*-Kitaake. The homozygous *OsSerk1Ri* line (#1602) derived from the T_0 line Ri4 (A-4) that expressed the lowest *OsSerk1* transcript level (Figure 3A) was used in subsequent morphological analysis. The *OsSerk1Ri* #1602 plants displayed significantly smaller lamina joint angles, but showed almost the same plant height and leaf width and length as the wild type *Xa21*-Kitaake (Figure 3B–E). Because *OsSerk1* has higher transcript levels in the rice flowers (Chen et al. 2014), we reasoned that it might regulate seed development. We measured the seed set of *OsSerk1Ri* #1602. We did not find significant differences between the seed sets of *OsSerk1Ri* #1602 and the wild type *Xa21*-Kitaake (Figure 3F). These results indicate that *OsSerk1* is mainly involved in the development of the angle of the lamina joint but does not affect traits controlling plant stature or seed set.

Silencing of *OsSerk1* does not affect XA21-mediated immunity or rice basal resistance to *Xoo*

To test whether *OsSerk1* is involved in XA21-mediated immunity to *Xoo*, we inoculated T_1 plants from each of the

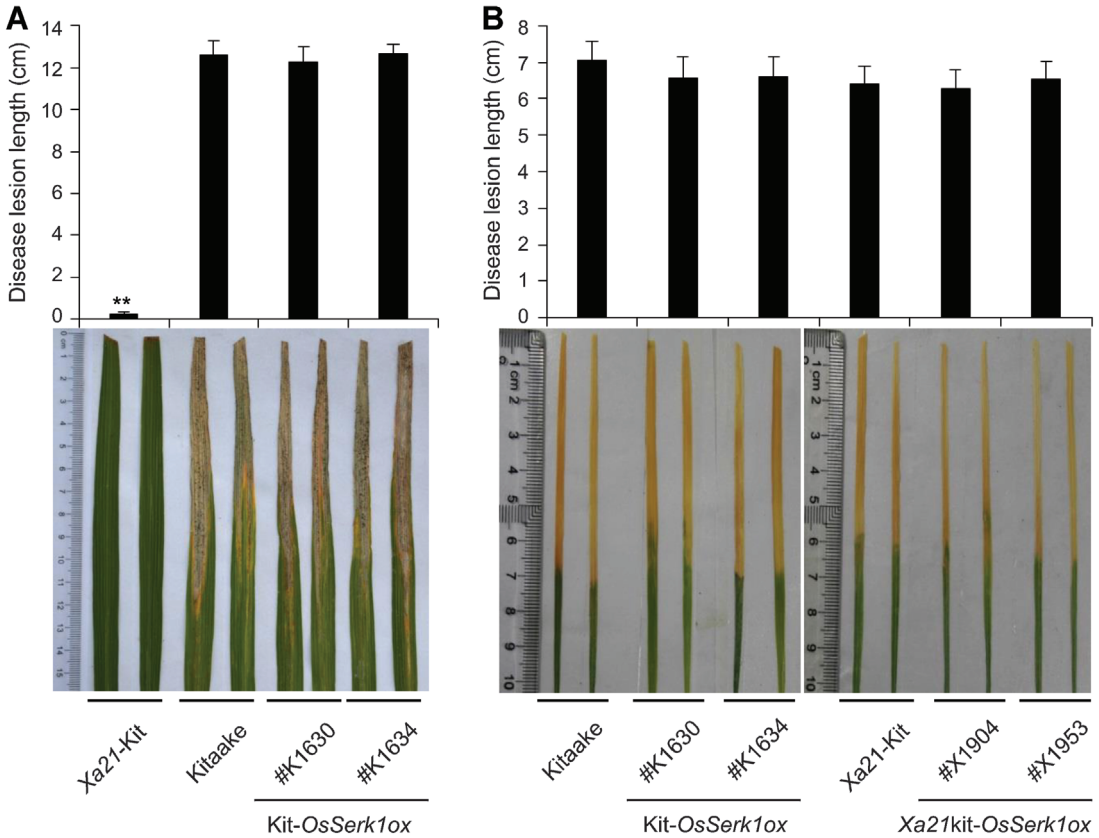

Figure 2. *OsSerk1* overexpression does not affect rice basal resistance to *Xoo*

(**A**) Lesion length of *Xa21* Kitaake, Kitaake, and Kit-OsSerk1ox #K1603 and #K1634 plants inoculated with *Xoo* at the adult stage (6 weeks old). The lines #K1603 and #K1634 were homozygous for *OsSerk1ox* in Kitaake background, which derived from independent T_0 plants Kit-*OsSerk1ox* #14 and Kit-*OsSerk1ox* #17, respectively. All plants were inoculated with the PXO99 *Xoo* strain. Lesion lengths were measured at 15 d after inoculation (DAI) from 10 independent plants. Photographs depict representative symptom development in leaves at 15 DAI. (**B**) Lesion length of *Xa21* Kitaake, Kitaake, *Xa21*kit-*OsSerk1ox* #X1904 and #X1953 plants inoculated at the seedling stage (3 weeks old). All plants were inoculated with PXO99 at 3 weeks old. Lesion lengths were measured at 10 DAI from 10 independent plants. Photographs depict representative symptom development in leaves at 10 DAI. Statistical significance comparison was conducted with ANOVA, where the mark "**" on the column indicates difference with $P \leqq 0.01$.

four *Xa21*kit-*OsSerk1Ri* lines with PXO99 at 6 weeks old, and found all were resistant to PXO99, showing no significant differences on lesion lengths between the plants carrying or lacking the *OsSerk1Ri* transgene (Figure S5). To further confirm this result, we inoculated two *OsSerk1Ri* homozygous lines (#1602 and #1603) at 6 weeks old (Figure 4A). These two lines showed similar resistance levels as the *Xa21*-Kitaake plants. Both *Xa21*kit-*OsSerk1Ri* and *Xa21*-Kitaake plants had significantly shorter lesions than those of the *Xa21*kit-*OsSerk2Ri* #A814 and Kitaake plants. Bacterial growth curve analysis revealed that *Xa21*kit-*OsSerk1Ri* plants harbored similar *Xoo* bacterial populations as the *Xa21*-Kitaake plants at 0, 10, and 20 d after inoculation (Figure 4B).

To test if *OsSerk1* is involved in rice basal resistance to *Xoo*, we inoculated the transgenic plants with the *Xoo-4* strain, which is virulent on plants carrying *Xa21*. We found that the two lines (#1602 and #1603) homozygous for *OsSerk1Ri* showed similar susceptibility to *Xoo-4* as the *Xa21*-Kitaake control (Figure S4), indicating that *OsSerk1* is not involved in rice basal resistance to *Xoo*. Taken together, we conclude that unlike *OsSerk2*, specific silencing of *OsSerk1* affects neither XA21-mediated immunity nor rice basal resistance to *Xoo*.

OsSerk1* is not involved in rice resistance to *M. oryzae

OsSerk1 does not function in rice immunity to biotrophic pathogen *Xoo*. We then tested whether it regulates rice resistance to hemi-necrotrophic pathogen *M. oryzae*. We inoculated the two *Xa21*kit-*OsSerk1Ri* lines (#1602 and #1603), the two *Xa21*kit-*OsSerk1ox* lines (#X1904 and #X1953), the two Kit-*OsSerk1ox* lines (#X1630 and #X1634), and controls with *M. oryzae* strains, ZB13 and ZB25 (Figures 5, S6). Both Kitaake and *Xa21*-Kitaake are susceptible to ZB25 but resistant to ZB13. We found that all lines tested showed similar susceptibility to ZB25, except for the resistant control Digu (Figure 5). On the contrary, all lines showed resistance to ZB13, except for the susceptible control Lijiang (Figure S6). These results demonstrate that *OsSerk1* does not regulate rice resistance to *M. oryzae*.

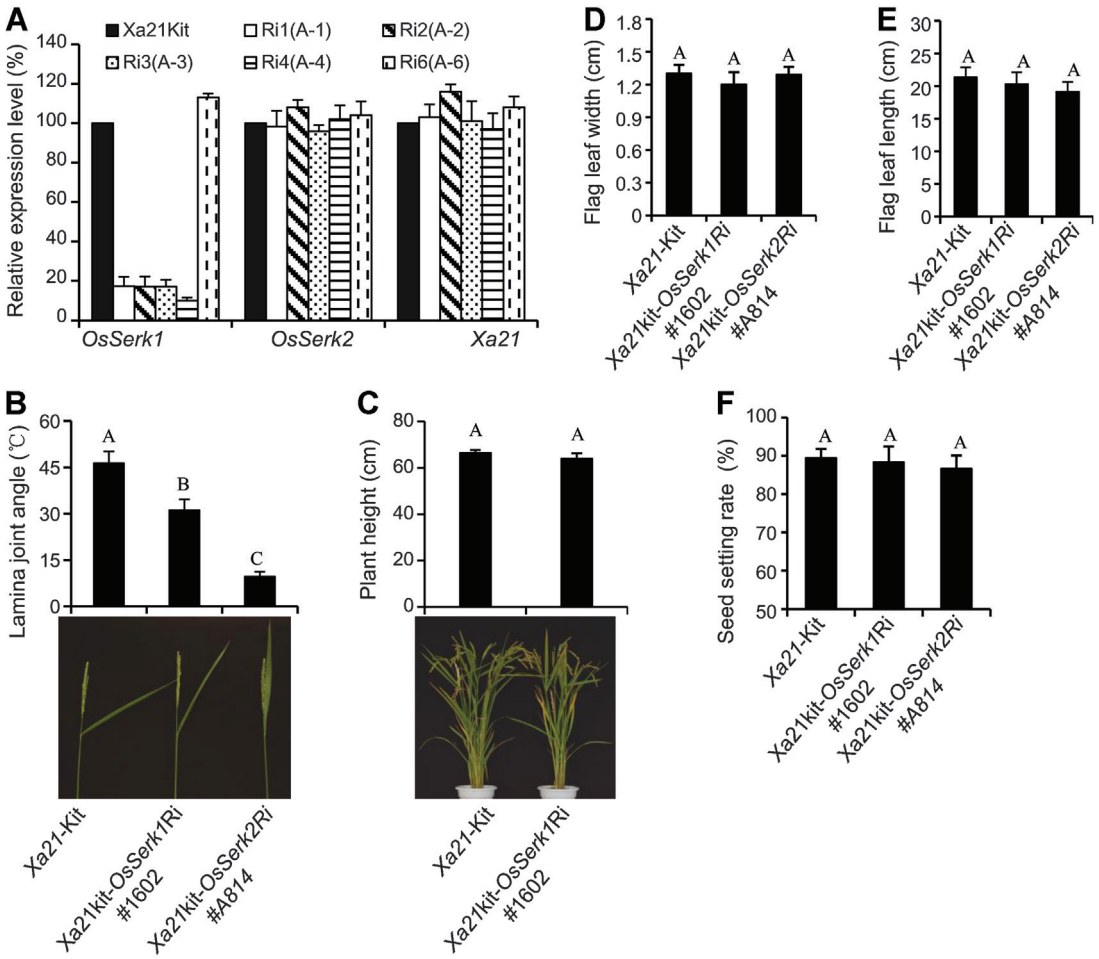

Figure 3. Identification of *Xa21*kit-*OsSerk1Ri* transgenic lines with reduced expression specific to *OsSerk1*
(**A**) Transcript levels of *OsSerk1*, *OsSerk2*, and *Xa21* in wild-type *Xa21*-Kitaake and five independent *Xa21*kit-*OsSerk1Ri* lines as revealed by real-time reverse transcription-polymerase chain reaction (RT-PCR). (**B**) Photographs and measured lamina joint angles of *Xa21* Kitaake, *Xa21*kit-*OsSerk1Ri* #1602 and *Xa21*kit-*OsSerk2Ri* #A814 plants at 15 d after heading. (**C**) Photographs and measured plant heights of *Xa21* Kitaake and *Xa21*kit-*OsSerk1Ri* #1602 at 25 d after heading. (**D**–**F**) Flag leaf width and length and seed set rates of *Xa21*-Kitaake and *Xa21*kit-*OsSerk1Ri* #1602 plants. Statistical significance comparison was conducted with ANOVA, where the different capital letters above the column indicate differences with $P \leqq 0.01$, whereas the same letter indicates no significant differences.

OsSerk1 cannot restore XA21-mediated immunity to *Xoo* in the *OsSerk2* silenced line

OsSERK2 is a regulatory co-receptor kinase of XA21. Silencing of *OsSerk2* in the *Xa21*-Kitaake genetic background severely compromises XA21-mediated immunity to *Xoo* strain PXO99 (Chen et al. 2014). Because *OsSerk1* is expressed in rice leaves at very low levels, we tested if overexpression of *OsSerk1* in the *Oserk2*-silenced line (*Xa21*kit-*OsSerk2Ri* #A814) would complement the mutant and restore XA21-mediated resistance. For this purpose, we generated several hybrid F_1 plants by crossing three independent *Xa21*kit-*OsSerk1ox* lines (#3, #7, and #18, as pollen donor) with *Xa21*kit-*OsSerk2Ri* #A814 line (as recipient) to obtain *OsSerk1oxOsSerk2Ri* plants in *Xa21*-Kitaake background (called *Xa21*kit-*OsSerk1oxOsSerk2Ri*). F_1 plants were inoculated at 6 weeks old. We found no significant differences in lesion length between the F_1 plants carrying both *OsSerk1ox* and *OsSerk2Ri* and those carrying only *OsSerk2Ri* (Figure S7A, B).

We next tested the resistance of two F_2 populations, including 57 and 48 individual plants derived from the crosses of *Xa21*kit-*OsSerk2Ri* #A814/*Xa21*kit-*OsSerk1ox* #7 and #A814/*Xa21*kit-*OsSerk1ox* #18, respectively. The two transgenes (*OsSerk1ox* and *OsSerk2Ri*) segregated in this population based on the genotyping results (Figure S8A, B). Nine F_2 plants with different combinations of *OsSerk1ox* and *OsSerk2Ri* were chosen to detect the expression levels of *OsSerk1* and *OsSerk2* by real time RT-PCR (Figure 6A). We found that *OsSerk2* expression was significantly reduced in plants with the transgene *OsSerk2Ri*. The *OsSerk1* transcription levels in the plants with only *OsSerk1ox* were higher than in those carrying both *OsSerk2Ri* and *OsSerk1ox* transgenes, indicating that the transgene *OsSerk2Ri* affects the overexpression level of *OsSerk1* to a certain extent. However, even in the presence of *OsSerk2Ri*, the expression levels of *OsSerk1* increased at least 10-fold compared with the wild type, and reached or slightly

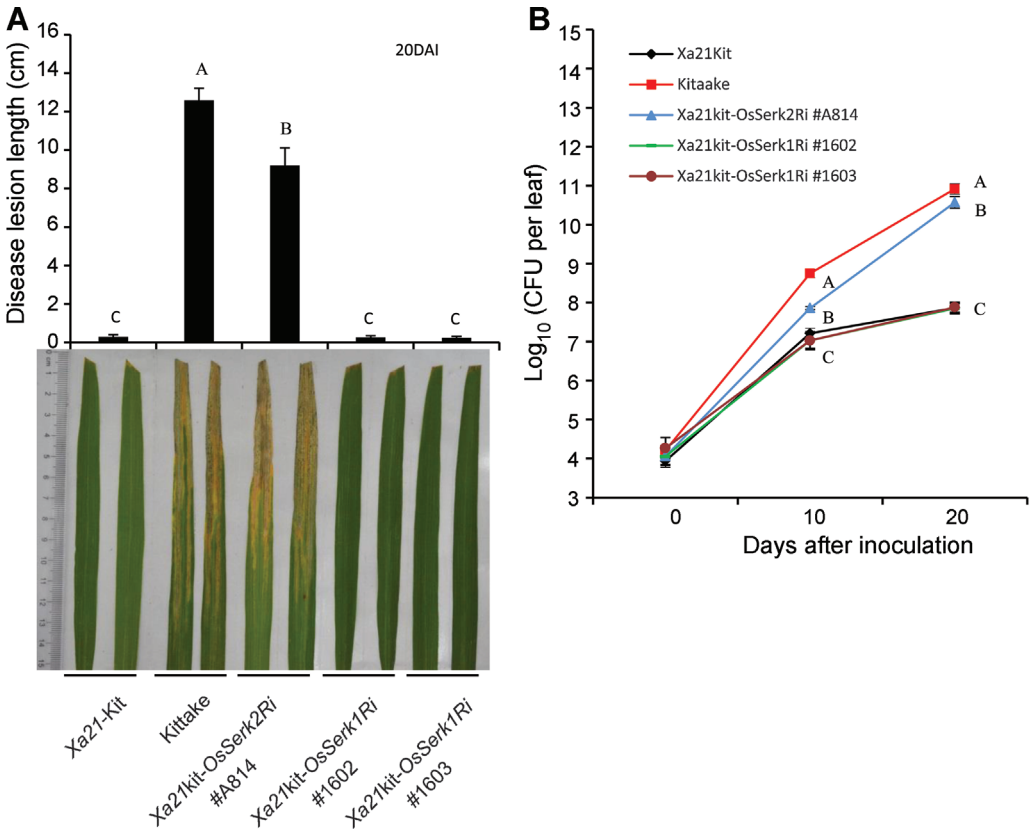

Figure 4. Silencing of *OsSerk1* does not affect XA21-mediated immunity to *Xoo*

(**A**) Disease lesion lengths of *Xa21* Kitaake, Kitaake, Xa21kit-*OsSerk1Ri* #1602 and #1603 plants at 20 d after inoculation (DAI). Lines #1602 and #1603, derived from independent T_0 plants Ri1(A-1) and Ri4(A-4), respectively, are homozygous for the transgene *OsSerk1Ri*. All plants were inoculated at 6 weeks old in the field. Lesion lengths were measured at 20 DAI for 10 independent plants. The photograph depicts representative symptom development in leaves at 20 DAI. (**B**) Bacterial populations of *Xa21* Kitaake, Kitaake, *Xa21*kit-*OsSerk2Ri* #A814, *Xa21*kit-*OsSerk1Ri* #1602 and #1603 lines at 0, 10, and 20 DAI. Each data point represents the average ± SD of six leaves from three independent plants. Statistical significance comparison was conducted with ANOVA, where the different capital letters above the columns and around the point indicate differences with $P \leqq 0.01$.

exceeded the *OsSerk2* transcript levels in the wild type. This result indicates that the transcript level of *OsSerk1* is strongly enhanced in the *OsSerk2Ri* background. All F_2 plants were inoculated with PXO99 at 6 weeks old. We found that the *Xa21*kit-*OsSerk1oxOsSerk2Ri* plants showed similar susceptible phenotype (showing an average lesion length of 14 cm) as the *Xa21*kit-*OsSerk2Ri* plants, while the *Xa21*kit-*OsSerk1ox* plants displayed similar resistant phenotype (average lesion length 2.5 cm) as the *Xa21*-Kitaake control (Figures 6B, S8A, B). These results demonstrate that overexpression of *OsSerk1* is not able to complement the function of *OsSerk2* in the XA21-mediated immune response. Taken together, we conclude that unlike *OsSerk2*, *OsSerk1* is not involved in XA21-mediated immunity.

Overexpression of *OsSerk1* is able to suppress the *bri1*-like phenotype caused by the *OsSerk2* knockdown

In previous studies, *OsSerk2* was shown to be required for *OsBRI1*-mediated signaling (Hu et al. 2005; Chen et al. 2014). The *Xa21*kit-*OsSerk2Ri* #A814 plants with reduced expression of *OsSerk2* show a typical *bri1*-like phenotype, including erect leaves and semi-dwarfism (Chen et al. 2014). We measured the plant height of the *Xa21*kit-*OsSerk1oxOsSerk2Ri* plants to investigate whether overexpression of *OsSerk1* is able to suppress the *bri1*-like phenotype of *Xa21*kit-*OsSerk2Ri*. We found that all *Xa21*kit-*OsSerk1oxOsSerk2Ri* plants were significantly taller (with a range from 66.5 ± 2.4 to 73 ± 3.4 cm) than those carrying only *OsSerk2Ri* (52.5 ± 5.1 cm) in hybrid F1 populations and the *Xa21*kit-*OsSerk2-Ri*#A814 plants (55.2 ± 4.1 cm) (Figure 7A). Compared with the *Xa21*-Kitaake control (74.2 ± 6.3 cm), the *Xa21*kit-*OsSerk1oxOsSerk2Ri* plants from two crosses (#A814/*Xa21*kit-*OsSerk1ox* #3 and #A814/*Xa21*kit-*OsSerk1ox* #18) almost restored the semi-dwarf phenotype of #A814 (Figure 7A, B) to the normal level of *Xa21*-Kitaake. Furthermore, we observed that the angles of the lamina joints of all *Xa21*kit-*OsSerk1oxOsSerk2Ri* plants (ranging from 22.06 ± 7.02° to 26.52 ± 6.35°) increased by at least 17°compared with those

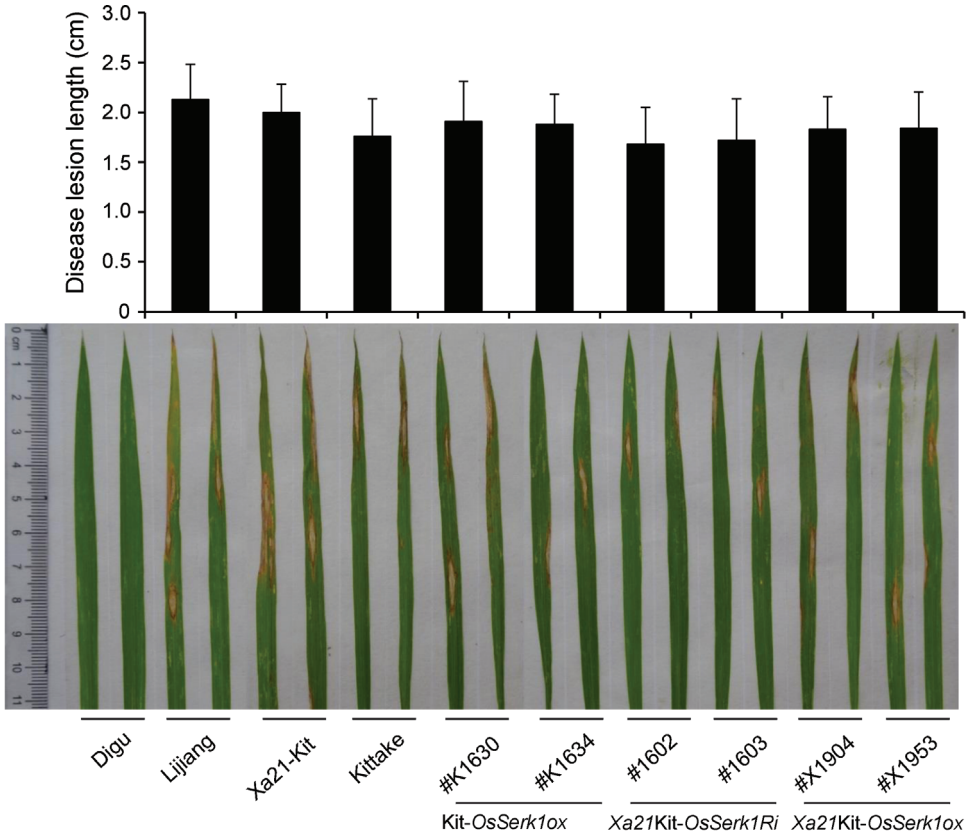

Figure 5. Altered expression of *OsSerk1* does not affect rice resistance to *Magnaporthe oryzae*
Digu and Lijiang are cultivars carrying broad-spectrum resistance and susceptibility to blast strain ZB25, respectively. Two-week-old rice plants were used for inoculation with ZB25. The lesion length was measured and pictures were taken at 7 d after inoculation.

of the *Xa21*kit-*OsSerk2Ri* (5.95 ± 1.78°) and *OsSerk2Ri* plants (Table S1). However, complementation with *OsSerk1*ox did not fully restore the lamina joint angles to the level of the wild type *Xa21*-Kitaake plants (38.07 ± 10.01°) (Figure 7C; Table S1). These results indicate that *OsSerk1* overexpression can suppress the semi-dwarf phenotype of the *Xa21*kit-*OsSerk2Ri* #A814 line and partially complements its erect-leaf phenotype.

We next investigated whether OsSERK1 directly interacts with OsBRI1 by using a yeast two-hybrid assay. The truncated versions of OsSERK1 (OsSERK1JMK) and OsBRI1 (OsBRI1K735), both containing the whole intra-cellular domain and the entire juxtamembrane (JM) domain, were used as bait and prey, respectively. OsSERK2JMK was included as a positive control because it can directly interact with OsBRI1K735 in yeast (Chen et al. 2014). Indeed OsSERK1JMK and OsBRI1K735 directly interact in the yeast-two hybrid assays as indicated by the blue colony coloration specific for this combination and its absence in the respective control reactions (Figure 7D).

Taken together, we suggest that *OsSerk1* encodes a similar function as *OsSerk2* with regard to regulation of rice development and that this function is most likely exerted via its direct interaction with OsBRI1.

OsSERK1 interacts with itself and with OsSERK2 *in vitro* and is a functional protein kinase

Because OsSERK1 and OsSERK2 both can interact with OsBRI1, we tested if the two OsSERKs can directly interact with each other in the yeast two-hybrid assay (Figure S9). We found that yeast cells containing both OsSERK2JMK and OsSERK1JMK in either orientations display a light blue coloration. This indicates OsSERK1 weakly interacts with OsSERK2, suggesting they may form heterodimer *in vitro*. While BD-OsSERK1JMK and AD-OsSERK1JMK interact with each other in the yeast two-hybrid system, BD-OsSERK2JMK and AD-OsSERK2JMK do not (Figure S9). This indicates that OsSERK1 is capable of homodimerization *in vitro*, but OsSERK2 cannot.

Because *OsSerk1* encodes a predicted protein kinase, we next tested whether it possesses kinase activity. We expressed and purified a GST-OsSERK1JMK fusion protein and its catalytically inactive kinase variant GST-OsSERK1JMK^{KE}, generated by mutating lysine (K) 329, which is conserved in all plant active kinase and is required for ATP binding and the kinase catalytic activity, to glutamic acid (E). The *Escherichia coli*-expressed XA21 kinase His-Nus-XA21K668 and its kinase inactive variant His-Nus-XA21K668^{KE} (Chen et al. 2014) were used as positive and negative controls, respectively, in the kinase assays. All four proteins contain the part of their

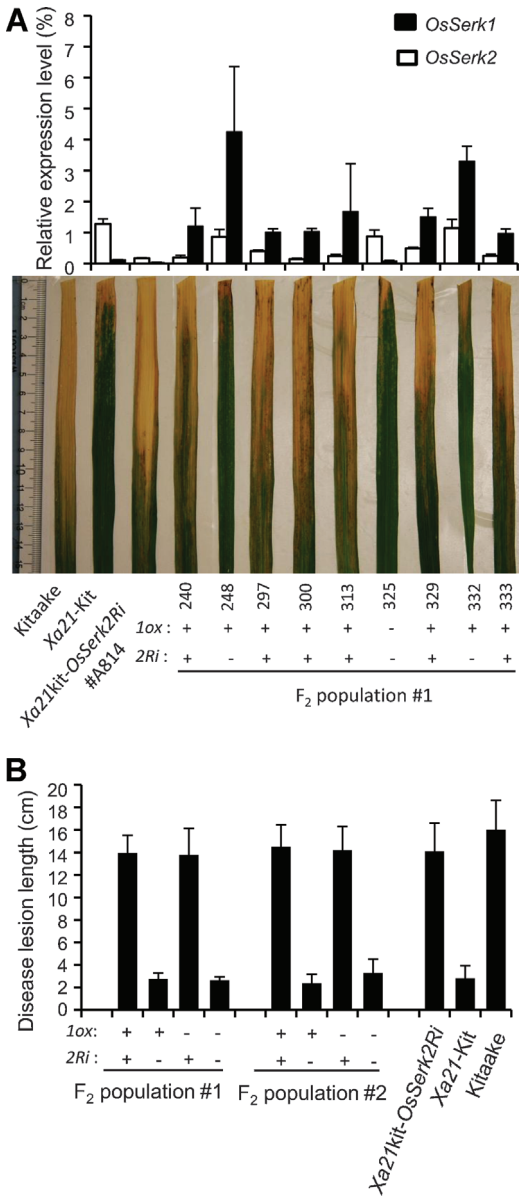

Figure 6. *OsSerk1* overexpression cannot suppress the *Xoo* susceptibility of *OsSerk2*-knockdown plants in *Xa21*-Kitaake background

(**A**) F_2 population #1 was derived from the cross of *Xa21*kit-*OsSerk2Ri* #A814/*Xa21*kit-*OsSerk1ox* #18. Transcript levels of *OsSerk1* and *OsSerk2* revealed by real-time reverse transcription-polymerase chain reaction (RT-PCR) in plants carrying both transgenes *OsSerk1ox* and *OsSerk2Ri*, either of them, or none of them, which were genotyped by specific PCR primers 1ox for *OsSerk1ox* and 2Ri for *OsSerk2Ri*. The photograph depicts representative symptom development in leaves at 15 d after inoculation (DAI). (**B**) Lesion lengths of *Xa21*kit-*OsSerk2Ri* #A814, *Xa21* Kitaake, Kitaake, and F_2 plants with both transgenes *OsSerk1ox* and *OsSerk2Ri*, either of them, or none of them. The F_2 plants were derived from two F_1 crosses, #A814/*Xa21*kit-*OsSerk1ox* #7 (F_2 population #2) and #A814/Xkit-*OsSerk1ox* #18 (F_2 population #1). All plants were inoculated with PXO99 at 6 weeks old, and lesion lengths were measured at 15 DAI for at least five leaves from three or more independent plants.

transmembrane domains (TM) and full JM and kinase domains, as depicted in Figure 8A. These proteins were subjected to *in vitro* kinase assays using [^{32}P]-γ-ATP. We found that the GST-OsSERK1JMK and His-Nus-XA21K668 fusion proteins were capable of auto-phosphorylation, whereas their respective kinase-inactive proteins failed to be autophosphorylated (Figure 8B). Notably, the OsSERK1 fusion protein showed much stronger kinase activity than the XA21 fusion protein (Figure 8B). We conclude that OsSERK1 is a functional protein kinase capable of auto-phosphorylation.

DISCUSSION

Orthologs of *Arabidopsis* SERK proteins in rice

In *Arabidopsis*, there are five SERK proteins that have evolved into two groups (Schmidt et al. 1997; Hecht et al. 2001). Group I consists of AtSERK1 and AtSERK2 that play redundant roles in regulation of plant development, while group II includes AtSERK3/BAK1 and AtSERK4/BKK1 that function redundantly in regulation of both plant immunity and development (Colcombet et al. 2005; Roux et al. 2011; Schwessinger and Ronald 2012). In contrast to the multiple SERK proteins in *Arabidopsis*, the cotton genome has only evolved three SERK orthologs. One of these is the ortholog of AtSERK1/SERK2 and the other two are the counterparts to AtSERK3/BAK1 (Gao et al. 2013). These studies illustrate the divergent evolution of *SERK* genes between species (Gao et al. 2013).

Through phylogenetic analysis, we identified two rice genes (*OsSerk1* and *OsSerk2*) that encode proteins with typical structural characteristics of SERK proteins (Schmidt et al. 1997; Hecht et al. 2001; Chen et al. 2014). OsSERK1 and OsSERK2 cluster with AtSERK1 and AtSERK2 but not AtSERK3/BAK1 and AtSERK4/BKK1 (Chen et al. 2014). OsSERK1 shows slightly higher identity (69.1%) with AtSERK3/BAK1 than OsSERK2 (61.2% identity), and can partially rescue the *Arabidopsis bri1–5* mutant phenotype. For this reason, OsSERK1 was hypothesized to be OsBAK1 by Li et al. (2009). Consistent with these observations, we found that OsSERK1 interacts with OsBRI1 (Figure 7D) and overexpression of *OsSerk1* can suppress the *bri1*-like phenotype of transgenic *OsSerk2Ri* plants (Figure7A–C). Based on these results, we hypothesize that OsSERK1 possesses a similar function in rice development as AtSERKs proteins, including AtSERK3/BAK1. Previous studies have demonstrated that simultaneous silencing of two *OsSerk* genes and others *OsSerk*-like genes increased expression levels of pathogenesis-related gene and enhance susceptibility to *M. oryzae*, suggesting the involvement of *OsSerk* or *OsSerk*-like genes in rice immunity (Park et al. 2011). However, these reports did not provide evidence that *OsSerk1* was involved in regulation of rice immunity. Our study reveals that neither overexpression nor silencing of *OsSerk1* affects rice resistance or susceptibility to *M. oryzae* (Figure 5, S6).

In our previous study, we showed that silencing of *OsSerk2* disrupts XA21-mediated immunity to *Xoo* and that OsSERK2 physically associated with XA21 *in vivo* and served as a regulatory receptor kinase of XA21 (Chen et al. 2014). In addition, *OsSerk2* also plays a pivotal role in regulating rice development through BR signaling. In summary, *OsSerk2* has a dual function in rice development and immunity, similar to AtSERK3/BAK1 and AtSERK4/BKK1 in *Arabidopsis* (He et al.

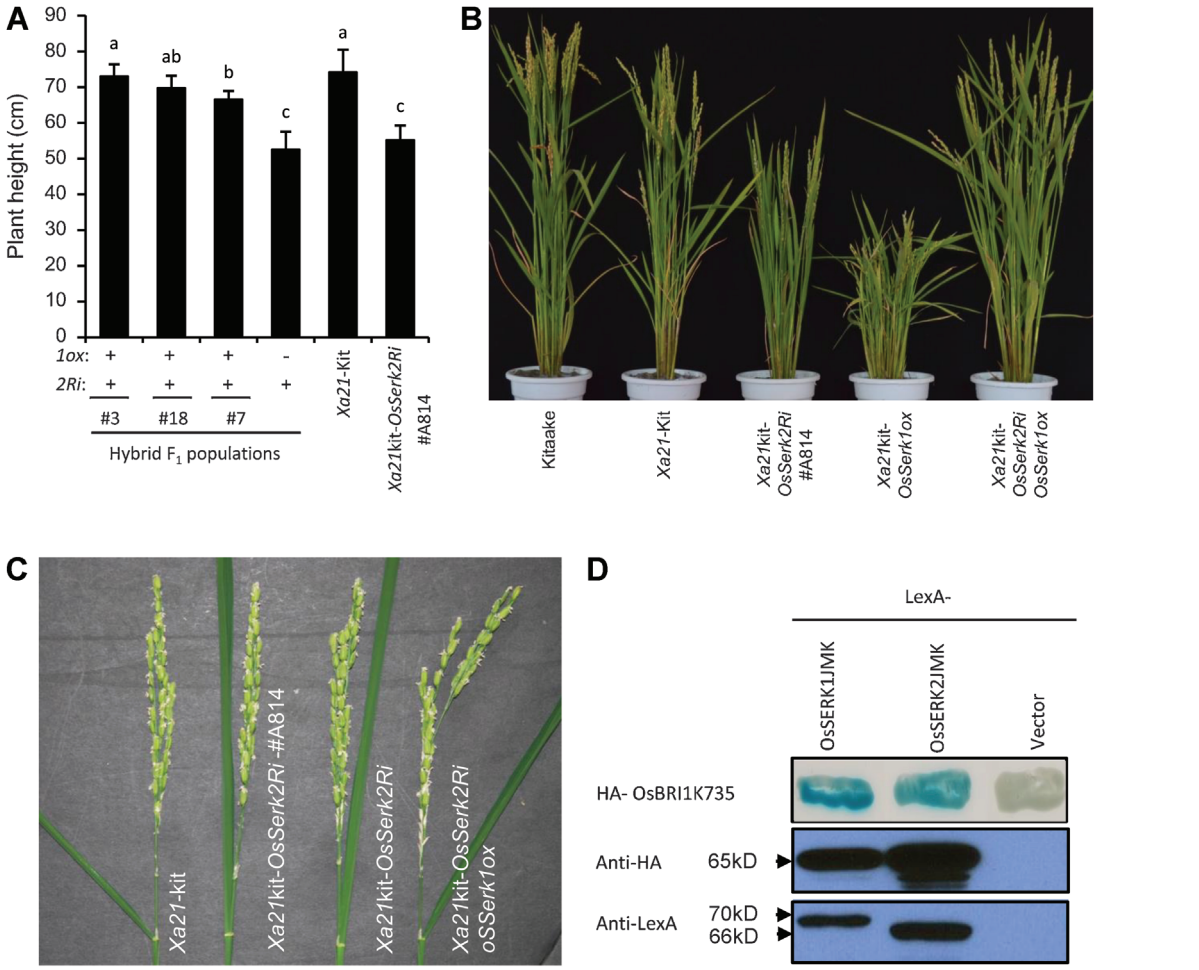

Figure 7. *OsSerk1* overexpression can suppress the *bri1*-like phenotype of *OsSerk2*-knockdown plants
(**A**) Plant heights of F_1 plants with or without the *OsSerk1ox* transgene and the control plants *Xa21*-Kitaake and *Xa21*kit-*OsSerk2Ri* #A814. Three hybrid F_1 populations, generated by crossing #A814 (pollen recipient) and each of three *Xa21*kit-*OsSerk1ox* independent lines (#3, #7, and #18), were included. (**B**) The photograph depicts plant height of different genotypes at 20 d after heading. The *Xa21*kit-*OsSerk2Ri* and *Xa21*kit-*OsSerk2RiOsSerk1ox* plants were selected from the progeny of the #A814/Xa21kit-*OsSerk1ox*-#18 cross, that contain either only the *OsSerk2Ri* transgene or both transgenes *OsSerk2Ri* and *OsSerk1ox* (same for panel C). (**C**) The photograph depicts lamina joint angles of plants with different genotypes at 15 d after heading. (**D**) The OsSERK1 intracellular domain interacts with OsBRI1 in yeast-two hybrid system. The blue color indicates interaction between the two co-expressed proteins. The OsSERK1JMK and OsSERK2JMK were fused to the LexA tag, respectively, and OsBRI1K735 was fused to B42AD with HA tag. The expression of LexA and HA fusion proteins were confirmed by Western blot analyses using anti-LexA and anti-HA antibodies, respectively. This experiment was repeated three times with same results.

2007; Roux et al. 2011; Gou et al. 2012). Compared with *OsSerk2*, *OsSerk1* is expressed at much lower level in leaves (Chen et al. 2014). To investigate if *OsSerk1* has similar function as *OsSerk2* in regulating rice immunity to *Xoo*, we overexpressed *OsSerk1* in Kitaake and *Xa21*-Kitaake genetic backgrounds and found that overexpression of *OsSerk1* did not alter rice resistance to *Xoo* in either of the two genetic backgrounds (Figures 2, S3). Altered *OsSerk1* expression also did not influence the plant susceptibility to a *Xoo* strain that is able to evade XA21-mediated immunity (Figure S4). In addition, overexpression of *OsSerk1* in *Xa21*kit-*OsSerk2Ri* lines could not restore the compromised XA21-mediated immunity caused by *OsSerk2*-silencing (Figures 6, S8). These results clearly demonstrate that *OsSerk1* is not required for XA21-mediated immunity or for basal resistance to *Xoo*.

We found that overexpression of *OsSerk1* can suppress the erect leaf and semi-dwarf phenotype resulting from *OsSerk2*-silencing (Figure 7). This suggests that OsSERK1 possesses a similar function as OsSERK2 in regulation of plant development. Both *OsSerk1ox* plants and *OsSerk2Ri* plants displayed semi-dwarf phenotype, while their hybrid F_1 plants (harboring both *OsSerk1ox* and *OsSerk2Ri* transgenes) regained plant height similar to the wild type (Figure 7A, B). In wild type plants, *OsSerk1* is expressed at significantly lower levels than *OsSerk2* in leaf tissues. In *OsSerk1oxOsSerk2Ri* lines, the *OsSerk1* expression level reaches a level very similar to the *OsSerk2* level

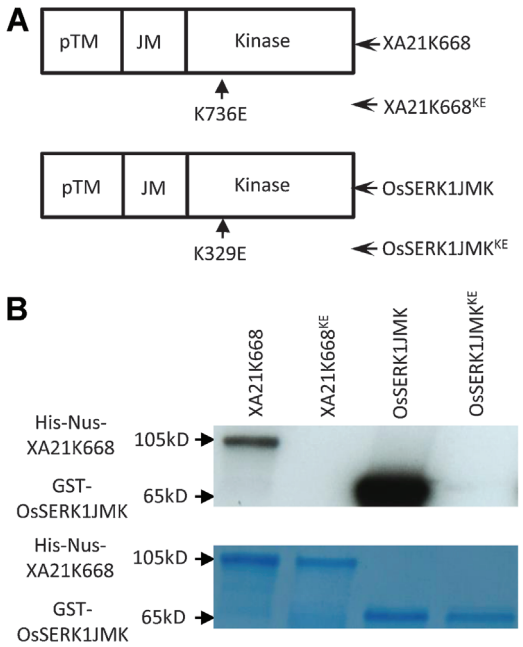

Figure 8. OsSERK1 is a functional protein kinase
(**A**) The truncated protein of each of OsSERK1 and XA21 contains part of the transmembrane (pTM) domain, full justxamembrane (JM) and kinase domains. The mutation site was labeled under the sketch of each truncated protein. (**B**) The *in vitro* kinase assay was performed by incubating with [^{32}P]-γ-ATP and each of the proteins, GST-OsSERK1JMK, GST-OsSERK1JMK^{KE}, His-Nus-XA21K668, and His-Nus-XA21K668^{KE}. Proteins were separated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by autoradiography in the top panel and stained by Coomassie blue (CBB) in the bottom panel, respectively.

in the wild type plants (Figure 6A). This may explain why *OsSerk1ox* can complement the semi-dwarf phenotype in *OsSerk2Ri* plants. To develop normal plant height, the optimum OsSERK protein (including both OsSERK1 and OsSERK2) level may be critical in order to properly regulate BRI1 function and maintain BR-signaling. This hypothesis is consistent with the observation that both OsSERK1 and OsSERK2 are able to directly interact with OsBRI1 in the yeast-two hybrid assay, suggesting that OsSERK1 and OsSERK2 may function interchangeably in modulation of BR signaling (Figure 7D). In the event that this optimum OsSERK protein level is shifted, either higher or lower, plants become dwarf or semi-dwarf due to inappropriate BR-signaling. Consequently, we conclude that the function of OsSERK1 in rice closely resembles the role of AtSERK1 and AtSERK2 in *Arabidopsis*, which function mainly in development, while OsSERK2 appears to be the true functional ortholog of AtSERK3/BAK1 and AtSERK4/BKK1, playing a major role in both immunity and development.

Potential use of *OsSerk1* in developing rice varieties with improved plant architecture

Plant architecture is a major factor affecting grain yield (Reinhardt and Kuhlemeier 2002; Jiao et al. 2010). Yield-related plant architecture includes plant height, tillering pattern, and leaf angle (Yang and Hwa 2008; Zhang et al. 2012). By using the semi-dwarf gene *sd-1*, the rice yield has experienced a remarkable increase, which was known as "The Green Revolution" (Spielmeyer et al. 2002). Recently, the rice ideotype approach has been used in breeding programs at the International Rice Research Institute (IRRI) and in China to further improve rice yield potential (Peng et al. 2008; Sharma et al. 2013). One of the most important characters for rice ideotype is erect leaves (or small leaf angles) (Peng et al. 2008; Jiao et al. 2010; Zhang et al. 2012), which can improve light penetration and canopy net photosynthesis rate and ultimately improve grain-yield (Zhang et al. 2012; Sharma et al. 2013). In addition, researchers may also increase yield by increasing the density of plants with erect leaves in the field (Sakamoto et al. 2006). In the present study, we found that the transgenic plants carrying *OsSerk1Ri* exhibited reduced lamina joint angles (Figure 3B). Notably, these plants do not show any obvious difference in other agronomic traits, grain-yield associated components, or resistance to *Xoo* compared with the parental *Xa21*-Kitaake plants (Figures 3C–F, 4). These results indicate that downregulating the expression of *OsSerk1* is able to improve the plant architecture without observable negative effects, which are consistent with the report of Li et al. (2009). Thus, modulating the expression level of *OsSerk1* may serve as a useful strategy to develop rice varieties with enhanced yield.

MATERIALS AND METHODS

Plant materials, growth, and pathogens inoculation conditions

Rice (*Oryza sativa* L.) lines used in this work included *japonica* cultivar Kitaake, transgenic *Xa21* line in Kitaake genetic background (hereafter called Xa21 Kitaake), and the transgenic line *Xa21*kit-Os*Serk2Ri* #A814 (Chen et al. 2014) with knockdown of *OsSerk2* in *Xa21*-Kitaake genetic background. The *Xa21*-Kitaake plants show robust resistance to *Xoo* due to the *Xa21* transgene, while the *Xa21*kit-Os*Serk2Ri* plants are fully susceptible to *Xoo* due to the reduced expression of *OsSerk2* (Chen et al. 2014). For adult rice plant inoculation, the plants were grown in the greenhouse until 6 weeks of age and transferred to the growth chamber before inoculation with the *Xoo* strain *PXO99* or *Xoo-4*. *PXO99* carries a genetic factor that triggers XA21-mediated immunity while *Xoo-4* lacks this genetic factor and can evade XA21-mediated immunity (*Xoo-4* was kindly provided by Dr Zhihui Xia from Hainan University, China). For seedling rice inoculation, the plants were grown in the greenhouse until 2.5 weeks of age before being transferred to the growth chamber for inoculation (Park et al. 2010). Growth chambers were set on 14 h light/10 h dark photoperiod, 28/26 °C temperature cycle, and 85%/90% humidity. *Xoo* bacterial suspension (OD_{600} of 0.5) was used to inoculate rice by the scissors-dip method. The disease lesion length and bacterial population accumulated in rice leaf were evaluated as reported before (Chern et al. 2005). The ANOVA (analysis of variance) program packaged in SPSS16.0 software was adopted to assess significance in statistics.

For rice blast inoculation, plants were grown in the growth chamber at 28 °C in 12 h light/12 h dark photoperiod with 75% humidity. Two-week-old rice plants were used for inoculation

with *M. oryzae* strains (ZB13 and ZB25) that were collected in Sichuan of China. The Digu and Lijiang rice varieties were used as the resistant and susceptible controls, respectively, to the two strains. The concentration of spore was 5×10^5/mL with 0.2% Tween-20. The fungal- and mock-inoculated rice seedlings were kept in dark inoculation chambers with 95% humidity at 28 °C. The lesion length was measured and pictures were taken at 7 d after inoculation.

Plasmid constructs

For RNAi construct, a 432 bp unique cDNA fragment of *OsSerk1* (amplified by primer pair OsSerk1Ri-1/-2: 5′-CACCATCCGTGCACTTGGTTTCAT-3/5′-AAGGGTTGTTGGCAAAACTG-3′) from japonica variety Nipponbare was cloned into the pENTR™/D-TOPO (Invitrogen Corporation, Carlsbad, CA, USA) vector and then put into pANDA (Kindly provided by Professor Ko Shimamoto, Nara Institute of Science and Technology, Japan) vector through LR recombination to generate *OsSerk1Ri* construct.

For overexpression construct, a 1,875 bp full-length cDNA fragment of *OsSerk1* (amplified by primer pair 07760cDNA-F/07760cDNA-R(Stop) (5′-CACCATGGCGGCGCATCGGTGGGCGGTG-3′/5′-TCACCTCGGCCCTGATAGCTCAACC-3′) from japonica cultivar Nipponbare was cloned into the pENTR/D-TOPO (Invitrogen) vector and then put into the Ubi-NC1300RFCA vector through LR recombination to generate *UbiC1300-OsSerk1* construct. The Ubi-NC1300RFCA vector was developed by introducing the 1,711 bp RFCA (reading frame cassette A) fragment into Ubi/NC1300 that has been reported previously (Chern et al. 2005). In the *UbiC1300-OsSerk1* construct, the *OsSerk1* gene is under control of the maize ubiquitin promoter.

For constructs used in yeast two-hybrid assay, the partial cDNA sequence of *OsSerk1* (named OsSERK1JMK), containing juxtamembrane and kinase domains (JMK) with stop codon, was amplified by primer pair OsSerk1G257-F/OsSerk1G257-R(w/stop) (5′-CACCATGGGTTTTGCATGGTATCGGCGC-3′/5′-TTATCATCTCGGCCCTGATAGCTCAACCG-3′) and cloned into pENTR/D-TOPO (Invitrogen) to create pENTR-OsSERK1JMK. The pENTR-OsSERK2JMK and pENTR-OsBRIK735 constructs were generated previously (Chen et al 2014). The pENTR-OsBRIK735 was recombined with the pB42AD vector to yield HA-tagged fusion protein. The pENTR-OsSERK1JMK and pENTR-OsSERK2JMK plasmids were recombined with the pLexA vector to produce LexA fusion proteins.

Development of rice transgenic lines and crossing

Through *Agrobacterium*-mediated transformation described previously (Chern et al. 2005), the overexpression construct of *OsSerk1* was introduced into *Xa21*-Kitaake and Kitaake plants, respectively. The RNAi construct of *OsSerk1* was introduced into *Xa21*-Kitaake plant. Because the transgenic *Xa21*-Kitaake plant is mannose resistant, transgenes *OsSerk1Ri* and *OsSerk1ox* were selected with hygromycin in the present study. The *Xa21*kit-*OsSerk2Ri* #A814 plants carrying reduced *OsSerk2* expression was used as the pollen recipient to cross with transgenic *OsSerk1ox* plants in *Xa21*-Kitaake background to obtain *OsSerk1oxOsSerk2Ri* plants. PCR-based genotyping was performed to determine the transgenic plants with or without the transgene(s) according to the description of previous study (Chen et al. 2010). The PCR-specific primer pairs used for genotyping transgenes *OsSerk1Ri*, *OsSerk1ox*, and *OsSerk2Ri* were Ubi-pro-F(5′-CATACGCTATTTATTTGCTTGG-3′)/OsSERK1Ri-2(5′-AAGGGTTGTTGGCAAAACTG-3′), Ubi-pro-F/OsSerk1oX-genotype-R(5′-GTATCGTTCCGCTTATGTTATT-3′), and Ubi-pro-F/OsSerk1Ri-R(5′-CCAATCGAGCAACATCACAT-3′), respectively.

RNA extraction and real time RT-PCR analyses

Total RNA was isolated from rice plant tissue using Invitrogen RNA isolation kit, TRIzol (Invitrogen), following the manufacturer's manual. Total RNA was treated with DNase I and used for the first strand cDNA synthesis using the Invitrogen reverse transcription kit (Invitrogen) following the provided manual. Quantitative real time PCR (qRT-PCR) was performed on a Bio-Rad CFX96 Real-Time System coupled to a C1000 Thermal Cycler (BIO-RAD Corporation, Hercules, CA, USA). For qRT-PCR reactions, the Bio-Rad SsoFast Eva Green Supermix was used. qRT-PCR primer pairs used were as follows: *OsSerk1*-Q1/-Q2 (5′-TGCATTGCATAGCTTGAGGA-3′/5′-GCAGCATTCCCAAGATCAAC-3′) for the *OsSerk1* gene, *Xak1*-Q1/Q2 (5′-TAGTCTGCGCCAAAGTCTGA-3′/5′-GCACCTGACAGTTGTGCATT-3′) for the *OsSerk2* gene, *Xa21*-Q1/-Q2 (5′-TGACACGAAGCTCATTTTGG-3′/5′-TTGATGGCATTCAGTTCGTC-3′) for the *Xa21* gene, and *actin*-Q1/-Q2 (5′-TCGGCTCTGAATGTACCTCCTA-3′/5′-CACTTGAGTAAAGACTGTCACTTG-3′) for the reference gene *Actin*. qRT-PCR reactions were run for 40 cycles with annealing at 56 °C for 12 s and denaturation at 95 °C for 8 s. The expression levels of *OsSerk1*, *OsSerk2* and *Xa21* were normalized to the *Actin* gene expression level.

Yeast two-hybrid assays

The Matchmaker LexA two-hybrid system (Clontech) was used for yeast two hybrid assays. Yeast pEGY48/p8op-lacZ (Clontech Laboratories, Mountain View, CA, USA) was co-transformed with the BD and AD vectors by using the Frozen-EZ yeast transformation II kit (Zymo Research Corporation, Irvine, CA, USA) and spread on an appropriate medium following the procedures described previously (Chen et al. 2010).

Immune-blotting

Total protein extraction from yeast cell and immuno-blotting (Western blotting) was performed as previously described (Chen et al. 2010). The anti-LexA antibody (Clontech) was used to detect LexA-fused protein and the anti-HA antibody (Covance Inc. Princeton, NJ, USA) used to detect HA-fused protein.

Purification of recombinant proteins and *in vitro* protein kinase assay

Recombinant fusion proteins were produced in *E. coli* BL21 (Novagen, Darmstadt, Germany). GST-tagged fusion proteins (GST-OsSERK1JMK, GST-OsSERK1JMK^{KE}) were enriched using Glutathione Sepharose Fast Flow (GE Healthcare Bio-Sciences, Piscataway, NJ, USA) according to the manufacturer's protocol. His-Nus-tagged fusion proteins (His-Nus-XA21K668, His-Nus-XA21K668^{KE}) were enriched using His-Bind Resin (Novagen) according to the manufacturer's protocol (Chen et al. 2014). After elusion, the fusion proteins were adjusted to the same concentration in 10% glycerol solution and stored at −70 °C until usage.

Two micrograms of each fusion protein was incubated in 30 μL kinase buffer (50 mmol/L Tris, pH 7.5, 10 mmol/L $MgCl_2$, 10 mmol/L $MnCl_2$, 1 mmol/L DTT) in the presence of 0.5 μL (5 mCui) [^{32}P]-γ-ATP for 30 min at 30 °C with shaking at

1,200 rpm. The reaction was stopped by adding 10 μL 4× LDS loading dye (Invitrogen) and immediately transferred to 80 °C for 10 min. The reaction mixture was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Post-electrophoresis, proteins were transferred onto polyvinylidene fluoride (PVDF) membranes followed by staining with 0.2% w/v ponceau S in trichloroacetic aci (TCA; 3% v/v). The membranes were dried at room temperature for 20 min and then followed by autoradiograph analysis as described previously (Chen et al. 2010).

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REFERENCES

Chen X, Chern M, Canlas PE, Ruan D, Jiang C, Ronald PC (2010) An ATPase promotes autophosphorylation of the pattern recognition receptor XA21 and inhibits XA21-mediated immunity. **Proc Natl Acad Sci USA** 107: 8029–8034

Chen XW, Zuo SM, Schwessinger B, Chern MS, Canlas PE, Ruan DL, Zhou XG, Wang J, Daudi A, Petzold CJ, Heazlewood JL, Ronald PC (2014) An XA21-associated kinase (OsSERK2) regulates immunity mediated by the XA21 and XA3 immune receptors. **Mol Plant** doi: 10.1093/mp/ssu003

Chern MS, Canlas PE, Fitzgerald H, Ronald PC (2005) NRR, a negative regulator of disease resistance in rice that interacts with arabidopsis NPR1 and rice NH1. **Plant J** 43: 623–635

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JDG, Felix G, Boller T (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. **Nature** 448: 497–500

Chinchilla D, Shan LB, He P, Vries S, Kemmerling B (2009) One for all: The receptor-associated kinase BAK1. **Trends in Plant Sci** 14: 535–541

Colcombet J, Boisson-Dernier A, Ros-Palau R, Vera CE, Schroeder JI (2005) *Arabidopsis* SOMATIC EMBRYOGENESIS RECEPTOR KINASES1 and 2 are essential for tapetum development and microspore maturation. **Plant Cell** 17: 3350–3361

Fradin EF, Abd-EI-Haliem A, Masini L, van den Berg GC, Joosten MH, Thormma BP (2011) Interfamily transfer of tomato Ve1 mediates Verticillium resistance in *Arabidopsis*. **Plant Physiol** 156: 2255–2265

Gao XQ, Li FJ, Li MY, Kianinejad AS, Dever JK, Wheeler TA, Li ZH, He P, Shan LB (2013) Cotton GhBAK1 mediates verticillium wilt resistance and cell death. **J Integr Plant Biol** 55: 586–596

Gou XP, Yin HJ, He K, Du JB, Yi J, Xu SB, Lin HH, Clouse SD, Li J (2012) Genetic evidence for an indispensable role of somatic embryogenesis receptor kinases in brassinosteroid signaling. **PLoS Genet** 8: e1002452

He K, Gou X, Yuan T, Lin H, Asami T, Yoshida S, Russell SD, Li J (2007) BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell-death pathways. **Curr Biol** 17: 1109–1115

Hecht V, Vielle-Calzada JP, Hartog MV, Schmidt ED, Boutilier K, Grossniklaus U, de Vries SC (2001) The *Arabidopsis* somatic embryogenesis receptor kinase 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in cultures. **Plant Physiol** 127: 803–816

Heese A, Hann DR, Gimenez-Ibanez S, Jones AM, He K, Li J, Schroeder JI, Peck SC, Rathjen JP (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. **Proc Natl Acad Sci USA** 104: 12217–12222

Hu H, Xiong L, Yang Y (2005) Rice SERK1 gene positively regulates somatic embryogenesis of cultured cell and host defense response against fungal infection. **Planta** 222: 107–117

Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X, Qian Q, Li J (2010) Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. **Nat Genet** 42: 541–544

Kemmerling B, Schwedt A, Rodriguez P, Mazzotta S, Frank M, Qamar SA, Mengiste T, Betsuyaku S, Parker JE, Müssig C, Thomma BP, Albrecht C, de Vries SC, Hirt H, Nürnberger T (2007) The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. **Curr Biol** 17: 1116–1122

Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC (2002) BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. **Cell** 110: 213–222

Li D, Wang L, Wang M, Xu YY, Luo W, Liu YJ, Xu ZH, Li J, Chong K (2009) Engineering *OsBAK1* gene as a molecular tool to improve rice architecture for high yield. **Plant Biotechnol J** 7: 791–806

Li J (2010) Multi-tasking of somatic embryogenesis receptor-like protein kinases. **Curr Opin in Plant Biol** 13: 509–514

Nam KH, Li J (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. **Cell** 110: 203–212

Park CJ, Lee SW, Chern MS, Sharma R, Canlas PE, Song MY, Jeon JS, Ronald PC (2010) Ectopic expression of rice *Xa21* overcomes developmentally controlled resistance to *Xanthomonas oryzae* pv. *oryzae*. **Plant Sci** 9: 66–71

Park HS, Ryu HY, Kim BH, Kim SY, Yoon IS, Nam KH (2011) A subset of OsSERK genes, including OsBAK1, affects normal growth and leaf development of rice. **Mol Cells** 32: 561–569

Peng SB, Khush GS, Virk P, Tang QY, Zou YB (2008) Progress in ideotype breeding to increase rice yield potential. **Field Crops Res** 108: 32–38

Reinhardt D, Kuhlemeier C (2002) Plant architecture. **EMBO Rep** 3: 846–851

Roux M, Schwessinger B, Albrecht C, Chinchilla D, Jones A, Holton N, Malinovsky FG, Tor M, de Vries S, Zipfel C (2011) The Arabidopsis leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. **Plant Cell** 23: 2440–2455

Sakamoto T, Morinaka Y, Ohnishi T, Sunohara H, Fujioka S, Ueguchi-Tanaka M, Mizutani M, Sakata K, Takatsuto S, Yoshida S, Tanaka H, Kitano H, Matsuoka M (2006) Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. **Nat Biotechnol** 24: 105–109

Santiago J, Henzler C, Hothorn M (2013) Molecular mechanism for plant steroid receptor activation by somatic embryogenesis co-receptor kinases. **Science** 341: 889–892

Schmidt EDL, Guzzo F, Toonen MAJ, de Vries SC (1997) A leucine-rich repeat containing receptor-like kinase marks somatic plant cells competent to form embryos. **Development** 124: 2049–2062

Schwessinger B, Roux M, Kadota Y, Ntoukakis V, Sklenar J, Jones A, Zipfel C (2011) Phosphorylation-dependent differential regulation of plant growth, cell death, and innate immunity by the regulatory receptor-like kinase BAK1. **PLoS Genet** 7: e1002046. doi: 10.1371/journal.pgen.1002046

Schwessinger B, Ronald PC (2012) Plant innate immunity: Perception of conserved microbial signatures. **Annu Rev Plant Biol** 63: 451–482

Sharma D, Sanghera GS, Sahu P, Sahu PS, Parikh M, Sharma B, Bhandarkar S, Chaudhari PR, Jena BK (2013) Tailoring rice plants for sustainable yield through ideotype breeding and physiological interventions. **Afr J Agric Res** 8: 5004–5019

Singla B, Khurana JP, Khurana P (2009) Structural characterization and expression analysis of the *SERK/SERL* gene family in rice (*Oryza sativa*). **Int J Plant Genomics** doi: 10.1155/2009/539402

Spielmeyer W, Ellis MH, Chandler PM (2002) Semidwarf (*sd-1*), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. **Proc Natl Acad Sci USA** 99: 9043–9048

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. **Science** 270: 1804–1806

Sun YD, Han ZF, Tang J, Hu ZH, Chai CL, Zhou B, Chai JJ (2013a) Structure reveals that BAK1 as a co-receptor recognizes the BRI1-bound brassinolide. **Cell Research** 23: 1326–1329

Sun YD, Li L, Macho AP, Han Z, Hu Z, Zipfel C, Zhou JM, Chai J (2013b) Structural basis for flg22-induced activation of the *Arabidopsis* FLS2-BAK1 immune complex. **Science** 342: 624–628

Wang X, Chory J (2006) Brassinosteroids regulate dissociation of BKI1, a negative regulator of BRI1 signaling, from the plasma membrane. **Science** 313: 1118–1122

Yang XC, Hwa CM (2008) Genetic modification of plant architecture and variety improvement in rice. **Heredity** 101: 396–404

Zhang C, Xu YY, Guo SY, Zhu JY, Huan Q, Liu HH, Wang L, Luo GZ, Wang XJ, Chong K (2012) Dynamics of brassinosteroid response modulated by negative regulator LIC in rice. **PLoS Genet** doi: 10.1371/journal.pgen.1002686

SUPPORTING INFORMATION

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Figure S1. Plant heights of transgenic T_o plants with *OsSerk1* overexpression

(**A**) Plant heights of the transgenic Kit-*OsSerk10x* T_o plants in Kitaake background. Total of 18 independent Kit-*OsSerk10x* T_o plants were obtained. Three of them were confirmed by polymerase chain reaction (PCR) not containing the *OsSerk10x* transgene, which was represented by "(−)" and white columns (same for the bottom panel). One of them did not show overexpression of *OsSerk1* by real-time RT-PCR, which was represented by grey columns (same for the bottom panel). (**B**) Plant heights of transgenic *Xa21*kit-*OsSerk10x* T_o plants in *Xa21* Kitaake background. Total of 30 independent *Xa21*kit-*OsSerk10x* T_o plants were obtained. one (shown as white bar) of them was confirmed not containing *OsSerk10x*, and two (grey bars) of them did not show overexpression of *OsSerk1*.

Figure S2. Morphological phenotypes of transgenic lines with *OsSerk1* overexpression

(**A**) The internode length and lamina joint angle of each line were measured from 10 independent plants at 25 d after heading. The control lines Xa21 Kitaake (*Xa21*-Kit) and Kitaake, and two lines homozygous for *OsSerk10x* from each background of *Xa21*-Kit and Kitaake. The two lines #X1904 and #X1953 homozygous for *OsSerk10x* were derived from *Xa21*kit-*OsSerk10x*-#3 and *Xa21*kit-*OsSerkox* #18 T_o lines, respectively. The lines #K1630 and #K1653 homozygous for *OsSerk10x* were derived from Kit-*OsSerk10x* #14 and Kit-*OsSerk10x* #17, respectively. (**B**) and (**C**) Photographs for seed size and lamina joint angle were taken at 25 d after heading.

Figure S3. Disease resistance determination of Kittake and *Xa21* kittake carrying *OsSerk10x* transgene after inoculation with *Xoo*

(**A**) Disease lesion lengths of *OsSerk10x* T_1 plants in Kitaake background (Kit-*OsSerk10x*) and the Xa21 Kitaake (*Xa21*-Kit) and Kitaake control plants at 15 d after inoculation (DAI) at the adult stage. All plants were inoculated with *Xoo* strain PXO99, which can trigger XA21 mediated immune response, at 6 weeks old. Lesion lengths were measured at 15 DAI for at least six leaves from three or more independent plants. "+" and "−" indicate presence or absence of the *OsSerk10x* transgene revealed by PCR with Hyg specific primers (same for below). (**B**) Disease lesion lengths of *OsSerk10x* T_1 plants and the control plants at 10 DAI at the seedling stage. All plants were inoculated with PXO99 at 3 weeks old. Lesion lengths were measured for at least eight leaves from three or more independent plants at 10 DAI. Statistical significance comparison was conducted with ANOVA, where the different "**" and "*" marks above the columns indicate differences with $P \leqq 0.01$ and $P \leqq 0.05$, respectively.

Figure S4. Disease resistance determination of *Xa21*kit-*OsSerk10x and Xa21*kit-*OsSerk1Ri* lines after inoculation with a virulent *Xoo* strain to *Xa21* gene

The rice lines, *Xa21* kitaake, *Xa21*kit-*OsSerk1Ri* (#1602 and #1603) and *Xa21*kit-*OsSerk10x* (#1904 and #1953) were inoculated with the *Xoo-4* strain, which is virulent on *Xa21* plants, at 6 weeks old. Lesion lengths were measured at 15 d after inoculation (DAI) for at least six leaves from three or more independent plants. The photograph depicts representative symptom development in leaves at 15 DAI.

Figure S5. Disease resistance determination of *Xa21* Kitaake carrying *OsSerk1Ri* transgeneto *Xoo*

Four independent *OsSerk1Ri* T_1 plants and *Xa21* Kitaake (*Xa21*-Kit), Kitaake, and *Xa21*kit-*OsSerk2Ri* were included in this experiment. All plants were inoculated at 6 weeks old. "+" and "−" indicate the presence and absence of the *OsSerk1Ri* transgene in T_1 plants, respectively. Lesion lengths were measured at 20 d after inoculation (DAI).

Figure S6. Altered expression of *OsSerk1* does not affect rice resistance to *Magnaporthe oryzae*

The Digu and Lijiang are the cultivars having broad-spectrum resistance and susceptibility to blast strain ZB13, respectively. Two-week-old rice plants were used for inoculation. The lesion length was measured and pictures were taken at 7 d after inoculation (DAI). This experiment was repeated three times with same results.

Figure S7. Disease resistance determination of *Xa21* kitaake plants carrying both *OsSerk1ox and OsSerk2Ri* transgenes after inoculation with *Xoo*

(**A**) Lesion lengths of the Xa21 Kitaake, Kitaake, and *Xa21*kit-*OsSerk2Ri #A814* plants, and the three F_1 hybrid lines (*Xa21*kit-*OsSerk2RiOsSerk1ox* #3, #7, and #14). Three F_1 hybrid populations were generated by crossing #A814 with each of the three independent *OsSerk1ox* lines, *Xa21*kit-*OsSerk1ox* #3, #7, and #14, respectively. The F_1 population consisted of plants with (represented by "+") *OsSerk1ox* and without *OsSerk1ox* (represented by "−") determined by specific polymerase chain reaction (PCR) primers. The numbers of *Xa21*kit-*OsSerk1oxOsSerk2Ri* versus *Xa21*kit-*OsSerk2Ri* F_1 plants obtained were 10/7, 29/34, and 29/24 from crosses, #A814/*Xa21*kit-*OsSerk1ox* #3, #A814/*Xa21*kit-*OsSerk1ox* #7, and #A814i/*Xa21*kit-*OsSerk1ox* #18, respectively. Inoculation with PXO99 was conducted at 6 weeks old. Lesion lengths were measured at 15 d after inoculation (DAI) for at least 14 leaves from 10 or more independent plants. Statistical significance comparison was conducted with ANOVA, where the different capital letters above the columns indicate differences with $P \leqq 0.01$. (**B**) The photograph depicts representative symptom development in leaves at 15 DAI. In each F_1 population, both *Xa21*kit-*OsSerk1oxOsSerk2Ri* (+) and *Xa21*Kit-*OsSerk2Ri* (-) F_1 plants were included in the photograph.

Figure S8. Long disease lesion lengths are correlated with the presence of *OsSerk2Ri* but not with *OsSerk1ox*

Two F_2 populations (**A** and **B**) were included in this experiment that were derived from two F_1 hybrids from crosses of *Xa21*kit-*OsSerk2Ri* #A814 with *Xa21*kit-*OsSerk1ox* #7 or *Xa21*kit-*OsSerk1ox* #18. All plants, including the Xa21 Kitaake (Xa21-Kit), #A814, and Kitaake controls, were inoculated with PXO99 at 6 weeks old. Lesion lengths were measured at 15 d after inoculation (DAI). The genotyping primers specific to *OsSerk2Ri* and *OsSerk1ox*, respectively, were used to determine the plants with *OsSerk2Ri* and/or *OsSerk1ox*. In both populations, the plants containing *OsSerk2Ri* all showed longer lesion lengths than those without this transgene. The plants with and without *OsSerk1ox* showed no correlation with the disease lesion lengths.

Figure S9. Interaction of OsSERK1 and OsSERK2 in yeast

The OsSERK1 and OsSERK2 intracellular domains were fused to B42AD with HA tag and to LexA tag, respectively. The blue colors indicate interaction between the two co-expressed proteins. This experiment was repeated three times with same results.

Table S1. Lamina joint angles of flag leaves of plants carrying different combinations of transgenes *OsSerk1ox* and *OsSerk2Ri*, and the control plants at the adult stage