A rice lectin receptor-like kinase that is involved in innate immune responses also contributes to seed germination

Xiaoyan Cheng, Yan Wu, Jianping Guo, Bo Du, Rongzhi Chen, Lili Zhu and Guangcun He*

State Key Laboratory of Hybrid Rice, College of Life Sciences, Wuhan University, Wuhan 430072, China

Received 9 April 2013; revised 14 August 2013; accepted 5 September 2013; published online 15 October 2013.

*For correspondence (e-mail gche@whu.edu.cn).

SUMMARY

Seed germination and innate immunity both have significant effects on plant life spans because they control the plant’s entry into the ecosystem and provide defenses against various external stresses, respectively. Much ecological evidence has shown that seeds with high vigor are generally more tolerant of various environmental stimuli in the field than those with low vigor. However, there is little genetic evidence linking germination and immunity in plants. Here, we show that the rice lectin receptor-like kinase OslecRK contributes to both seed germination and plant innate immunity. We demonstrate that knocking down the OslecRK gene depresses the expression of α-amylase genes, reducing seed viability and thereby decreasing the rate of seed germination. Moreover, it also inhibits the expression of defense genes, and so reduces the resistance of rice plants to fungal and bacterial pathogens as well as herbivorous insects. Yeast two-hybrid and co-immunoprecipitation experiments revealed that OslecRK interacts with an actin-depolymerizing factor (ADF) in vivo via its kinase domain. Moreover, the rice adf mutant exhibited a reduced seed germination rate due to the suppression of α-amylase gene expression. This mutant also exhibited depressed immune responses and reduced resistance to biotic stresses. Our results thus provide direct genetic evidence for a common physiological pathway connecting germination and immunity in plants. They also partially explain the common observation that high-vigor seeds often perform well in the field. The dual effects of OslecRK may be indicative of progressive adaptive evolution in rice.

Keywords: rice, lectin receptor-like kinase, seed germination, innate immunity, pleiotropy, progressive fitness.

INTRODUCTION

It is commonly observed that sowing high-vigor seeds tends to result in good seedling establishment and increases the seedlings’ ability to withstand various environmental stresses in the field. Germination is the first stage in the plant lifecycle, and events that occur during germination have strong effects on post-germination traits, as indicated by numerous lines of ecological and evolutionary evidence (Donohue et al., 2010). Genetic investigations have revealed extensive inter-connections between germination and post-germination processes, including numerous interactions between various aspects of growth or development and immune responses (Donohue et al., 2010; Alcázar et al., 2011). These are generally considered to represent evolutionary cost–benefit trade-offs that strike a balance between the capacity to acclimatize quickly to unfavorable environments and the capacity for rapid growth and development (Chiang et al., 2009; Donohue et al., 2010; Albrecht et al., 2011; Alcázar et al., 2011).

Seed germination may be regarded as resumption of the temporarily suspended growth of the plant embryo. It is initiated when the quiescent dry seed imbibes water. This is followed by the reactivation of metabolic processes, and concludes with elongation of the embryonic axis (Bewley, 1997). In cereals, germination is dependent on the degradation of storage reserves in mature seeds. Specifically, the enzymatic hydrolysis of starch into metabolizable sugars is the primary source of energy for the emerging seedling (Beck and Ziegler, 1989). The major enzyme involved in hydrolyzing starch into glucose is α-amylase (EC 3.2.1.1), which cleaves the α-1,4-glycosidic bonds: it attacks starch granules directly and accounts for 40-60% of the de novo protein synthesis in grains (Beck and Ziegler, 1989; Mitsunaga et al., 2001). β-amylase (EC 3.2.1.2) is responsible for the remaining amylase activity during seed germination, and catalyzes the conversion of starch into maltose by cleaving the second α-1,4 glycosidic bond; the maltose
produced in this way is subsequently converted into glucose (Mitsunaga et al., 2001; Smith et al., 2005). Many plant hormones and sugars function as signaling agents and thereby contribute to the regulation of \( \alpha \)-amylase gene expression (Yu et al., 1996; Peng and Harberd, 2002; Barreto et al., 2013). However, because germination is an intricate and multi-stage process that involves numerous cellular and metabolic changes (Bewley, 1997; Yang et al., 2007; Nonogaki et al., 2010), it is reasonable to assume that there are a range of other factors that also influence expression of the amylase genes.

Plants have co-evolved with a wide range of natural enemies such as fungi, bacteria and insects. As a result, they have developed a degree of innate immunity based on a range of immune responses that are activated to repel specific attacks (Rausher, 2001). The immune responses are accomplished by defense-related genes associated with signal transduction, hypersensitive cell death, and downstream defense responses (Reymond and Farmer, 1998). The expression of defense-related genes is usually up-regulated in response to attacks by pathogens and insects. Many of these genes have dual or multiple functions in various physiological processes (van Loon et al., 2006). Together, they are involved in and define a series of cellular and metabolic changes (Bewley, 1997; Yang et al., 2007; Nonogaki et al., 2010), which are known to be involved in diverse physiological processes, including pollen development and pathogen resistance (Wan et al., 2008; Bouwmeester et al., 2011; Singh et al., 2012). Here, we show that a G-type lectin receptor kinase gene from rice, *OslecRK*, promotes seed germination, enhancing seed vigor by inducing \( \alpha \)-amylase gene expression. Evidence is presented showing that OslecRK influences the expression of defense-related genes that affect biotic stress tolerance in rice. We also observed direct interactions between the kinase domain of OslecRK and an actin-depolymerizing factor (OsADF). The rice mutant *adf*, in which the *OsADF* gene is silenced, was found to have a reduced rate of seed germination and depressed immune responses to the fungal blast pathogen *Magnaporthe grisea*, the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo), and the herbivorous insect brown plant hopper (*Nilaparvata lugens*, BPH). Quantitative PCR analyses showed that OsADF is a component of two signaling pathways mediated by OslecRK that affect two distinct physiological processes. All the results have thus demonstrated that the defense-related gene OslecRK is important in the regulation of \( \alpha \)-amylase gene expression during germination. This represents an important insight into the relationship between seed vigor and plant resilience to environmental stresses. Moreover, the beneficial effects of OslecRK on seed germination and plant innate immunity suggest that rice may have undergone progressive adaptive evolution.

**RESULTS**

**Characteristics of OslecRK**

The existence of OslecRK was predicted by the Rice Genome Automated Annotation System (RiceGAAS)
OslecRK was cloned; its open reading frame (ORF) is 2880 bp long. After rapid amplification of 3' and 5' cDNA ends (RACE) within the target region by means of PCR with appropriate primers, the whole length of OslecRK was cloned; its open reading frame (ORF) is 2880 bp long.

Analyses of transcripts from various tissues revealed that OslecRK has a distinctive pattern of expression. Semi-quantitative RT–PCR experiments showed that OslecRK fragments were only amplified from plumule, radicle and panicle samples; no fragments were detected in coleoptile, root, leaf and stem samples (Figure 1a). It is thus expressed specifically in the plumule, radicle and panicle, which is similar to the expression pattern predicted based on an analysis using the NCBI UniGene dbEST database (Benson et al., 2005) (Figure S1). It was also found that OslecRK is only expressed during germination and flowering rather than during the vegetative phase. However, quantitative PCR analysis revealed that OslecRK expression was up-regulated when rice plants in the vegetative phase were treated with the fungus M. grisea or the bacterium Xoo, or exposed to the herbivorous insect BPH. This suggests that stresses induce OslecRK expression (Figure 1b), which is not consistent with the expression pattern predicted based on massively parallel signature sequencing of the rice genome (Nakano et al., 2006) (Table S1).

Southern blot experiments using DNA extracted from the rice varieties Hejiang 19 and B5 were performed to determine the genomic copy number of OslecRK. In both cases, only one strong band was obtained with the selected probe (Figure S2). A BLAST (Altschul et al., 1990) search against a rice genome database (http://www.gramene.org/) also identified only one copy of OslecRK.

Previous reports have characterized many lectin receptor-like kinases from other plants, including Arabidopsis thaliana, Nicotiana benthamiana and Medicago truncatula (Navarro-Gochicoa et al., 2003; Kanzaki et al., 2008; Bouwmeester and Govers, 2009). An unrooted phylogenetic tree for OslecRK was constructed, showing that it occupies a branch by itself rather than sharing one with other lectin receptor-like kinases such as ACR15163 (P1-d2) from rice (Chen et al., 2006) (Figure 1c and Table S2).

Transgenic OslecRK-silenced rice plants show inhibited germination

To study the function of OslecRK in plants, RNA interference (RNAi) knockdown experiments were performed in rice (Chen et al., 2007). We found that the seeds of the RNAi lines had low germination rates in 3-day germination tests, ranging from 23.3 to 43.3%. This is appreciably lower than the rate of 83.3% observed for wild-type (WT) seeds (Figure 2a). However, there were no statistically significant phenotypic differences between the RNAi line and the WT during the later vegetative and reproductive periods. The germination rate of the homozygotic RNAi strain Ri2–10 (Ri) (Figure 2b) was monitored over three successive generations, confirming that its low rate of germination was due to the suppression of OslecRK expression (Figure 2c). This clearly demonstrates that OslecRK influences the rate of seed germination.

The inhibition of germination in OslecRK-silenced plants is due to repression of α-amylase expression

Seed vigor refers to the sum total of those properties of the seed that determine its activity and performance during germination and seedling emergence (Copeland and McDonald, 2001). Mature seeds from WT and Ri rice were treated with the redox indicator TTC (2,3,5-triphenyltetrazolium chloride) to assess their viability (Porter et al., 1947; Towill and Mazur, 1975). Parts of the plumules, hypocotyls and scutellae from the Ri embryos were stained pink, while the radicles were barely stained at all. Conversely, the radicles, plumules, hypocotyls and scutellae of WT embryos were stained deep red (Figure 3a). This clearly shows that the Ri embryos were less viable than their WT counterparts, consistent with the lower germination rates of the Ri seeds. The seeds of Ri plants are thus less vigorous than WT seeds and so have a lower rate of germination.

Figure 1. The unique characteristics of the OslecRK gene.
(a) OslecRK is expressed differently in the various rice organs examined by RT-PCR. Its transcripts were detected only in plumules, radicles and panicles. The β-actin gene was used as a reference control to normalize the samples.
(b) OslecRK expression is induced by biotic attacks, as demonstrated by quantitative PCR. The biotic stresses examined include inoculation with the fungal pathogen M. grisea and the bacterial pathogen Xoo, and infestation with the herbivorous insect BPH. This suggests that stresses induce OslecRK expression (Figure 1b), which is not consistent with the expression pattern predicted based on massively parallel signature sequencing of the rice genome (Nakano et al., 2006) (Table S1).
(c) Unrooted phylogenetic tree including all of the lectin receptor-like kinases that have been isolated from plants to date. Bootstrap values are shown at each branch point (percentage of 1000 bootstrap samples). The black circle indicates that OslecRK is in a branch of its own. All of the relevant accession numbers are provided in Table S2.

© 2013 The Authors.
The Plant Journal © 2013 John Wiley & Sons Ltd, The Plant Journal, (2013), 76, 687–698
To determine whether the depressed vigor of the Ri seeds was due to any structural abnormality in the embryos, paraffin sections were taken and examined by microscopy. As shown in Figure 3(b), there were no morphological differences between the coleorhiza, radicles, coleoptiles, plumules or hypocotyls of the Ri and WT seed embryos. This implies that suppression of OslecRK does not affect seed germination rates by inducing formation of embryos with abnormal structural phenotypes.

In rice, amylase activity has been identified as an important index of seed vigor during germination (Karrer et al., 1992; Nandi et al., 1995). The α-amylases encoded by OsAmy1A, OsAmy1B, OsAmy1C, OsAmy2A, OsAmy3D and OsAmy4A, together with the β-amylases encoded by OsBmy1, OsBmy2 and OsBmy3, are considered to be essential for starch degradation (Hwang et al., 1999; Kossmann and Lloyd, 2000; Lao et al., 2002). A quantitative PCR study was therefore performed to compare the
expression of these genes in Ri and WT seeds. It was found that the level of α-amylase gene expression in Ri seeds was significantly lower than that in WT seeds, whereas the expression of β-amylase genes appeared identical in both cases (Figure 3c). We also investigated the amylase activity of the seeds during early germination, and found that, while Ri seeds exhibited substantially lower α-amylase activity than WT seeds, the levels of β-amylase activity were similar in both cases (Figure 3d). These results demonstrate that knocking down OslecRK has adverse effects on expression of α-amylase genes and thereby reduces seed vigor, but does not affect expression of β-amylase genes.

To confirm the regulation of α-amylase genes by OslecRK during germination, seed embryos were separated from their endosperms and grown on media containing various sugars. When the embryos were grown on half-strength MS medium containing sucrose (RM), glucose (RM-G) or maltose (RM-M), both Ri and WT embryos exhibited similar germination rates. Neither embryo type grew when placed on half-strength MS medium without sucrose (RM–). However, when starch-containing medium (RM–) was used, the WT embryos developed small shoots but the Ri embryos did not (Figure 3e and Figure S3). Both monosaccharides such as glucose and disaccharides such as sucrose and maltose may be directly absorbed from the medium and used to fuel germination without requiring α-amylase activity. However, the starch from the RM– medium requires hydrolysis by α-amylases before being used in this way, which is why the Ri embryos failed to germinate in this case. These results are consistent with the hypothesis that OslecRK promotes α-amylase gene expression and thereby facilitates seed germination.

**OslecRK is a component of the innate immune system in plants**

OslecRK was cloned from the region of chromosome 4 with a Bph15 locus for BPH resistance in rice (Huang et al., 2001; Yang et al., 2004). We compared the performance of BPH on OslecRK-silenced (Ri) and WT rice plants to determine whether OslecRK is important for resistance to attacks by this herbivorous insect in rice (Du et al., 2009; Kloth et al., 2012). As shown in Figure 4(a) and Figure S4 (a,b), more insects congregated on the Ri plants than on the WT plants, indicating that Ri plants were less resistant to BPH than WT plants. The preference of BPH for the Ri plants suggests that OslecRK confers resistance to BPH attacks via anti-xenosis, which is known to be an important component of plant innate immunity against insect attacks (Walling and Thompson, 2012).

It has been noted that plants’ immune responses towards plant pathogens are analogous to those induced by microbial pathogens (Kaloshian and Walling, 2005; Cheng et al. 2013). Therefore, we also challenged the Ri and WT plants with two important rice pathogens: the causative agents of fungal blast (M. grisea) and bacterial blight (Xanthomonas oryzae pv. oryzae, Xoo). We observed that more fungal spores grew on Ri plants than on WT plants (Figure 4b) following inoculation with the fungus M. grisea, which suggests that OslecRK expression confers enhanced resistance to blast disease (Figure S4c,d). Similarly, when Ri and WT rice plants were inoculated with the bacterium Xoo, bacterial growth was faster on the Ri seedlings (Figure 4c), and the Ri plants displayed more substantial lesions (Figure S4e,f). These results clearly show that Ri plants are less resistant to pathogens than their WT counterparts, suggesting that OslecRK is important for resistance to both fungal disease and bacterial infection in rice.

Gene expression analysis revealed that defense-related response genes such as PR1a (basic pathogenesis-related gene 1), LOX (encoding a lipoxygenase) and CHS (encoding a peroxidase) (Buell and Somerville, 1995; Qi et al., 2007; Du et al., 2009) were expressed less strongly in Ri plants than in WT plants following exposure to biotic

---

**Figure 4. Rice immune responses to biotic invasions after inoculation.**

(a) Congregation of BPHs on WT and Ri rice plants after infestation in the host selection test. BPHs congregate on the OslecRK-silenced rice plants to a greater extent than on WT plants. This shows that BPHs prefer OslecRK-silenced rice plants to WT plants.

(b) Growth of blast fungus on Ri and WT rice plants. M. grisea grows more extensively on the OslecRK-silenced rice plants than on WT plants after inoculation at a spore suspension concentration of 5 × 10⁶ conidia ml⁻¹.

(c) Growth of blight bacterium on Ri and WT rice seedlings. Xoo grows more quickly on the OslecRK-silenced rice plants than on WT plants following treatment at a dose of 9 × 10⁸ bacteria ml⁻¹.

(d) Expression of defense-related genes in rice after exposure to biotic stresses. PR1a, LOX and CHS are induced less strongly in OslecRK-silenced rice plants than in WT plants. M. plants inoculated with M. grisea; X, plants inoculated with Xoo; B, plants incubated with brown planthoppers.

In all panels, the error bars represent SD values for three biological replicates. Asterisks indicate statistically significant differences relative to WT (*P < 0.05, one-way ANOVA). WT, Hejiang 19; Ri, OslecRK-silenced rice line R12–10.
stress (Figure 4d). This indicates that OslecRK contributes to the innate immune responses of rice to attacks by herbivorous insects and plant pathogens, and that it participates in multiple defense signaling pathways.

**OslecRK interacts with OsADF via its cytosolic kinase domain in vivo**

To determine how OslecRK affects two different biological processes, OslecRK was used as bait to screen a rice cDNA expression library (Hu et al., 2011) established in a yeast two-hybrid (Y2H) system. After sequencing, we found that a prey clone encoding an actin-depolymerizing factor (ADF) interacted with OslecRK. ADFs are multi-faceted molecules that are involved in various physiological processes and immune responses to biotic stresses (Miklis et al., 2007; Porter et al., 2012). The complete coding sequence of the prey clone was isolated and named OsADF. BLAST analysis showed that OsADF exhibits 75% sequence identity with actin-depolymerizing factor 3 from Zea mays (ZmADF3) (Lopez et al., 1996), 62% identity with Arabidopsis actin-depolymerizing factor 4 (AtADF4) (Tian et al., 2009) and 59% identity with Arabidopsis actin-depolymerizing factor 2 (AtADF2) (Clément et al., 2008).

To further investigate the interactions between OslecRK and OsADF in vivo, two recombinant proteins, Lec and Kp, were created that consist only of the lectin and kinase domains, respectively. An additional protein, LecKp, was created that consists of both the lectin domain and the kinase domain. The interaction between OslecRK and OsADF was examined by cloning the sequences encoding Lec, Kp, LecKp and OslecRK into the pGBK7T7 vector. In addition, the sequence encoding OsADF was cloned into the pGADT7 vector. The clones were then used in a Y2H screen, which revealed that Kp, LecKp and OslecRK interacted with OsADF but Lec did not (Figure 5a). This implies that OslecRK binds to OsADF via its kinase domain rather than the lectin domain. An in vivo co-immunoprecipitation experiment was then performed to confirm the interaction between OslecRK and OsADF. The proteins Lec, Kp, LecKp and OslecRK were fused with hemagglutinin (HA) to give fusion proteins named Lec:HA, Kp:HA, LecKp:HA and RK:HA, respectively, while OsADF was fused with Myc (OsADF:Myc). No signal was detected when Lec and OsADF were co-expressed in *Nicotiana benthamiana* in tobacco leaves (Figure 5b). These results verify that OslecRK interacts with OsADF in vivo, and that the kinase domain of OslecRK is necessary for the interaction.

The rice afd mutant exhibits a reduced germination rate and resistance to biotic stresses

To further explore the regulatory effects of OslecRK, we examined the phenotype of a mutant strain generated by T-DNA insertion into the OsADF gene in Dongjin (DJ) rice (Jeon et al., 2000; Jeong et al., 2006). The homogenous mutant line TA2–5, which carries the *adf* mutation (Figure S5), was compared to wild-type DJ rice. The germination rate of TA2–5 was lower than that of DJ during the early stages of germination (Figure 6a), consistent with previous reports concerning the importance of ADF for plant viability (Augustine et al., 2008). The expression of amylase genes involved in germination in the two lines was investigated using quantitative PCR. It was found that the three α-amylase genes *OsAmy1A*, *OsAmy1C* and *OsAmy3D* were expressed less strongly in TA2–5 than in DJ, but the expression of other α-amylase genes (*OsAmy1B*, *OsAmy2A* and *OsAmy2C*) were expressed more strongly in TA2–5 than in DJ.
OsBmy1 and β-amylase genes (OsBmy1, OsBmy2 and OsBmy3) in the two lines did not differ significantly (Figure 6b). We also observed that there were no significant differences in the expression of OslecRK in the two lines during germination, but that the expression of OsADF was inhibited after OslecRK silenced (Figure S6). These results indicate that OsADF may influence the expression of α-amylase genes during seed germination.

We also investigated the immune responses of the two rice lines to biotic stresses. Host selection experiments showed that the TA2-5 plants were more attractive to adult BPH than DJ plants (Figure 7a), demonstrating that OsADF affects plant resistance to this herbivore. Similarly, when inoculated with the fungal pathogen M. grisea, TA2-5 plants were less resistant than DJ (Figure S7a), and more fungal spores grew on TA2-5 plants than on DJ plants (Figure 7b). This suggests that OsADF is also involved in resistance to blast disease. After inoculation with the bacterial pathogen Xoo, the TA2-5 plants exhibited more lesions than DJ (Figure S7b), and the bacterium grew more rapidly on the TA2-5 plants than on DJ plants (Figure 7c). This shows that OsADF is contributing factor in resistance to bacterial blight disease. Overall, these results suggest that OsADF plays a role in the plant’s defense against a wide range of biotic attacks, consistent with previous reports that ADF proteins play important roles in mediating plant defense responses (Miklis et al., 2007; Clément et al., 2009; Tian et al., 2009).

We also investigated the expression of the defense-related genes PR1a, LOX and CHS in the TA2-5 and DJ lines after exposure to the above biotic stresses. All of these genes were expressed much less strongly in the TA2-5 line (Figure 7d). In addition, OslecRK was expressed at similar levels in both lines, whereas OsADF expression during immune responses to biotic stresses was down-regulated in plants where OslecRK expression was knocked down (Figure S6). These results support the hypothesis that OsADF affects the expression of downstream defense-related genes that are important for basal defense responses to attacks by insects and pathogens. Overall, our results indicate that OsADF influences the expression of both amylase genes and defense-related genes, thereby promoting both seed germination and basal defense responses.

**DISCUSSION**

**OslecRK exhibits unique traits that are not found in other G-type lectin receptor-like kinases**

OslecRK is a G-type lectin receptor-like kinase isolated from rice. It has the characteristic structure of proteins belonging to this family, featuring an extracellular G-type lectin domain, a transmembrane region, and an intracellular kinase domain (Hervé et al., 1996; Bouwmeester and Govers, 2009). However, it has some unique traits that are not present in other G-type kinases. One is that it has a
unique spatio-temporal pattern of expression (Figure 1a,b); other members of the family are expressed constitutively in plants (Chen et al., 2006; Gilardoni et al., 2011). Another is that OslecRK does not lie on the same branch of the phylogenetic tree as the other characterized members of the family from rice and Arabidopsis (Figure 1c). These unique characteristics are consistent with the seemingly unusual role of OslecRK in the plants.

OslecRK has roles in both seed germination and innate immunity

While the Arabidopsis lecRKs have previously been reported to affect germination via the abscisic acid signaling pathway (Deng et al., 2009; Xin et al., 2009), our results show that knocking down OslecRK does not damage the physical structure of seed embryos. Instead, it directly reduces seed vigor. Our results also show that OslecRK promotes the expression of α-amylase genes, which increases seed vigor. In addition, OslecRK is involved in rice immune responses to BPH as well as blast disease and leaf blight disease (Figure 4 and Figure S5). In contrast, other lecRK genes are involved in resistance to specific individual threats such as fungi, oomycetes, bacteria or herbivores (Chen et al., 2006; Bouwmeester et al., 2011; Singh et al., 2012). The knockdown of OslecRK also reduces the expression of defense-related genes such as PR1a, LOX and CHS, which suggests that it may affect multiple defense signaling pathways. These findings clearly show that OslecRK has dual roles in seed germination and plant immunity, i.e. it is a pleiotropic gene (Stearns, 2010).

The identification of these dual functions of OslecRK partially explains the observation that seeds with high levels of vigor generally produce robust seedlings that can withstand diverse environmental stresses, while seeds of low vigor usually produce weak seedlings that are particularly susceptible to various stresses. This seems to contradict the common view that plants must make trade-offs between factors that favor growth or development on the one hand and those that confer defense against external stresses on the other (Alcázar et al., 2011).

Generally speaking, limitations on the availability of endogenous resources mean that, in order to maintain effective defenses against invasive pathogens and herbivores, plants must alter their patterns of resource allocation in a way that hampers growth or reproduction, and vice versa (Albrecht et al., 2011; Alcázar et al., 2011). Deleterious interactions of this kind are important components of the evolutionary model of compensatory adaptive fitness under natural selection (Alcázar et al. 2011; Pavlicev and Wagner, 2012). However, OslecRK functions to increase seed vigor during the early stages of germination. The energy used in this process comes from starch granules that were laid down by the plant that produced the seed rather than by the metabolic processes of the embryo (Beck and Ziegler, 1989; Mitsunaga et al., 2001). Therefore, OslecRK can promote growth in a way that does not require the reallocation of limited resources and is not deleterious to robust immune responses. Our results suggest that, rather than evolving as usually described by the theory of constrained adaptation (Alcázar et al., 2011; Pavlicev and Wagner, 2012), plants may also evolve in a progressive adaptive fashion, which suggests the development of rapid evolutionary solutions to environmental challenges.

OslecRK interacts directly with an actin-depolymerizing factor that acts on two different physiological processes

Since it was first demonstrated that there is a link between depolymerization of the actin cytoskeleton and the activation of defense responses (Kobayashi and Kobayashi, 2007), a growing body of evidence has been uncovered suggesting that actin-depolymerizing factors play crucial roles in plant defenses against biotic attacks (Miklis et al., 2007; Clément et al., 2009; Tian et al., 2009; Porter et al., 2012; Wang et al., 2013). ADF4 contributes to plant–bacterium interactions via its role as a key regulator in the AvrPphB–RPS5 signaling pathway, which links pathogen perception and the regulation of host defense genes (Tian et al., 2009; Porter et al., 2012). In barley, the ADF3 gene (HvADF3) plays an important role in plant–fungus interactions, contributing to both non-host and race-specific defense responses following recognition of powdery mildews by the MLO (barley mildew resistance locus o) protein (Miklis et al., 2007; Wang et al., 2013). In addition, root-knot nematode infections have been shown to up-regulate ADF2 expression, which in turn promotes cell maturation and thereby facilitates nematode development and reproduction. This suggests that, in some cases, actin depolymerization in plant cells may enhance parasitic infectivity (Clément et al., 2009). Overall, these results clearly indicate the importance of the actin cytoskeleton, and the regulation of its dynamic behavior by ADFs, for signal transduction in plant innate immune responses (Day et al., 2011).

We have shown that OslecRK interacts with an actin-depolymerizing factor (OsADF) in vivo, and that OsADF is important in both seed germination and plant immunity. Our results may be used to establish a preliminary outline of OslecRK’s mechanism of action (Figure 8). First, its expression is stimulated by signals relating to germination or biotic attacks. The kinase domain of OslecRK then interacts with OsADF to transduce these signals. Finally, the signals increase the expression of α-amylase genes (OsAmy1A, OsAmy1C and OsAmy3D) to promote seed germination or the expression of defense-related genes (PR1a, LOX and CHS) in order to strengthen the plant’s immune response. Our findings therefore imply that the actin cytoskeleton may participate in signal transduction mediated by OslecRK during both germination and immune responses to pathogen and insect attacks (Day et al., 2011).
Moreover, we observed that OslecRK affected six β-amylase genes while OsADF affected only three genes during germination (Figures 3c and 6b). Therefore, the influence of OslecRK on the β-amylase genes OsAmy1B, OsAmy2A and OsAmy4A does not occur through activation of OsADF. In our Y2H screening, we did not find any direct interaction of β-amylase with OslecRK. These results may indicate that OslecRK interacts with cellular factors other than OsADF to affect the expression of other β-amylase genes in early seedlings. In addition, the resistance conferred by OsADF activation is somewhat weaker than that conferred by OslecRK. These findings suggest that OslecRK may also activate other molecules during the immune response, and further investigations aimed at identifying these putative partners are clearly warranted.

EXPERIMENTAL PROCEDURES

Materials

Rice (Oryza sativa cv. Hejiang19) was transformed with Agrobacterium as described previously elsewhere (Chen et al., 2007). The Hejiang 19 variety was also used as a source of extracted DNA and RNA, and as a control in reverse genetic (OslecRK silencing) analyses. Rice adf mutant lines were isolated from rice mutants that were generated by a T-DNA insertion in the Dongjin variety of japonica rice using gG2717 (Jeon et al., 2000; Jeong et al., 2008). The mutant TAJ2-5 was a homogenous line with T-DNA inserted next to the first exon of OsADF (Figure S5). All of the experimental plants were grown at Wuhan University, China. BPH was collected from Zhejiang Province, China, and maintained on the variety TN1 (Du et al., 2009). All the primers used in gene cloning, plasmid construction, mutant characterization and quantitative PCR are listed in Table S3.

RACE cloning

Total RNA isolated from panicles was used to prepare special cDNA using a Takara (http://www.takara-bio.com/) full RACE kit according to the manufacturer’s instructions. 3′ full RACE was performed using a 3′ full RACE kit (Takara) with the gene-specific outer primer 3′GSO and the gene-specific inner primer 3′GSI. 5′ full RACE was performed by means of nested PCR in which the first round of amplification was achieved using the 5′ RACE outer primer and the gene-specific outer primer 5′GSO. Subsequent rounds of amplification were performed using the 5′ RACE inner primer and the gene-specific inner primer 5′GSI. The primers (3′ GSP1, 3′GSP2, 5′GSP1 and 5′GSP2) are detailed in Table S3.

RT–PCR and quantitative PCR

Various rice tissues were collected and stored at −80°C. RT–PCR was performed using the following thermal cycle: initial denaturation at 95°C for 3 min, 40 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 30 sec, with a final extension period at 72°C for 3 min. The β-actin gene was amplified over 22 cycles for sample normalization. The amplified products were separated on a 1% agarose gel and visualized using a molecular imager (Bio-Rad, http://www.bio-rad.com/).

Total RNA was extracted from the samples stored at −80°C, and was treated to remove DNA as described before (Du et al., 2009). DNA-free RNA (5 μg) was used to synthesize the first-strand cDNA according to the procedure supplied with the RevertAid first-strand cDNA synthesis kit (Fermentas, http://www.thermoscientificbio.com/fermentas/). The resulting new templates were amplified by quantitative PCR using SYBR Green Supermix (Bio-Rad) in the CFX96 real-time system (Bio-Rad).

Phylogenetic tree

Alignment was performed using CLUSTALX1.8 (Thompson et al., 1994) with default settings. The phylogenetic tree was created using PHYLIP3.69 (Felsenstein, 1989) with 1000 bootstrap replicates by the neighbor-joining method (Saitou and Nei, 1997).

Seed germination

Fifty seeds of each tested line were allowed to imbibne tap water at 28°C for 3 days, and then transferred to Petri dishes containing water-saturated filter papers to germinate. Seeds were regarded as having germinated when the radicle was longer than 1 mm. Germination rates were scored every day after transferred to dishes. The test was performed in triplicate and each biological replicate was performed using 50 such seed.

TTC testing

Seeds were peeled off the hulls and cut longitudinally with a scalpel. The half-seeds were soaked in a 0.2% solution of TTC at
28°C for 2 h in the dark, and then rinsed with distilled water until the water ran clear. The seeds were then observed under a light microscope. Embryos that were completely stained pink or red were considered viable, and uncolored embryos were considered non-viable. Deeper staining was assumed to reflect greater viability (Porter et al., 1947; Towill and Mazur, 1975).

**Histochemistry and microscopy**

Rice seeds were soaked in water at 28°C for 3 h and then peeled off the hulls. The mature embryos were separated carefully from the seeds, and then quickly fixed overnight at 4°C in formalin/acetate acid/alcohol fixative solution. Samples were dehydrated using an ethanol/water dilution series and embedded in paraffin (Sigma, http://www.sigmaaldrich.com/sigma-aldrich/home.html). The tissues were then cut into 10 μm sections using a microtome, stained with 1% eosin in 95% ethanol for 5 min, and examined using an Olympus (http://www.olympus-global.com/en/) BX51 microscope (Chen et al. 2007).

**Inoculation with pathogens and BPH host selection test**

*Magnaporthe grisea* strain 97–17–2 and Xoo PXO145 were cultured on tomato/ oat/agar medium and potato dextrose agar (PDA) medium, respectively (Chen et al., 2006; Qiu et al., 2007).

When the plants had grown to the four-leaf stage, they were inoculated by spraying with *M. grisea* 97–17–2 at a spore suspension concentration of 5 × 10^6 conidia ml^{-1}, according to the National Standard for assessing resistance to blight disease as described previously (Chen et al., 2006). The inoculated plants were stored in the dark at 25°C and 99% humidity for 24 h, and then transferred to a greenhouse. Their response to the disease was examined after 15 days, including evaluation of the plants’ resistance scores and testing for the growth of fungal spores (Chen et al., 2006).

To assess the rice plants’ resistance to bacterial blight, leaves from plants at the four-leaf stage were inoculated by leaf clipping and treated with PXO145 at a dose of 9 × 10^9 bacteria ml^{-1} (Kauffman et al., 1973). The severity of the resulting disease was then scored by measuring the lesion area as a fraction of the total leaf surface area 15 days after inoculation. Mock-inoculated (control) plants were treated in the same way but using water instead of the pathogen suspension.

In the host selection test, four 30-day-old rice plants were grown in one plastic bucket. Two are the control WT, others are from the OslecRK-silenced rice lines. And the plants from the same line were in opposition to each other. Twenty third- or fourth-instar BPH nymphs were then placed in the buckets, and the number of nymphs that settled on each plant was recorded daily after release (Qiu et al., 2010). Each biological replicate was performed using ten plastic buckets.

**Y2H analysis**

The Y2H assay was performed using the Matchmaker GAL4 yeast two-hybrid system 3 (Clontech, http://www.clontech.com/) according to the manufacturer’s instructions. The OslecRK cDNA and its domain derivatives were sub-cloned into pGBK7T, while the OsADF cDNA was ligated into pGADT7. The hybrid yeast cells were incubated at 28°C on selection plates containing SD medium with Aureobasidin A and X-α-gal (a chromogenic substrate for yeast galactosidase) but lacking Leu, Trp, Ade and His. SD medium is synthetically defined as medium which is the minimal media that is routinely used for culturing yeast. It is comprised of a nitrogen base, a carbon source and a dropout supplement.

**In vivo co-immunoprecipitation assay**

The agroinfiltration assay for *Nicotiana benthamiana* was performed as described previously (Voinnet et al., 2003). The tobacco leaves were then homogenized in 2 ml protein extraction buffer (25 mM Tris/HCl, pH 7.5, 50 mM NaCl, 0.2% Triton X-100, 10 mM EDTA, 1 mM phenylmethyisulfonlfyl fluoride, and 1× protease inhibitor cocktail (Sigma)) per 1 g of the material (Mackey et al., 2002). After centrifugation at 20 000 g for 15 min at 4°C, the supernatant was transferred to new tube; 1 ml of this material was combined with 40 μl of uMACS anti- HA microbeads (Miltenyi Biotec, https://www.miltenyibiotech.com/en/), and the resulting mixture was incubated for 1 h at 4°C. The precipitated samples were collected according to the manufacturer’s instructions (uMACS™ epitope tag protein isolation kit; Miltenyi Biotec).

**Accession numbers**

Sequence data for OslecRK may be found in the GenBank database under accession number KC131131.

**ACKNOWLEDGEMENTS**

We thank Professor Shiping Wang (Huazhong Agricultural University, China) for kindly providing rice disease pathogens 97–17–2 and PXO145, and Professor Zhiyong Gao (Wuhan University, China) for technical assistance with *in vivo* co-immunoprecipitation. This work was supported by the National Special Key Project on Functional Genomics and Biochip of China (grant number 2012AA10A303), the National Natural Science Foundation of China (grant number 30730062), the National Program of Transgenic Variety Development of China (grant number 2011ZX08009004) and funds from the Ministry of Agriculture of China (grant number 2011ZX08009-003-001).

**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article.

**Figure S1.** The expression pattern for OslecRK predicted based on an analysis using the NCBI UniGene dbEST database.

**Figure S2.** Southern blot of OslecRK in two rice varieties (B5 and Hejiang 19).

**Figure S3.** Percentage of germinating shoots that developed from seed embryos grown on media containing various sugars.

**Figure S4.** The OslecRK-silenced rice plants exhibit reduced resistance to pathogens and herbivorous insects.

**Figure S5.** Characterization of the rice *adf* mutant.

**Figure S6.** Expression of OslecRK in the rice *adf* mutant and OsADF in *Ri* plants during germination, and immune responses to invasions.

**Figure S7.** Resistance of the *adf* mutant to disease-causing pathogens.

**Table S1.** OslecRK expression patterns predicted by massively parallel signature sequencing.

**Table S2.** Accession numbers used in phylogenetic analysis.

**Table S3.** Oligonucleotide primers used in cloning, plasmid construction and quantitative PCR.

**Methods S1.** Plasmid construction and Southern blotting.
The dual physiological functions of rice OslecRK

Deng, K., Wang, Q., Zeng, J., Guo, X., Zhao, X., Tang, D. and Liu, X. (2009) A lectin receptor kinase positively regulates ABA response during seed germination and is involved in salt and osmotic stress response. J. Plant Biol. 52, 493-500.

Desclos-Theyenviau, M., Arnaud, D., Huang, T.Y., Lin, G.J.C., Chen, W.Y., Lin, Y.C. and Zimmerli, L. (2012) The Arabidopsis lectin receptor kinase LecR-K-V5 represses stomatal immunity induced by Pseudomonas syringae pv. tomato DC3000. PLoS Pathog. 8, e1002513.

Donohue, K., Rubio de Casas, R., Burghardt, L., Kovach, K. and Willis, C.G. (2010) Germination, postgermination adaptation, and species ecological ranges. Annu. Rev. Ecol. Evol. Syst. 41, 293-319.

Du, B., Zhang, W., Liu, B. et al. (2009) Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. Proc. Natl Acad. Sci. USA 106, 22163-22168.

Durst, S., Nick, P. and Maisch, J. (2013) Nicotiana tabacum actin-depolymerizing factor 2 is involved in actin-driven, auxin-dependent patterning. J. Plant Physiol. 170, 1057-1066.

Felsenstein, J. (1989) PHYLIP – phylogeny inference package (version 3.2). Cladistics, 5, 164-166.

Gilaridi, P.A., Hettenhausen, C., Baldwin, I.T. and Bonaventure, G. (2011) Nicotiana attenuata LECTIN RECEPTOR KINASE1 suppresses the insect-mediated inhibition of induced defense responses during Manduca sexta herbivory. Plant Cell, 23, 3512-3532.

Heng, J.J., Bledsoe, S.W., Khurana, P., Meagher, R.B., Day, B., Blanchon, L. and Staiger, C.J. (2011) Arabidopsis actin depolymerizing factor4 modulates the stochastic dynamic behavior of actin filaments in the cortical array of epidermal cells. Plant Cell, 23, 3711-3726.

Hervé, C., Dabos, P., Galaud, J.-P., Rouge, R. and Lescure, B. (1996) Characterization of an Arabidopsis thaliana gene that defines a new class of putative plant receptor kinase with an extracellular leucine-like domain. J. Mol. Biol. 258, 778-788.

Hervé, C., Serres, J., Dabos, P., Canut, H., Barre, A., Rouge, P. and Lescure, B. (1999) Characterization of the Arabidopsis lecRK-a genes: members of a superfamily encoding putative receptors with an extracellular domain homologous to legume lectins. Plant Mol. Biol. 39, 671-692.

Hu, J., Zhou, J., Peng, X., Xu, H., Liu, C., Du, B., Yuan, H., Zhu, L. and He, G. (2011) The Bph008a gene interacts with the ethylene pathway and transcriptionally regulates MAPK genes in the response of rice to brown planthopper feeding. Plant Physiol. 156, 858-872.

Huang, Z., He, G., Shu, L., Li, X. and Zhang, Q. (2001) Identification and mapping of two brown planthopper resistance genes in rice. Theor. Appl. Genet. 102, 929-934.

Hwang, Y.S., Thomas, B.R. and Rodriguez, R.L. (1999) Differential expression of rice α-amylase genes during seedling development under anoxia. Plant Mol. Biol. 40, 911-920.

Jeon, J.-S., Lee, S., Jung, K.-H. et al. (2000) T-DNA insertion mutagenesis for functional genomics in rice. Plant J. 22, 561-570.

Jeong, D.-H., An, S., Park, S. et al. (2006) Generation of a flanking sequence-tag database for activation-tagging lines in japonica rice. Plant J. 45, 123-132.

Joshi, A., Dang, H.Q., Vaid, N. and Tuteja, N. (2009) Pea lectin receptor-like kinase promotes high salinity stress tolerance in bacteria and expresses in response to stress in plants. Glycoconj. J. 27, 133-150.

Kaloushon, I. and Walling, L.L. (2005) Hemipterans as plant pathogens. Annu. Rev. Phytopathol. 43, 491-521.

Kandasamy, M.K., Burgos-Rivera, B., McKinney, E.C., Ruzicka, D.R. and Meagher, R.B. (2007) Class-specific interaction of profiling and ADF/COIL genes: potential role inactivation in the development of plant disease. Plant Cell, 19, 3111-3126.

Kanzaki, H., Saitoh, H., Takahashi, Y., Berberich, T., Ito, A., Kamoun, S. and Terauchi, R. (2011) The Arabidopsis lectin receptor kinase gene conferring rice blast resistance. Mol. Plant Pathol. 12, 959-973.

Keller, E.E., Chandler, J.M., Foolad, M.R. and Rodriguez, R.L. (2011) The pathogen-

REFERENCES
Albrecht, C., Boutrot, F., Segonzac, C., Schwessinger, B., Gimenez-Ibanez, S., Chinchilla, D., Rathjen, J.P., de Vries, S.C. and Zipfel, C. (2011) Brassi-
norsteroids inhibit pathogen-associated molecular pattern-triggered immune signaling independent of the receptor kinase BAK1. Proc. Natl Acad. Sci. USA, 109, 303-308.
Alcazar, R., Reymond, M., Schmitz, G. and de Meaux, J. (2011) Genetic and evolutional perspectives on the crosstalk between plant immunity and development. Curr. Opin. Plant Biol. 14, 378-384.
Ali, G.M. and Komatsu, S. (2006) Proteomic analysis of rice leaf sheath during drought stress. J. Proteome Res. 5, 396-403.
Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. J. Mol. Biol. 215, 403-408.
Augustine, R.C., Vidal, L., Kleinman, K.P. and Beazanilla, M. (2008) Actin depolymerizing factor is essential for viability in plants, and its phosphore-
gulation is important for tip growth. Plant J. 54, 863-875.
Barreto, J.M., Mrva, K., Talbot, M.J., White, R.G., Taylor, J., Gubler, F. and Mares, D.J. (2013) Genetic, hormonal, and physiological analysis of late matur-
ity α-amylase in wheat. Plant Physiol. 161, 1265-1277.
Beck, E. and Ziegler, P. (1989) Biosynthesis and degradation of starch in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 95-117.
Benson, D.A., Karches-Mizrachi, I., Lipman, D.J., Gest J. and Wheeler, D.L. (2011) GenBank. Nucleic Acids Res. 33, D33-D38.
Bewley, J.D. (1997) Seed germination and dormancy. Plant Cell, 9, 1055-1066.
Bouwmeester, K. and Govers, F. (2009) Arabidopsis L-type lectin receptor kinase: phylogeny, classification, and expression profiles. J. Exp. Bot. 60, 4383-4396.
Bouwmeester, K., de Sain, M., Weide, R., Gouget, A., Klamer, S., Canut, H. and Govers, F. (2011) The lectin receptor kinase LeckR-I.9 is a novel Phy-
tothphthora resistance component and a potential host target for a RXLR effector. PLoS Pathog. 7, e1001327.
Buell, C.R. and Somerville, S.C. (1995) Expression of defense-related and
Buell, C.R. and Somerville, S.C. (1995) Expression of defense-related and

The Plant Journal © 2013 John Wiley & Sons Ltd, The Plant Journal (2013), 76, 687-698.

© 2013 The Authors.

The Plant Journal © 2013 John Wiley & Sons Ltd, The Plant Journal (2013), 76, 687-698.
Kloth, K.J., Thoen, M.P.M., Bouwmeester, H.J., Jongsm, M.A. and Dicke, M. (2012) Association mapping of plant resistance to insects. Trends Plant Sci. 17, 311-319.

Kobayashi, Y. and Kobayashi, I. (2007) Depolymerization of the actin cytoskeleton induces defense responses in tobacco plants. J. Gen. Plant Pathol. 73, 360-364.

Kessmann, J. and Lloyd, J. (2000) Understanding and influencing starch biochemistry. Crit. Rev. Plant Sci. 19, 171-226.

Lao, N.T., Schoneveld, O., Mould, R.M., Hibberd, J.M., Gray, J.C. and Kavanagh, T.A. (2002) An Arabidopsis gene encoding a chloroplast-targeted β-amylase. Plant J. 29, 519-527.

Liao, D.Q., Zhang, H.L., Bennett, J. and Li, Z.C. (2010) Characterization of evolution and tissue-expression of rice (Oryza sativa L.) α-amylase genes. Acta Agron. Sin. 36, 17-27.

van Loon, L.C., Rep, M. and Pieterse, C.M.J. (2006) Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol. 44, 135-162.

Lopez, I., Anthony, R.G., Maciver, S.K., Jiang, C.J., Khan, S., Weeds, A.G., van Loon, L.C., Rep, M. and Pieterse, C.M.J. (2006) Plant MPSS databases: signature-based transcriptional signatures of defense-related proteins in infected plants. Nucleic Acids Res. 34, D731-D737.

Nakano, M., Nobuta, K., Vemaraju, K., Tej, S.S., Skogen, J.W. and Meyers, R.G., Kossmann, J. and Lloyd, J. (2007) Barley MLO modulates actin-dependent and actin-independent antifungal defense pathways at the cell periphery. Plant Physiol. 144, 1132-1143.

Mitsunaga, S., Kawakami, O., Numata, T., Yamaguchi, J., Fukui, K. and Mikut, M., Consonni, C., Bhat, R.A., Lipka, V., Schulze-Lefert, P. and Panstruga, R. (2007) Barley MLO modulates actin-dependent and actin-indepenent antifungal defense pathways at the cell periphery. Plant Physiol. 144, 1132-1143.

M. (2005) Starch degradation. Annu. Rev. Plant Biol. 56, 73-98.

Steams, F.W. (2010) One hundred years of pleiotropy: a retrospective. Genetics, 186, 767-773.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673-4680.

Tian, M., Chaufray, F., Ruzicka, D.R., Meaghr, R.B., Staiger, C.J. and Day, J. (2009) Arabidopsis actin-depolymerizing factor ADF14 mediates defense signal transduction triggered by the Arabidopsis syngene effector AvrPphB. Plant Physiol. 150, 815-824.

Towill, L.E. and Mazur, P. (1975) Studies on the reduction of 2,3,5-triphenyl-tetrazolium chloride as a measure of seed germinability. J. Gen. Plant Physiol. 351.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F. and Shen, S. (2007) Proteomic analysis of rice (Oryza sativa L.) Theor. Appl. Genet. 110, 192-191.