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Research Article

Lectin receptor-like kinase LecRK-VIII.2 is a missing link in MAPK signaling-mediated yield control

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W.X. and S.H. performed the expression profiles analyses of the *LecRK* gene family, genotyped *lecrk-VIII.2* T-DNA mutants and generated OE lines, measured the seed size, and carried out the observation of developing seeds. W.X. and R.C. performed the GUS staining and quantitative PCR (qPCR) assays. W.X., S.H., R.C., and R.L. performed the yield-related traits analyses and the observation of seed shape. S.H., W.X., and X.Z. conducted the reciprocal cross experiment and measured the F1 seed size. W.X., S.H., and R.L. performed the FB28 staining assays. W.X., R.C., and R.L. determined the content of storage materials and protein profiles of seeds. W.X., S.H., and R.C. performed the analyses of LecRK-VIII.2-MPK3/6 genetic association. W.X. contributed to the detection of pMPK3/6, performed homologs analyses of LecRK-VIII.2, and drafted the manuscript. W.X., R.L., and X.Z. conducted the subcellular location assay. W.X., S.H., X.G., and R.Y. designed the experiments and analyzed the data. All authors reviewed and approved the manuscript.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (https://academic.oup.com/plphys/pages/general-instructions) are: Ruifeng Yao (ryao@hnu.edu.cn) and Xinhong Guo (gxh@hnu.edu.cn).

Abstract

The energy allocation for vegetative and reproductive growth is regulated by developmental signals and environmental cues, which subsequently affects seed output. However, the molecular mechanism underlying how plants coordinate yield-related traits to control yield in changing source–sink relationships remains largely unknown. Here, we discovered the lectin receptor-like kinase LecRK-VIII.2 as a specific receptor-like kinase that coordinates silique number, seed size, and seed number to determine seed yield in Arabidopsis (*Arabidopsis thaliana*). The *lecrk-VIII.2* mutants develop smaller seeds, but more siliques and seeds, leading to increased yield. In contrast, the plants overexpressing *LecRK-VIII.2* form bigger seeds, but less siliques and seeds, which results in similar yield to that of wild-type plants. Interestingly, *LecRK-VIII.2* promotes the growth of the rosette, root, and stem by coordinating the source–sink relationship. Additionally, *LecRK-VIII.2* positively regulates cell expansion and proliferation in the seed coat, and maternally controls seed size. The genetic and biochemical analyses demonstrated that *LecRK-VIII.2* acts upstream of the mitogen-activated protein kinase (MAPK) gene *MPK6* to regulate silique number, seed size, and seed number. Collectively, these findings uncover LecRK-VIII.2 as an upstream component of the MAPK signaling pathway to control yield-related traits and suggest its potential for crop improvement aimed at developing plants with stable yield, a robust root system, and improved lodging resistance.

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Introduction

The seed size/number trade-off is an important concept in life-history theory, which contributes to evolutionarily comprehending the astonishing diversity of seed sizes in plant communities (Lord et al., 1995; Moles et al., 2005). The seed size/number trade-off has been observed in different environments and studied for decades in a variety of species, but the molecular mechanism underlying how plants perceive and transduce extracellular signals to organize this process remains obscure. In plants, if the resources supplied for reproduction are fixed, the trade-off is inevitable. The increase in seed size must be compensated by a decrease in seed number (Smith and Fretwell, 1974), whereas several previous studies have shown a large variability, with negative, positive, or even uncorrelated relationships between both variables (GlendA and Mike, 1998; Eriksson, 1999; Richards, 2000; Baker et al., 2006; Koenig et al., 2009; Sõber and Ramula, 2013; Brancalion and Rodrigues, 2014; Lazaro and Larrinaga, 2018). These studies suggest that plants may possess both common and distinct mechanisms to determine their trade-off. Additionally, for seed crops, reproductive output is an accumulated outcome of both source and sink strength during plant growth and development. When the net photosynthetic rate and the rate of photoassimilate remobilization from source tissues to sink fluctuate due to abiotic and biotic stresses, the seed size/number trade-off and seed yield are subsequently affected (reviewed in Smith et al. 2018). However, our understanding about the molecular mechanism underlying how the source–sink relationship influences the trade-off remains limited.

Seed size is determined by the coordinated growth of embryo, endosperm, and maternal tissues. Up to now, numerous signaling pathways that act maternally or zygotically to regulate seed size have been identified, including the ubiquitin-proteasome pathway (Li et al., 2008; Xia et al., 2013; Du et al., 2014; Peng et al., 2015; Dong et al., 2017), G-protein signaling (Fujisawa et al., 1999; Fan et al., 2006; Huang et al., 2009; Mao et al., 2010; Chakravorty, 2011; Roy et al., 2014), mitogen-activated protein kinase (MAPK) cascade signaling (Lukowitz et al., 2004; Duan et al., 2014; Liu et al., 2015; Zhang et al., 2017), the HAIKU pathway (Luo et al., 2005; Zhou et al., 2009; Wang et al., 2010; Cheng et al., 2014), phytohormones (auxin, abscisic acid, brassinosteroid (BR), and cytokinin), and some of their downstream transcriptional factors (reviewed in Li et al., 2019). Seed size is the final result that is coordinated by complex networks involving many different developmental and environmental signals. Many regulators and pathways have been characterized, but our understanding of the regulatory networks of seed size is fragmented. Mainly as a result of this, the key factors upstream of these signaling pathways are still missing, which could integrate some of them as a network.

The receptor-like kinases (RLKs) are believed to act as sensors to mediate the cellular response toward various environmental cues, hormonal signals, and stress (De Smet et al., 2009). The *Arabidopsis thaliana* genome encodes over 600 RLKs (Shiu and Bleecker, 2001a, 2001b), and these include 75 lectin receptor-like kinases (LecRKs) that are divided into three types: C-type, G-type, and L-type (Vaid et al., 2012). LecRKs are designated by the presence of a legume–lectin protein-like extracellular domain that is structurally similar to carbohydrate-binding proteins and exhibits glucose/mannose specificity (Hervé et al., 1999). LecRKs play important and versatile roles in pollen development (Wan et al., 2008; Peng et al., 2020), seed germination (Cheng et al., 2013), fiber development (Zuo et al., 2004), legume-rhizobia symbiosis (Hirsch, 1999; Navarro-Gochicoa et al., 2003; Labbé et al., 2019), hormone signaling (Deng et al., 2009; Xin et al., 2009; Luo et al., 2017; Tripathi et al., 2018; Zhang et al., 2018), defense against pathogens and insect pests (Chen et al., 2006; Bonaventure, 2011; Gilardoni et al., 2011; Singh et al., 2012; Wang et al., 2016; Gouhier-Darimont et al., 2019; Wang et al., 2019; Luo et al., 2020; Pham et al., 2020; Woo et al., 2020; Xu et al., 2020), and responses to abiotic stresses (He et al., 2004; Joshi et al., 2010; Vaid et al., 2015; Liu et al., 2017). More importantly, recent studies revealed that LecRK-I.9 and LecRK-I.5 act as plant receptors for extracellular ATP (eATP), which are required for eATP-induced calcium response, MAPK activation, and gene expression (Choi et al., 2014; Tripathi et al., 2018; Wang et al., 2018; Pham et al., 2020). Equally, LecRK-I.8 and LecRK-VI.2 function as potential extracellular nicotinamide adenine dinucleotide ($eNAD^+$) receptors that are essential for basal resistance against bacterial pathogens (Wang et al., 2017, 2019). As pentose sugar derivatives, ATP and NAD^+ are two of the most universal energy currencies and common signaling molecules in all organisms (Choi et al., 2014; Wang et al., 2017, 2018, 2019; Pham et al., 2020). Considering that the trade-off and the energy allocation between vegetative growth and reproductive output could be involved in carbohydrate (or saccharide derivative)-based signal transduction and depend on RLKs to organize cellular activities between the inside and outside of a cell, we therefore hypothesized that LecRKs could be good candidates to dissect the molecular mechanism coordinating the process.

In this study, we report that *LecRK-VIII.2* acts upstream of *MPK6* to determine yield by coordinating silique number, seed size, and seed number. The genetic results show that LecRK-VIII.2 increases the organ size of the rosette, stem, and root, and affects their source–sink relationship. *LecRK-VIII.2* promotes cell expansion and proliferation to regulate the development of the embryo and seed coat. In general, our findings reveal the roles of LecRK-VIII.2 in regulating yield-related traits and plant growth and development.

Results

LecRK-VIII.2 exhibits specific expression in developing seeds

To test our hypothesis about the roles of LecRKs in coordinating the three interactive, yield-related traits, we performed global expression analyses of all 75 Arabidopsis

LecRKs during plant development and responses to various environmental cues. *LecRK-VIII.2* showed specific high expression during stage 4–stage 7 of developing seeds, while few transcripts were detected in other tissues (Schmid et al., 2005; Nakabayashi et al., 2005; Klaas and Francine, 2009). The analyses of the microarray data on seed development revealed that *LecRK-VIII.2* was predominantly expressed in the seed coat, but less in the embryo and endosperm (Le et al., 2010; Supplemental Figure S1, A and B). Interestingly, both independent datasets showed that *LecRK-VIII.2* transcripts exhibited accumulation from the globular to torpedo stage and displayed a common transcriptional peak in the torpedo stage of developing seeds. The patterns of *LecRK-VIII.2* were confirmed by our reverse transcription quantitative PCR (RT-qPCR) results, showing its high expression in developing seeds and flowers (Supplemental Figure S1, C and D).

To further define the *LecRK-VIII.2* functions during plant development at the protein level, we constructed *pLecRK-VIII.2::LecRK-VIII.2-GUS* transgenic plants. In 7-d-old seedlings, no β-glucuronidase (GUS) signal was detected in cotyledons, while LecRK-VIII.2-GUS was expressed in the shoot apical meristem, adventitious root primordia, lateral root (elongation zone), and primary root tip (Figure 1, A–D). In flowers, GUS activity was only observed in the pistil, ovule, filament, and receptacle but not in the sepal, carpel, anther, and pollen (Figure 1, E–G and K). After fertilization, LecRK-VIII.2-GUS was expressed in the suspensor, embryo, developing seed, and seed coat, but not in mature seed (Figure 1, H and L–O). In summary, the transcription and GUS staining data suggest that *LecRK-VIII.2* could play an important role in seed growth and development.

LecRK-VIII.2 coordinates seed size, silique number, and seed number to determine seed yield

To further investigate the function of *LecRK-VIII.2*, we identified two T-DNA insertion mutants, *lecrk-VIII.2-1* (Salk_051706) and *lecrk-VIII.2-2* (Salk_053278), and constructed two *LecRK-VIII.2* overexpression lines (OE3 and OE7). After genotyping of the T-DNA events (Figure 2, B, primers listed in Supplemental Table S1), a RT-qPCR assay was performed to check the *LecRK-VIII.2* expression level. The transcripts of *LecRK-VIII.2* were hardly detected in the T-DNA mutants, while the OE7 plant exhibited a nearly 10-fold transcriptional increase (Figure 2, C and Supplemental Figure S2, B).

The reproductive output of plants is directly determined by the silique (or spikelet) number, seed size, and seed number (Li et al., 2019). Given the expression patterns of *LecRK-VIII.2*, we examined the function of *LecRK-VIII.2* in controlling seed growth and development. Compared with the dry seeds from wild-type (WT, Columbia-0) plants, the *lecrk-VIII.2* mutant seeds exhibited significantly reduced length and width, resulting in seeds with a rounded appearance, while the seeds from the OE7 line were obviously bigger and heavier than the WT seeds (Figure 2, D–F and Supplemental Figure S2, A–D). This demonstrates that *LecRK-VIII.2* positively controls seed size and weight. Moreover, the *lecrk-VIII.2* mutants produced less seeds per silique, while the OE7 line formed more than WT plants (Figure 2, G and H), indicating that *LecRK-VIII.2* increases the number of seeds in each silique. Interestingly, when the number of siliques and total seed yield per plant were measured, we observed that the *lecrk-VIII.2* plants obviously formed more siliques and the OE7 lines but produced less than WT plants. The seed output of the *lecrk-VIII.2* mutants was significantly higher than that of the WT, whereas OE7 plants exhibited a similar reproductive output to the WT (Figure 2, I and J). In summary, *lecrk-VIII.2* mutants develop small seeds, but increase seed yield by forming more siliques and seeds. Plants overexpressing *LecRK-VIII.2* produce big seeds, but stabilize their reproductive output by reducing the amount of siliques and seeds. This reveals that *LecRK-VIII.2* acts as a key regulator to determine seed yield by coordinating seed size, silique number, and seed number.

LecRK-VIII.2 coordinates the source–sink relationship among shoots, roots, and seeds

Generally, source is defined as the capacity of net carbohydrate export in mature leaves, while sink means seeds, tubers (stems), and fruits that accumulate and consume carbohydrates. Seed yield is the cumulative result of both the strength of source and sink for photoassimilates and nutrients during seed development (reviewed in Smith et al., 2018). Given that the reproductive output was controlled by *LecRK-VIII.2*, we further asked whether it affected the energy flow between the source and the sink. Thus, we measured the organ size and biomass of the rosette, root, and stem in the different genotypes.

Interestingly, the *lecrk-VIII.2* mutants exhibited reduced size of rosettes, roots, and stems, whereas the plants overexpressing *LecRK-VIII.2* developed bigger rosettes, more robust roots, and thicker stems (Figure 3, A–F), indicating that *LecRK-VIII.2* promoted the growth of these organs. In this study, we focused on its roles in seed output (Figures 2, J and 3, C). Thus, in order to dissect the source–sink relationship, we conducted multiple comparisons of the weight ratio among shoots, roots, and seeds. The *lecrk-VIII.2* mutants showed a significantly higher ratio of seed to shoot, seed to root, and seed to total weight, suggesting that loss of function of *LecRK-VIII.2* induced energy from photosynthesis to be stored in seeds. On the contrary, the OE lines displayed a prominently decreased ratio of the three pairwise comparisons compared with WT plants, demonstrating that overexpressing *LecRK-VIII.2* resulted in more energy to be allocated for the growth of rosettes and roots, but less for seeds (Figure 3, G–K). Moreover, no differences from the ratio of root to shoot were observed between the mutants and the WT (Figure 3, L). In general, these results demonstrate that *LecRK-VIII.2* promotes biomass accumulation and regulates the source–sink relationship among shoots, roots, and seeds.

Additionally, given the altered rosette area and total biomass of the mutants and OE lines (Figure 3, D and H), we further measured cell size and chlorophyll content of

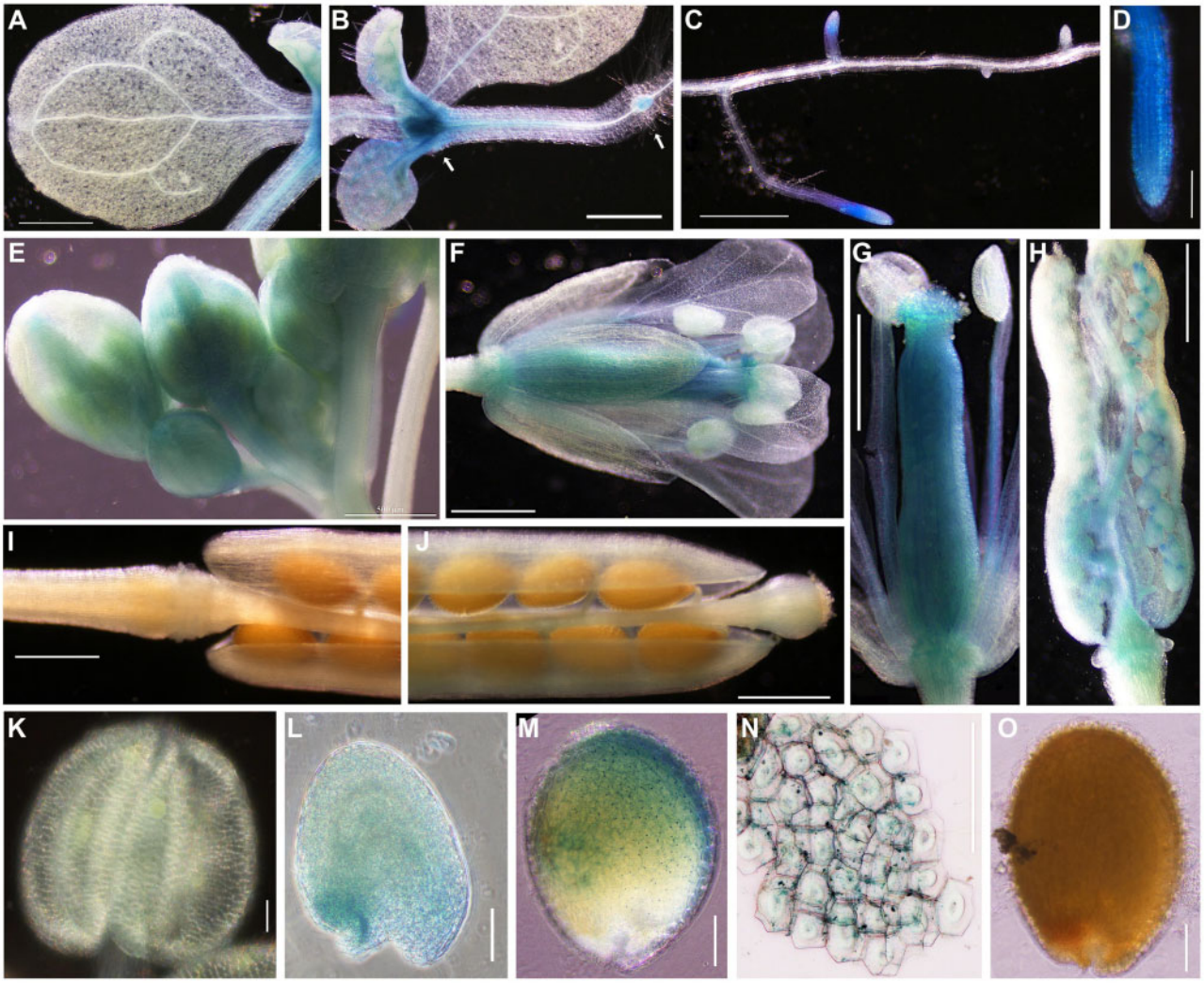

Figure 1 Tissue-specific expression of *pLecRK-VIII.2::LecRK-VIII.2-GUS*. A–O, The expression of *pLecRK-VIII.2::LecRK-VIII.2-GUS* in cotyledon (7-d-old seedling, A), shoot apical meristem (left arrow, B) and adventitious root primordia (right arrow, B), lateral root (7-d-old seedling, C), primary root tip (7-d-old seedling, D), inflorescence (E), flower (F), pistil and stamen (G), silique (2 DAF, H), mature silique (I, J), pollen (K), embryo (L), developing seed (M), seed coat (N), mature seed (O). Bar = 500 μm (A–C, E, F, G–J), 100 μm (D, K–O).

rosettes. The *lecrk-VIII.2* mutants developed smaller epidermal cells, and showed lower levels of chlorophyll a and chlorophyll b, while the OE lines exhibited opposite phenotypes (Figure 3, M–O). This suggests that *LecRK-VIII.2* promotes cell expansion to increase leaf area, and positively regulates the chlorophyll level in rosettes.

LecRK-VIII.2 positively regulates the size of the integument and embryo

In flowering plants, a seed is comprised of an embryo, endosperm, and seed coat, and seed size is determined by the coordinated growth of maternal sporophytic and/or zygotic tissues (Lopes and Larkins, 1993; Reiser and Fischer, 1993). The integument develops into the seed coat after fertilization, which can restrict seed growth physically to determine the final seed size in Arabidopsis (Schruff et al., 2006; Adamski et al., 2009; Fang et al., 2012). Thus, we observed the development of the seed coat and embryo after clearing treatment. The OE lines displayed faster growth of seeds after the globular stage, while the *lecrk-VIII.2* mutants showed a significantly decreased size of seeds compared with WT plants starting from the torpedo stage (Figure 4, A and C). The *lecrk-VIII.2* mutants exhibited reduced seed coat thickness at the beginning of the heart stage, while the seed coat of OE7 plants was thicker compared with that of the WT (Figure 4, D). Starting from the torpedo embryo stage, the difference of embryos size between WT and OE7 plants became significant, while the difference between the *lecrk-VIII.2* and WT embryos did not emerge until the mature green stage (Figure 4, E). In summary, these results demonstrate that *LecRK-VIII.2* promotes the growth of the seed coat and embryo.

LecRK-VIII.2 promotes cell expansion and proliferation, and maternally controls seed size

Organ size is determined by cell number and cell size. The GUS profile shows the specific expression of *LecRK-VIII.2* in

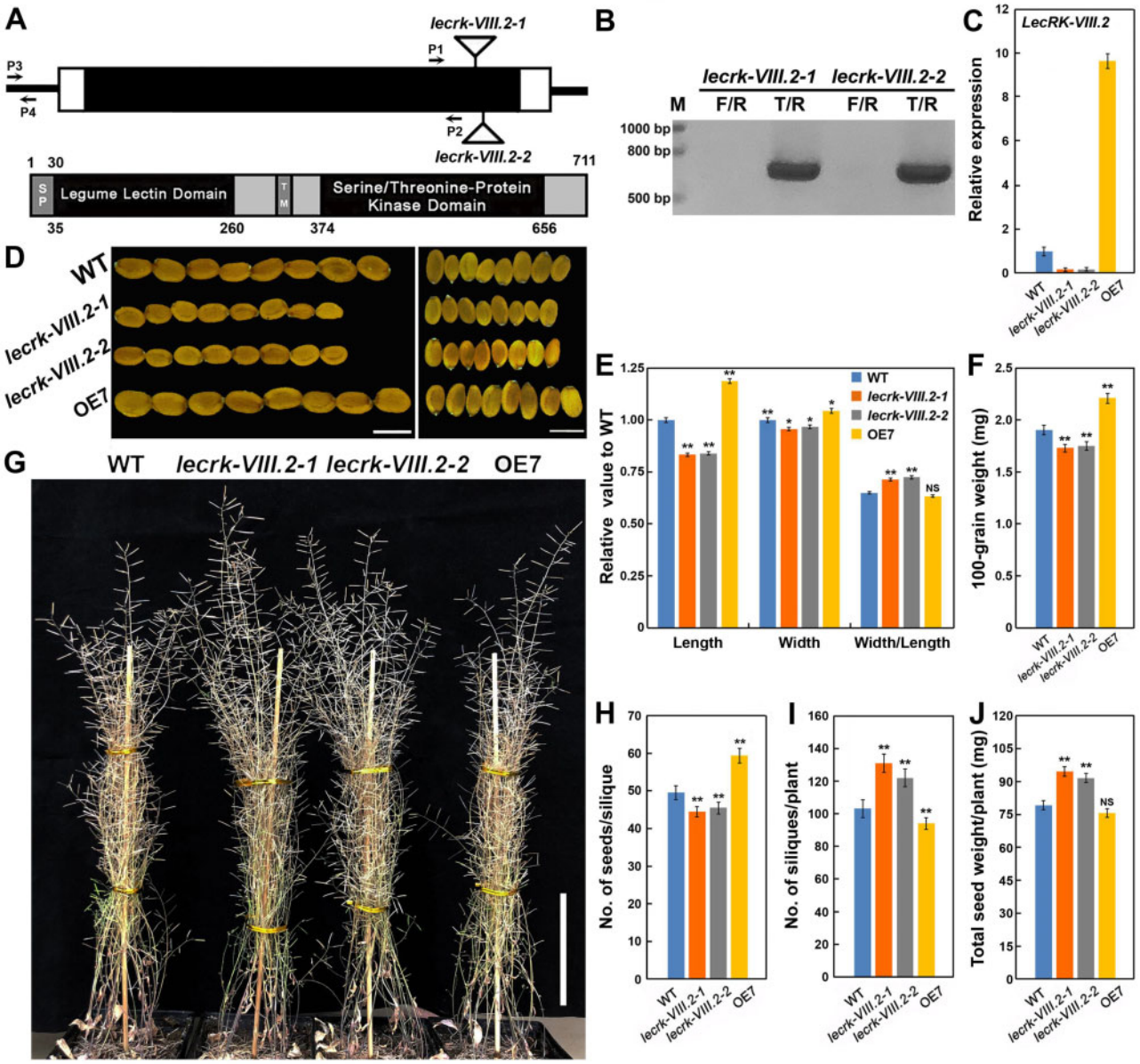

Figure 2 *LecRK-VIII.2* coordinates silique number, seed size, and seed number to determine seed yield. A, *LecRK-VIII.2* gene structure. Black box represents the exon and white boxes represent the 5′- and 3′-untranslated regions, and lines indicate intergenic regions. The T-DNA insertions sites of the *lecrk-VIII.2-1* and *lecrk-VIII.2-2* mutants are shown. The arrows of P1/P2 and P3/P4 show the positions of primers. The LecRK-VIII.2 protein contains a signal peptide (SP), legume lectin domain, transmembrane (TM), and serine/threonine protein kinase domain. B, Genotyping T-DNA insertion events in the *lecrk-VIII.2-1* and *lecrk-VIII.2-2* mutants by PCR. The band from the primers T/R means the T-DNA insertion in *LecRK-VIII.2*. The primers (*lecrk-VIII.2*-F/R/T) are listed in Supplemental Table S1. C, Relative expression level of *LecRK-VIII.2* in 7-d-old seedlings of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7. The values are based on three biological replicates. The primers (*LecRK-VIII.2*-qF/R-(P1/2)) are shown in Supplemental Table S1. D, Mature seeds of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7, bar = 500 μm. E, Seed length, width and length/width ratio of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 are shown after being normalized to the mean value of WT. Values are means ± SE ($n > 500$ seeds) relative to the WT value that is set at 1. F, 100-grain weight of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7. G, 90-d-old plants of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7, bar = 10 cm. H–J, Number of seeds per silique, number of siliques per plant, and total seed weight per plant of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7. Values are means ± SE (E, $n > 500$; F, $n > 500$ * 5 seeds; H, $n > 30$ siliques; I, $n = 12$ plants; J, $n = 12$ plants). *$P < 0.05$, **$P < 0.01$ compared with the WT, NS means no significance (Student's *t* test).

the epidermal cells of the seed coat (Figure 1, N). Therefore, we investigated the cellular basis on altered seed size of the mutants using Fluorescent Brightener 28 staining. The *lecrk-VIII.2* mutants produced significantly smaller cells than WT plants, while OE7 lines formed larger cells, indicating that *LecRK-VIII.2* positively controls cell expansion in the maternal integument. Further detection of the number of epidermal cells showed that the *lecrk-VIII.2* mutant developed slightly less cells, but OE7 lines produced more cells than WT plants (Figure 5, A–C and

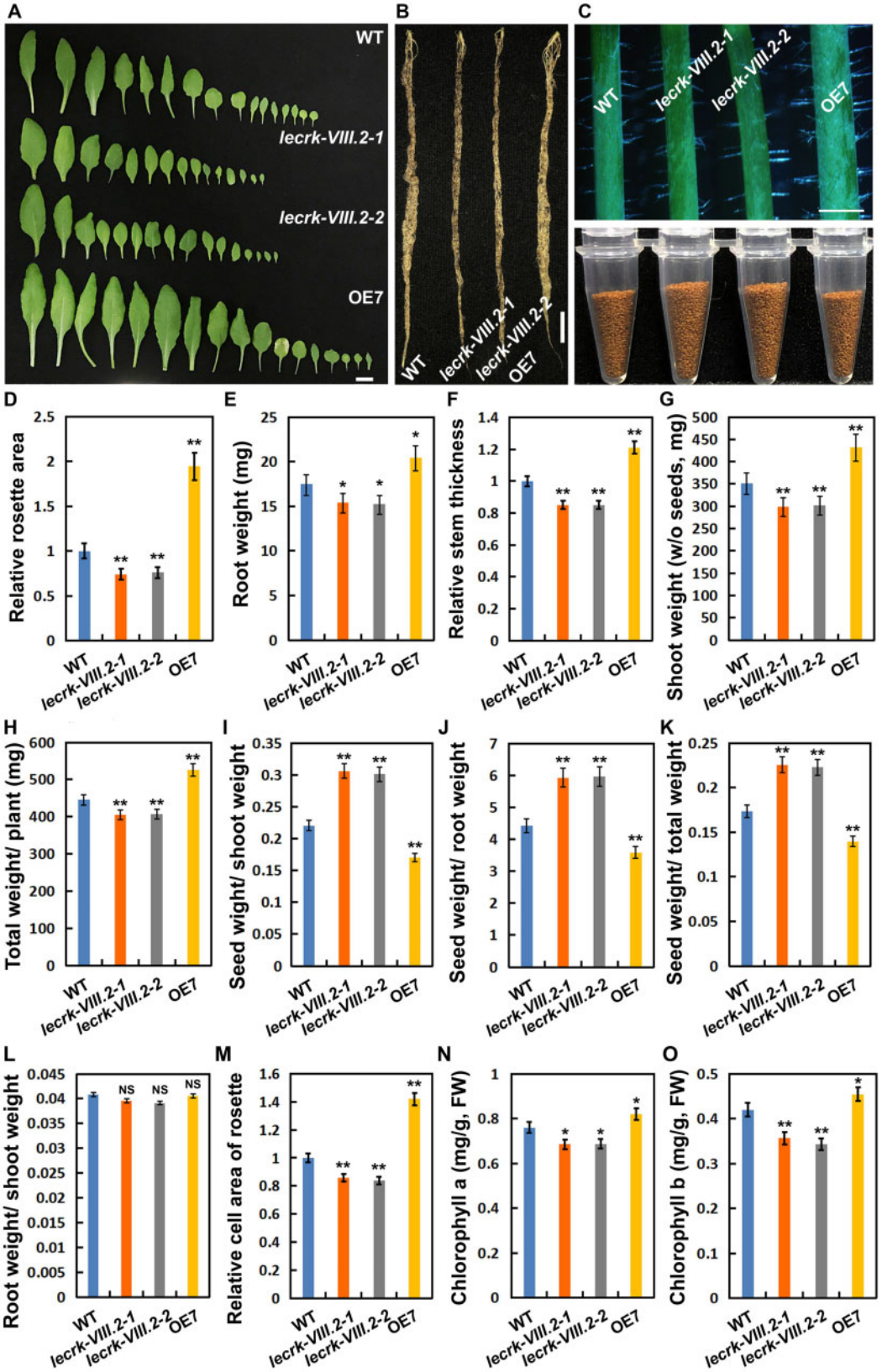

Figure 3 *LecRK-VIII.2* promotes the growth of rosettes, roots, and stems by coordinating the source–sink relationship. A, Rosettes from 35-d-old plants of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7, bar = 1 cm. B, Roots from 35-d-old plants of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7, bar = 1 cm. C, Primary stems from 35-d-old plants of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7, bar = 1 cm (upper panel). Total seeds from each plant of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 (bottom panel). D, Relative rosettes size (35-d-old plants, $n = 8$) of *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 to WT. Values are means ± SE relative to the WT value that is set at 1. E, Root dry weight per plant of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 (82-

Supplemental Figure S2, E). These results reveal that *LecRK-VIII.2* promotes cell expansion and slightly affects cell proliferation in maternal tissue to determine seed size.

Furthermore, we attempted to figure out the molecular mechanism of *LecRK-VIII.2* in promoting cell expansion. In Arabidopsis, expansins (AtEXPAs) are identified as cell-wall-loosening proteins that mediate pH-dependent extension of

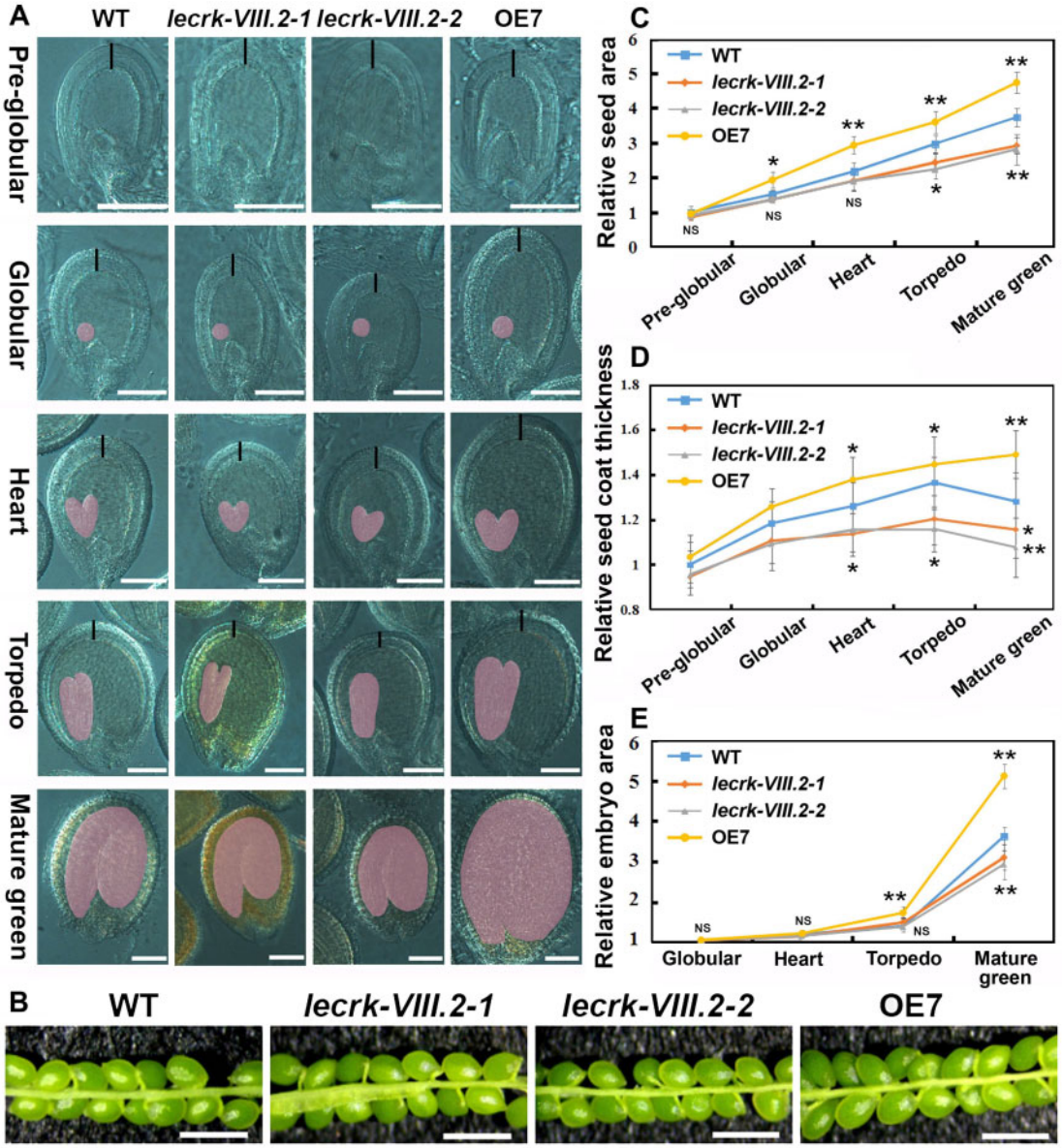

Figure 4 *LecRK-VIII.2* positively regulates the size of the integument and embryo. A and B, Developing seeds of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 lines were collected at various stages (Pre-globular, Globular, Heart, Terpedo and Mature green), and observed by a differential interference contrast microscope after fixation and clearing treatment as described in the "Materials and methods" section, bar = 50 μm A and 1 mm B. C–E, Relative size of *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 lines to WT. Values are means ± SE ($n > 100$ developing seeds) relative to the WT value that is set at 1. *$P < 0.05$, **$P < 0.01$ compared with the WT, NS means no significance (Student's *t* test).

Figure 3 Continued

d-old plants, $n = 12$). F, Relative stem thickness of *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 to WT ($n = 12$). G, Shoot dry weight (without seeds) per plant of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 (82-d-old plants, $n = 12$). H, Total dry weight per plant of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 (82-d-old plants, $n = 12$). I and L, Seed weight to shoot weight ratio (I), seed weight to root weight ratio (J), seed weight to total weight ratio (K), root weight to shoot weight ratio (L). M, Relative size of epidermal cells in rosettes from WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 (35-d-old plants, $n > 100$). N and O, Chlorophyll content in rosettes from 35-d-old plants of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7. FW means fresh weight. Values are means ± SE, n = three biological repeats. The dry weight of shoots and roots was scored after incubation at 80°C for 12 h, while the seed weight was scored after incubation at 37°C for 3 d. Values are means ± SE (E–H, $n = 12$). *$P < 0.05$, **$P < 0.01$ compared with WT (Student's *t* test).

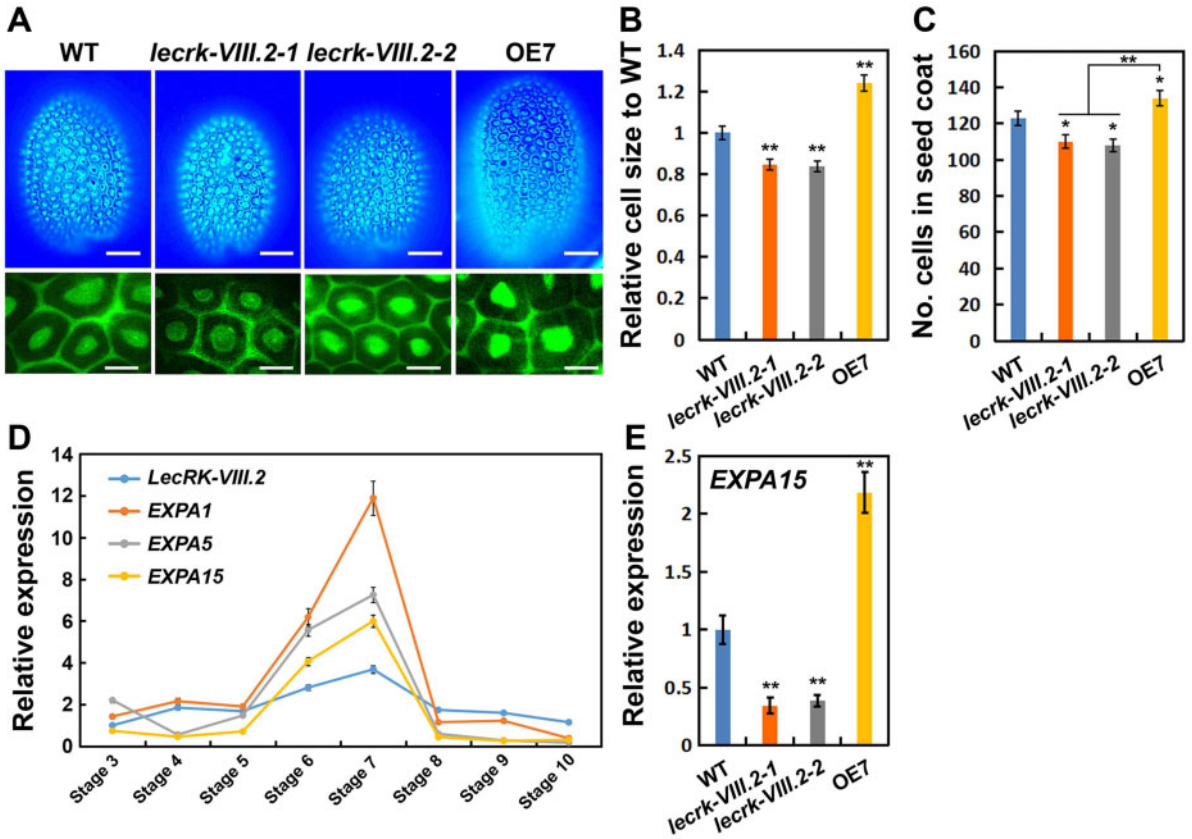

Figure 5 *LecRK-VIII.2* promotes cell expansion and proliferation in the seed coat. A, The size and number of cells in the seed coat were investigated by Fluorescent Brightener 28 staining, bar = 100 μm (upper panel) or 20 μm (lower panel). B, Relative cell size of *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 to WT. Values are means ± SE ($n > 120$ cells) relative to the WT value that is set at 1. C, The number of cells in the seed coat of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7. Values are means ± SE ($n > 120$ seeds). D, The expression level of *LecRK-VIII.2*, *EXPA1*, *EXPA5*, and *EXPA15* in different stages of developing seeds. Values are means ± SE relative to the *LecRK-VIII.2* value that is set at 1. E, The expression level of *EXPA15* in the developing seeds of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7. Values are means ± SE (n = three biological replicates). *$P < 0.05$, **$P < 0.01$ compared with the WT (Student's *t* test).

the cell wall and induce cell expansion (Lee et al., 2001; Cosgrove, 2015; Nardi et al., 2015). Considering the potential association based on co-localization between EXPAs and LecRK-VIII.2, we compared the expression profiles of all 36 *AtEXPAs* during seed development. Interestingly, *EXPA1*, *EXPA5*, and *EXPA15* exhibited similar patterns to *LecRK-VIII.2*, showing a common transcriptional peak in stage 7 of developing seeds (Figure 5, D). Next, we performed a RT-qPCR assay to investigate their relationship. The significantly reduced level of *EXPA15* was detected in the *lecrk-VIII.2* mutants, while overexpressing *LecRK-VIII.2* obviously induced *EXPA15* transcription (Figure 5, E). However, evidence of *EXPA1* and *EXPA5* regulated by *LecRK-VIII.2* was not observed in developing seeds. In general, this suggests that *LecRK-VIII.2* probably promotes cell expansion of the seed coat by regulating the expression of *EXPA15*.

Additionally, we conducted reciprocal cross experiments to test whether *LecRK-VIII.2* acted maternally. We pollinated *lecrk-VIII.2* (or OE7) plants with WT pollen. The length and width of F1 seeds from such crosses were comparable to that of self-pollinated *lecrk-VIII.2* mutants (or OE7). In contrast, the size of seeds from WT plants pollinated by *lecrk-VIII.2* mutant (or OE7) pollen was undistinguishable from that of the self-pollinated WT plants (Figure 6, A–C). This indicates that *LecRK-VIII.2* functions maternally to determine seed size. We further investigated the size of *lecrk-VIII.2-2*/Col-0 F2 and Col-0/*lecrk-VIII.2-2* F2 seeds. Seeds from *lecrk-VIII.2-2*/Col-0 F1 and Col-0/*lecrk-VIII.2-2* F1 plants contain WT, *lecrk-VIII.2*, and *lecrk-VIII.2*/+ embryos within *lecrk-VIII.2*/+ seed coats. The size of *lecrk-VIII.2*/Col-0 F2 and Col-0/*lecrk-VIII.2* F2 seeds was comparable with that of WT seeds (Figure 6, D). These results indicate that the embryo and endosperm genotypes for *LecRK-VIII.2* have no effect on seed size, and *LecRK-VIII.2* is essential in sporophytic tissue of the mother plant to determine seed size.

LecRK-VIII.2 regulates yield-related traits in a *MPK6*-dependent manner

MAPKs cascades play essential roles in plant development and stress responses (Xu and Zhang, 2015). Recent studies indicate that MPK3 and MPK6, as conserved downstream factors of RLKs, regulate inflorescence architecture, ovule integument development, and seed development (Bush and Krysan, 2007; Wang et al., 2008; López-Bucio et al., 2013;

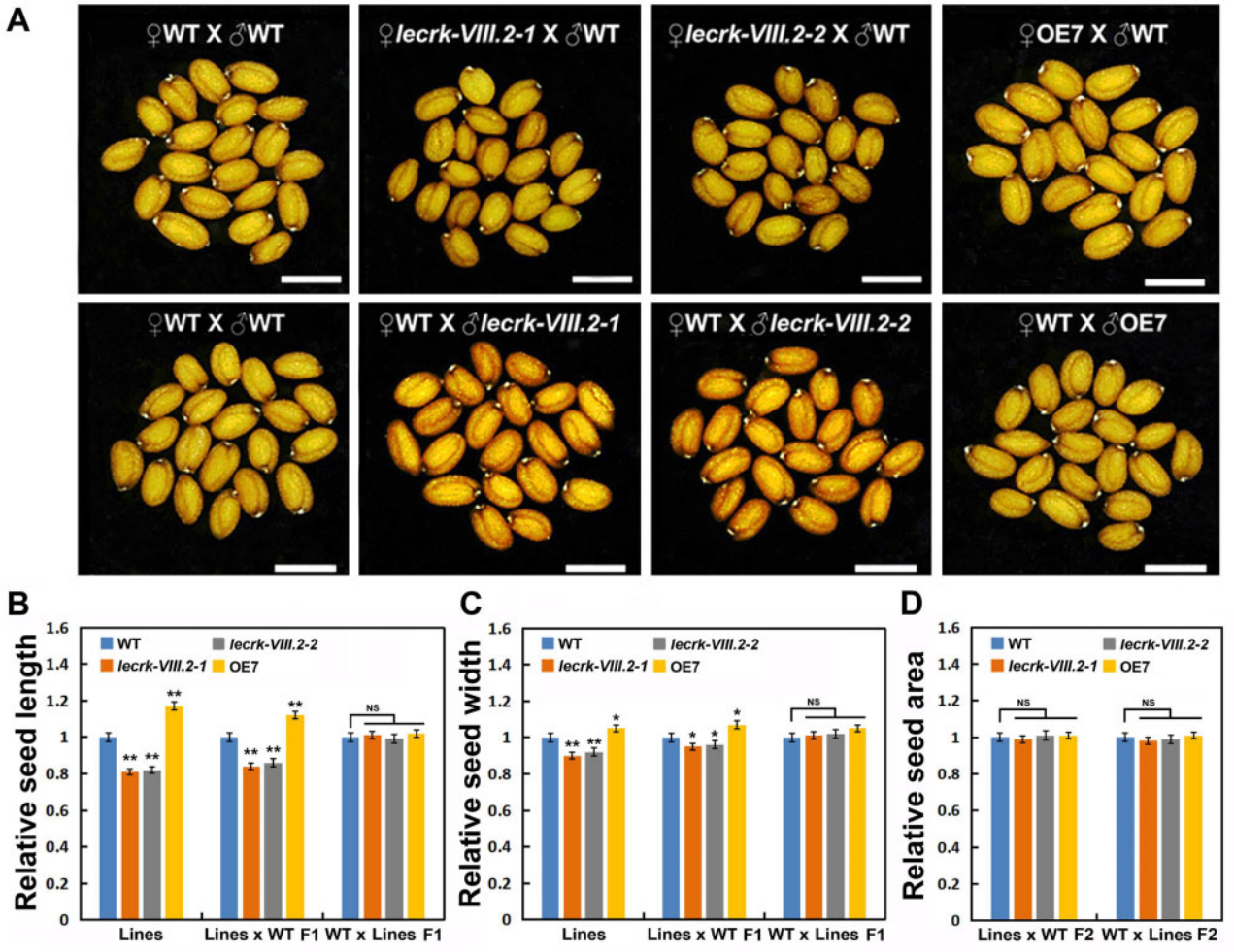

Figure 6 *LecRK-VIII.2* maternally regulates seed size. A, Mature F1 seeds from the reciprocal cross experiments among WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, and OE7 lines, bar = 500 μm. B and C, Relative seed length and width of the F1 seeds of reciprocal cross experiments. D, Relative seed area of the F2 seeds of reciprocal cross experiments. Values are means ± SE ($n > 500$ seeds) relative to the WT value that is set at 1. $^{**}P < 0.01$ compared with the WT (Student's *t* test).

Zhang et al., 2017). In light of this, we constructed *lecrk-VIII.2 mpk6-2*, OE7 *mpk6-2*, *lecrk-VIII.2 mpk3-1*, and OE7 *mpk3-1* lines by crossing (Supplemental Figure S3). Interestingly, seeds from the *mpk6-2* mutant display a range of phenotypes, ∼67% WT-like bigger seeds (*mpk6wb* seeds), ∼25% raisin-like seeds, and ∼6% seeds with burst-out embryo (burst seeds; Bush and Krysan, 2007; López-Bucio et al., 2013; Zhang et al., 2017; Creff et al., 2019), indicating the complex function of MPK6 in controlling seed size and development. In order to remove the interference of the embryo/seed development defect on seed size, we only selected the WT-like seeds to explore the genetic relationship between LecRK-VIII.2 and MPK6. Surprisingly, the length and width of *lecrk-VIII.2-2 mpk6-2* and OE7 *mpk6-2* seeds were undistinguishable from those of *mpk6wb* seeds (Figure 7, A and B), suggesting that *mpk6* was epistatic to *lecrk-VIII.2*. Additionally, the genetic results also reveal that *LecRK-VIII.2* acts upstream of *MPK6* to control silique number and seed yield (Figure 7, C and D). In general, LecRK-VIII.2 functions in a common pathway with MPK6 to regulate seed size, seed number, and silique number. In addition, the epistatic role of *MPK6* to *LecRK-VIII.2* in controlling flower size by promoting cell expansion was observed (Supplemental Figure S4, A and B), also confirming that *LecRK-VIII.2* acts upstream of *MPK6* to determine organ size.

To further investigate the molecular basis of interplay between LecRK-VIII.2 and MPK6, we detected the phosphorylation status of MPK6 in developing seeds. We found reduced phosphorylated MPK6 (pMPK6) levels in the *lecrk-VIII.2* mutant, while overexpressing *LecRK-VIII.2* increased pMPK6 levels (Figure 7, E and F). Considering the genetic relationship between them, this demonstrates that LecRK-VIII.2 regulates the three yield-related traits by adjusting the phosphorylation level of MPK6. Interestingly, the OE lines exhibited a significantly increased level of pMPK3, but no alteration of pMPK3 was observed in *lecrk-VIII.2* mutants. Previous studies indicate that MPK6 and MPK3 usually work redundantly to regulate plant growth and development

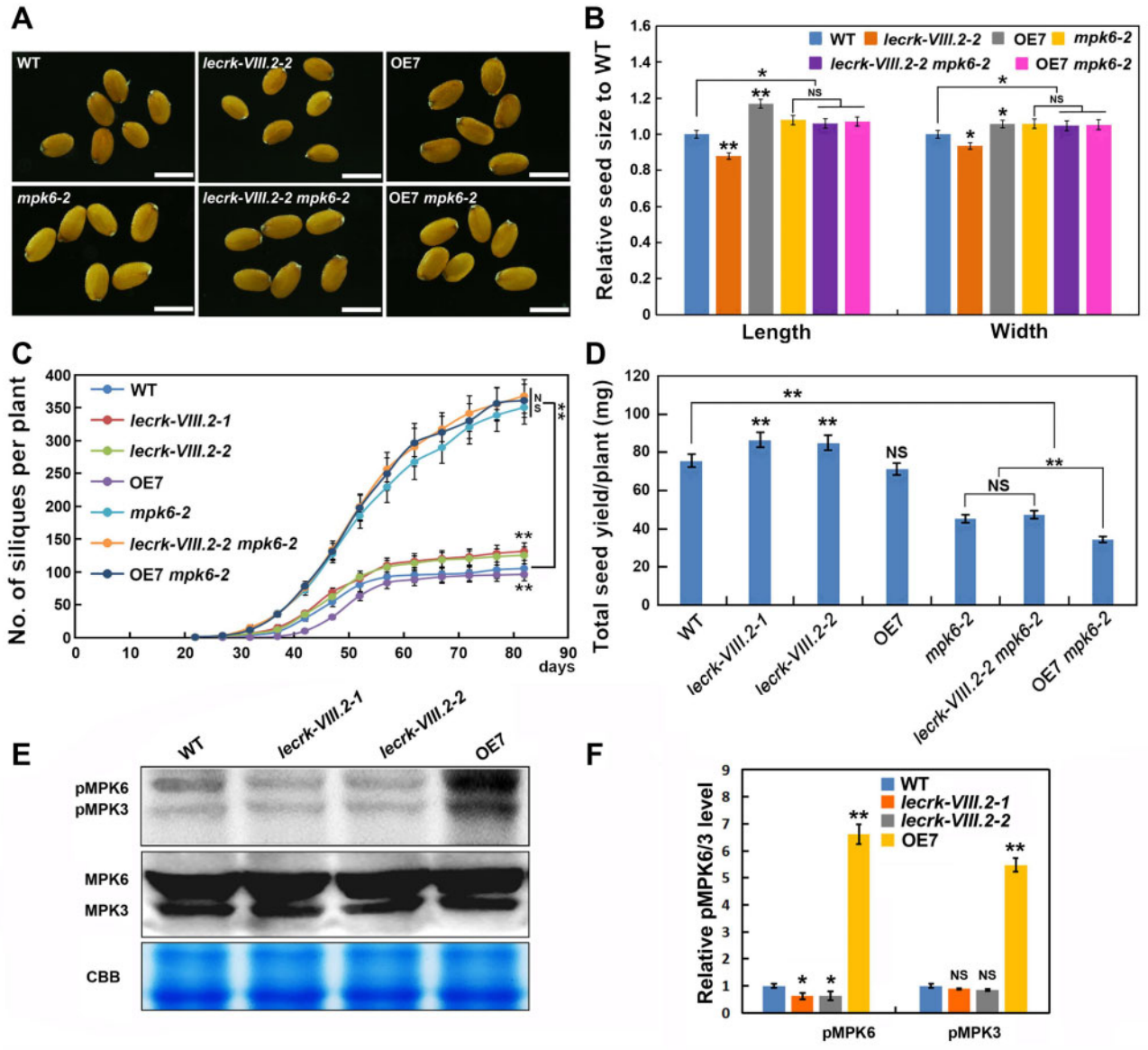

Figure 7 *LecRK-VIII.2* acts upstream of *MPK6* to control yield-related traits. A, WT-like mature seeds of WT, *lecrk-VIII.2-2*, OE7, *mpk6-2*, *lecrk-VIII.2-2 mpk6-2*, and OE7 *mpk6-2*, bar = 500 μm. B, Relative seed length and width of the mutants to WT. Values are means ± SE ($n > 500$ seeds) relative to the WT value that is set at 1. C, The number of siliques per plant of WT, *lecrk-VIII.2-2*, OE7, *mpk6-2*, *lecrk-VIII.2-2 mpk6-2*, and OE7 *mpk6-2*. The number of siliques was counted every 5 d, starting from the bolting of the *mpk6-2* mutant (the earliest flowering line among analyzed plants) to the 82nd d. Values are means ± SE ($n = 12$ plants). D, Total seed yield per plant of WT, *lecrk-VIII.2-2*, OE7, *mpk6-2*, *lecrk-VIII.2-2 mpk6-2*, and OE7 *mpk6-2*. Values are means ± SE ($n = 12$ plants). E and F, Phosphorylation level of MPK3 and MPK6 in developing seeds (~10 DAF) of WT, *lecrk-VIII.2-1*, *lecrk-VIII.2-2*, OE7, *mpk3-1*, and *mpk6-2*. Values are means ± SE (n = three biological replicates) relative to the WT value that is set at 1. *$P < 0.05$, **$P < 0.01$ compared with the WT, NS means no significance (Student's *t* test).

(reviewed in Xu and Zhang, 2015). Thus, we compared the size of seeds and flowers from *mpk3-1*, OE7 *mpk3-1*, and *mpk3-1* plants. The results showed there was no significant change in the size of seed and flower of the *mpk3-1* mutant compared with that of the WT, indicating that *MPK3* may not play an important role in seed development. However, the seed and flower size of the OE7 *mpk3-1* line was significantly bigger than those of the *mpk3-1* mutant, suggesting that *MPK3* may not be the downstream factor of *LecRK-VIII.2* to regulate seed and flower size (Supplemental Figure S4, C and D). As a result, *LecRK-VIII.2* controls the size of seeds and flowers upstream of *MPK6*, but independent of *MPK3*.

LecRK-VIII.2 regulates the development of the pistil and ovule

Recent studies revealed that the development of the flower organ is closely linked with yield-related traits. For example, inflorescence architecture is implicated in the number of spikelets or siliques (reviewed in Li et al., 2019). Therefore, we

dissected the flowers and observed the development of the pistil, stamen, and pollen. The *lecrk-VIII.2* mutants developed shorter pistils with less ovules, whereas the OE plants formed longer pistils containing more ovules than the WT. The knockout mutants showed reduced length of siliques with less seeds, while the OE lines exhibited opposite phenotypes (Supplemental Figure S5). Nevertheless, the knockout and OE plants all displayed no developmental defects of the pistil, ovule, stamen, and pollen, and were undistinguishable in pollen size and pollen activity (Supplemental Figures S5, S6). In summary, these results demonstrate that *LecRK-VIII.2* positively regulates pistil length and ovule number per pistil, but does not affect anther and pollen development. This is consistent with the GUS pattern that *LecRK-VIII.2* is expressed in the pistil and ovule, but not in the anther or pollen (Figure 1, G–K). Importantly, the difference of pistil length and ovule number per pistil could have a direct effect on silique length and seed number per silique.

LecRK-VIII.2 regulates the accumulation of storage protein and carbohydrate in seeds

Larger seeds usually show increased germination activity and fitness, mainly as a result of higher storage protein and carbohydrate in seeds (Michelle and Richards 2000; Li et al., 2019). Therefore, we determined the content of storage protein and carbohydrate in dry seeds. *LecRK-VIII.2* loss-of-function mutants exhibit reduced content of total protein per seed, whereas OE lines contain much more than WT plants (Figure 8, B), indicating that *LecRK-VIII.2* promotes the accumulation of storage protein. Next, in order to further check the profiles of seed storage protein, total protein extracted from dry seeds was separated on 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels. The gels showed that *lecrk-VIII.2* mutant developed seeds with significantly less 12S-α and 12S-β (12S globulin subunits) than WT plants, while they contained a comparable content of 2S albumin subunits (2S-L and 2S-S). OE7 seeds accumulated a remarkably higher level of 12S and 2S subunits than WT (Figure 8, A and C). Additionally, the loss of function of *LecRK-VIII.2* decreased the content of sucrose and starch per seed, whereas OE lines accumulated more sucrose and starch in seeds (Figure 8, E and F). This indicates that *LecRK-VIII.2* positively regulates the carbohydrate content in seeds. In general, *LecRK-VIII.2* promotes the accumulation of protein and carbohydrate in seeds, and regulates the relative proportion and content of the subunits of storage protein.

Discussion

The energy allocation between vegetative and reproductive organs is regulated by developmental signals and environmental cues, which subsequently affect the seed size/number trade-off to determine seed yield. This phenomenon has been observed and studied for decades in a variety of species (reviewed in Smith and Fretwell, 1974; Lazaro and Larrinaga, 2018; Li et al., 2019), but the regulatory mechanism underlying how plants perceive and transduce extracellular or environmental signals to organize this process remains largely obscure. The major reason is that the key factors, especially RLKs upstream of the pathways, are still undiscovered, which could connect the signals and integrate some identified signaling into a network.

In this study, we report that LecRK-VIII.2 is a specific RLK to determine seed yield by coordinating silique number, seed size, and seed number. The *lecrk-VIII.2* mutants form smaller seeds, but produce an increased number of siliques and seeds, leading to significantly increased yield. In contrast, the OE lines develop bigger seeds, but less siliques and seeds, which results in a similar yield to that of the WT. Interestingly, the OE lines exhibit an elevated rosette area, more robust roots, and thicker stem compared with WT plants (Figures 2, 3, A–F, and 9). This indicates that *LecRK-VIII.2* has potential as a promising target for crop improvement, which aims to cultivate crops with stable grain yield, enhanced use efficiency of water and fertilizer, and improved lodging resistance.

Additionally, in OE plants, the elevated total biomass could result from increased leaf area and chlorophyll level, which subsequently lead to increased photosynthesis. The higher amount of photoassimilates is allocated for the growth of rosettes and roots, rather than accumulated in seeds (Figure 3, G–O). It should be noted that the *lecrk-VIII.2* mutants show a reduced size of rosette, root, and stem, but increased yield. This indicates that the *LecRK-VIII.2* loss-of-function plants store more energy in seeds at the cost of the growth of rosettes and roots. Given that LecRK-VIII.2 function as a potential receptor, we hypothesize the existence of a ligand(s) to control the energy allocation between vegetative and reproductive growth. The energy fluctuation inputted to reproductive growth could lead to the alteration of silique number, seed size, and seed number, which subsequently determine seed output.

A previous study revealed that the legume-lectin protein-like extracellular domain of L-type LecRKs is structurally similar to that of carbohydrate-binding proteins and exhibited glucose/mannose specificity (Hervé et al., 1999). Interestingly, no GUS activity was observed in 7-d-old cotyledons (Figure 1, A), whereas strong signal was detected after mannose and glucose treatment. Additionally, previous studies revealed that in developing seeds, a low sucrose to hexose (fructose and glucose) ratio is closely associated with cell division at the early stage, while a high ratio is required for cell elongation later (Weber et al., 1996, 1997; Ohto et al., 2005). Generally, considering the roles of LecRK-VIII.2 both in cell expansion and proliferation, we therefore infer that the potential ligands of LecRK-VIII.2 are some kind of hexose or sugar derivatives. Furthermore, recent studies indicate that in the LecRK family, LecRK-I.9 functions as a receptor for eATP, and LecRK-I.8 acts as an $eNAD^+$ sensor (Choi et al., 2014; Wang et al., 2017, 2018; Tripathi et al., 2018). Thus, nucleotides, as signal substances and/or energy molecules, also should be considered as the potential ligands of LecRK-VIII.2. Metabolomics study on saccharides and

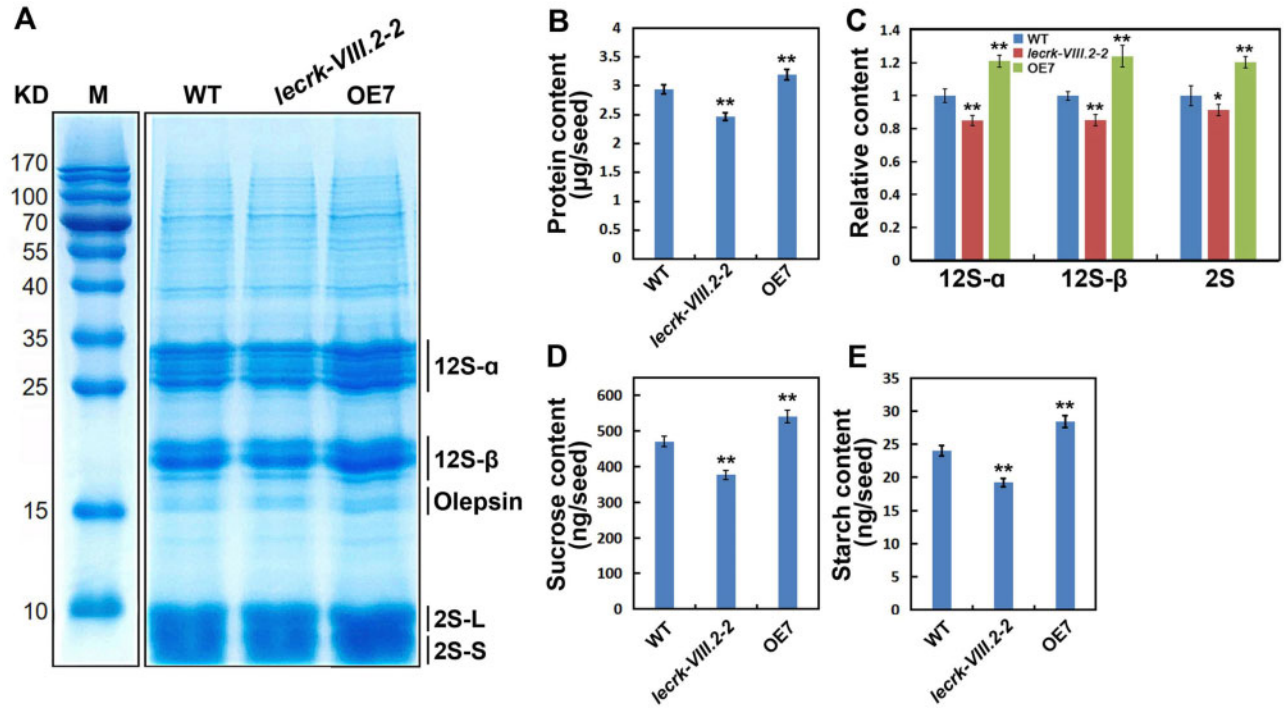

Figure 8 *LecRK-VIII.2* promotes the accumulation of storage protein and carbohydrate in seeds. A, Profiles of storage protein in dry seeds from WT, *lecrk-VIII.2-2*, and OE7 plants. Each lane contains protein extracted from 10 seeds. Total proteins extracted from dry seeds were separated on 15% SDS-PAGE and stained with Coomassie Blue. 12S-α and 12S-β, 12S globulin subunits; 2S-L and 2S-S, 2S albumin subunits. B, Protein content per seed of WT, *lecrk-VIII.2-2*, and OE7 plants. C, Relative content of 12S-α, 12S-β, and 2S in the seeds from WT, *lecrk-VIII.2-2*, and OE7 plants. D, Sucrose content per seed of WT, *lecrk-VIII.2-2*, and OE7 plants. E, Starch content per seed of WT, *lecrk-VIII.2-2*, and OE7 plants. Values are means ± SE of three (B–E) biological replicates. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the WT, NS means no significance (Student's *t* test).

nucleotides during seed development or response to sugar stimulus could be required for fully understanding the molecular mechanism of signal transduction and energy allocation in a LecRK-VIII.2-dependent manner. Moreover, it would be interesting to study the action of LecRK-VIII.2 in photosynthesis.

The investigation of seed development shows that *LecRK-VIII.2* promotes the growth of the integument and embryo (Figure 4). Given that the difference of embryo size emerges later than that in the integument, we speculate that the reduced thickness of the integument in *lecrk-VIII.2* mutants sets the physical limitation, and this inhibition emerges after fertilization and constantly accumulates, which subsequently leads to the observable suppression of embryo expansion starting from the torpedo stage. Interestingly, the OE lines display faster growth of seeds beginning at the globular stage, while *LecRK-VIII.2* loss-of-function mutants show decreased seed size compared with WT plants beginning at the torpedo stage (Figure 4, C). Similarly, the *LecRK-VIII.2-GUS* expression profiles showed high activity in the pistil (valve), but 2 d after fertilization, the signal emerged from the embryo, along with decreased expression in the valve (Figure 1). These asynchronous phenotypes suggest the roles of *LecRK-VIII.2* to regulate the timing of key events during pistil and seed development. Further studies are needed to dissect the regulatory mechanism of *LecRK-VIII.2* before and after fertilization.

The genetic and biochemical results demonstrate that *LecRK-VIII.2* works upstream of *MPK6* to determine yield by coordinating silique number, seed size, and seed number (Figures 7, 9). Previous studies reveal that the YDA(MAPKKK4)-MKK4/MKK5-MPK3/MPK6 cascade plays important roles in embryo development, localized cell division, root development, and seed size (Bergmann, 2004; Lukowitz et al., 2004; Wang et al., 2007; Meng et al., 2013; Musielak and Bayer, 2014; Xu and Zhang, 2015). Given that the mutants of *YDA*, *MKK4/MKK5*, *MPK3/MPK6* develop seeds with a burst embryo, it is possible that LecRK-VIII.2 is involved in the YDA-MKK4/5-MPK3/6 signaling pathway. Interestingly, LecRK-VIII.2, MKK4/5, and MPK6 all exhibit maternal control of seed development, further suggesting the possibility. However, we were not able to identify YDA as an interaction factor with LecRK-VIII.2-3xFLAG fusion protein by a co-immunoprecipitation (Co-IP)/mass spectrometry (MS) assay. LecRK-VIII.2 could interact with other MAPKKKs to transduce the signals via a phosphorylation cascade. A recent study demonstrated that the G-protein beta subunit, AGB1 acts as a linker between RLKs and the YDA-MKK4/5-MPK3/6 cascade to control zygote and fruit development (Yuan et al., 2017), and AGB1 plays an essential role in regulating seed size (Li et al., 2012). Thus, it would be interesting to investigate whether LecRK-VIII.2 is linked to the MAPK pathway by recruiting the G-protein complex as a scaffold. Interestingly, we identified some BR-

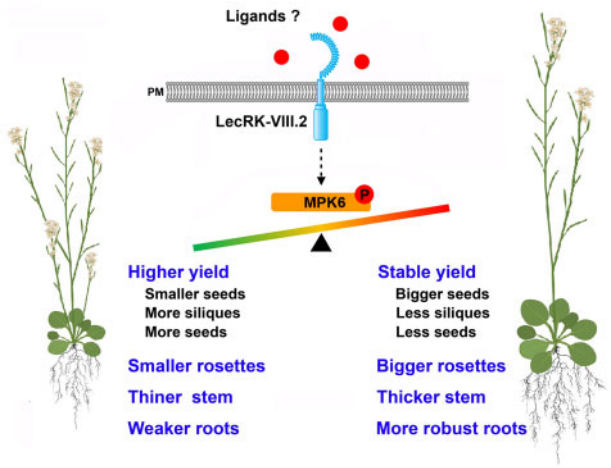

Figure 9 A genetic and molecular framework for LecRK-VIII.2/MPK6-mediated regulation of yield-related traits and organ size. In the presence of unknown ligands as developmental signals, LecRK-VIII.2 phosphorylates MPK6, and coordinates silique number, seed size, and seed number to determine reproductive output. *lecrk-VIII.2* mutants with reduced pMPK6 level exhibit increased seed yield, but decreased size of rosettes, stems, and roots. Plants overexpressing *LecRK-VII.2* with an elevated pMPK6 level can maintain stable yield, and develop bigger rosettes, thicker stems, and more robust roots, compared with WT plants. Like some RLKs, LecRK-VIII.2 is able to form a homodimer, suggesting that the signaling downstream of LecRK-VIII.2 could start from its autophosphorylation. LecRK-VIII.2 and its homolog LecRK-VIII.1 can compose a heterodimer, suggesting that they could play similar roles during plant development.

responsive leucine-rich repeat receptor like kinases (LRR-RLKs) as interaction proteins with LecRK-VIII.2 by Co-IP/MS and yeast two-hybrid (Y2H) assays. Given that BRASSINOSTEROIDS INSENSITIVE 1 (BRI1) and BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) have been implicated to interact with G proteins and regulate sugar-responsive growth and development (Peng et al., 2018), further studies are needed to identify the association among the LecRK-VIII.2-MAPKs module, G proteins, and BR signaling.

Recent studies revealed that rice (*Oryza sativa*) ERECTA1 (OsER1) and the dual-specific MAPK PHOSPHATASE1 (OsMKP1) regulate the OsMKKK10-OsMKK4-OsMPK6 cascade to coordinate the trade-off between grain number per panicle and grain size in rice (Guo et al., 2018, 2020; Xu et al., 2018). This greatly extends our understanding of the molecular mechanism of the trade-off. Considering the conserved role of MPK6 in Arabidopsis and rice (Bush and Krysan, 2007; López-Bucio et al., 2013; Yi et al., 2016; Zhang et al., 2017; Guo et al., 2018, 2020; Xu et al., 2018), and the potential of AtLecRK-VIII.2 for crop improvement, we therefore searched against the dataset of Viridiplantae in EggNOG v4.5.1 (Jaime et al., 2015). We found that Os05g0125200 and Os06g0285400 shared high sequence similarity and conserved domains with AtLecRK-VIII.2, and more homologs of AtLecRK-VIII.2 in crops are shown in Supplemental Figures S7, S8. Some of them in rice, soybean (*Glycine max*), maize (*Zea mays*), and potato (*Solanum tuberosum*) exhibit common high expression during grain (or fruit) development (Supplemental Figure S9), suggesting the conserved roles of *LecRK-VIII.2* in the crops. It would be interesting to dissect their functions in grain (or fruit) yield and plant development, making it a candidate for crop improvement or design.

In summary, we discovered that LecRK-VIII.2 is a specific RLK that regulates seed yield and the source–sink relationship. Further studies on uncovering the ligands and new component(s) in the LecRK-VIII.2-MPK6 signaling pathway would further contribute to clarifying their regulatory mechanism of energy allocation and promoting crop improvement.

Materials and methods

Plant growth conditions and plant materials

Arabidopsis thaliana ecotype Col-0 was used as the WT. Mutants *lecrk-VIII.2-1* (Salk_051706) and *lecrk-VIII.2-2* (Salk_053278) were obtained from the Arabidopsis Biological Resource Center. The *mpk3-1* (Salk_151594) and *mpk6-2* (Salk_073907) mutants were obtained from the Nottingham Arabidopsis Stock Centre (NASC). The double mutants *lecrk-VIII.2-2 mpk6-2*, OE7 *mpk6-2*, *lecrk-VIII.2-2 mpk3-1*, and OE7 *mpk3-1* were identified from the F2 generation after crossing. Seeds were plated on half-strength Murashige and Skoog (1/2 MS) medium supplemented with 1% (w/v) sucrose and kept at 4°C for 3 d before transferring to a 22°C chamber under long day conditions (16-h light/8-h dark) for 7 d. Then, seedlings were transplanted into soil and cultured in a greenhouse (22°C, 16-h/8-h light cycle). The LecRK-VIII.2 coding sequence (CDS) was amplified by the primers listed in Supplemental Table S1 and was recombined into pLEELA (Invitrogen), driven by the CaMV 35S promoter. Then, the *35S::LecRK-VIII.2* recombinant vector was transformed into *Agrobacterium tumefaciens* strain GV3101 and the positive colony was cultured for the subsequent floral dipping transformation into WT plants. Basta-resistant T1 transgenic plants were screened and the homozygous OE lines with a single-copy insertion were obtained after self-cross and Basta screening.

GUS histochemical staining

GUS activity was analyzed by staining various tissues of *pLecRK-VIII.2-2::LecRK-VIII.2-2-GUS* transgenic plants for 24 h at 37°C in staining solution (1.5 mg/mL X-GlcA, 50 mM sodium phosphate buffer, pH 7, 0.1% (v/v) Triton X-100, 0.5 mM potassium ferricyanide, and 0.5 mM potassium ferrocyanide). Samples were photographed using the NIKON ECLIPSE Ti2 microscope after destaining by ethanol.

Phenotype observation and analyses

For observing seeds and flowers, green seeds were separated from siliques (before turning brown), dry seeds were dehydrated in a 37°C incubator for 7 d, and flowers at stage 15 were photographed using a stereo microscope equipped

with an Olympus DP12 digital camera. The pistils and stamens from flowers at stage 15 were dissected and observed by the same lens. The Digimizer software (https://www.digimizer.com/index.php) was used for organ size measurement.

Differential interference contrast analyses

Seeds were collected at various development stages and dipped in fixative solution (methanol/acetic acid 3:1, v/v) at 4°C overnight. Fixed seeds were then incubated in Hoyer's solution for 40 min and observed by differential interference contrast optics of a Zeiss Scope A1 microscope equipped with an Olympus DP12 digital camera. Embryo area, and integument perimeter and thickness were measured using Digimizer software as described above.

To count the ovule number, flowers of stage 12 that had not been fertilized were cleared in a chloralhydrate/glycerol/water solution (8 g:1 mL:3 mL) overnight, and dissected under the Nikon Inverted Microscope Eclipse Ti-S and the ovule number was counted directly.

Pollen size and germination assay

Pollens from stage 15 flowers were harvested and observed by a Nikon inverted microscope eclipse Ti-S. Pollen size was measured using Digimizer software as described above. For pollen germination in vitro, pollens were collected from the stage 15 flowers and placed onto medium containing 0.36 mg/mL $CaCl_2$, 0.01 mg/mL myo-inositol, 1% (w/v) gelatin, 0.08 mg/mL H_3BO_3, 20% (w/v) sucrose, and 1% (w/v) agar. The plates were cultured at 22°C under moist conditions in the dark. The same Nikon microscope was used to observe pollen grains. The pollen germination rate was calculated by dividing the number of germinated tubes by the number of grains (Zhu et al., 2014).

Cell size observation

For petal cells observation, petals were separated from flowers at stage 14 and fixed in fixative solution, then photographed by using a Zeiss Scope A1 microscope. For seed coat cell measurement, mature seeds were stained using a 1% (w/v) solution of Fluorescent Brightener 28 (Sigma) according to the method (Ursache et al., 2018). Seeds were then photographed under the excitation of UV light using a Zeiss Scope A1 microscope. Cell size and number were measured via Digimizer software.

RNA isolation and RT-qPCR analyses

Total RNA was extracted from the plant tissues (homogenized by multi-sample tissuelyser, Jingxin Technology), using the plant RNA extraction kit (R401-01, Vazyme Biotech Co., Ltd), and the first chain of cDNA was synthesized by cDNA synthesis superMix (AE301-02, Transgen Biotech). Quantitative PCR was performed with a QuantStudio 5 Real-Time PCR System (Applied Biosystems) using SYBR RT-PCR Master Mix (-21, Transgen Biotech). Primers used in the qPCR are shown in Supplemental Table S1 and the mRNA level of genes in each sample (three technical replicates and three biological replicates) was normalized to that of WT (*ACTIN2* was used as internal control).

MAPK phosphorylation analyses

Plant tissues were collected and ground with liquid nitrogen by a multi-sample tissuelyser (Jingxin Technology). Then total protein was extracted with homogenization buffer (50 mM HEPES pH 7.5, 250 mM sucrose, 15 mM EDTA, 5% (w/v) glycerol, 0.5% (w/v) polyvinylpyrrolidone, 3 mM DTT, and 1× protease inhibitor cocktail) and quantified with Brandford reagent (E211-01, Vazyme Biotech Co., Ltd). Equal protein samples were separated using 10% (W/V) SDS-PAGE and transferred to polyvinylidene difluoride membranes. After blocking with block buffer (PBS buffer, 3% (w/v) BSA, 0.01% (v/v) Tween 20), transblots were incubated in Rabbit Anti-Phospho-p44/p42 MAPK antibody (1:2000, Cell Signaling Technology, #9101) diluted with PBS buffer at 4°C overnight, and immunoreactive bands were visualized with FluorChem system (Protein Simple) after incubation in PBS buffer with 1:1000 anti-rabbit-IgG-HRP antibody (Cell Signaling Technology, #7074) at room temperature for 1 h. The protein level was represented by immunoblot analysis using anti-MPK3 (M8318) and anti-MPK6 (A7104) antibodies from Sigma. Coomassie brilliant blue (CBB) staining was used to confirm equal protein loading.

In order to quantify the relative level between pMPK6 or pMPK3 and MPK6 or MPK3, Image J was used to measure the mean gray value of the bands. The value of pMPK6/MPK6 or pMPK3/MPK3 in WT plants was set to 1.

Chlorophyll content determination

Fresh leaves (0.1 g) were homogenized by tissuelyser in liquid nitrogen. Total chlorophyll was extracted using 95% (v/v) ethanol by vortex for 10 min in darkness. After centrifuging at 15,000 rpm for 10 min at 4°C, the supernatant was transferred to a new tube and diluted 10-fold with 95% ethanol. The absorbance of the diluted solution was determined at 665 and 649 nm using a Shimadzu 1800 spectrophotometer. The chlorophyll content was calculated according to the following formula: Chl-a = $(13.95A_{665} - 6.88A_{649})$ * $V/1000W$ and Chl-b = $(24.96*A_{649} - 7.32*A_{665})*V/1000W$.

Quantification of protein and carbohydrate in seeds

Forty mature dry seeds from WT plants or mutants were homogenized with 80 μL of extraction buffer (100 mM Tris–HCl, pH 8, 0.5% [v/v] SDS, 10% [v/v] glycerol, and 20% [v/v] 2-mercaptoethanol) by tissuelyser, followed by boiling for 5 min and centrifugation for 10 min at 15,000 rpm. Five microliters of the supernatant was used for total protein quantification (E211-01, Vazyme Biotech Co., Ltd) according to the manufacturer's protocol. Twenty microliters of each extract was used for 15% SDS-PAGE (Carine et al., 2015; Di et al., 2018). Samples of 20 dry seeds were used for carbohydrate analyses according to the previous protocol

(Baud et al., 2002). Seeds were ground twice in 1000 μL of 80% (v/v) ethanol at 4°C. After centrifugation (15,000 *g* for 10 min at 4°C), the supernatant containing the soluble carbohydrates was collected, evaporated at room temperature under vacuum, and then dissolved in 200 μL of water before analysis. The pellet left from the extraction was used for starch determination. The sucrose and starch measurements were performed with kits (#BC2465 and #BC0700 from Solarbio Life Sciences).

Analyses of expression profiles and *AtLecRK-VIII.2* homologs

The expression profiles of 75 *LecRKs* during plant development were retrieved and analyzed by Arabidopsis eFP browser of the Bio-Array Resource (BAR) website (http://bar.utoronto.ca/; Winter et al., 2007) and AtGenExpress Visualization Tool (Redman et al., 2004; Schmid et al., 2005; Kilian et al., 2007). To identify homologs of AtLecRK-VIII.2, its amino acid sequence was searched against the dataset of Viridiplantae in EggNOG v4.5.1. The analysis of alignment, phylogenetic tree, and conserved domain was performed by the online tools curated by EggNOG v4.5.1 (Jaime et al., 2015).

Statistical tests

Three biological repeats were used in all RT-qPCR and western blot assays. To score the embryo/seed phenotypes, we randomly picked at least five siliques, phenotyped all embryos/seeds (>100), and calculated the percentages of each phenotype. Triplicates were performed to obtain mean values with standard deviations. *P* values were calculated by two-sided Student's *t* test. Asterisks above the columns were used to indicate differences that are statistically significant ($^{*}P < 0.05$) and very significant ($^{**}P < 0.01$), respectively.

Accession numbers

Sequence data used in this article can be obtained from The Arabidopsis Information Resource (TAIR) or GenBank/EMBL datasets by using the following accession numbers: *LecRK-VIII.2* (AT5G03140), *LecRK-VIII.1* (AT3G53380), *MPK6* (AT2G43790), *MPK3* (AT3G45640), *EXPA1* (AT1G69530), *EXPA5* (AT3G29030), *EXPA15* (AT2G03090), *ACTIN2* (AT3G18780).

Supplemental data

Supplemental Figure S1. Transcription patterns of *LecRK-VIII.2* during plant growth and development.

Supplemental Figure S2. Overexpression of *LecRK-VIII.2* affects organ size and seed development.

Supplemental Figure S3. *LecRK-VIII.2* acts upstream of *MPK6* to control the growth of the rosette and stem.

Supplemental Figure S4. *LecRK-VIII.2* controls the size of seeds and flowers in an *MPK6*-dependent manner, but independent of *MPK3*.

Supplemental Figure S5. *LecRK-VIII.2* regulates the development of flowers and siliques.

Supplemental Figure S6. *LecRK-VIII.2* exhibits no effect on the development of stamen and pollen.

Supplemental Figure S7. Phylogenetic analysis of AtLecRK-VIII.2 homologs in crops.

Supplemental Figure S8. Alignment of amino acid sequences of AtLecRK-VIII.2 and its homologs in higher plants.

Supplemental Figure S9. The expression profiles of the homologous genes of *AtLecRK-VIII.2*.

Supplemental Table S1. Primers used in this work.

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