The SnRK2-APC/C^{TE} regulatory module mediates the antagonistic action of gibberellic acid and abscisic acid pathways

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Abscisic acid (ABA) and gibberellic acid (GA) antagonistically regulate many developmental processes and responses to biotic or abiotic stresses in higher plants. However, the molecular mechanism underlying this antagonism is still poorly understood. Here, we show that loss-of-function mutation in rice Tiller Enhancer (TE), an activator of the APC/C^{TE} complex, causes hypersensitivity and hyposensitivity to ABA and GA, respectively. We find that TE physically interacts with ABA receptor OsPYL/RCARs and promotes their degradation by the proteasome. Genetic analysis also shows OsPYL/RCARs act downstream of TE in mediating ABA responses. Conversely, ABA inhibits APC/C^{TE} activity by phosphorylating TE through activating the SNF1-related protein kinases (SnRK2s), which may interrupt the interaction between TE and OsPYL/RCARs and subsequently stabilize OsPYL/RCARs. In contrast, GA can reduce the level of SnRK2s and may promote APC/C^{TE}-mediated degradation of OsPYL/RCARs. Thus, we propose that the SnRK2-APC/C^{TE} regulatory module represents a regulatory hub underlying the antagonistic action of GA and ABA in plants.
A
bscисic acid (ABA) is a stress responsive phytohormone that inhibits seed germination and seedling growth to adapt to unfavourable environmental conditions while gibberellic acid (GA) is a major growth promoting phytohormone that promotes seed germination, seedling growth, flowering and leaf expansion\textsuperscript{1-3}. Recent studies have portrayed a conceptual ABA signalling pathway in which ABA binds to its receptor PYL/PYR/RCARs, subsequently the PYL/PYR/RCAR-ABA complex binds to PP2C phosphatases that repress the SnRK2s, releasing the activated SnRK2s to phosphorylate downstream targets to activate ABA responses\textsuperscript{4-11}. In the GA signalling pathway, the receptor GID1 and E3 ligase SCF\textsubscript{SLY1/GID2} together promote the degradation of the DELLA repressor proteins in a GA-dependent manner to relieve their repression of GA action\textsuperscript{12-14}. Although recent studies have shown that ABA can antagonize GA-promoted degradation of DELLA proteins\textsuperscript{15}, the regulatory mechanism underlying the antagonism of GA on ABA signalling pathway remains largely unknown.

In a previous study, we reported that rice Tiller Enhancer (TE) encodes an activator of the APC/CTE E3 ubiquitin ligase complex that helps to repress tillering (branching) by promoting the degradation of MOC1, a master regulator of plant architecture and tiller number\textsuperscript{16}. In this study, we find that the loss-of-function te mutant displays increased sensitivity to ABA, but reduced sensitivity to GA. We show that APC/CTE can repress the ABA signalling by mediating the degradation of ABA receptors. Conversely, ABA can inhibit the APC/CTE-mediated degradation of ABA receptors by interrupting the interaction between TE and ABA receptors through activating the SnRK2-mediated phosphorylation of TE. In contrast, GA can promote the APC/CTE-mediated degradation of ABA receptors by enhancing the interaction between TE and ABA receptors through reducing the protein levels of SnRK2s. Our results unravel a novel mechanism underlying the antagonistic action of GA on ABA signalling pathway.

Results
Contrary responses of te mutant to ABA and GA. In addition to increased tillers, the te mutant displays a pleiotropic phenotype, including reduced height, twisted flag leaf and panicles, suggesting that TE regulates multiple developmental processes\textsuperscript{16}. In this study, we found that compared with wild-type (WT) plants, te mutant also displayed delayed seed germination and seedling growth in the presence or absence of exogenously supplied ABA; in contrast, the TE overexpression lines (OE17 and OE65) displayed accelerated seed germination and seedling growth compared with WT when grown on Murashige and Skoog (MS) medium supplemented with 10 \mu M ABA (Fig. 1a-f), suggesting that TE may play a role in regulating ABA responses. Consistent with this notion, te mutants were also more tolerant to drought stress, compared with WT plants (Supplementary Fig. 1a,b). Quantitative reverse transcriptase–PCR (qRT–PCR) analyses showed that the expression of representative ABA-responsive genes LEA3 and RAB16A was slightly increased in te plants, but the LEA3, LIP9 and RAB16A mRNAs were clearly reduced in the OE17 plants (Supplementary Fig. 1c-e). ABA levels were not significantly different in te, OE17 and WT plants (Supplementary Fig. 1f). These results suggest that TE likely acts as a repressor of ABA signalling.

Conversely, we found that the te mutant displayed a reduced response to GA\textsubscript{3} in seed germination and seedling growth, as well as GA-promoted \alpha-amylase activity, compared with WT and the TE overexpression lines, OE17 and OE65 (Fig. 1a-g and h and Supplementary Fig. 2a). qRT–PCR assay showed that expression of the \alpha-amylase gene RAMy1A was dramatically induced in OE17 plants (Supplementary Fig. 2b–d), indicating that overexpression of TE conferred a hypersensitivity to GA. Thus, TE appears to play a positive role in regulating GA signalling. Further assays showed that ABA treatment inhibited the germination and growth of te more significantly than WT, OE17 and OE65, and this inhibition was more effectively blocked by 2 \mu M GA\textsubscript{3} in WT, OE17 and OE65 than in te mutant (Fig. 1a,i and j). These results suggest that TE is involved in mediating the antagonistic action of ABA and GA.

APC/CTE targets ABA receptors for degradation. Previous studies have reported that Cdh1 (TE) mainly recognizes the destruction-box (D-box; RxxL) and KEN-box (Lys (K)-Glu (E)-Asn (N)) in substrate proteins to target them for ubiquitination and proteasome-mediated degradation\textsuperscript{16-18}. To test whether TE may regulate ABA and GA signalling via targeted degradation of known ABA and GA signalling components, we searched for D-box and KEN-box sequences in key positive components of ABA signalling and negative components of GA signalling. Interestingly, we found a typical D-box region in the Lid loop one of both rice and Arabidopsis PYR/PYL/RCAR proteins (Supplementary Fig. 3). A cell-free degradation assay showed that all of the eight PYL/PYRs examined were effectively degraded by WT extracts, but their degradation was slowed down in the te mutant extracts. Strikingly, OsPYL/RCAR2, OsPYL/RCAR9 and OsPYL/RCAR10 were very stable in te plant extracts. Further, treatment with proteasome inhibitor MG132 effectively blocked their degradation (Supplementary Fig. 4).

To verify whether these OsPYL/RCARs proteins are authentic substrates of APC/CTE, we selected OsPYL/RCAR10 (abbreviated as R10 hereafter) for further detailed analyses. R10 shares the highest homology to Arabidopsis PYL1 and PYR1 (ref. 5) and is widely expressed in all rice tissues (Supplementary Fig. 5a). Similar to the Arabidopsis RCAR1 (ref. 4) and PYR1 (ref. 5) proteins and rice TE protein\textsuperscript{16,18}, the R10-GFP fusion protein was localized in both the nucleus and cytoplasm (Supplementary Fig. 5b). As expected, the R10-GFP overexpression lines displayed an ABA hypersensitive phenotype, while reducing R10 expression via RNA interference (RNAi) caused a decreased sensitivity to ABA (Supplementary Fig. 5c–i). Thus, R10 likely functions as an authentic ABA receptor in rice, like its Arabidopsis counterpart\textsuperscript{5}. Both bimolecular fluorescence complementation (BiFC) and Co-IP assays showed that TE physically interacted with R10 (Fig. 2a,b). In addition, we found that degradation of R10 or R10-GFP could be effectively blocked by two different protease inhibitors, MG132 or MG115 (Fig. 2c), whereas the mutant His-R10-m protein (with a mutated D-box; RLDL→ALDA) remained relatively stable in WT plant extracts compared with the WT His-R10 protein (Fig. 2d). Moreover, an in vitro ubiquitination assay showed that His-R10 was polyubiquitinated more efficiently by WT plant extracts than by te plant extracts (Fig. 2e). Further, a western blot with anti-R10 antibody showed that R10 over accumulated in OE17 and OE65 plants but was reduced in OE17 and OE65 plants, compared with WT plants (Fig. 2f).

Finally, reducing R10 expression via RNAi in te plants rescued the seedling height and seed germination defects but not the tillering phenotype of te mutant (Fig. 2g–j and Supplementary Fig. 6), indicating that R10 acts downstream of te and is specifically involved in TE-mediated ABA responses. Together, these results support the notion that APC/CTE represses ABA signalling by targeted proteasomal degradation of R10 (and possibly some other OsPYL/RCARs proteins as well).

SnRK2 kinases inhibit the activity of APC/CTE. An integral positive component of ABA signalling, the SnRK2 kinases,
phosphorylate S/T residues in the RXXS/T domain of their substrates. We found two conserved SnRK2s recognition sites (S77 and T457) in the TE protein (Fig. 3a,b). Notably, it has been shown that the kinase activities of three rice SnRK2s (SAPK10, SAPK8 and SAPK9) were induced by ABA. BiFC assays showed that TE physically interacted with SAPK8, SAPK9 and SAPK10 and yeast two-hybrid assays showed that TE interacted with SAPK8 and SAPK10 (Supplementary Fig. 7). Further, an in vitro phosphorylation assay with MBP-fusion proteins showed that the WT N-terminal peptide of TE (TE_{N195}) containing the S77 site could be clearly phosphorylated by SAPK10, SAPK8 or SAPK9, but TE_{N195}(S77A) in which S77 was mutated, was only slightly phosphorylated by SAPK10 (Fig. 3c). Further, LC-MS/MS analysis detected a phosphate group on S77 of MBP-TE_{N195} protein phosphorylated in vitro (Fig. 3d). These results suggest that TE is a substrate of SnRK2s and that S77 is a likely phosphorylation site.

To test whether TE phosphorylation affects its association with R10, we performed BiFC assay and in vitro pull-down. We found that MBP-TE, but not the phosphomimetic mutant MBP-TE(S77D), bound to R10 (Fig. 3e,f), suggesting that phosphorylating S77 of TE may block its interaction with R10.
Consistent with this, the SAPK8-GFP, SAPK9-GFP and SAPK10-GFP overexpression lines all exhibited delayed germination and reduced seedling growth, and accumulated more R10 compared with WT plants (Fig. 3g,h). Intriguingly, we also found that SAPK8-GFP, SAPK9-GFP and SAPK10-GFP overexpression lines accumulated slightly higher levels of ABA compared with WT plants (Fig. 3i). On the basis of these results, we suggest that SnRK2s positively regulate ABA signalling by stabilizing the OsPYL/RCAR receptors via phosphorylation of TE on one hand, and upregulating ABA biosynthesis on the other hand.

**Opposite effects of ABA and GA on the degradation of R10.** Next, to explore how APC/CTE mediates the antagonism between ABA and GA, we compared the effect of GA treatment on R10 degradation in WT and te plants. All samples were pre-treated with 1 mM cycloheximide for 1 h to inhibit de novo protein biosynthesis and then treated with GA3 or ABA, respectively. Notably, GA3 effectively induced degradation of R10 in WT seeds and plants; but R10 remained stable in te seeds and plants (Fig. 4a,b and Supplementary Fig. 8a,b), and became less stable in OE17 seeds regardless of the treatments (Fig. 4a and j).
Supplementary Fig. 8a). In contrast, ABA treatment stabilized R10 in WT plants (Fig. 4c). Similarly, GA3 induced the degradation of R10-GFP, while ABA stabilized R10-GFP proteins in the transgenic plants overexpressing R10-GFP (Fig. 4d,e). qRT–PCR analysis showed that after 12 h treatment, ABA reduced while GA3 did not substantially affect R10 mRNA expression (Fig. 4f). A degradation kinetics assay further showed that GA3 markedly accelerated the degradation of R10 in WT seeds but not in te seeds (Fig. 4g). Moreover, we found that the induced degradation of R10 by GA3 could be gradually inhibited by application of increased amounts of ABA, and conversely, the induced accumulation of R10 by ABA could be reduced gradually by application of increased amounts of GA3 (Fig. 4h,i). These results suggest that ABA and GA3 act antagonistically to stabilize and destabilize the R10 protein, respectively, and APC/C<sup>TE</sup> is required for GA-promoted degradation of R10. Consistent with this notion, we found that ABA inhibited while GA3 promoted the interaction between TE and R10 (Fig. 4j).

To further investigate whether the antagonistic action of GA and ABA on the interaction between TE and R10 is mediated by SnRK2s, we analysed their effects on SnRK2 activity. We found that GA3 treatment reduced the accumulation of SAPK10, SAPK8 and SAPK9 proteins (Fig. 5a and Supplementary Fig. 9), while ABA treatment increased the accumulation of SAPK10, SAPK8 and SAPK9 proteins (Fig. 5a). Consistent with this, SnRK2 protein levels were also higher in the GA-insensitive dwarf1 mutant (gid1) and GA biosynthesis mutant dwarf 18 (d18) compared with WT plants (Fig. 5b,c). Further, treatment with the
GA biosynthesis inhibitor Paclobutrazol also caused over-accumulation of SnRK2 proteins (Supplementary Fig. 9). In addition to stabilizing SAPK10, SAPK8 and SAPK9 proteins, ABA treatment also promoted the expression of SAPK10 and SAPK8 mRNA (Supplementary Fig. 10). On the basis of these results, we propose that GA promotes the interaction between TE and ABA receptors, thus stabilizing the OsPYL/RCAR receptors and further enhancing ABA signalling. Together, these results suggest that ABA mainly positively regulates ABA signalling through a positive feedback mechanism (Fig. 5d).

**Discussion**

Previous studies have shown that DELLA proteins represent a regulatory hub that mediates the repression of ABA on GA signalling in plants. On the basis of the results presented in this study, we suggest that the SnRK2s-APC/CTE regulatory module represents a new signalling hub mediating the antagonistic action of GA on ABA signalling in higher plants. As perception of ABA by the OsPYL/RCAR receptors activates SnRK2s, leading to phosphorylation of TE and disruption of the interaction between TE and ABA receptors, thus stabilizing the OsPYL/RCAR receptors and further enhancing ABA responses through a positive feedback mechanism (Fig. 5d).

Although ABA can repress the expression of R10 mRNA by a negative feedback mechanism (Fig. 4f), it does not destabilize R10 and SnRK2 proteins. Instead, R10 is destabilized by GA in the WT plants or by overexpression of TE in OE17 seeds. Further, R10 protein is stable in te plants, GA signalling and biosynthetic mutants (Figs 4a–c and 5b,c). Together, these results suggest that ABA mainly positively regulates ABA signalling through a phytohormone treatment in...
post-transcriptional regulatory mechanism. On the other hand, we suggest that GA can reduce ABA signalling by promoting the interaction between TE and OsPYL/RCARs by reducing SnRK2 activity, and causing subsequent proteasomal degradation of OsPYL/RCARs (Fig. 5d). The self-enhancing effect of ABA on its biosynthesis23 and signalling and desensitization by GA is in sharp contrast with other known signalling pathways in both plants and animals reported so far27–34. In most reported cases, organisms have adopted a self-repression mechanism to attenuate many signalling pathways after they are activated by corresponding signal molecules, and long-term activation of these signalling pathways will have catastrophic consequences29–31,35. Unlike mobile animals, sessile plants cannot evade but have to tolerate unfavourable environmental conditions such as drought, salinity or cold. Thus, it is conceivable that the self-enhancing mechanism of ABA responses might offer an advantageous tactic for plants to survive in long-term stressful conditions. In line with this proposition, it was recently reported that proteasome-mediated degradation of the ABA receptor PYL8 is also counteracted by ABA in Arabidopsis36, suggesting conservation of such a regulatory mechanism in higher plants. When the environmental conditions became more favourable, the GA pathway may be activated to promote the degradation of ABA core signalling components (ABA receptors and SnRK2s) and DELLA proteins13, which would allow plants to resume normal growth and development. Antagonistic action of GA and ABA thus may serve as a ‘rheostat’ to fine tune plant growth and development in response to the fluctuating environments.

It is worth noting that among the eight rice ABA receptors examined in this work, OsPYL/RCA2, OsPYL/RCA9 and R10 are very stable in te plant extracts (Supplementary Fig. 4), suggesting that APC/C<sup>TE</sup> is the major E3 ligase for their degradation; but, other five OsPYL/RCARs were still reduced in te plant extracts compared with ‘Input’ (Supplementary Fig. 4), suggesting that besides APC/C<sup>TE</sup>, other E3 ligases are likely involved in the degradation of these five ABA receptors. Consistent with this proposition, recent studies reported that in Arabidopsis, the RPN10 subunit of 26S proteasome, the substrate adaptors DDA1 of a multi-subunit E3 ligase and a single subunit E3 ligase RSL1 all target specific PYL proteins for proteasomal degradation36–39. The employment of multiple E3 ligases for proteasomal degradation of the ABA receptor proteins possibly enables the plants to more effectively respond to different developmental or external signals and adds additional complexity of ABA signalling regulation in higher plants. Identification and functional studies of other unknown E3 ligases will lead to a better understanding of ABA signalling mechanism and its crosstalk with other signalling pathways.

**Methods**

**Plant materials and growth conditions.** The WT, te mutant and TE over-expression transgenic lines OE17 and OE65 used in this study were described previously16. Except when indicated otherwise, rice plants were cultivated in an experimental field at Beijing in the natural growing seasons. For qRT–PCR assays, phytohormone treatments, ABA analyses and Co-IP assays, the seedlings of WT, te, OE17 and OE65, were grown in climate chambers (HP1500GS, Ruihua) at 70% humidity, under long-day conditions with a photocycle of 14.5 h light (30 °C) and 9.5 h darkness (25 °C). Light was provided by fluorescent white-light tubes (400–700 nm, 225 μmol m<sup>–2</sup> s<sup>–1</sup>).

**Germination and seedling growth assay.** For the seed germination assay, dehulled rice (Oryza sativa) seeds from WT, various mutant and transgenic rice lines were first surface sterilized in 70% ethanol for 1 min and washed once with sterilized water. Then, seeds were immersed in NaClO for 30 min, and subsequently were washed at least five times with sterilized water. Rinsed seeds were planted on half-strength MS medium (pH 6.0) (M524, Phyto Technology Ltd) supplemented with 0.4% Gelzan (G3251, Sino Industrial) and various concentrations of (±) ABA or GA3. The seeds were then placed in a growth chamber (Ruihua) with a 14.5/9.5-h light/dark cycle at 30/25 °C. Germination was considered complete when the coleoptile was 5 mm long. Every experiment was repeated three times, with 30 seeds per sample.

For drought stress, WT and te mutant plants were grown in small pots with the same amount of soil. After growth for 3 weeks, drought was imposed by withholding irrigation for 10 days. On the eleventh day after withdrawing irrigation, surviving seedlings were photographed and counted.

**Vector construction and plant transformation.** To generate the DNA constructs, p<sub>UBI</sub>-OsPYL/RCAR10-GFP, p<sub>UBI</sub>-SAPK10-GFP, p<sub>UBI</sub>-SAPK8-GFP and p<sub>UBI</sub>-SAPK9-GFP, we amplified full-length coding sequence (CDS) of OsPYL/RCAR10, SAPK10, SAPK8 and SAPK9 with the primers shown in Supplementary Table 1. The PCR products were then cloned into the binary vector, pCUBI1390, which has a GFP insertion, with the In-Fusion Advantage PCR Cloning Kit (Cat: PT065, Clontech). To generate the OsPYL/RCAR10 RNAi construct, the OsPYL/RCAR10 CDS was amplified with the primers shown in Supplementary Table 1, and the PCR product was inserted into the LHI-FAD2-1390 RNAi vector. The resultant constructs were introduced into the rice Nipponbare variety or te mutant by Agrobacterium tumefaciens-mediated transformation.

**Antibody preparation and western blot analysis.** To produce the DNA constructs, p<sub>UBI</sub>-OsPYL/RCAR10-GFP, p<sub>UBI</sub>-SAPK10-GFP, p<sub>UBI</sub>-SAPK8-GFP and p<sub>UBI</sub>-SAPK9-GFP, we amplified full-length coding sequence (CDS) of OsPYL/RCAR10, SAPK10, SAPK8 and SAPK9 with the primers shown in Supplementary Table 1. The PCR products were then cloned into the binary vector, pCUBI1390, which has a GFP insertion, with the In-Fusion Advantage PCR Cloning Kit (Cat: PT065, Clontech). To generate the OsPYL/RCAR10 RNAi construct, the OsPYL/RCAR10 CDS was amplified with the primers shown in Supplementary Table 1, and the PCR product was inserted into the LHI-FAD2-1390 RNAi vector. The resultant constructs were introduced into the rice Nipponbare variety or te mutant by Agrobacterium tumefaciens-mediated transformation.

**Figure 5 | Antagonism of GA on ABA signalling by GA-promoted degradation of SnRK2s.** (a) Treatment for 3 h with 100 μM GA<sub>3</sub> reduces, while treatment with 10 μM ABA increases the levels of SAPK10, SAPK8 and SAPK9 proteins in OE17 plants. Left panel shows a representative western blot. Right panel shows the SAPKs/HSP82 relative protein level corresponding to left panel. Values are means ± s.d. (n = 3 replicates). Student’s t-test analysis indicated a significant difference (compared with control (CK)). *P < 0.05, **P < 0.01. Note: The seeds or seedlings were pre-treated for 1 h with 1 mM cycloheximide before phytohormone treatment. (b) and (c) Western blot analysis showing the levels of SAPK10, SAPK8, SAPK9 and R10 proteins in WT Taichung 65 (T65) and its GA-insensitive dwarf1 mutant (gid1) (b) or in WT Nipponbare (Nip) and its GA biosynthesis mutant dwarf 18 (d18) (c). (d) A model shows that the SnRK2-APC/C<sup>TE</sup> regulatory module underlies the antagonism between GA and ABA. Dashed arrows or bars represent indirect action and solid arrows or bars represent direct action. The ‘α’HSP82 signals in a–c show that roughly equal amounts of total plant extracts were used.
GST-SAPK10, GST-SAPK8 or GST-SAPK9 fusion proteins, respectively, and then affinity purified with corresponding GST-OsPYL/RCAR10, GST-SAPK10, GST-SAPK8 or GST-SAPK9 fusion proteins. Western blots were performed with the purified antibodies at 1:2,000 dilution and visualized with enhanced chemiluminescence reagent (GE Healthcare). The antibody against OsCDC27 (at 1:2,000 dilution) was described previously16. The antibodies of anti-His (Cat: D291-7, at 1:2,000 dilution), anti-GFP (Cat: 598-7, at 1:2,000 dilution), anti-Flag (Cat: M185-7, at 15,000 dilution) and anti-z-tubulin (Cat: P0574-1, at 1:1,000 dilution) were purchased from Medical & Biological Laboratories Co., Ltd. (MBL) and the antibodies against HSP28 (Cat: AbM51099-31-PU, at 1:1,000 dilution) were purchased from Beijing Protein Innovation. The uncleaved full scans of all western blot/gel images are presented in Supplementary Figs 11 and 12.

All western blot experiments were repeated at least three times, essentially with the same conclusions, and representative results are shown. Quantification of western blots was conducted according to Saito et al.44. Briefly, band intensities of R10, input (start quantity) and HSP28 (loading control for total lysates), were measured. The intensities were then calculated using the ratio of R10/input or R10/HSP28 for each western blot panel.

**Construction of protein expression plasmids.** The CDS encoding the N-terminal 193aa (TE(N258)) and the C-terminal 155 aa (TE(C155)) of TE, as well as the mutant forms, TE-N195(S77A) TE-C155(T457A), TE(N77A) and TE(C176T) were amplified using the primers shown in Supplementary Table 2. The PCR products were cloned into the pMAL-c2x vector (NEB) with the In-Fusion Advantage PCR Cloning Kit (Cat: PT4045, Clontech) to generate the following plasmids: pMAL-C2x::MBP-TE(N195)-(S77A)-His, pMAL-C2x::MBP-TE-(C155)-T457A-His, pMAL-C2x::MBP-TE(N77A)-His, pMAL-C2x::MBP-TE-C155(T457A)-His, pMAL-C2x::MBP-TE-(C176T)-His, pMAL-C2x::MBP-TE-(C176A)-His and pMAL-C2x::MBP-TE-(C176C)-His. The CDS for OsPYL/RCAR1 to OsPYL/RCAR10 or the mutant, OsPYL/RCAR10-m (with a D-box mutation), were amplified with the primers shown in Supplementary Table 2. The PCR products were inserted into the pET28a vector to express the D-box mutation), were amplified with the primers shown in Supplementary Table 2; the vectors were created with the In-Fusion Advantage PCR Cloning Kit (Takara). The proteins were expressed in E. coli and purified according to the user’s manual. Phosphorylation assays were performed with 1 µg of recombinant fusion protein, MBP-TE-N195-His, MBP-TE(C155)77A-His, MBP-TE-(C155)135-His or MBP-TE-(C155)135-His and 2 µg of His-SAPK’ protein in 30 µl of reaction buffer (20 mM HEPES, pH 7.5, 20 mM MgCl2, 2 mM DTT, 10 µg [32P]ATP, 1 x proteinase inhibitor cocktail and 1 x phosphatase inhibitor cocktail), incubated at 30°C for 1.5 h. The reaction was terminated by adding 6 µl sample buffer and heating at 100°C for 1 min. After separation on 10% SDS-PAGE gel, the gels were stained with Coomassie blue and imaged with a BIO-RAD Gel Doc XR + imaging system. Then, the gel was exposed to GE Amersham hyperfilm MP film for detecting phosphorylated proteins.

**BIFC assays.** The TE-eYFP plasmid was reported previously16. The CDS of SAPK10, SAPK8 and SAPK9 were amplified with primers listed in Supplementary Table 3 and cloned into the vector pSPYCE(M) (eYCE). For transient expression, A. tumefaciens strains (EHA105) carrying the BIFC constructs were used together with the p19 strain and ER marker, mCherry ER-nk CDY-959 (ref. 44), for infiltration of 5-week-old Nicotiana benthamiana leaves as described in Waadt and Kuuda45. Infiltrated leaves were observed 48–72 h after infiltration using a laser confocal scanning microscope (ZEISS Microsystems LSM 700). The eYFP and mCherry fluorescent signals from the expressed fusion constructs were monitored sequentially. The excitation and detection wavelengths for eYFP and mCherry were 514 and 587 nm for excitation and 527 and 610 nm for detection, respectively.

**UPLC-MS/MS Analysis of ABA.** Frozen rice samples were ground in liquid nitrogen with mortar and pestle. The internal standards 45 pmol 2H6-ABA (OIChemMf (Olomouc, Czech Republic)) were added to 100 mg of ground powder. The powder was extracted with 2 ml methanol and kept overnight at ~20°C, then centrifuged at 4°C for 15 min at 18,000 r.p.m. The supernatant was collected, dried under nitrogen, then dissolved in 10 µl ammonium solution (5%). The crude extracts were further purified by Oasis MAX solid phase extraction (SPE) column (Waters, Milford, MA), which had been sequentially preconditioned with 4 ml methanol, 4 ml water and 4 ml ammonium solution (5%). After the samples were loaded, SPE columns were sequentially washed with 4 ml ammonium solution (5%), 4 ml water and 4 ml ammonium solution (1%). The eluates were collected, evaporated to dryness using a nitrogen stream, redissolved in 50 µl of acetonitrile, which was then increased to 40% in 5 min and further increased to 80% in the next 0.5 min. The injection volume for all samples was 5 µl and the column temperature was 35°C. The injection volume was 0.5 µl for each sample. The mass spectrometer was a Quadrupole Premier XE mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ionization source. The electrospray capillary voltage was operated at 2.80 kV in the negative ion mode. The optimized parameters with electrospray ionization source output obtained by infusion of the standard solution of 10 pmol 1 µl1-4 acetoinol-water (95:5, v/v) at 10µl min1 were the following: source temperature, 110°C; desolvation temperature, 350°C; desolvation gas flow, 600 l/min; cone gas flow, 60 l/min; multiplier, 650 V. Quantitative analysis was performed in multiple reaction monitoring (MRM) mode with four timedesigned scans as reported previously46.

**qRT-PCR analyses.** RNA was extracted from frozen samples with the RNAprep Pure Plant Kit (Tiangen) according to the manufacturer’s instructions. qRT-PCRs were performed with the SYBR Premix Ex Taq RT–PCR Kit (Takara), according to the manufacturer’s instructions, with the primers listed in Supplementary Table 4.

**Phytohormone treatment analyses.** One-week-old seedlings of WT, te, Nipponbare and OsPYL/RCAR10-GFP, SAPK10-GFP, SAPK8-GFP, SAPK9-GFP transgenic lines were grown in a growth chamber (Ruihua) under a 14:5:5.5 h light/dark cycle at 30°C. These plants were subjected to ABA and GA3 treatment to determine their effects on the stability of OsPYL/RCAR10 and SnRK2 proteins. For phytohormone treatments, seedlings were placed in 50 ml tubes containing 5 ml of half-strength MS liquid medium supplemented with 1 mM cycloheximide (Inagro), 5 µl ethanol (control), 0.5 µl 100 mM ABA or 5 µl 100 mM GA3. Subsequently, the tubes were placed in a growth chamber (Ruihua) under a 14:5:5.5 h light/dark cycle at 30°C for the indicated times. After phytohormone treatment, the seedlings were frozen in liquid nitrogen for further analysis. Western blots were performed with antibodies against OsPYL/RCAR10, SAPK10, SAPK8, SAPK9, GFP or HSP92, and signals were visualized with enhanced chemiluminescence reagent (GE Healthcare).
Co-immunoprecipitation assays. For Fig. 2b, no phytohormone treatment was done. For Fig. 4j, 1-week-old OE17 plants were washed several times with water. Then, they were placed in 50 ml tubes containing 5 ml of half-strength MS liquid medium supplemented with either 5 µl ethanol, 0.5 µl 100 mM ABA or 5 µl 100 mM GAs. Subsequently, the tubes were placed in a growth chamber (Ruhua) under a 14.5/9.5-h light/dark cycle at 30°C for 3 h. After phytohormone treatment, the seedlings were frozen in liquid nitrogen for further analysis.

For Co-IP assays, total proteins were extracted from 1-week-old and OE17 seedlings in a RIPA buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10 mM MgCl2, 1 mM EDTA, 10% Glycerol, 50 µM MG132, 1x complete proteinase inhibitors (Roche), 1x PhosSTOP phosphatase inhibitors (Roche), 2 µM Staurosporine (Cell Signaling). Then, 200 µl HisBind resin (Novagen) were incubated with 800 µl total or OE17 extracts (containing 3 mg total proteins) for each assay. The mixture was gently shaken at 4°C for 15 min and then loaded on the HisBind Columns (Novagen). The first eluate was collected for western blot analysis. Then the columns were washed two times with 1 ml RIPA buffer. The total protein extracts, first eluate and final resins were resolved in 1xSDS-PAGE sample buffer and western blot was conducted with anti-His or anti-OpyV1/RCAR10 antibodies at 1:2,000 dilution.

LC-MS/MS analysis of phosphorylated TE peptides. The bands of in vitro phosphorylated MBP-TEN195 protein were excised from the PAGE gels, reduced with 25 mM DTT, and alkylated with 55 mM iodoacetamide. In-gel digestion was performed with trypsin (Promega) at 37°C overnight, then sample was heated at 60°C for 1 h to inactivate trypsin and further digested with Asp-N (Promega) at 37°C overnight. The peptides were extracted twice with 0.1% (v/v) trifluoroacetic acid (TFA) in acetonitrile aqueous solution for 30 min. The extracts were centrifuged in a speed-vac to reduce the volume. After that, they were resolved in 0.1% trifluoroacetic acid water solution for Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) analysis.

For LC-MS/MS analysis, the digested peptides were separated by a 60-min gradient elution at a flow rate of 0.3 µl min⁻¹ with the Dionex Ultimate 3000 Nano HPLC system that was directly interfaced with the Thermo Q Exactive mass spectrometer. The analytical column was performed with a Thermofisher Acclaim HPLC system that was directly interfaced with the Thermo Q Exective mass spectrometry/Mass Spectrometry (LC-MS/MS) analysis.

For LC-MS/MS analysis, the digested peptides were separated by a 60-min gradient elution at a flow rate of 0.3 µl min⁻¹ with the Dionex Ultimate 3000 Nano HPLC system that was directly interfaced with the Thermo Q Exactive mass spectrometer. The analytical column was performed with a Thermofisher Acclaim HPLC system that was directly interfaced with the Thermo Q Exective mass spectrometry/Mass Spectrometry (LC-MS/MS) analysis.
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Author contributions
J.W. supervised the project. J.W., H.W., Q.L. and F.W. designed the research and wrote the paper. Q.L. and F.W. performed most of the experiments, P.S. performed some of the qRT-PCR analysis and yeast two-hybrid assay, Z.Z. performed the BiFC assay, X.Z. and X.G. generated the transgenic plants, J.L.W., Z.C. and Jie, W. cultivated the transgenic plants in the field.

Additional information
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