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Calcium-dependent protein kinase 21 phosphorylates 14-3-3 proteins in response to ABA signaling and salt stress in rice

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ABSTRACT

The calcium-dependent protein kinases (CDPKs) are a class of plant-specific kinase that directly bind Ca^{2+} and mediate the calcium-signaling pathways to play important physiological roles in growth and development. The rice genome contains 31 CDPK genes, one of which, OsCPK21, is known to modulate the abscisic acid (ABA) and salt stress responses in this crop; however, the molecular mechanisms underlying this regulation are largely unknown. In the present study, we performed yeast two-hybrid screening, glutathione S-transferase pull-down, co-immunoprecipitation, and bimolecular fluorescence complementation assays to confirm the interaction between OsCPK21 and one of its putative targets, Os14-3-3 (OsGF14e). We used an in vitro kinase assay and site-directed mutagenesis to verify that OsCPK21 phosphorylates OsGF14e at Tyr-138. We used real-time PCR to reveal that several ABA and salt inducible genes were more highly expressed in the OsCPK21-OE and OsGF14e WT-OE plants than in the mutant OsGF14e Y138A-OE and wild-type plants. These results suggest that OsCPK21 phosphorylates OsGF14e to facilitate the response to ABA and salt stress.

1. Introduction

Calcium-dependent protein kinases (CDPKs), which are widely and specifically expressed in plants, are calcium-binding Ser/Thr protein kinases that mediate calcium-signaling pathways [1,2]. CDPKs have variable N-terminal domain sequences and lengths, but all contain myristoylation sites that are important for membrane association. A junction domain connects the CDPK protein kinase catalytic domain with a C-terminal calmodulin-like calcium-binding domain, which contains an autoinhibitory pseudosubstrate sequence and a binding site for intramolecular interactions with the C-terminal domain [3]. Upon Ca^{2+} binding, the CDPKs undergo a conformational change that exposes the active site, and they become prone to autophosphorylation and/or they phosphorylate their downstream targets, such as transcription factors, metabolic enzymes, and ion channels [4–6].

The CDPKs play a crucial role in the response to abiotic stresses, including drought, salt, and cold, often regulating stress tolerance through the modulation of abscisic acid (ABA) signaling [7]. One of the 31 CDPK genes in rice (Oryza sativa), OsCPK14, encodes a protein that regulates the ABA-response genes through the phosphorylation of OsDi19-4,which is a C2H2 type zinc finger protein and plays an important role in abiotic stress [8]. Furthermore, OsCPK9, OsCPK12, and OsCPK21 have been demonstrated to be positive regulators of abiotic stress tolerance and the response to ABA treatment [9–11]. By contrast, OsCPK12 also negatively modulates resistance to rice blast (Magnaporthe grisea) [10].

CDPKs can phosphorylate several 14-3-3 proteins targets. 14-3-3 protein is a conserved protein family in eukaryotes. It can be used as a molecular scaffold with two different proteins together participating in a variety of biological events [12]. However, there is mounting evidence that the 14-3-3 scaffolding proteins can also be directly targeted for post translational modification. In *Arabidopsis thaliana*, AtCPK3 phosphorylates At14-3-3ζ at Ser-58, which in turn affects its interaction with AtCPK3, leading to AtCPK3 degradation [13]. Furthermore, site-directed mutagenesis of CDPK phosphorylation sites on At14-3-3χ (converting Ser-72 to Asp) can inhibit the inactivation of nitrate reductase [14]; however, the effect of OsCDPKs phosphorylation of Os14-3-3s on signal transmission has

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not been reported.

Previous studies have shown that OsCPK21 responds to ABA signaling and salt stress to regulate the expression of several stress-response genes in rice [11]. In this study, we demonstrate that OsCPK21 interacts with Os14-3-3 (OsGF14e), specifically phosphorylating it at Tyr-138. Our findings suggest that OsCPK21 is involved in the response to ABA signaling and salt stress through its phosphorylation of OsGF14e.

2. Materials and methods

2.1. Plant materials and growth conditions

Rice plants (Oryza sativa spp. japonica cv. Zhonghua11 (ZH11)) were grown under natural conditions in a field at Beijing Normal University (Beijing, China). The young rice seedlings used for treatment were grown in a growth chamber, using previously described growth conditions [15]. Wild-type (WT) *Arabidopsis thaliana* (Col-0) for the Arabidopsis mesophyll protoplast transformations was grown in growth chambers, under previously described growth conditions [16].

2.2. Plasmid construction

The coding sequences of OsCPK21 and OsGF14e were amplified using PCR with the primers listed in Supplemental Table 1. To construct the yeast two-hybrid (Y2H) vectors, the coding region of OsCPK21VK (amino acids 1–363) was cloned into the Y2H 'prey' vector pGADT7 (Clontech Laboratories), and the coding region of OsGF14e was cloned into the Y2H 'bait' vector pGBKT7 (Clontech Laboratories). To generate the in vitro expression constructs and pENTR constructs, the PCR products were digested with restriction enzymes (listed in Supplemental Table 1) and cloned into the corresponding vectors. The pENTR (Addgene) constructs were inserted into the destination vector pMDC32 (http://www.arabidopsis.org/) using the Gateway LR II Kit (Thermo Fisher Scientific). To produce the bimolecular fluorescence complementation (BiFC) constructs, the coding region of OsCPK21 was cloned into the pSAT1-cCFP-N vector to generate the plasmid OsCPK21-cCFP, and OsGF14e was cloned into the pSAT1-nVenus-C vector to generate the plasmid nVenus-OsGF14e.

2.3. RNA extraction and quantitative real-time PCR

Total RNA was isolated from different rice tissues using the TRIzol reagent. Approximately 3.5 μg of the isolated RNA was reverse-transcribed using the Revert-Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) and used as a template for qRT-PCR, which was performed using a Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific) with the Power SYBR Green PCR Master Mix (Thermo Fisher Scientific). OsActin1 was amplified and used to normalize the expression of the target genes. Three technical repeats were performed for each qRT-PCR assay. The primers used in the qRT-PCR assays are listed in Supplemental Table 1.

2.4. Yeast two-hybrid assay

A yeast two-hybrid screening assay was performed according to the "Matchmaker™ GAL4 Two-Hybrid System 3 & Libraries User Manual" (Clontech Laboratories). Total RNA was extracted from rice tissues at the vegetative stage (including the coleoptile, root, shoot, and tiller) and the reproductive stage (including the anther, pistil, and growing embryo). Transformed colonies were grown for 3–8 days at 28 °C on a yeast SD medium lacking leucine (Leu), tryptophan (Trp), adenine (Ade), and histidine (His) (SD-LWAH). The plasmid DNA was recovered from positive yeast colonies, transformed into *Escherichia coli* (strain DH5α), and extracted for sequencing.

For the Y2H assay, pGADT7-OsCPK21VK and pGBKT7 OsGF14e were co-transformed into AH109. The transformed colonies were grown on yeast SD medium lacking Leu and Trp (SD-LW) for three days at 28 °C, then grown in a liquid medium lacking Leu and Trp until $OD_{600} = 1$. A 6-μL aliquot of undiluted cell suspension was plated on yeast SD medium lacking His, Leu, and Trp (SD-LWH) with 25 mM 3-AT to identify protein interactions. Yeast strains containing pGADT7-OsCPK21VK or pGBKT7-OsGF14e in combination with the pGADT7 or pGBKT7 empty vectors were used as controls.

2.5. Glutathione S-transferase pull-down assay

For the in vitro glutathione S-transferase (GST)-OsGF14e pull-down assay, 7.5 μg recombinant GST-OsGF14e or 3.5 μg GST was incubated with 12 μg His-OsCPK21 at 4 °C for 30 min in 200 μL binding buffer (25 mM Tris-HCl (pH 7.5), 2.5 mM β-mercaptoethanol, 100 mM NaCl, and 10% glycerol). After incubation, 20 μL glutathione beads (GE Healthcare) were added and incubated at 25 °C for 15 min, then washed five times with washing buffer (25 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 0.5% Triton X-100). His-OsCPK21 was detected by anti-His antibody (1:2500; Cell Signaling Technology).

2.6. Co-immunoprecipitation assay

The Ubi:OsCPK21-3xHA/Ubi:6xMyc-OsGF14e WT and Ubi:EYFP-3xHA/Ubi:6xMyc-OsGF14e WT constructs were independently transiently expressed in Arabidopsis mesophyll protoplasts, as previously described [16]. After incubation in the dark for 16 h at 22 °C, the total proteins were extracted. OsCPK21-3xHA was immunoprecipitated using an anti-HA antibody (1:250; Cell Signaling Technology), and the co-immunoprecipitated proteins were then detected using an anti-Myc antibody (1:2500; Cell Signaling Technology).

2.7. Subcellular localization of OsCPK21 and OsGF14e using BiFC

The subcellular localization of OsCPK21 and OsGF14e was observed using a transient transformation assay in Arabidopsis mesophyll protoplasts. To identify the interactions between OsCPK21 and OsGF14e (WT or Y138A), OsCPK21-cCFP and nVenus-OsGF14e were co-transformed into Arabidopsis mesophyll protoplasts, and the transformed cells were analyzed for fluorescence. Enhanced yellow fluorescent protein (EYFP), mCherry, and Venus were observed under a confocal laser scanning microscope (LSM 700; Carl Zeiss).

2.8. In vitro kinase assay and Phos-tag SDS-PAGE

For the kinase assay, 2.5 μg His-OsGF14e as a substrate was mixed with 100 μL kinase assay buffer (25 mM Tris-HCl (pH 7.5), 10 mM $MgSO_4$, 2 mM DTT), 100 μM ATP, and 5 μg purified Trx-OsCPK21. For the calcium-dependence assay, either $CaCl_2$ (1 mM) or EGTA (2 mM) were added to the reaction buffer. Reactions were performed for 30 min at 30 °C, then terminated by adding 20 μL 6xSDS-PAGE loading buffer. The samples were boiled at 100 °C for 4 min, 13000 g, centrifuged for 2 min at room temperature, then subjected to SDS-PAGE.

Fluorescent staining of SDS-polyacrylamide gels using Pro-Q® Diamond Phosphoprotein Gel Stain (Thermo Fisher Scientific) was

performed as previously described [17]. The gel was fixed in 50% methanol, 10% acetic acid solution twice, for 30 min per fixing, then washed three times with ultrapure water for 10 min per wash. The gel was incubated in Pro-Q® Diamond phosphoprotein gel stain for 90 min at room temperature, then destained three times using 15% 1, 2-propanediol in 50 mM sodium acetate (pH 4.0) for 30 min each time. Images were acquired using a Molecular Imager FX (Bio-Rad Laboratories) at an excitation wavelength of 532 nm and using a 580-nm band pass emission filter for Pro-Q Diamond dye detection. After image acquisition, the gel was stained using Coomassie Brilliant Blue to quantify the amount of protein loaded.

Phosphoprotein detection was performed using Phos-tag SDS-PAGE, as previously described [18]. Phos-tag gels can separate phosphorylated proteins by the number of phosphate groups attached; proteins containing more phosphorylated residues have a lower migration rate through the Phos-tag gel. His-OsCPK21, His-OsGF14e, and their phosphorylated forms were detected from the Phos-tag gel using an anti-His antibody (1:2500; Cell Signaling Technology).

2.9. Generation of overexpression plants

The overexpression constructs of OsCPK21-EYFP, 6xMyc-OsGF14e WT and 6xMyc-OsGF14e Y138A were introduced into ZH11 separately by Agrobacterium tumefaciens-mediated transformation, as described previously [19]. Western blotting was used to identified these transgenic rice plants. OsCPK21-EYFP was detected by anti-GFP antibody (1:5000; Cell Signaling Technology), 6xMyc-OsGF14e WT and 6xMyc-OsGF14e Y138A were detected by anti-Myc antibody (1:2500; Cell Signaling Technology), and the loading quantity of protein samples was detected by anti-Actin antibody (1:10000; Cell Signaling Technology).

2.10. ABA and NaCl treatment

The seeds of WT and transgenic plants were germinated on ½-strength MS liquid medium for five days, after which the seedlings were transferred to ½-strength MS liquid medium containing 1 μM ABA and grown for two weeks. The ABA solution was changed twice a week. And 2 weeks seedlings were transferred to ½-strength MS liquid medium containing 200 mM NaCl and grown for two days. Harvested the roots as the sample for qRT.

2.11. Accession number

Sequence data from this article can be found in the GenBank/EMBL data libraries under the following accession numbers: OsCPK21 (Os08g42750), OsGF14e (Os02g36974), OsLEA3 (Os05g46480), Osbzip23 (Os02g52780), OsP5CS (Os01g62900), and OsUBIQUITIN5 (Os01g22490).

3. Results and discussion

3.1. OsCPK21 interacts with OsGF14e

To investigate the molecular mechanisms by which OsCPK21 regulates salt stress tolerance in rice, we screened for putative OsCPK21-interacting proteins using a Y2H assay (Table 1). One 14-3-3 scaffold protein, OsGF14e, was of particular interest, and we performed an additional Y2H assay to confirm its interaction with OsCPK21. As the kinase activities of the CDPKs are regulated by the concentration of Ca^{2+}, we removed the C-terminal fragment of OsCPK21, including the calcium-sensing EF-hand motifs (the calmodulin-like regulatory domain) and the junction domain, to obtain a truncated protein, OsCPK21VK, which acts as a constitutively active kinase. The Y2H assay showed that OsCPK21VK could physically interact with OsGF14e (Fig. 1A). Our in vitro GST pull-down assay also revealed a direct physical interaction between OsCPK21 and OsGF14e (Fig. 1B). We next performed a co-immunoprecipitation assay, in which Arabidopsis mesophyll protoplasts were transformed to co-express OsCPK21-3xHA and 6x-Myc-OsGF14e WT, and OsGF14e was again found to interact with OsCPK21 (Fig. 1C).

Table 1
Putative OsCPK21-interacting proteins identified using a yeast two-hybrid screen.

Locus ID	Function description
Os01g56680	photosystem II reaction center W protein, chloroplast precursor
Os01g59150	tubulin/FtsZ domain containing protein
Os01g62610	peptidyl-prolyl cis-trans isomerase, FKBP-type
Os02g02410	DnaK family protein
Os02g16040	ubiquitin-conjugating enzyme
Os02g25640	glutelin
Os02g36974	**14-3-3 protein, OsGF14e**
Os02g37150	expressed protein
Os02g38920	glyceraldehyde-3-phosphate dehydrogenase
Os02g39850	transferase family protein
Os02g47140	L11 domain containing ribosomal protein
Os03g07880	nuclear transcription factor Y subunit
Os03g12010	expressed protein
Os03g22180	60S ribosomal protein L 18-3
Os03g22180	CS domain containing protein
Os03g40270	alpha-1,4-glucan-protein synthase
Os05g02070	expressed protein
Os05g13940	retrotransposon protein, putative, inclassified
Os05g33380	fructose-bisphospate aldolase isozyme
Os05g45950	outer mitochondrial membrane porin
Os07g07670	expressed protein
Os08g07670	expressed protein
Os08g41830	sulfite oxidase
Os08g41810	ribosomal protein L22
Os08g42010	nodulin
Os09g12750	Myb-like DNA-binding domain containing protein
Os10g02880	O-methyltransferase
Os11g01510	ubiquitin-activating enzyme
Os12g44140	expressed protein

The candidate interaction protein of OsCPK21 is shown in bold.

To identify whether OsCPK21 and OsGF14e interact in rice, we first observed their subcellular localization. The proper localization of most CDPKs on the plasma membrane is known to require N-terminal acylation, such as myristoylation and palmitoylation [20,21]. Our transient expression of EYFP-tagged OsCPK21 and OsGF14e in the mesophyll protoplasts of Arabidopsis revealed that OsCPK21FL (full length)-EYFP was localized to the plasma membrane, while EYFP-OsGF14e was located in the cytoplasm (Fig. 1D). We then performed a BiFC assay to investigate the interaction between OsCPK21 and OsGF14e in planta. When nVenus-OsGF14e and OsCPK21-cCFP were co-expressed in Arabidopsis mesophyll protoplasts, the complemented Venus signal was observed in the cytoplasm where OsGF14e, but not OsCPK21, was localized (Fig. 1E). This result may have two explanations: (1) OsCPK21 interacts with OsGF14e in the cytoplasm, or (2) OsCPK21 might be captured in the cytoplasm when it meets OsGF14e, and the resulting complex might influence the N-terminal acylation of OsCPK21 and alter its localization. These data collectively indicate that OsCPK21 interacts with OsGF14e.

3.2. OsCPK21 phosphorylates OsGF14e at Tyr-138

The finding that OsCPK21 interacts with OsGF14e suggests that OsGF14e is a substrate of OsCPK21. To confirm this hypothesis, we performed an in vitro kinase assay. The results showed that OsCPK21 phosphorylates the general substrate Histone IIIS, and the

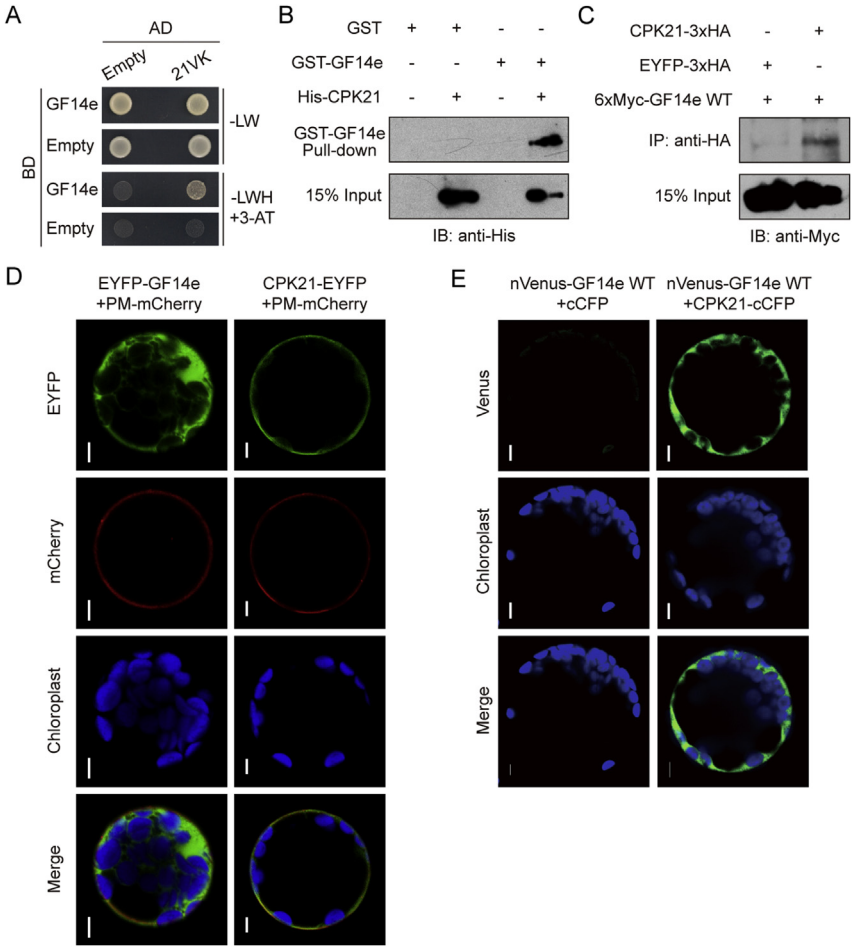

Fig. 1. OsCPK21 interacts with OsGF14e.
(A) Interaction of OsCPK21VK and OsGF14e detected using a yeast two-hybrid assay. Yeast transformants were grown on the control medium (SD-Leu/-Trp (-LW)) and the selective medium (SD-Leu/-Trp/-His (-LWH) plus 25 mM 3-AT). AD, activating domain; BD, binding domain; SD, synthetic dropout.
(B) Interaction of OsCPK21 and OsGF14e in vitro detected using a glutathione S-transferase (GST) pull-down assay. Resins containing GST-OsGF14e or GST were used to pull down His-OsCPK21. The proteins were analyzed by performing an immunoblot assay using anti-His antibody. Protein input (15% input) for His-OsCPK21 was also detected.
(C) Interaction of OsCPK21 and OsGF14e detected using a co-immunoprecipitation assay in Arabidopsis mesophyll protoplasts. OsCPK21-3xHA or EYFP-3xHA was immunoprecipitated using an anti-HA antibody, and co-immunoprecipitated 6xMyc-OsGF14e WT was detected using anti-Myc antibody. Protein input (15% input) for 6xMyc-OsGF14e WT was also detected.
(D) Subcellular localization of OsGF14e (left) and OsCPK21(right) in Arabidopsis mesophyll protoplasts. The fluorescence of PM-mCherry indicates the plasma membrane location. Bars = 5 μm.
(E) Bimolecular fluorescence complementation analysis of the interaction between OsCPK21 and OsGF14e in *Arabidopsis* mesophyll protoplasts. nVenus, N terminus of Venus; cCFP, C terminus of CFP. Bars = 5 μm.

extent of phosphorylation was influenced by the concentration of Ca^{2+} (Fig. 2A). His-OsGF14e was also phosphorylated by His-OsCPK21 in a calcium-dependent manner (Fig. 2B).

We further determined which Ser/Thr/Tyr residues in OsGF14e can be phosphorylated by OsCPK21. We chose six Ser/Thr/Tyr residues as potential OsCPK21 phosphorylation sites based on the conserved sequences of the 14-3-3s (Fig. 2C); these residues were reported to be potential phosphorylation sites in other 14-3-3s (in maize or Arabidopsis), according to phosphoproteomic data [22]. Using site-directed mutagenesis, these candidate sites were substituted with Ala. By comparing the phosphorylation levels of these mutated proteins with WT OsGF14e, one Tyr residue (Tyr-138) emerged as the site of OsCPK21 phosphorylation (Fig. 2D). We then used a Phos-tag SDS-PAGE gel (Phos-tag gel) to further confirm this; after phosphorylation by His-OsCPK21, three mobility-shifted bands of phosphorylated His-OsGF14e were detected in a Phos-tag gel, and these shifted bands vanished when Tyr-138 was substituted with Ala (Fig. 2E). Although CDPKs are classified as serine/threonine protein kinases, not tyrosine protein kinases, recent studies have shown that autophosphorylation at Tyr-24 occurs in soybean (Glycine max) CDPKβ (GmCDPKβ), and the site-directed mutation of GmCDPKβ (Y24F) resulted in a weaker kinase activity [23]. Autophosphorylation of Tyr residues was also observed for several Arabidopsis CDPKs, such as AtCPK4, AtCPK34, and AtCPK28 [14,23]. These data suggest that several CDPKs may be dual-specificity kinases and play an important role in phosphotyrosine signaling. The Tyr-138 residue of OsGF14e is conserved in all Arabidopsis 14-3-3 proteins, and corresponds to Tyr-137 of GF14-6 in the monocotyledon maize (Zea mays). GF14-6 was reported to be phosphorylated by insulin-like growth factor receptor 1 (IGFR-1) at Tyr-137, which reduced its affinity for H^+-ATPase [24]. These results suggest that OsCPK21 phosphorylates Tyr-138 on OsGF14e.

The site mutation of OsGF14e may influence its interaction with OsCPK21, and then influence the OsGF14e phosphorylation by OsCPK21. In order to examine this possibility, we performed

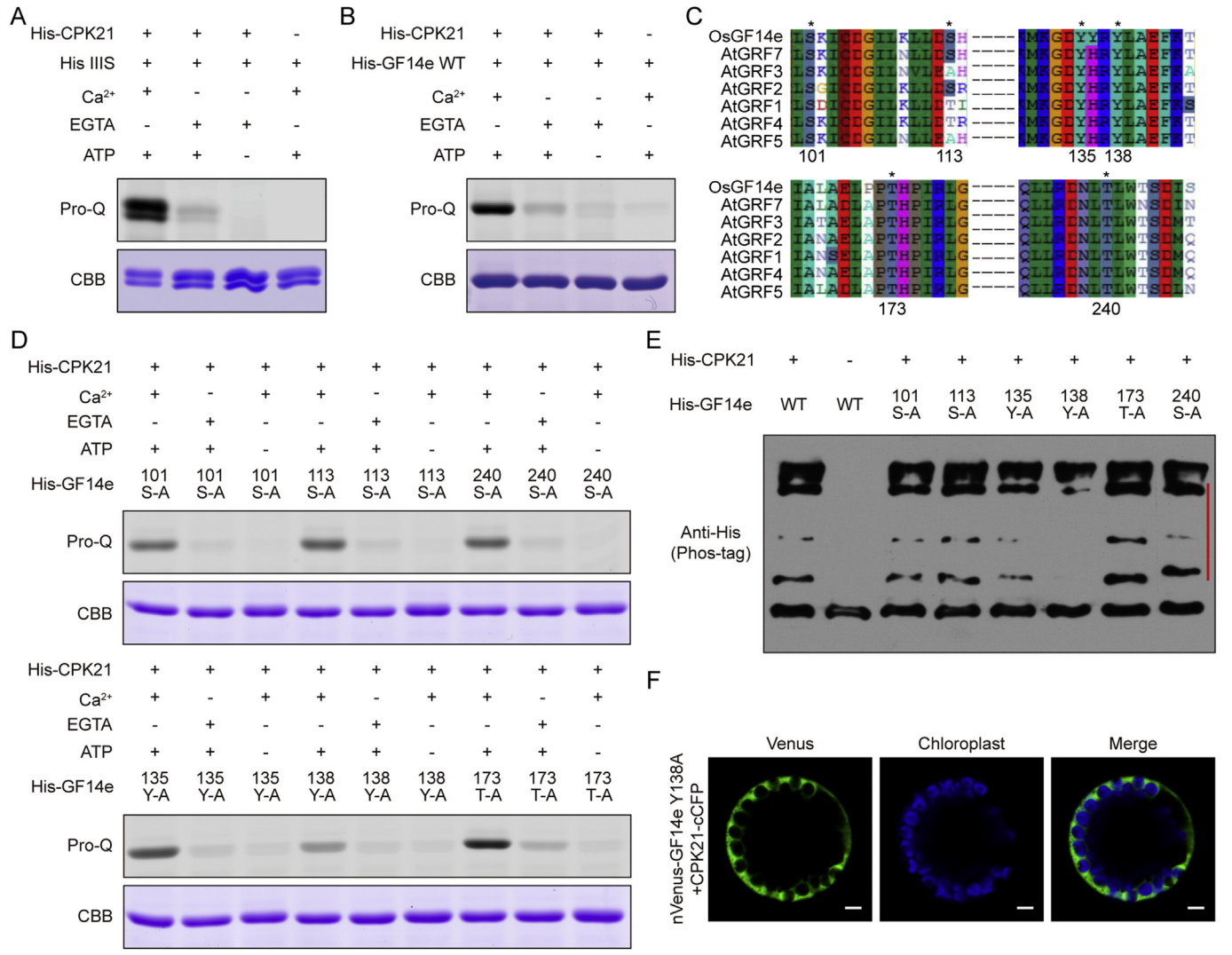

Fig. 2. OsCPK21 phosphorylates OsGF14e at Tyr-138.

(A) In vitro kinase assay of OsCPK21 showing the phosphorylation of Histone IIIS by His-CPK21 in a calcium-dependent manner. The polyacrylamide gel was stained with Pro-Q® Diamond phosphoprotein gel stain, and imaged on a laser scanner (top). Coomassie Brilliant Blue staining was used as the control to indicate the loading quantity of the sample (bottom). His IIIS represents Histone IIIS.

(B) In vitro kinase assay of OsCPK21 showing the calcium-dependent phosphorylation of His-OsGF14e WT by His-OsCPK21.

(C) Six Ser/Thr/Tyr residues as potential OsCPK21 phosphorylation sites on OsGF14e. Multiple sequence alignments of OsGF14e and several AtGRFs (14-3-3s in *Arabidopsis* with high homology to OsGF14e) were performed. Stars indicate the conserved sites selected as potential OsCPK21 phosphorylation sites. Numbers indicate the locations of these six Ser/Thr/Tyr residues on OsGF14e.

(D) In vitro kinase assay of OsCPK21 showing the calcium-dependent phosphorylation of His-OsGF14e and its site-specific mutants by His-OsCPK21.

(E) In vitro kinase assay of OsCPK21 showing the phosphorylation of His-OsGF14e and its site-specific mutants by His-OsCPK21. The phosphorylation states of His-OsGF14e were tested using a phospho-specific Phos-tag gel, and His-OsCPK21 and His-OsGF14e were detected by immunoblotting (WB) with anti-His antibody. Red line marks shift bands, which indicate phosphorylated His-OsGF14e.

another BiFC assay. When nVenus-OsGF14e Y138A and OsCPK21-cCFP were co-expressed in the mesophyll protoplasts of Arabidopsis, the complemented Venus signal was also observed in the cytoplasm (Fig. 2F), as was nVenus-OsGF14e WT and OsCPK21-cCFP. This suggests that the site-directed mutations of OsGF14e do not influence the interaction between OsCPK21 and OsGF14e WT and OsCPK21-cCFP. This suggests that the site-directed mutations of OsGF14e do not influence the interaction between OsCPK21 and OsGF14e.

3.3. OsCPK21 phosphorylates OsGF14e in response to ABA signaling and salt stress in rice

To further explore whether OsGF14e phosphorylation by OsCPK21 is involved in the ABA and salt response in rice, we generated the OsCPK21-OE (overexpress) lines, OsGF14e WT-OE (ProOsCPK21: OsGF14e WT) and OsGF14e Y138A-OE (ProOsCPK21: OsGF14e Y138A) transgenic plants (Fig. 3A) to analyzed the ABA and NaCl responsive genes transcription levels. OsLEA3, a late embryogenesis abundant protein gene; OsP5CS, D1-pyrroline-5-carboxylate synthetase gene for proline biosynthesis and Osbzip23, a basic leucine zipper (bZIP) transcription factor gene have been tested after ABA and NaCl treatment [11]. After ABA and NaCl treatment, the transcript levels of OsLEA3 were higher in the OsCPK21-OE lines than in the WT, as was previously reported. Meanwhile, these genes were all upregulated in the OsGF14e-OE plants compared with the WT, and partially downregulated in OsGF14e Y138A-OE relative to OsGF14e-OE, almost to their levels in

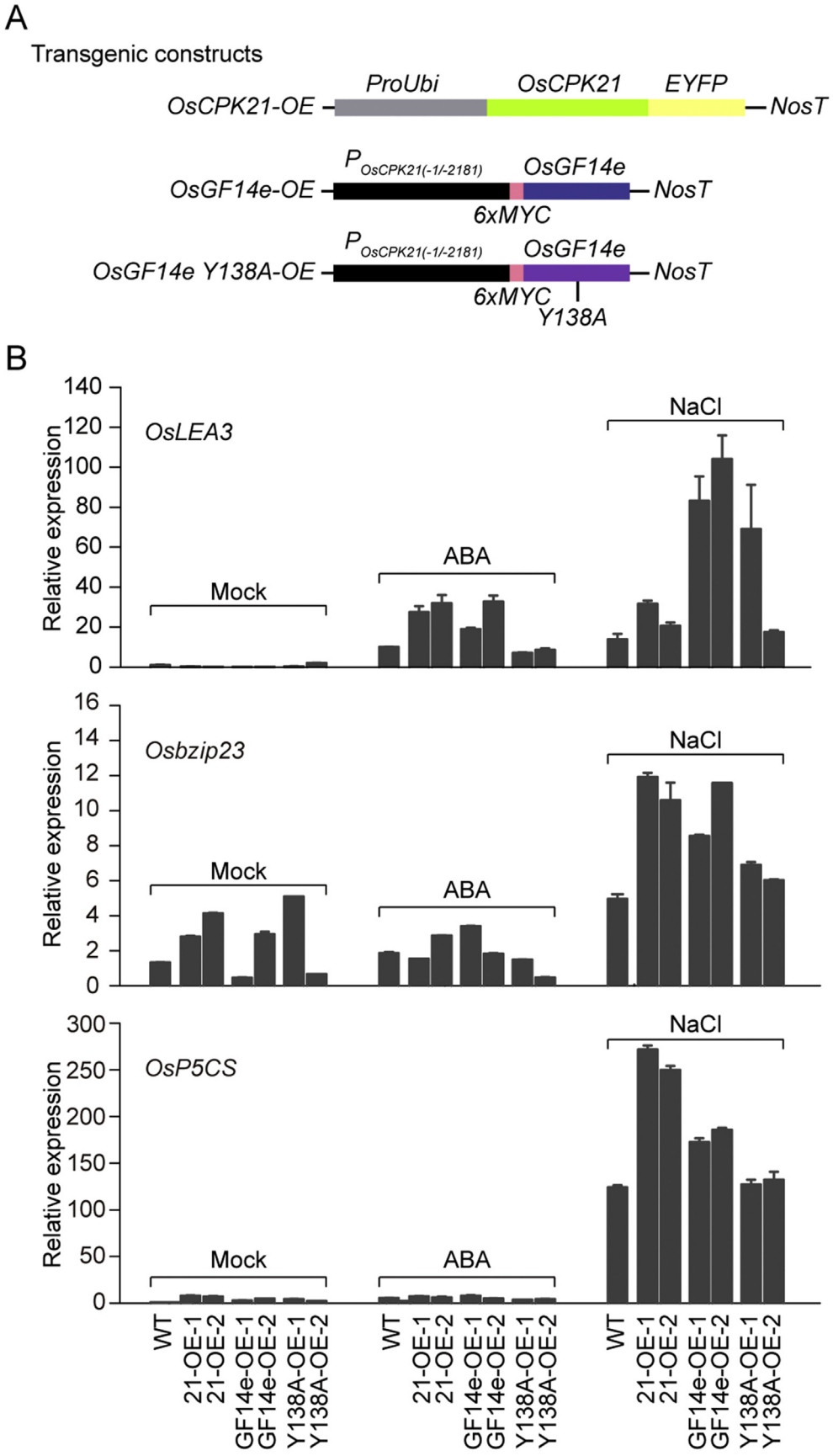

Fig. 3. OsCPK21 phosphorylates OsGF14e in response to ABA and salt.
(A) Schematic maps for transgenic constructs of OsCPK21-OE, OsGF14e WT-OE, and OsGF14e Y138A-OE transgenic plants. *ProUbi, UBIQUITIN5* promoter; $P_{OsCPK21(-1/-2181)}$, putative full length promoter of *OsCPK21*.
(B) Expression analysis of selected ABA and salt response genes in OsCPK21-OE, OsGF14e WT-OE, and OsGF14e Y138A-OE transgenic plants. Data are means ± SE of three independent measurements.

the WT plants. But when the expression level of Oszip23 and OsP5CS have been tested in these plants, the similar regulation only existed in the NaCl treatment not ABA (Fig. 3B). There are at least four rice 14-3-3/GF14 genes (GF14b, GF14c, GF14e, and GF14f) are induced by abiotic stress accumulated in roots that interact with several stress response-related proteins [25]. Previous study had reported OsCPK1 could regulate the transcript level of OsGF14c induced by abiotic stress [26]. But OsCPK21 interact with OsGF14e and phosphorylate at Tyr-138 is the post transcriptional regulation reported firstly. Collectively, these results suggested that OsCPK21 interacts with OsGF14e to positively participate in ABA signaling and salt stress, partially by phosphorylating Tyr-138.

In conclusion, this is the first report showing that OsCPK21 can interact with OsGF14e by Y2H screening; and phosphorylate OsGF14e at Tyr-138. OsCPK21 response to ABA signaling and salt stress by post transcriptional regulation of OsGF14e in rice. In Arabidopsis, the cold-responsive protein kinase CPRK1 phosphorylates 14-3-3 proteins to enable them to interact with C-repeat/DRE-binding factor (CBF) in the nucleus during the cold response. This suggests that transcription factors may receive the signal from OsCPK21 via post transcriptional regulation of OsGF14e during the ABA and salt response. However, several OsCPK21 phosphorylation sites may exist on OsGF14e, because the mutation of Tyr-138 to Ala only partially reverted the expression levels of the ABA and salt inducible genes in OsCPK21-OE to the WT level.

Conflict of interest

We declare that there are no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2017.09.166.

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