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AtSIEK, an EXD1-like protein with KH domain, involves in salt stress response by interacting with FRY2/CPL1

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ABSTRACT

Abiotic stress has great impacts on plant germination, growth and development and crop yield. Therefore, it is important to understand the molecular mechanism of plants response to abiotic stress. In this study, we identified a plant specific protein AtSIEK (stress-induced protein with EXD1-like domain and KH domain) response to salt stress. *AtSIEK* encodes a hnRNP K homology (KH) protein localized in nucleus. Amino acid sequences analysis found that SIEK protein is specific in plants, containing two domains with EXD1-like domain and KH domain, while SIEK homolog in animals only had EXD1-like domain without KH domain. Physiology experiments revealed that *AtSIEK* was significantly induced under salt stress and the *siek* mutant shows sensitive to salt stress, indicating that AtSIEK was a positive regulator in stress response. Further, molecular, biochemical, and genetic assays suggested that AtSIEK interacts with FRY2/CPL1, a known regulator in response to abiotic stress, and they function synergistically in response to salt stress. Taken together, these results shed new light on the regulation of plant adaption to abiotic stress, which deepen our understanding of the molecular mechanisms of abiotic stress regulation in plants.

1. Introduction

In nature, plants are constantly subjected to miscellaneous abiotic stresses such as soil salinity, drought, extreme temperatures. The abiotic stresses have caused a lot of adverse effects on the growth and development of plants, leading to reduction of world crop food yield. It is estimated that the yield of major food crops in the world may be reduced by 50 % or even more due to the threat of abiotic stresses [1]. To coordinate growth and defense, plants have expanded elaborated mechanisms of stresses perception and signal transduction.

Abiotic stresses can be sensed on the plasma membrane by stress sensors. Osmotic stress, which can be induced by drought and salinity, is perceived by hyperosmolarity-gated calcium channel OSCA1 [2–4]. Besides hyperosmotic stress, Soil salinity can also cause Na^+ ionic stress for plants. MOCA1-dependent GIPC (glycosyl inositol phosphorylceramide) binds to Na^+ cation and perceives the variation of sodium abundance [5]. The perception of environmental stimuli excites downstream signal transduction including activation of second messengers, transcriptional regulation and transcripts processing, post-translational and epigenetic regulation [6,7]. Except for signaling cascades which can change the status of components in cytosol and nucleus such as nucleic acids, proteins, carbohydrates, lipids, ROS and metabolites, cellular organelles, for example ER, chloroplast, mitochondrion, peroxisome and cell wall, can also be involved in sensing and responding to abiotic stresses [8].

To adapt with harsh environment, plant have developed sophisticated strategies of which RNA transcription takes intermediate hub position. The quality control of RNA molecules is especially important for plant survival under abiotic stresses [9,10]. A large number of distinct ribonucleases, 3′ to 5′ exoribonucleases in particular, were required for almost all RNA metabolism. There are six exoribonucleases families in plants which consist of the RNR, DEDD, RBN, PDX, RRP4 and 5PX. The DEDD family can be subdivided into DEDDh and DEDDy based on the conserved amino acid residues in motif [11].

In *Saccharomyces cerevisiae*, RRP6 exoribonuclease with a DEDDy catalytic domain, is a core component of RNA exosomes and has

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function in RNA supervision, processing and degradation [12–14]. MUT-7 in *Caenorhabditis. Elegans*, interacts with RDE-2 to participate in siRNA generation during RNA interference [15]. Egalitarian and Nibbler are well characterized DEDDy exoribonucleases in *Drosophila*. Egalitarian is essential for oocyte specification and axis determination by linking mRNA localization signals to the dynein motor [16,17]. Nibbler acts as not only a trimmer of miRNA at 3′ end, but also a cutter of piRNA precursor in piRNA maturation [18–20]. In mammalian, EXD1, with a Like-Sm domain at N-terminal, core DEDDy exoribonuclease domain and a long tail C-terminal, can form a complex with TDRD12 to participate in MIWI2 piRNA biosynthesis [21,22]. In *Arabidopsis*, Atrimmer 2, which is a DEDDy type 3′ to 5′ exoribonuclease, can trim miRNA* rather than miRNA with interacting with Argonaute proteins [23].

In the process of transcription and post transcriptional modification, mRNA is closely related to RNA binding proteins (RBPs), which have one or more RNA binding domains, namely RNA recognition motif (RRM) and K-homology (KH) domains [24,25]. Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is the first protein found to contain KH domain [26]. The typical function of KH domain is to recognize RNA and bind single strand DNA. The proteins containing KH domain are mainly involved in transcription, mRNA stability, translation silencing and mRNA localization [27]. KH domain containing proteins have been found in human disease research. For example, fragile X mental retardation syndrome is caused by the loss of function of fragile X mental retardation protein (FMRP) [28]. Several KH domain containing proteins have been found in *Arabidopsis* and HOS5 is the most characterized KH protein. HOS5 regulates mRNA splicing and miRNA biogenesis in response to abiotic stress by interacting with splicing factors RS40 and RS41 [29–31]. HEN4, which contains four KH domains, promotes the processing of pre-mRNA and plays a key role in flower morphogenesis [31]. Another KH protein in *Arabidopsis thaliana* is FLK (Flowering Locus KH Domain), which has three KH domains and acts as an inhibitor of FLC gene to regulate flowering time [32].

Here we report that a plant specific gene *SIEK* (stress-induced protein with EXD1-like domain and KH domian) was identified as salt stress regulator in plant adaptation to environmental stimuli, by interacting with stress-related protein FRY2/CPL1. With the single mutant of SIEK and FRY2/CPL1 has similar phenotype under abiotic stresses, *siek cpl* double mutant showed much stronger phenotype than single mutant. Our results provided the molecular mechanism of plant specific protein SIEK involving in plant abiotic stress response.

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis thaliana plants were growth on Murashige and Skoog (1/2 MS) plates and MS plates including abiotic factors under LDs (16-h light/8-h dark) at 23 ± 2 °C. The mutant seeds of *siek-1*, *siek-2* and *cpl1–9* in the Columbia (Col) background were obtained from the Arabidopsis Biological Resource Center (ABRC). Double mutant *siek-2 cpl1–9* was generated by genetic crossing.

2.2. RNA extraction and qRT-PCR analyses

Total RNA was extracted from leaves of *Arabidopsis thaliana* under the treatment of 150 mM NaCl for each sample using an RNAprep pure kit (Tiangen) and then reverse transcribed using HiScript II Q RT SuperMix (Vazyme). qRT-PCR analyses were performed in the CFX96 Real-Time System (Bio-Rad) with ChamQ Universal SYBR qPCR Master Mix (Vazyme). The *Arabidopsis thaliana Actin* gene was used as an internal control. Primers used in this assay are listed in Supplemental Table 1.

2.3. Physiological experiments and germination rate statistics

The mutants of *siek-1*, *siek-2*, *cpl1–9* and *siek-2 cpl1–9* were first germinated on the plates of 1/2 MS medium (0.75 % Agar, placed horizontally). Then after 3 days, germination healthy seedings were transplanted onto 1/2 MS medium plates (1.2 % Agar, placed vertically) containing different factors including 90 mM and 150 mM NaCl, 20 mM LiCl, 20 μM MeJA, 300 mM Sorbitol, and 60 μM MMS. These plates were cultured under the same suitable conditions then periodically observe the recording phenotype.

2.4. Subcellular localization of AtSIEK

The full-length coding sequences of *AtSIEK* were amplified and cloned into the transient expression vector CD3–688-GFP to produce *pro35S:AtSIEK-GFP* fusion construction. This vector was introduced into EHA105 then resuspend the transferred cells with the infective buffer, including 10 mM $MgCl_2$, 10 mM MES, and 150 μM acetosyringone (AS), which infected by disposable syringes and expressed in young tobacco leaf cells. After 48 h, the fluorescence signals were observed using a Zeiss LSM710 laser scanning confocal microscope. Primers used in this assay are listed in Supplemental Table 2.

2.5. GUS staining

The promoter sequences of *AtSIEK* were amplified and cloned into the expression vector CD3–693-GUS to produce *proAtSIEK:GUS* fusion construction. This vector was transferred into *Col* to obtain GUS-positive seeds. Positive seedings of 4-day-old seedling, 5-day-old seedling, 6-day-old seedling, 10-day-old seedling, Matured leaf, flower structure, and silique and stalk were selected for GUS staining. These samples were staining by GUS stain Kit (Coolaber) and the stained sites were observed under stereoscopy. Primers used for this vector construction are listed in Supplemental Table 2.

2.6. Yeast two-hybrid and BiFC assays

For the Y2H assays, the full-length coding region, the SIEK-Egl fragment (1–270 aa), the SIEK-KH fragment (251–343 aa) of SIEK were cloned into pGBKT7 and the full-length of FRY2/CPL1 were cloned into pGADT7. Various combinations of plasmids were co-transformed into the yeast strain AH109 using the Yeast Transformation Kit (Coolaber) according to the instruction manual. Yeast strains with plasmid combinations were spread onto SD/−Trp/−Leu/+X-gal plates and incubated at 30 °C until colonies appeared. Colonies were then plated onto SD/−Ade/-His/−Leu/−Trp dropout screen medium to test protein interactions.

For the BiFC assay, the full-length of SIEK and FRY2 were cloned into the expression vector of NYFP and CYFP respectively. These NYFP and CYFP plasmids were co-transformed into the protoplasts of *Arabidopsis thaliana*. Fluorescence signals were observed using a Zeiss LSM710 laser scanning confocal microscope. Primers used in this assay are listed in Supplemental Table 2.

2.7. Measurement of ROS

Four-day-old seedlings of Col and mutants *siek-1*, *siek-2*, *cpl1–9*, and *siek-2 cpl1–9* were treated in 150 mM NaCl solution for 5 h before the seedlings were stained. For diaminobenzidine (DAB) staining to detect H_2O_2, the seedlings were incubated in 0.3 mg/mL DAB (Sigma-Aldrich) dissolved in 50 mM Tris-HCl (pH 5.0) for 8 h.

For nitroblue tetrazolium (NBT) staining to detect superoxides, the seedlings were incubated in a reaction buffer containing 1 mM NBT (Sigma-Aldrich), 20 mM K-phosphate, and 0.1 M NaCl at pH 6.2 for 15 min. The seedlings stained by DAB or NBT were then washed three times with water. For clearing, seedlings were incubated in acidified methanol

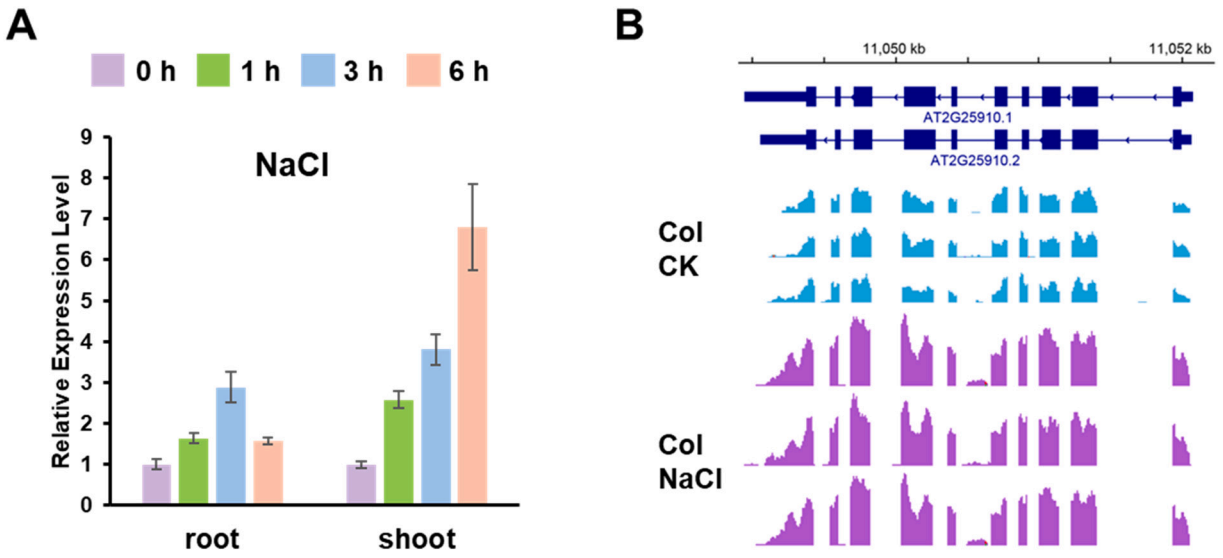

Fig. 1. Expression of *AtSIEK* was induced by salinity stress
A. Expression level of *AtSIEK* under the treatment of 150 mM NaCl. *AtActin2* was used as an internal control. Values are presented as means ± SD. B. The RNA-Seq data of *AtSIEK* expression under salt treatment visualized by IGV browser.

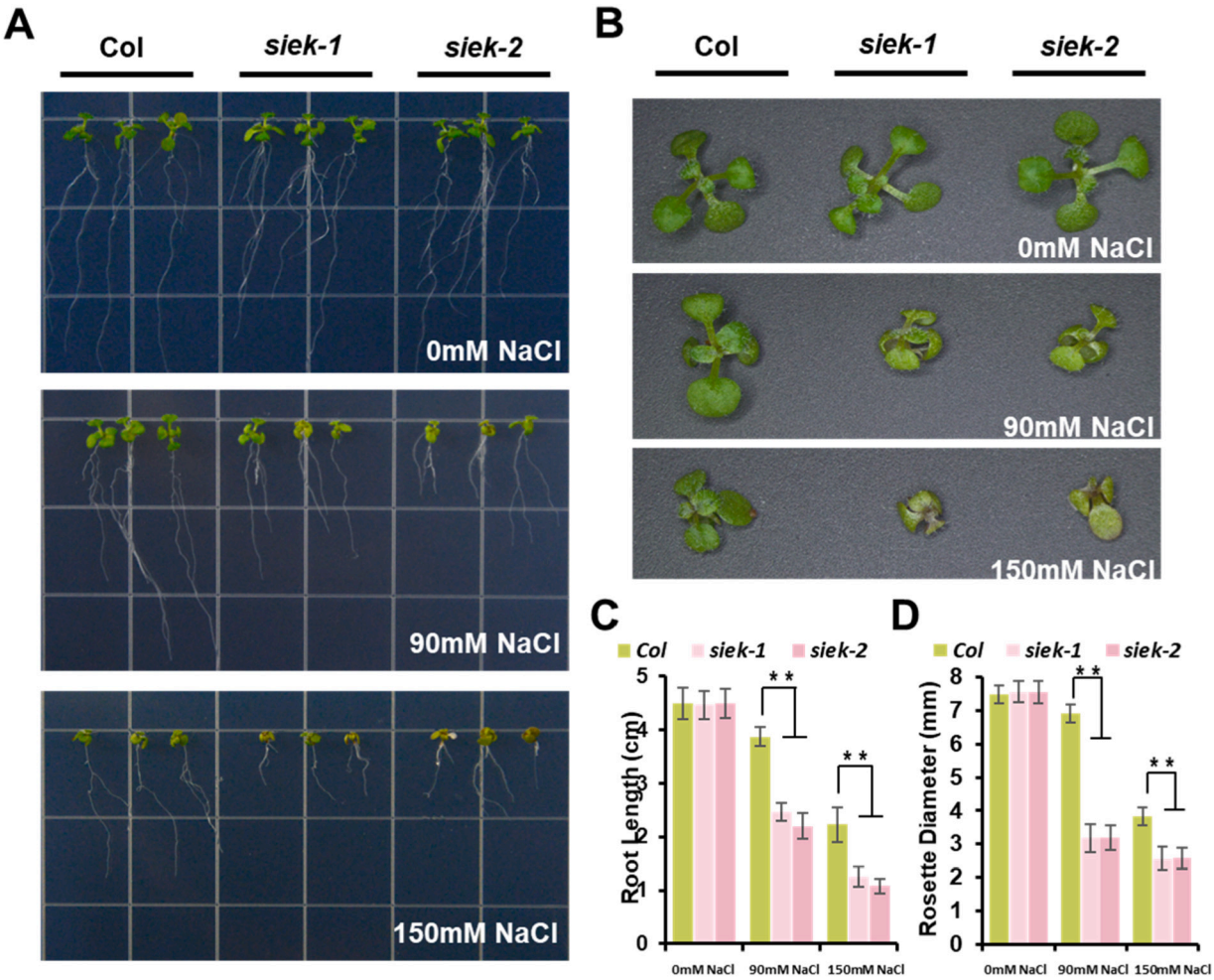

Fig. 2. *siek* mutants were sensitive to salt stress
A. The growth phenotypes of Col, *siek-1* and *siek-2* on 1/2 MS, 90 mM and 150 mM NaCl plates. B. Rosette of Col, *siek-1* and *siek-2* under the salt stress. C and D. Root length statistics (C) and rosette diameter statistics (D) of Col, *siek-1* and *siek-2* under the salinity stress. Values are presented as means ± SD ($n = 3$, $^{**}P < 0.01$; Student's *t*-test).

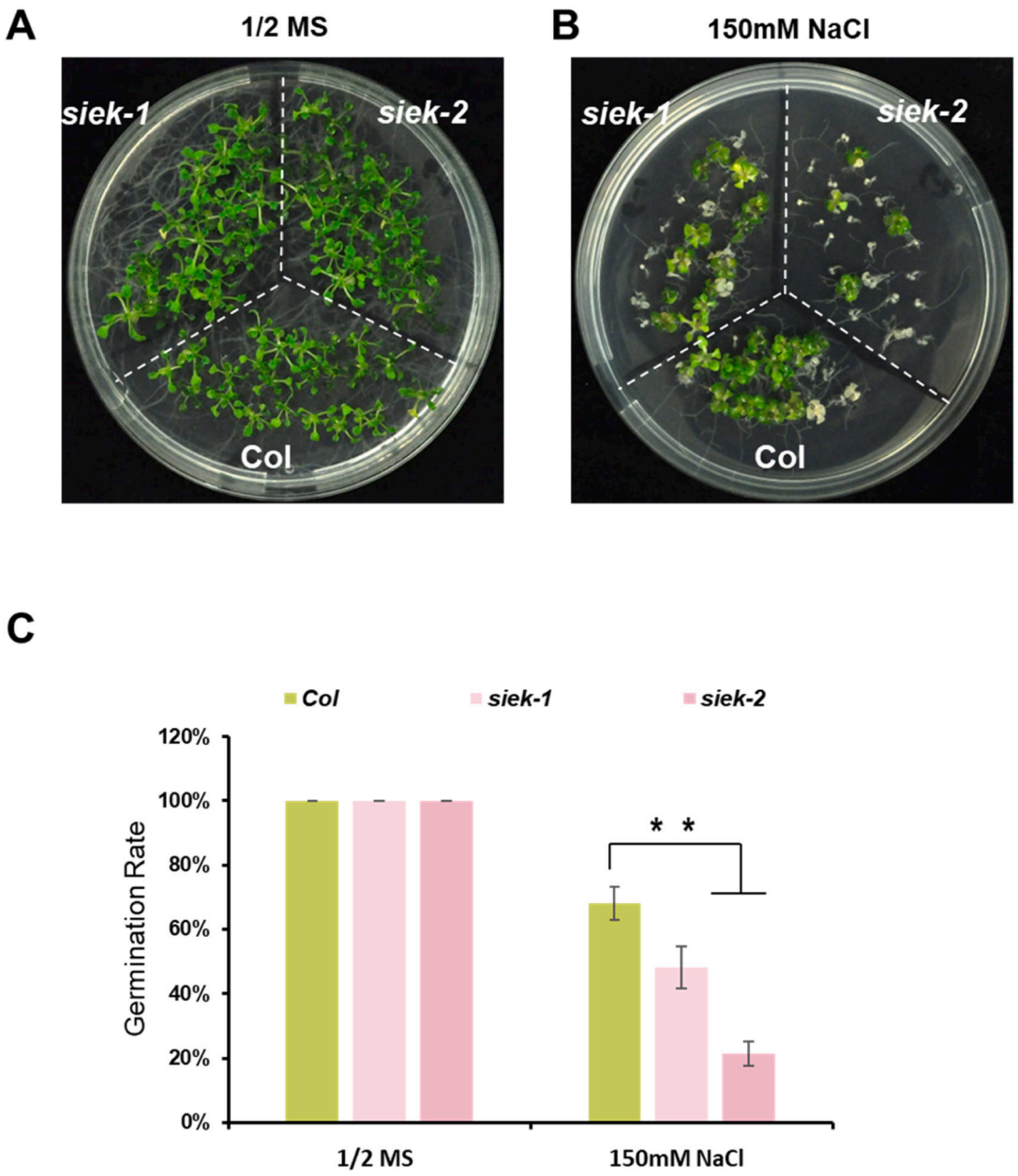

Fig. 3. Germination of *siek* mutants under salt stress
A and B. Plant germination phenotypes of Col, *siek-1*, and *siek-2* on 1/2 MS plates (A), 150 mM NaCl (B). C. Germination rate of Col, *siek-1*, and *siek-2* under the treatment of NaCl. Values are presented as means ± SD ($n = 20$, $^{**}P < 0.01$; Student's *t*-test).

buffer (10 mL of methanol, 2 mL of HCl, 38 mL of water) at 57 °C for 15 min and then in a basic solution (7 % NaOH in 60 % ethanol) for 15 min at room temperature. The seedlings were incubated 10 min at each step in the following series: 40 % ethanol, 20 % ethanol, 10 % ethanol, 5 % ethanol, and 25 % glycerol. The seedlings were then examined in 50 % glycerol with an Olympus BX53 microscope.

For 2′,7′-dichlorodihydrofluorescin diacetate (DCFH-DA) staining to detect H_2O_2, the seedlings were incubated in a buffer containing 50 μM DCFH-DA (Sigma-Aldrich) and 20 μM K-phosphate at pH 6.0 in darkness for 10 min. The roots were then photographed using a Zeiss LSM710 laser scanning confocal microscope with an excitation at 488 nm.

3. Results

3.1. AtSIEK responds to salt stresses

To identity genes involving in salt stresses, *Arabidopsis thaliana* (Col) treated with NaCl were used for RNA extraction and qRT-PCR. We found that the expression of *AtSIEK* (*At02g25910*) showed strong changes under stress. To test this, we detected the expression of *AtSIEK* under treatment with NaCl for 1 h, 3 h, 6 h , respectively. Results showed that the expression of *AtSIEK* was significantly induced after salt stress (Fig. 1A). The RNA-Seq data of Col under salt stress [33] also showed that the expression of *AtSIEK* was significantly increased under NaCl treatment, which confirmed our data (Fig. 1B). These results suggested that *AtSIEK* may play an important role in responding to abiotic stress.

To further confirm the function of *AtSIEK* involving in salt stress response, two T-DNA mutants *siek-1* and *siek-2* (Supplemental Fig. 1)

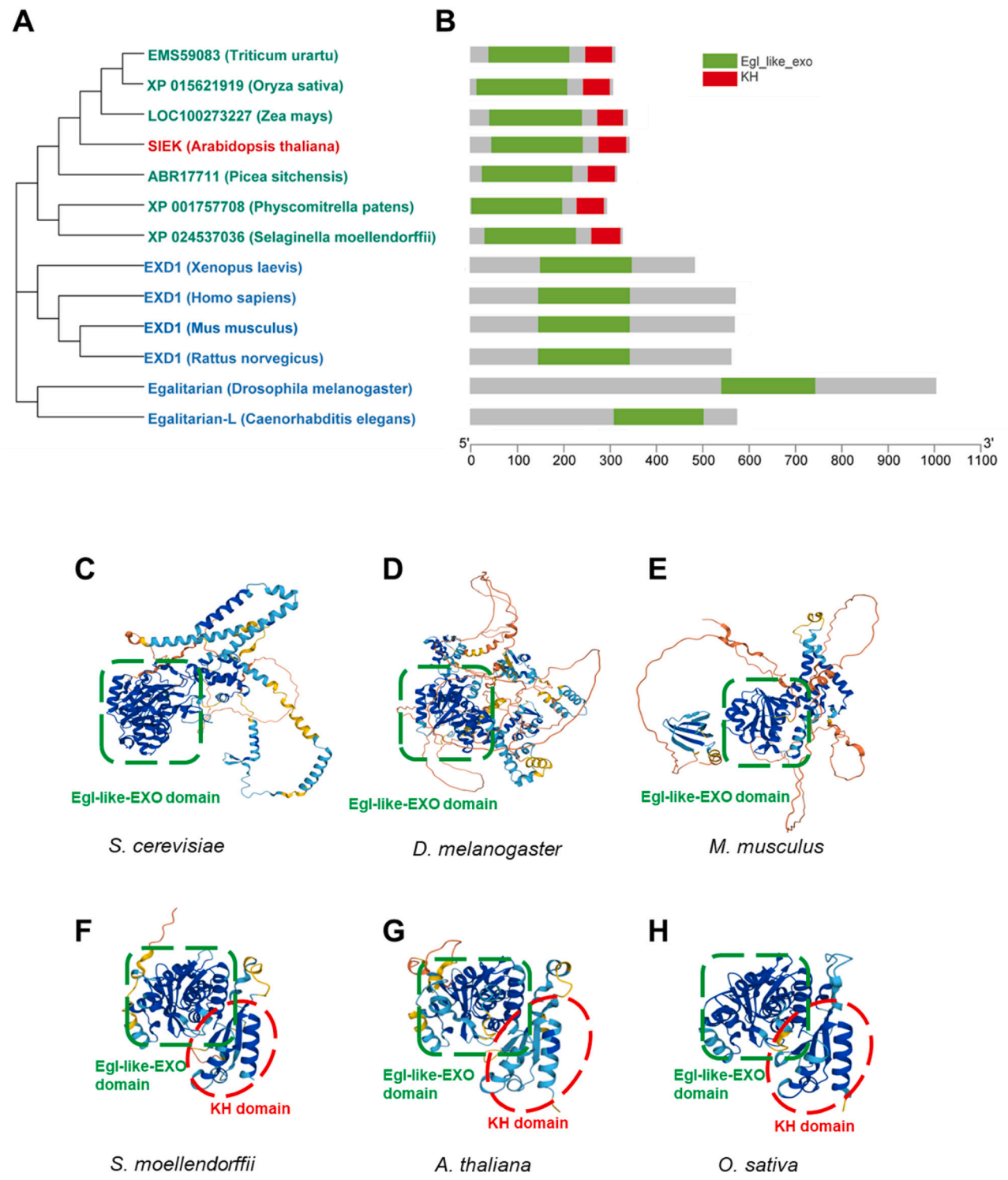

Fig. 4. Phylogenetic tree and conserved domain analysis of SIEK
A. Phylogenetic tree of 13 species was constructed using MEGA 11 by neighbor-joining method with 1000 bootstrap replications. Animals and plants were labeled with different colors, blue represents animals, green represents plants, red is AtSIEK. B. Conserved domain analysis of these 13 species. Green and red boxes represent Egl-like-EXO and KH domain, respectively. C—H. Structure of typically SIEK homologous protein in animals (C-E) and in plants (F—H). Green and red dotted diamonds frame the Egl-like-EXO and KH domain, respectively.

were used for 90 mM and 150 mM NaCl treatment. Data showed that *siek* mutants were sensitive to salt stress comparing with wild type (Col) (Fig. 2). The root length of *siek-1* and *siek-2* were significantly shorter than Col (Fig. 2A and C), and the rosette diameter of *siek-1* and *siek-2* were obviously smaller than Col (Fig. 2B and D). Besides, the mutants *siek-1* and *siek-2* were sensitive to other abiotic stress, including sorbitol, LiCl, MeJA and MMS (Supplemental Fig. 2). These results confirmed that *AtSIEK* participates in response to abiotic stress.

To study whether the germination of *siek* mutants is affected by salt stress, *siek-1* and *siek-2* seeds were cultured separately in 1/2 MS, and the MS containing 150 mM NaCl. Under NaCl treatment, the germination rate of *siek-1* and *siek-2* were 48 % and 21 %, respectively, which

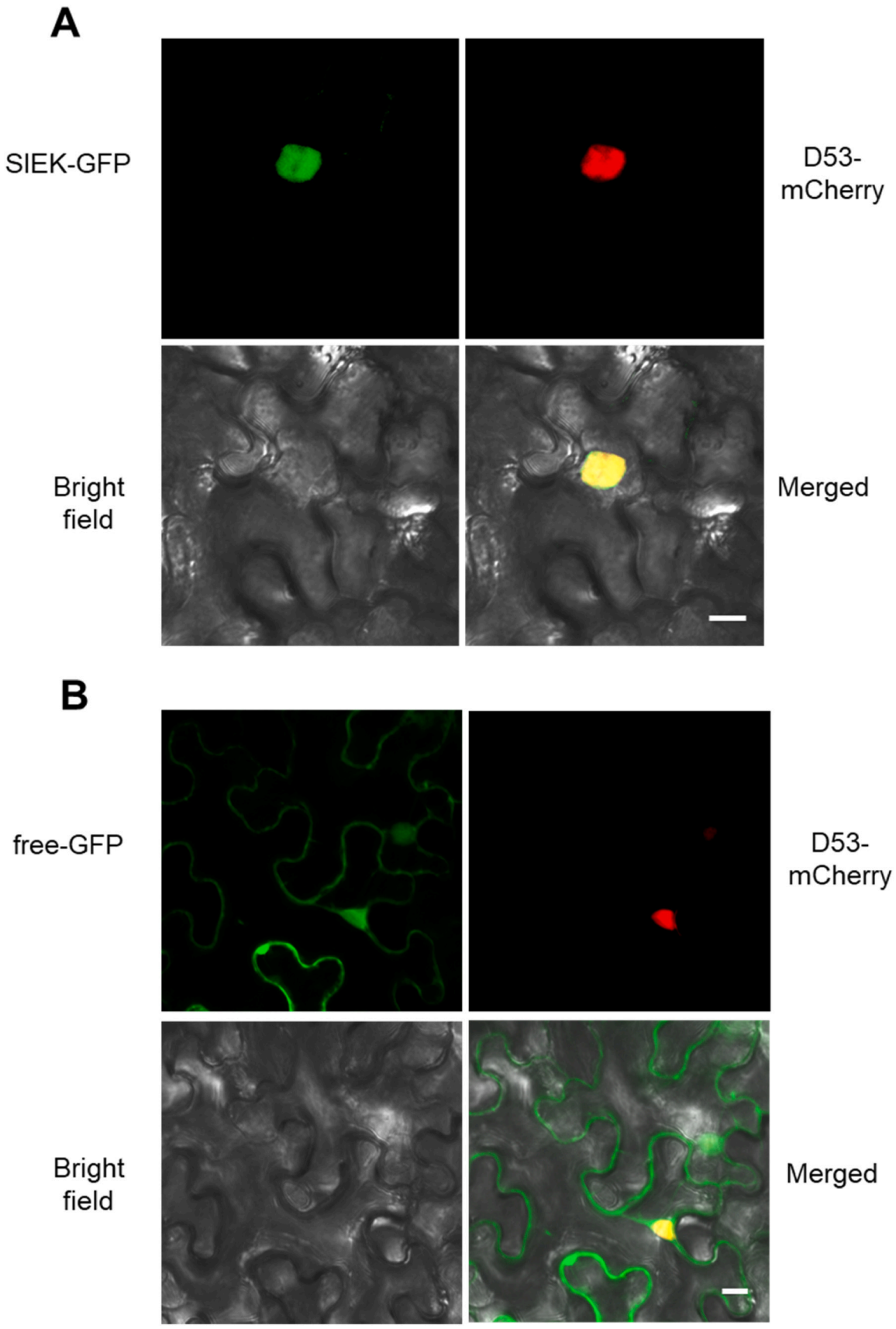

Fig. 5. Subcellular localization of the SIEK–GFP fusion protein
A. Subcellular localization of the AtSIEK–GFP fusion protein in tabacco. D53–mCherry was used as a nuclear marker. Bar, 5 μm. B. Free GFP was used as the control. Bar, 5 μm.

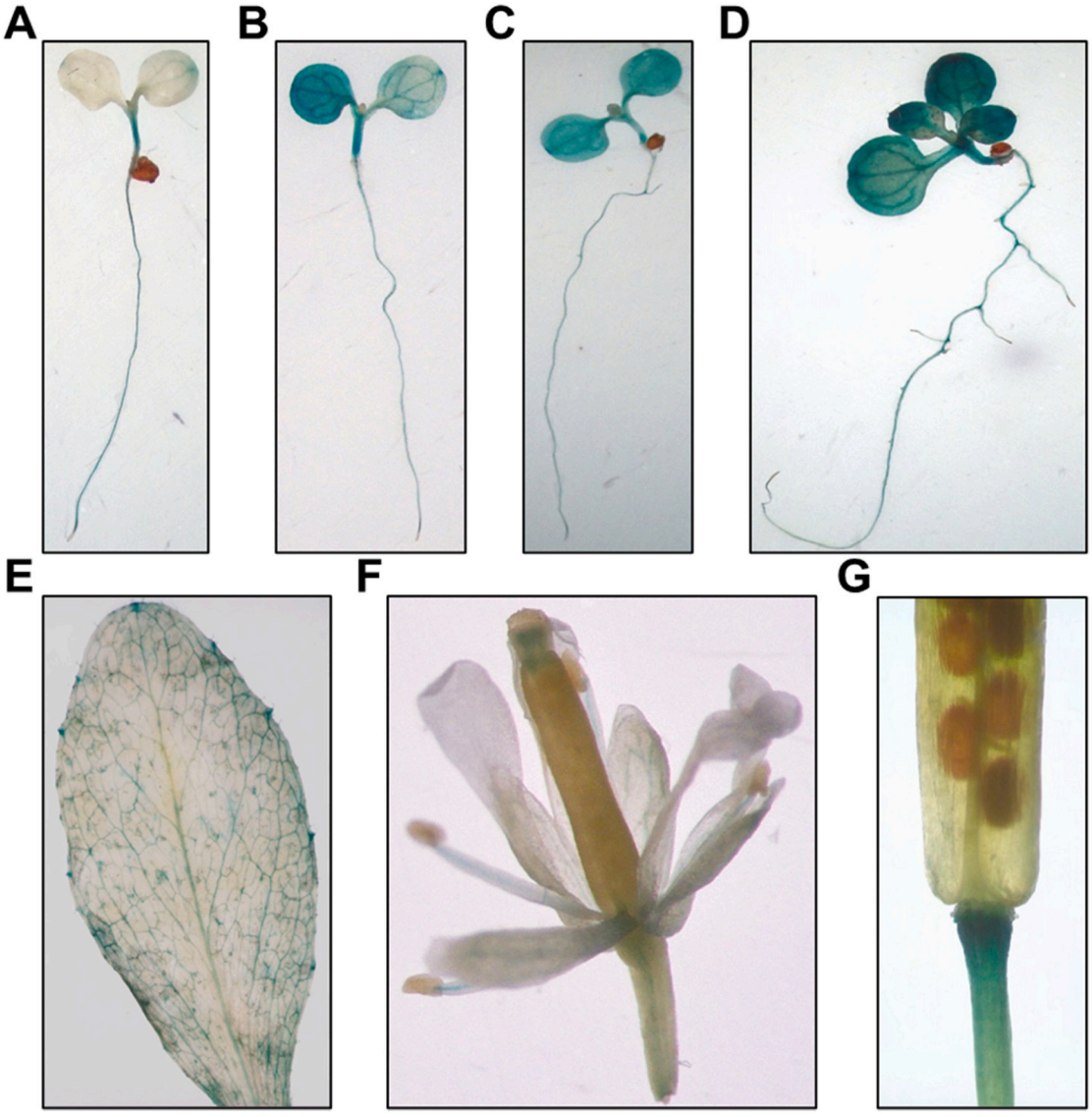

Fig. 6. Expression pattern of *AtSIEK*
A-F. GUS staining of a representative *proAtSIEK-GUS* transgenic line in 4-day-old seedling (A), 5-day-old seeding (B), 6-day-old seeding (C), 10-day-old seeding (D), Matured leaf (E), flower structure (F), silique and stalk (G).

were obviously lower that Col (68 %) (Fig. 3). These Data indicated that the mutants *siek-1* and *siek-2* were sensitive to abiotic stress at the seed germination stage. All these results suggested that *AtSIEK* plays a vital role in response to abiotic stress.

3.2. AtSIEK encoding a hnRNP K homology (KH) protein localized in nucleus

Sequence analysis revealed that AtSIEK contains two conserved domains including Eql 3′-5′ exoribonuclease (EXO) domain and KH domain (Supplemental Fig. 3). To study the evolution relationship of SIEK, the sequences of 13 SIEK proteins were used for phylogenetic trees construction and results indicated that the homologous protein of SIEK is relatively conservative in animals and plants (Fig. 4A). Interestingly, conserved domain and protein structure analyze of SIEK found that the protein structure of SIEK in plants were specific with EXD1-like domain and KH domain, which were evidently different from the SIEK in animals with only one EXD1-like domain (Fig. 4B-H).

To analyze the localization of AtSIEK, the transient expression assay in *Nicotiana tabacum* were performed and data showed that the green fluorescent protein (GFP) signal of the AtSIEK–GFP fusion protein was mainly localized in the nucleus and overlapped closely with the mCherry signal of a reported nuclear protein, the D53–mCherry fusion protein [34] (Fig. 5).

3.3. AtSIEK is highly expressed in seedling

To determine the expression pattern of *AtSIEK*, we generated the *AtSIEK-GUS* reporter lines, in which *AtSIEK* was translationally fused to the β-glucuronidase (GUS) reporter gene. GUS staining results indicated that *AtSIEK* preferentially expressed in seedlings (Fig. 6A-D), and with lower expression in flowers and siliques at mature stage (Fig. 6E-G). These results indicated that *AtSIEK* is mainly expresses and play functions in leaf and root at seeding stage, which is consistent with phenotype of *siek* mutants under abiotic stress at seedling stage.

3.4. AtSIEK interacts with FRY2/CPL1

To study the molecular mechanism of AtSIEK in response to abiotic stress, the full-length protein of AtSIEK without transcriptional

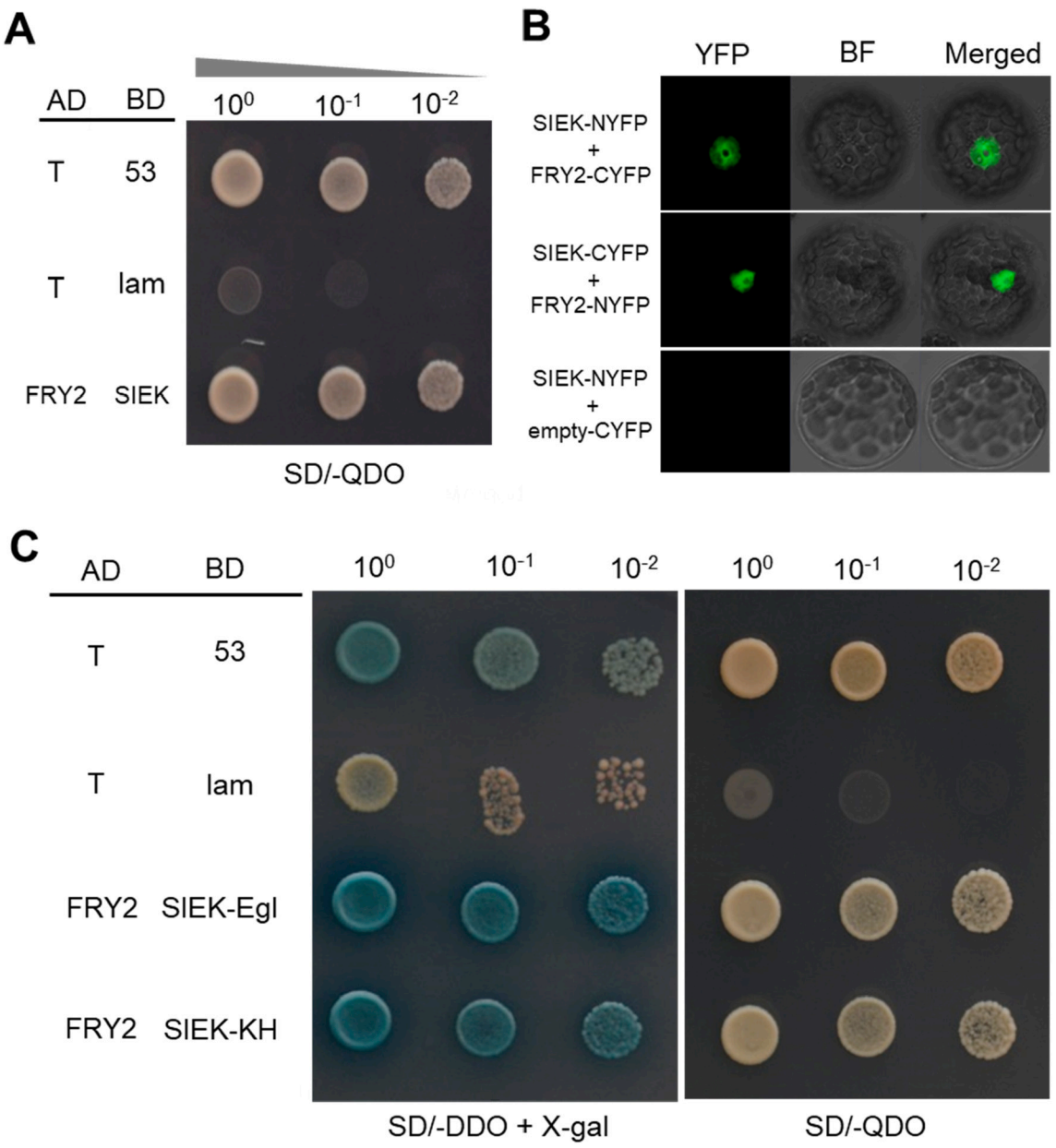

Fig. 7. AtSIEK interacts with FRY2/CPL1

A. Yeast-two hybrid assay verified the interaction between AtSIEK and FRY2/CPL1. The transformed yeast cells were plated on QDO (SD/−Trp/−Leu/-His/−Ade). T-53 and T-lam combinations were positive and negative controls, respectively. B. Bimolecular fluorescence complementation (BiFC) assay verified the interaction between AtSIEK and FRY2/CPL1. C. Yeast-two hybrid assay verified the disrupted interaction between AtSIEK and FRY2/CPL1. The transformed yeast cells were plated on DDO (SD/−Trp/−Leu/+X-gal) and QDO (SD/−Trp/−Leu/-His/−Ade). T-53 and T-lam combinations as positive and negative controls, respectively.

activation activity were used as a bait to screen its interacting protein by yeast two-hybrid method. An RNA polymerase II C-terminal domain phosphatase-like protein (FRY2, also called C-TERMINAL DOMAIN PHOSPHATASE-LIKE 1, CPL1) was identified. To confirm this interaction, the full-length amino sequences of FRY2/CPL1 were fused to pGADT7, which was co-transformed to AH109 with pGBKT7-AtSIEK, and results showed that AtSIEK interacts with FRY2/CPL1 in yeast (Fig. 7A). The interaction between AtSIEK and FRY2/CPL1 was further verified by bimolecular fluorescence complementation (BiFC) assay, which revealed that they interact in the nucleus (Fig. 7B). To determine the interaction domain between AtSIEK and FRY2/CPL1, the two domains (Eql like 3′-5’ EXO domain and KH domain) were fused to pGBKT7, respectively. and we found that both Eql like 3′-5’ EXO and KH domain can interact with FRY2/CPL1 (Fig. 7C).

3.5. AtSIEK and FRY2/CPL1 act synergistically in response to salt stress

Previous study had reported that FRY2/CPL1 plays important role in response to salt stress [35,36]. To detect its function, we ordered the FRY2/CPL1mutant (*cpl1–9*) from ABRC (Arabidopsis Biological Resource Center, Ohio State University, Columbus OH, U.S.A.) (Supplemental Fig. 4), and results showed that *cpl1–9* is sensitive to salt stress, which is similar to the phenotypes of *siek-1* and *siek-2* (Fig. 8A and B). To further investigate the meaning of their interaction, *siek-2* and *cpl1–9* were crossed to obtain a double mutant *siek-2 cpl1–9*. The germination rates experiment under salt treatment revealed that *siek-2*, *cpl1–9* and *siekl-2 cpl1–9* all had a lower germination rate than Col (Fig. 8C and D), indicating that AtSIEK functions in same pathway with FRY2/CPL1 in response to salt stress. To finally confirm the salt stress response of AtSIEK and FRY2/CPL1, the expression of four known

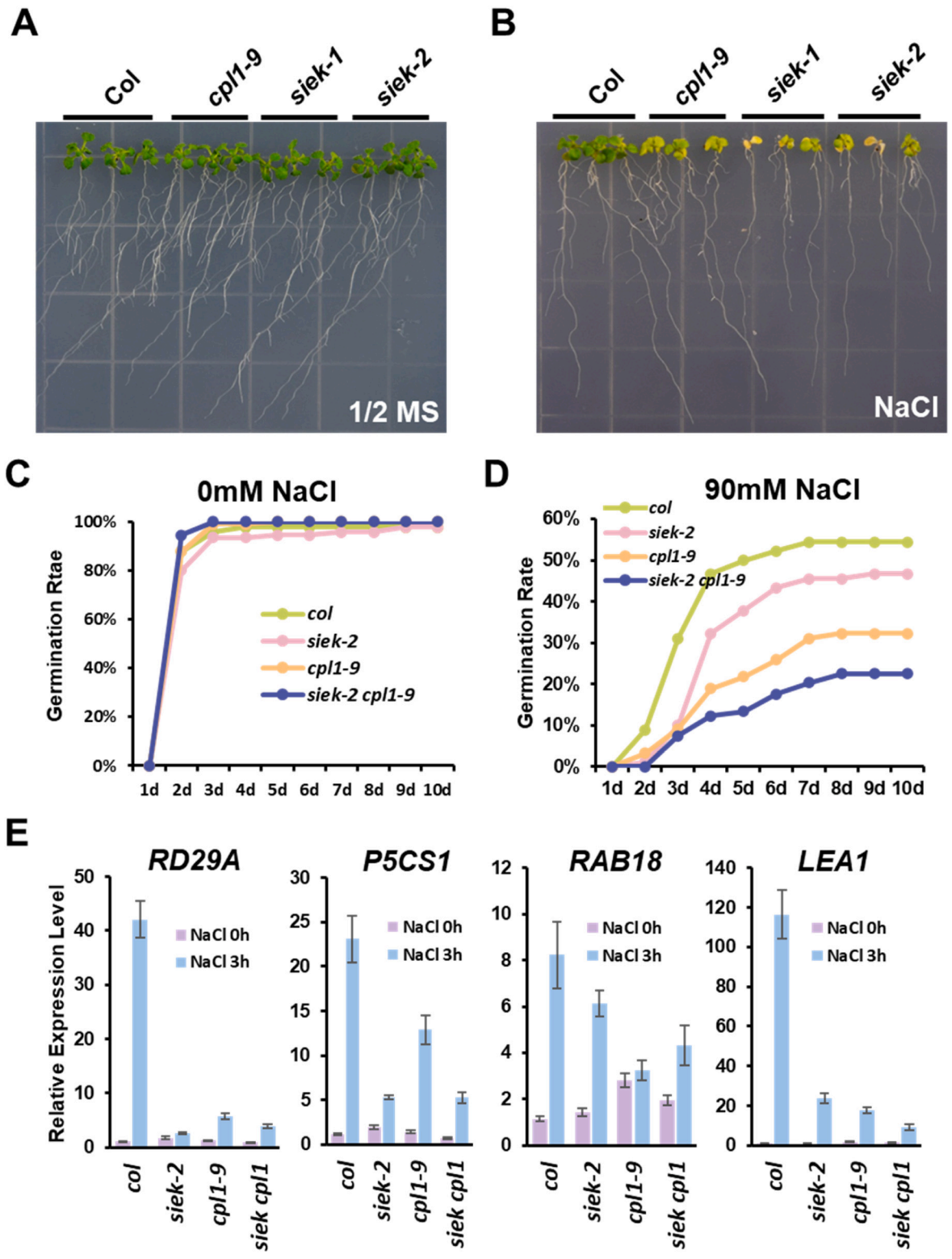

Fig. 8. AtEXD1 and FRY2/CPL1 act Synergistically in response to salt stress
A and B. Growth phenotypes of Col, *cpl1–9*, *siek-1*, and *siek-2* plated on 1/2 MS (A) and 1/2 MS containing 90 mM NaCl (B). C and D. Germination rate of Col, *siek-2*, *cpl1–9*, and *siek-2 cpl1–9* under the treatment of 90 mM NaCl. Values are presented as means ± SD E. Expression of *RD29A*, *P5CS1*, *RAB18* and *LEA1* (abiotic stress marker genes) after the treatment of NaCl 3 h. Values are presented as means ± SD.

abiotic stress marker genes, including *RD29A*, *P5CS1*, *RAB18* and *LEA1*, were detected in these mutants under salt stress. Data showed that, the increased expression of the four genes in these mutants under salt stress were all significantly lower than that Col, indicating that the loss-function of these genes reduces the ability for plants response to salt stress (Fig. 8E). These results confirmed that AtSIEK and FRY2/CPL1 played vital roles in response to salt stress. And we also found that *siek-2 cpl1–9* showed more sensitive to UV compared with *siek-2* and *cpl1–9*

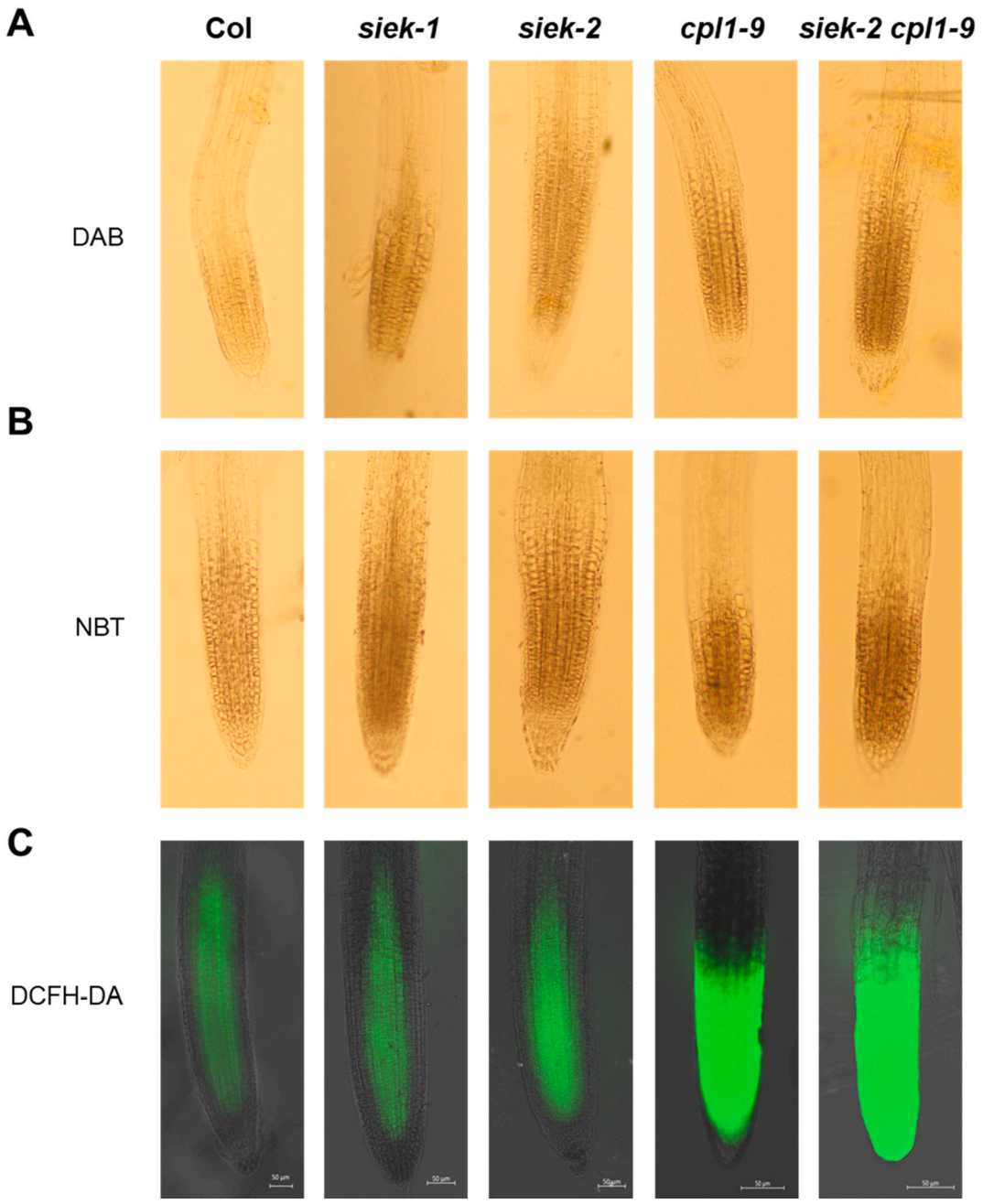

Fig. 9. ROS staining under the salt stress
A-C. Higher ROS levels in mutants *siek-1*, *siek-2*, *cpl1–9*, and *siek-2 cpl1–9* after the treatment of 150 mM NaCl. ROS was detected by DAB (A), NBT (B), and DCFH-DA (C) staining. Seedings of Col, and mutants *siek-1*, *siek-2*, *cpl1–9*, and *siek-2 cpl1–9* were immersed in DAB, NBT and DCFH-DA solution at room temperature.

(Supplemental Fig. 5). The similar results were observed under MMS treatment (Supplemental Fig. 6).

To further explain *AtSIEK* responding to salt stress, we measured the ROS (reactive oxygen species) levels of WT, *siek-1*, *siek-2*, *cpl1–9*, and *siek-2 cpl1–9*. Three staining methods were used to detect the level of ROS, including DAB, NBT, and DCFH-DA. All the results of three methods showed that the ROS levels of mutants were higher than Col, especially *siek-2 cpl1–9* had the strongest ROS level, which is higher than *siek* or *cpl1–9* (Fig. 9). These results indicated that the mutants of *siek-1*, *siek-2*, *cpl1–9*, and *siek-2 cpl1–9* were more sensitive to salt stress than Col, and demonstrated that AtSIEK and FRY2/CPL1 act synergistically in response to salt stress.

4. Discussion

4.1. SIEK is a plant specific stress-related RNA binding protein

In this study, SIEK was isolated since it was induced by salt stress (Fig. 1) and the structure of SIEK was unique. SIEK is the only protein in Arabidopsis having a 3′-5′ exoribonuclease domain and a KH RNA binding domain. Interestingly, there was only single SIEK homologs in other plant species but none in zoological species (Fig. 4). Two independent T-DNA insertion mutants showed similar stress-sensitive phenotypes comparing with Col, when plants suffered salt (Figs. 2 and 3).

4.2. The role of SIEK in plant abiotic stress response

We confirmed that SIEK participated in plant abiotic stress response by two allelic mutants, and demonstrated that *siek-1* and *siek-2* mutants were sensitive to salt, drought, stress hormones etc. These results implied that SIEK was a positive regulator in stress response signaling. In Arabidopsis, DEDDy type exoribonucleases RRP6L1 and RRP6L2 have multi-roles in plant development and stress responses with molecular mechanisms including poly(A)-mediated RNA degradation, RNA-directed DNA methylation through regulation of noncoding RNAs, antisense RNAs generation, DCL-independent siRNA biogenesis and surveillance of unmethylated miRNA/miRNA* duplexes [23,37–41]. In the protozoan parasite *Entamoeba histolytica*, EhRrp6 also protected cells against environmental stress [42]. KH domain proteins in plants, such as HOS5, HEN4, FLK and PEP, had been well characterized as developmental and stress regulator. HEN4, forming a complex with FLK and PEP, ensures normal processing of AG pre-mRNA. Loss-of-function hen4 mutant is sensitive to abiotic stress such as low temperature [31,43,44]. HOS5, the most studied KH protein, was identified by three research groups independently as abiotic stress response [29,30]. HOS5 controls plant growth under salt and ABA treatment, by alternative splicing of stress response genes and biogenesis of miRNA [30,45]. Since SIEK possesses both DEDDy type exoribonucleases domain and KH domain, it is supposed that both domains may contribute to SIEK function in abiotic stress response.

4.3. SIEK regulates plant abiotic stress response by interacting with FRY2/CPL1

To discover the signaling pathway that SIEK may involve in, yeast two hybrid screening was carried out with SIEK used as bait protein. One stress response regulator FRY2/CPL1 was isolated as strong interaction was detected by Y2H and BiFC (Fig. 7). FRY2/CPL1 was identified as a regulator in response to abiotic stress [35,36]. Since FRY2/CPL1 is the key C-terminal domain phosphatase of RNA Polymerase II, the phosphorylation status is the important junction of plant abiotic stress responses [46]. Beside the de-phosphorylation function to Pol II CTD, FRY2/CPL1 also play vital roles in RNA splicing, miRNA biogenesis, NMD decay by regulating phosphorylation status of different substrates [10,29,30,47,48]. Both *cpl* mutant and *siek* mutant had similar stress sensitive phenotypes, meanwhile the *cpl siek* double mutant had stronger phenotype than single mutant (Figs. 8 and 9). Here we revealed that SIEK regulated plant abiotic stress by interacting with FRY2/CPL1.

The advantage of this study is two-fold. On one hand, we confirmed that AtSIEK participates in salt stress via the physiological experiment of *siek* mutants. On the other hand, we explained the primary molecular mechanism of AtSIEK, which interacts with FRY2/CPL1 to involve in stress response, and *cpl*1 and *siek* mutants had similar stress sensitive phenotypes. This method also has two limitations. One is that we need more molecular biological evidences, such as kinase phosphorylation, SIEK-FRY2 interaction sites detection to clarify the significance of SIEK-FRY2 interaction in response to salt stress. The other is that we need to identify the downstream gene of *AtSIEK* to further illustrate the molecular mechanism of SIEK in participates in salt stress response.

5. Conclusions

In this study, we identified a plant specific protein having EXD1-like DEDDy exoribonuclease domain and KH domain playing important role in abiotic stress response. The T-DNA mutants *siek-1* and *siek-2* showed sensitive phenotypes to salt, drought, ABA, MeJA and MMS. SEIK protein was located mostly in nucleus. Spatial-temporal characteristics of *AtSEIK* expression demonstrated that *At*SEIK was ubiquitous expressed at seedling stage, and in leaves, vascular bundle of filament and stigma at mature stage. We identified a stress-related protein FRY2/CPL1 interacting with SEIK by Y2H screening. Both the EXD1-like domain and KH domain of SEIK can interact with FRY2/CPL1 in vivo. FRY2/CPL1 mutant showed similar phenotype with *siek-1* and *siek-2* under salt stress condition. Meanwhile, the *siek cpl* double mutant showed stronger phenotypes than single mutants under abiotic stresses. Taken together, our data elucidated the molecular mechanism of plant specific protein SEIK participating in plant adaptation to abiotic stress.

CRediT authorship contribution statement

Xiangxiang Zhang: Performing main experiments, Data curation, Writing-original Draft

Qinyu Xie: Performing some experiments, Data curation, Writing-original Draft

Lijun Xiang: paper editing

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2023.123369.

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