

Genome-wide identification of cysteine-rich receptor like kinases in sweet cherry and functional analysis of PaCRK1 to salt stress

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Abstract

Cysteine-rich receptor-like kinases (CRKs), a large subgroup of the receptor-like kinases, play an important role in plant development and stress response. However, knowledge about CRKs and its function against adverse environmental stresses in sweet cherry were lacking. In this study, 40 PaCRKs were identified from sweet cherry (*Prunus avium*) genome database. Phylogenetic analysis indicated that PaCRKs could be classified into six subgroups. Transcriptome analysis showed that the expression levels of most *PaCRKs* were changed under external environmental stresses. Functional study showed that PaCRK1 overexpression could enhance Arabidopsis and sweet cherry tolerance to salt stress. Moreover, biochemical analysis showed that PaCRK1 increased salt tolerance of sweet cherry by regulating the expression of antioxidation-related genes and their enzyme activities. This study provides a comprehensive understanding of PaCRKs in sweet cherry and elucidates the potential role of PaCRKs in response to various environmental stimuli.

Key Message

Forty PaCRKs have been identified from sweet cherry and overexpression PaCRK1 in sweet cherry enhances its resistance to salt stress.

Introduction

Plants encounter a variety of biotic and abiotic stresses in their lifespan, like high salinity, drought and various pathogens. Over the long time evolution, plants have developed complex mechanisms to defend themselves by arousing their immune system. Receptor-like kinases (RLKs) are a class of transmembrane proteins, which could sense and transduce external signals to intracellular environment for responding to stress (Jamieson, Shan et al. 2018). For now, more and more RLKs have been discovered, including more than 600 and 1000 RLKs in Arabidopsis and rice (Wu and Zhou 2013). RLKs were classified into diverse types according to their highly diverse extracellular regions, like leucine-rich repeats RLKs, cysteine-rich RLKs, wall associated RLKs, and S domain RLKs, *etc* (Shiu and Bleecker 2001).

Cysteine-rich RLKs (CRKs), a member of the receptor-like kinases, are mainly composed of extracellular DUF26 domain and intracellular kinase domain (Idänheimo, Gauthier et al. 2014). Studies reported that CRKs play important roles in responding external stresses. There are 44 CRK members in Arabidopsis, among which overexpression of CRK4, CRK13 and CK20 can enhance its resistance to biotic and abiotic stresses (Burdiak, Rusaczek et al. 2015, Lu, Liang et al. 2016). TaCRK2, identified from wheat, plays vital role in resistance to *Puccinia triticina* infection and positively regulates the death process of HR cells (Uno, Furihata et al. 2000). SCR96, a small cysteine-rich secreted protein of *Phytophthora cactorum*, plays a significant role in pathogenicity and antioxidant stress (Chen, Li et al. 2016). The transcription abundance of CRK1 in *Triticum aestivum* increased after *Fusarium gramineis* infection and exogenous

ABA treatment. OSRMC containing DUF26 extracellular domain has been reported to be involved in root bending and salt stress response in *Oryza sativa* L. (Serra, Figueiredo et al. 2013). Although some functions of CRK have been reported, the role of CRK in response to environmental stresses remains to be further studied.

Sweet cherry is a species with higher economic benefits among deciduous fruit trees in northern China. With the changes of environments, the growth of sweet cherry was strongly affected by biological stresses and abiotic stresses, such as salinity and drought (Wang, Liu et al. 2020). Thus, it is of great significance to improve the stress resistance of sweet cherry through molecular breeding.

In this study, 40 PaCRKs from sweet cherry were systematically identified by phylogenetic analysis, conserved domain and motif alignment. Transcriptome analysis showed that most of PaCRKs were affected by different environmental stresses. Functional studies showed that PaCRK1 could enhance the tolerance of Arabidopsis and sweet cherry to salt stress. Meanwhile, the antioxidant capacity of overexpressed PaCRK1 lines under salt stress was enhanced with the increase of enzyme activities and up-regulation of related genes. This study of PaCRK family will broaden our understanding of the signal transduction mechanism of receptor-like kinases in sweet cherry resistance with external circumstances.

Materials And Methods

Identification and phylogenetic analysis of PaCRKs family in sweet cherry

The identification and phylogenetic analysis of PaCRKs were based on the method of Sun *et.al*/with some modifications (Sun, Zhao et al. 2022). The HMM data of kinase domain (PF00069) or Pkinase_Tyr domain (PF07714) and stress-antifung domain (PF01657) were download. All candidate sequences were searched with the software of Pfam, SMART and ExPASy. The phylogenetic analysis was using the software of MEGA5. The conserved domains and motifs of PaCRKs were analyzed by PFAM and MEME.

Plant materials and treatments

Gisela 5 seedlings of sweet cherry rootstock were cultured in rooting medium at 25°C for 4 weeks. Then, fifteen seedlings with the same growth were selected from the rooting seedlings for subsequent stress treatment, with 3 seedlings in each group. And each group was treated with 100 mM NaCl, 10 µM ABA, 10% PEG₆₀₀₀ and 10⁷ agrobacterium for 4 h, respectively.

RNA-seq analysis

The root materials under different stresses and control were prepared for RNA extraction. The total RNA was isolated by plant RNA extraction kit. RNA integrity was analyzed by 2100 bioanalyzer. All samples had integrity values as high as 2. The quality and quantity of the sample library were evaluated by Agilent 2100 bioanalyzer. The cDNA library products were sequenced on Illumina HiSeqTM 2000 platform.

Validation by qRT-PCR

Fur PaCRK genes who were highly expressed under stresses were selected for qRT-PCR testing. The housekeeping gene β -tubulin of sweet cherry was used as an internal reference. SYBR® Premix ExTaq™ II kit was used for qRT-PCR analysis. Data analysis was performed based on the method with Bio-Rad quantitative PCR Application Guide.

Subcellular localization

PaCRK1 gene was constructed into the vector of pMDC83 and fused with the C-terminal GFP. The empty vector of pMDC83 with GFP was used as negative control. PMDC83 control and PMDC83-*PaCRK1* vectors were transformed into *A. tumefaciens* GV3101 cells, respectively, and the transformed bacterial solution was injected into tobacco epidermal cells. The localization of GFP signal was observed by confocal laser microscope.

Overexpression of PaCRK1 in Gisela 5 seedling roots

The pMDC83-*PaCRK1* transformed into *A. rhizogenes* strain C58C1 was expressed in Gisela5 callus by hairy root transformation as described previously (Sun, Zhao et al. 2022). Positive transgenic seedlings were detected by PCR amplification and qRT-PCR.

Determination the root vigor of PaCRK1 overexpression Gisela 5 seedling

The root vigor was measured with the methods as previously reported (Clemensson-Lindell 1994). Briefly, put the fresh Gisela 5 seedling roots (0.25 g) into 15 mL tube. 5 mL 0.1 M $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ buffer and 5 mL 0.4% TTC were added and incubated at 37°C for 1 h. Then 2 mL 2 M H_2SO_4 was added to stop the reaction. Put the root into a mortar and add 5 mL ethyl acetate and a small amount of quartz sand thoroughly grind extraction. The extraction buffer was converted to a new tube and measured at 485 nm.

Detection the antioxidant products and enzymes

The Gisela 5 seedlings roots were frozen in liquid nitrogen and ground into a fine powder. The contents of MDA, H_2O_2 and Vc were detected by the previously method (Sun, Zhao et al. 2022). The activities of catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD) were measured by previous method (Nakano and Asada 1981, Havir and McHale 1987, Cruz de Carvalho 2008).

Results

Identification and phylogenetic analysis of PaCRKs in sweet cherry

To obtain the full sequences of PaCRKs, the domains of DUF26 (PFAM domain PF01657) and kinases (PFAM domain PF00069 and PF07714) were used for searching the *Prunus avium* database by HMMER

and aligned by PFAM. All the candidates with two DUF26 domains and kinase domain were predicted to encode PaCRK proteins. The results showed that a total of 40 PaCRKs were identified in *Prunus avium* (Table 1). The number of amino acids (aa) in PaCRK protein sequence ranged from 331 aa (PaCRK21) to 810 aa (PaCRK29) with an average length of 589 aa. The predicted molecular weights (MWs) of coding proteins ranged from 36.14 kDa (PaCRK21) to 90.91 kDa (PaCRK29) with an average of 65.33 kDa. According to isoelectric point (pI) analysis, 26 PaCRKs with $pI < 7.0$ were acidic in nature, 5 PaCRKs with $7.0 < pI < 8.0$ were neutral in nature, whereas 9 PaCRKs with $pI > 8.0$ were basic in nature. To visualize the location of *PaCRKs* in the genome, the map of chromosomal localization was constructed. According to their relative chromosome locations, the 40 candidate *PaCRKs* were named as *PaCRK1* to *PaCRK40* from chromosome 1 to 8 (**Figure S1**). The result showed that the 40 *PaCRKs* were mapped to seven chromosomes (from 1 to 8, except 7).

To further classify these PaCRKs into subgroups, the maximum likelihood phylogenetic tree analysis was performed. The result showed that PaCRKs were grouped into six subgroups, of which VI subgroup was the largest and V subgroup was the second (Fig. 1A). We found that the PaCRKs clustered on the same chromosome also preferentially clustered on the same subgroup in the phylogenetic tree. For example, PaCRK2, PaCRK3 and PaCRK4 were clustered on chromosome 1 and they were also clustered together into group III in the phylogenetic tree. This phenomenon indicated that PaCRK genes may originate from gene duplication. In order to further study the classification and evolutionary relationship of PaCRKs, the CRK sequences of apple (*Malus domestica*), Arabidopsis and rice (*Oryza sativa* L.) were selected for phylogenetic tree construction (Fig. 1B). Group III, group X and group XIII contained all the members of CRKs from the four tested plants. Group VIII, group IX and group XII contained three tested plants (sweet cherry, apple and Arabidopsis) with CRK members. Some groups had only one tested plant, such as group I had CRK members of sweet cherry, group II, IV and XI were Arabidopsis, and group V was rice. These results indicated that CRKs have a distinct evolutionary process in plants.

Domain structure and conserved motif analysis of PaCRKs proteins

To further explore the characteristics of PaCRKs proteins with secondary structure, different domains were analyzed using SMART and InterproScan. As previously reported, PaCRKs proteins contained highly conserved domains. There were two DUF26 domains located at the N-terminal and one kinase domain appeared in the C-terminal end (Fig. 2A). Some unique structures were also found in PaCRKs amino acid sequences. For example, one PaCRK (PaCRK19) contained three DUF26 domains, two in the N-terminal and one in the C-terminal. Another PaCRK (PaCRK40) contained two DUF26 domains in the C-terminal, while kinase domain in the N-terminal. Besides that, one PaCRK (PaCRK21) contained only one DUF26 domain and one kinase domain. The motif analysis showed that the amino acid frequencies were conserved at each position in the homologous domain (Fig. 2B and **Figure S2**). For example, all members of PaCRKs family showed three conserved cysteine residues in DUF26 domains, which was a typical feature of DUF26 domain (C-X8-C-X2-C).

Gene expression pattern analysis of PaCRKs response to stresses

CRKs play a significant role in responding to external stresses during plant development. Thus, the expression pattern of *PaCRKs* in roots under four stresses were analyzed. The results showed that the expression levels of *PaCRKs* were also different under different stress conditions (Fig. 3A). Salt stress had the most obvious effect on *PaCRKs* expression, with 19 genes up-regulated and 8 genes down-regulated. Meanwhile, we found that *PaCRK1* and *PaCRK19* were up-regulated at high levels under the four stresses. All these results indicated that PaCRKs participated in response to external environmental changes.

To further validate the expression of *PaCRKs* under various stresses, four *PaCRKs* (*PaCRK1*, *PaCRK10*, *PaCRK19*, and *PaCRK35*), who were highly expressed under stresses by RNA-seq analysis, were selected for qRT-PCR verification. The results showed that the expression level of the four genes validated by qRT-PCR was similar with the data from RNA-seq (Fig. 3B). Under different stress conditions, the expression level of *PaCRK1* was most obviously up-regulated, which increased about 9 times under salt stress, 8 times under ABA stress, 4 times under drought and pathogen infection stresses compared with the control group. Given the high expression of *PaCRK1* under different stresses, we believed that PaCRK1 may play a significant role in responding to environmental changes. Thus, PaCRK1 was selected for further functional studies.

PaCRK1 was localized to plasma membrane

Conserved domain analysis showed that PaCRK1 contained a signal peptide (1–23 aa), two extracellular DUF26 domains (32–130 aa and 145–240 aa), one transmembrane domain (259–281 aa), and one kinase domain (322–594 aa) (Fig. 4A). To further identifying the location of PaCRK1, it was conjugated with a GFP protein and transiently expressed in tobacco. The results showed that the fluorescence signal of GFP-fused PaCRK1 protein was mainly located on the plasma membrane of tobacco cells, while the control GFP protein was distributed on the whole cell and nucleus (Fig. 4B). These results suggested that PaCRK1 was localized to the plasma membrane and may play a vital role in mediating signal transduction.

PaCRK1 overexpression in Arabidopsis enhanced salt and ABA tolerance

To investigated the function of PaCRK1 with stress tolerance in plants, we overexpressed it in Arabidopsis. The DNA band of *PaCRK1* was detected with PCR amplification in Arabidopsis T2 transgenic line, while no target bands were amplified in the wild type (WT) (**Figure S3A**). Furthermore, qRT-PCR validation also confirmed the successful overexpression of *PaCRK1* in Arabidopsis (**Figure S3B**).

The stress tolerance assay was used to detect the function of PaCRK1 overexpression in Arabidopsis under salt and hormone stresses. The results showed that PaCRK1 overexpression increased the tolerance of Arabidopsis under stresses compared with WT (Fig. 5). Root length and whole seedling fresh weight were significantly enhanced in PaCRK1 overexpressed Arabidopsis compared to WT under 50 μ M

and 100 μ M ABA stresses (Fig. 5A and C). Similarly, under 100 and 150 mM NaCl treatments, the root length and whole shoot fresh weight of PaCRK1-overexpressing lines were significantly higher than those of WT (Fig. 5B and D). These results indicated that PaCRK1 played significant roles in coping with external environmental stresses.

PaCRK1 enhanced the antioxidant capacity of Gisela 5 under salt stress

To study the role of PaCRK1, we overexpressed PaCRK1 in the roots of sweet cherry rootstock Gisela 5. The CDS sequence of *PaCRK1* was inserted into the pMDC83 expression vector promoted by the 35S promoter (Figure S4A). A fragment of 1938 bp was amplified by PCR validation in three transgenic plants (O2, O3 and O4), but not in control plants (WT) (Figure S4B). qRT-PCR results showed that *PaCRK1* was overexpressed in Gisela 5 roots (Figure S4C).

To further explore the ability of overexpression of PaCRK1 on Gisela 5 seedlings to cope with salt stress, the physiological and biochemical indexes were detected. In terms of phenotype, the transgenic lines grew better than control under salt stress (Fig. 6A). And also PaCRK1 overexpression enhanced root activity and chlorophyll content (Fig. 6B). Analysis of antioxidant products and related enzymes showed that compared with WT, the accumulation of MDA and H_2O_2 in PaCRK1 overexpressing roots was significantly decreased, while Vc content was increased under salt stress (Fig. 6C). At the same time, the activities of ROS scavenging enzymes APX and CAT in roots overexpressing PaCRK1 were significantly higher than WT under salt stress (Fig. 6D). These results indicated that PaCRK1 could improve the antioxidant capacity of Gisela 5 seedlings under salt stress.

Antioxidant-related genes were regulated by PaCRK1

In order to elucidate the effect of PaCRK1 overexpression on sweet cherry growth at the molecular level, we analyzed the differentially expressed genes (DEGs) between PaCRK1 overexpression and WT by transcriptome. In all, a total of 6,237 DEGs were identified, including 1,875 up-regulated and 4,362 downregulated genes (Figure S5A). In order to assign functional information to the DEGs between WT and PaCRK1 overexpression, Gene Ontology (GO) analysis was performed. These genes can be divided into three main categories by GO analysis: biological process (BP), cellular component (CC) and molecular function (MF). In the BP classification, these genes are mainly involved in cell metabolism, stress and biological regulation. In CC classification, they are mainly located on the cell membrane, which is closely related to their signal transduction. In MF classification, these up-regulated genes are mainly involved in catalytic activity and binding processes (Figure S5B).

Previous studies have found that antioxidant-related genes such as *APX*, *POD*, *CAT* and *ANX* were regulated in stress-tolerant plants (Bassil, Tajima et al. 2011, Calzone, Cotrozzi et al. 2021). Thus, we analyzed the expression of antioxidant-related genes under the condition of PaCRK1 overexpression. The results showed that a total of 46 DEGs were identified, of which 21 genes were significantly up-regulated and 25 genes were significantly down-regulated (Fig. 7A). To further verify the expression changes of

these differential genes under salt stress, we selected three significantly up-regulated genes (*APX2*, *CAT1* and *POD4*). The results showed that PaCRK1 overexpression could significantly up-regulate the expression level of *APX2* under stress, followed by *CAT1* and *POD4* (Fig. 7B). These results suggested that PaCRK1 enhanced the salt tolerance of sweet cherry seedlings by up-regulating the expression of antioxidant-related genes.

Discussion

For now, the classification of CRKs have been reported in some herbaceous plants, such as *Arabidopsis* (Wrzaczek, Brosché et al. 2010), rice (Chern, Xu et al.), soybean (Delgado-Cerrone, Alvarez et al.) and some woody plants like apple (Zuo, Liu et al. 2020) and cotton (Zhang ZhongQi 2018). The number of CRKs varies greatly among species. For example, there were 44 CRKs in *Arabidopsis*, 40 CRKs in rice, while in the woody plants, there were 36 CRKs in apple, 70 CRKs in upland cotton. In our study, we have found 40 PaCRKs in sweet cherry (Fig. 1). These results indicated that there were significant differences in the evolutionary history of CRKs from herbaceous plants to woody plants. According to theoretical isoelectric point analysis, most of the pI values of PaCRKs were slightly acidic, while the rest were alkaline (Table 1). Since plants can change their gene expression according to external pH (Lager, AndréAsson et al. 2010), changes in isoelectric points of PaCRKs contribute to the functional diversity of these proteins. Splicing variations in these gene families have been reported to have different isoelectric points, and the presence of isomers with different isoelectric points may help to adapt to changes in external pH (Brew-Appiah, York et al.). Isoelectric points can also affect protein localization, so different pI values can help CRK family to perform better subcellular localization and function (Kiraga, Mackiewicz et al. 2007). Most PaCRKs possess two copies of DUF26 in their extracellular domain in our study, except PaCRK19 who have three DUF26 domains (Fig. 2). These results were also reported by Quezada (Quezada, García et al.), who have found 4 DUF26 domains in *Phaseolus* with CRK9.

It is reported that CRKs play a significant role in coping with plant development and innate immunity. Therefore, we analyzed the expression of 40 *PaCRKs* genes in sweet cherry under different stresses such as salt, drought, pathogenic bacteria and ABA (Fig. 3A). In *Arabidopsis*, CRKs were involved in regulating seed germination and in response to abiotic stress induced ABA signaling. Studies on *Arabidopsis* showed that overexpression of CRK20, CRK45 and CRK36 enhanced salt, drought and ABA tolerance and inhibition of seed germination, respectively (Ederli, Madeo et al. 2011, Zhang, Han et al. 2013, Lee, Kim et al. 2017). Overexpression of CRK5, CRK13 and CRK28 in *Arabidopsis* can enhance resistance to *Pseudomonas syringae* (Chen, Du et al. 2003, Acharya, Raina et al. 2007, Yadeta, Elmore et al. 2017). While, in our study, we found that the expression level of *PaCRK1* was significantly up-regulated by salt and ABA stresses (Fig. 3B). The above results suggested that the PaCRKs family in sweet cherry, and particularly PaCRK1, plays a positive role in responding to external stimuli.

Based on the subcellular localization analysis of GFP fusion, PaCRK1 was likely to be localized on the plasma membrane (Fig. 4). Studies reported that CRK4, CRK6 and CRK36 in *Arabidopsis* (Yeh, Chang et al. 2015) and GbCRK18 in cotton (Li, Zhang et al. 2018) were also found to be localized on cell

membranes. Therefore, the transient expression analysis clearly indicated that PaCRK1 was a membrane-targeted protein that plays a role in extracellular signal reception.

Salinity affects the entire growth and development process of plants (García-Sánchez, Simón-Grao et al. 2020). It is reported that CRK2 in Arabidopsis is required for salt-induced callose deposition (Hunter, Kimura et al. 2019). We also found that PaCRK1 overexpression enhanced the tolerance of Arabidopsis and Gisela 5 to salt stress (Fig. 5 and Fig. 6). Reactive oxygen species (ROS) are produced in large quantities in the process of external stresses, which greatly affect the metabolic activities of plant cells. To avoid producing excessive ROS, plants could improve their scavenging capacity through regulating the activities of related oxidases (APX, CAT and POD), thus avoiding the oxidative damage they cause (Das and Roychoudhury 2014). In our study, the results showed that compared with the WT, the activity of APX and CAT in the transgenic lines overexpressing PaCRK1 was higher, resulting in the low accumulation of ROS (Fig. 6). Meanwhile, the genes related to oxidation were more elevated in PaCRK1 overexpression transgenic lines under salt stress than WT control, such as *APX2*, *CAT1* and *POD4* (Fig. 7). CAT plays an important role in plant response to salt stress. Study reported that MAP kinase MEK1 positively regulate the expression of three Arabidopsis *CATs* (*CAT1*, *CAT2*, and *CAT3*) (Xing, Jia et al. 2007). Under salt stress, catalase is regulated not only by transcription but also by its interacting proteins. Calcium-dependent protein kinase 8 (CPK8) interacted with CAT3 and phosphorylated it, activating its catalase activity and enhancing Arabidopsis tolerance to drought stress (Zou, Li et al. 2015). These results suggest that CAT changes are influenced not only by changes in transcription level, but also by posttranslational modifications. Our study found that PaCRK1 overexpressed lines not only increased the enzyme activity of CAT, but also regulated the up-regulation of *CAT1* gene expression, suggesting that PaCRK1 may play an important role in both the regulation of CAT. Subsequent experiments will further verify the specific pathway of PaCRK1 affecting CAT function and how it works.

Conclusion

In this study, we have found 40 PaCRKs in sweet cherry. Transcriptome analysis showed that the expression of *PaCRKs* was regulated by stresses, such as drought, salt, pathogens and hormones. Moreover, overexpression of PaCRK1 in Arabidopsis and Gisela 5 roots improved their tolerance to salt stress by enhancing their antioxidant capacity. The excavation of the PaCRKs family may further reveal the potential function of sweet cherry in salt stress resistance.

Declarations

Data availability

The RNA-Seq data has been deposited to the SRA of NCBI (<https://www.ncbi.nlm.nih.gov/biosample>) with the data set Submission ID SUB11195318 and BioProject ID PRJNA816669.

Author Contributions

FLW and HYS conceived and designed the project. XHZ and DHQ performed most of the experiments. LW, YHG, NNA, APW and YXL contributed reagents or provided laboratory assistance. FLW interpreted results and wrote the manuscript. HYS and JJY commented on the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1 is available in the Supplementary Files section.

Figures

Figure 1

Phylogenetic tree analysis the PaCRKs classification in sweet cherry. **A.** The phylogenetic tree was constructed using MEGA 7.0. Different groups of PaCRKs are marked by different colors. **B.** The phylogenetic tree was constructed using the full length of CRKs protein amino acid sequences in sweet cherry (*Prunus avium*), apple (*Malus domestica*), rice (*Oryza sativa* L.) and *Arabidopsis thaliana*.

Figure 2

Conserved domains and motifs analysis of PaCRKs. Conserved domains (A) and motifs (B) of PaCRK proteins were determined using PAFM and MEME.

Figure 3

Analysis the expression levels of *PaCRKs* under different stresses. **A.** The heatmap diagram showed the relative expression levels of 40 *PaCRKs* in Gisela 5 roots after 4 hours treatment with four stresses, including NaCl, PEG₆₀₀₀, Agrobacterium and ABA, compared to control. **B.** qRT-PCR expression analysis of four highly up-regulated *PaCRKs* genes (*PaCRK1*, *PaCRK10*, *PaCRK19* and *PaCRK35*) subjected to PEG₆₀₀₀, NaCl, Agrobacterium, and ABA stresses. “*” means p-value < 0.05, “**” means p-value < 0.01, “***” means p-value < 0.001..

Figure 4

Protein structure model and subcellular localization of *PaCRK1*. **A.** Conserved domains of *PaCRK1* were analyzed by PAFM. **B.** The CDS sequence of *PaCRK1* was constructed to the vector of pMDC83 and fused with GFP. The pMDC83-*PaCRK1* and GFP empty control were transformed into *Agrobacterium tumefaciens* GV3101 and injected into tobacco cells, respectively.

Figure 5

***PaCRK1* overexpression enhanced the tolerance of Arabidopsis to salt and ABA stresses.** **A.** Phenotypes of *PaCRK1* overexpression and WT lines under 0, 50 μ M and 100 μ M ABA stresses. **B.** The phenotypes of *PaCRK1* overexpression and WT lines were analyzed under 0, 100 and 150 mM NaCl treatments. **C and D.** Root length and fresh weight changes in *PaCRK1* overexpression and WT seedlings under ABA and NaCl stresses, respectively. “*” means p-value < 0.05.

Figure 6

***PaCRK1* overexpression enhanced the antioxidant ability of Gisela 5 under salt stress.** **A.** Comparison the phenotype of *PaCRK1*overexpressed lines and WT with two weeks under 200 mM NaCl. **B.** The root activity and chlorophyll contents were analyzed with *PaCRK1*overexpression. **C.** The contents of MDA, H₂O₂, and Vc were detected between *PaCRK1*overexpressed lines and WT. **D.** The activities of APX, CAT and POD were measured between *PaCRK1*overexpressed lines and WT. “*” means p-value < 0.05, “**” means p-value < 0.01.

Figure 7

Expression analysis of the antioxidant-related genes under salt stress in PaCRK1 overexpressed Gisela 5.

A. The antioxidant-related DEGs were identified between PaCRK1 overexpression and WT using transcriptome analysis. **B.** The expression levels of antioxidant-related genes were detected by qRT-PCR. “*” means p-value < 0.05, “**” means p-value < 0.01.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)
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