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Genome-wide analysis of the *PYL-PP2C-SnRK2s* family in the ABA signaling pathway of pitaya reveals its expression profiles under canker disease stress

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Abstract

Background Abscisic acid (ABA) plays a crucial role in seed dormancy, germination, and growth, as well as in regulating plant responses to environmental stresses during plant growth and development. However, detailed information about the *PYL-PP2C-SnRK2s* family, a central component of the ABA signaling pathway, is not known in pitaya.

Results In this study, we identified 19 pyrabactin resistance-likes (*PYLs*), 70 type 2 C protein phosphatases (*PP2Cs*), and 14 SNF1-related protein kinase 2s (*SnRK2s*) from pitaya. In pitaya, tandem duplication was the primary mechanism for amplifying the *PYL-PP2C-SnRK2s* family. Co-linearity analysis revealed more homologous *PYL-PP2C-SnRK2s* gene pairs located in collinear blocks between pitaya and *Beta vulgaris L.* than that between pitaya and *Arabidopsis*. Transcriptome analysis showed that the *PYL-PP2C-SnRK2s* gene family plays a role in pitaya's response to infection by *N. dimidiatum*. By spraying ABA on pitaya and subsequently inoculating it with *N. dimidiatum*, we conducted qRT-PCR experiments to observe the response of the *PYL-PP2C-SnRK2s* gene family and disease resistance-related genes to ABA. These treatments significantly enhanced pitaya's resistance to pitaya canker. Further protein interaction network analysis helped us identify five key *PYLs* genes that were upregulated during the interaction between pitaya and *N. dimidiatum*, and their expression patterns were verified by qRT-PCR. Subcellular localization analysis revealed that the *PYL* (*Hp1879*) gene is primarily distributed in the nucleus.

Conclusion This study enhances our understanding of the response of *PYL-PP2C-SnRK2s* to ABA and also offers a new perspective on pitaya disease resistance.

Keywords *PYL-PP2C-SnRK2s*, Abscisic acid, Pitaya, *N. dimidiatum*, Pitaya canker

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Background

Abscisic acid (ABA) is widely recognized as a key phytohormone and signaling molecule involved in a broad range of plant growth and developmental processes, including seed germination, flowering, color transformation, stomatal movement, and plant senescence. In addition, it mediates the effects of adverse conditions, such as pest and pathogen attacks [1–3].

Three regulatory sensing and transduction mechanisms exist for the activation of the ABA signaling pathway: the PYR/PYL/RCAR receptors, type 2 C protein phosphatase (PP2C) enzymes, which act as negative regulators of the pathway, and SNF1-RELATED PROTEIN KINASE 2s (SnRK2s), which serve as positive regulators of signal transduction [4]. In the absence of ABA, PYL proteins fail to bind to PP2C, resulting in high PP2C activity, which in turn prevents the activation of SnRK2 and its downstream factors. Activation occurs when ABA is present. The interaction between the transduction components is primarily mediated by the receptor of the *PYR*/*PYL*/*RCAR* genes binding to the plant hormone molecule ABA. This binding leads to the inhibition of *PP2Cs* activity, which in turn activates the *SnRK2s* and enables their participation in the transcription factor known as the ABA-responsive element binding factor/protein. The phosphorylation of the transcription factor/protein (*ABF*/*AREB*) and downstream transcription factors and ion channels initiates a response to stress [5–8].

A total of 14 proteins encoding ABA receptor PYLs have been identified in the genome of *Arabidopsis thaliana* with conserved amino acid sequences [9]. Among these, *PYR1* and *AtPYL2* are involved in ABA-induced stomatal closure, *AtPYL4* and *AtPYL5* are essential for stomatal response to CO_2 [10]. *AtPYL6* interacts with the JA signaling regulator *MYC2* in an ABA-enhanced manner [11]. *AtPYL8* binds to the transcription factor *MYB77* and synergistically regulates lateral root growth with growth factors. Lastly, *AtPYL9* can regulate the phosphorylation of ABA-responsive element-binding factors (ABFs) and Related to ABA-Insensitive 3/VP1 (RAV1) transcription factors to promote leaf senescence [12, 13]. Thirteen homologs of PYL receptors have been identified in rice, of which 10 are functional [14, 15]. For example, *PYL1*, *PYL4*, and *PYL6* promote rice growth and increase rice yield under natural conditions, while also maintaining seed dormancy. Seventy-six *PP2Cs* have been identified in *Arabidopsis*, and some are linked to the inhibition of ABA signaling, but the functions of most are still unknown [8, 16]. For example, ABA-insensitive 1 (*ABI1*), *ABI2*, ABA-hypersensitive 1 (*HAB1*), *HAB2*, and ABA-hypersensitive germination 1 (*AHG1*) are involved in the negative regulation of phytohormone signaling pathways. *PP2Cs* activate or inactivate ABA signaling by phosphorylating and dephosphorylating *SnRK2s* and *SnRK1s*. In addition, they can phosphorylate *SLAC1*, which releases anions from guard cells. This leads to the release of intracellular potassium ions to the outside, lowering the expansion pressure of the guard cells and resulting in the closure of plant stomata [17]. This closure reduces plant transpiration and also defends against pathogenic microorganisms that invade through plant stomata. The SnRK2 kinase is classified as a serine/threonine kinase that is activated and acts as a positive regulator in the ABA signaling pathway. *SnRK2* is capable of phosphorylating the corresponding ion channels and plasma membrane-localized respiratory burst oxidase homologs (RBOH) [18].

Numerous experiments have shown that phytohormones also play a crucial role in defending against biotic stresses, such as pathogens [19, 20]. The roles of phytohormones such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ETH) in plant defense responses are well understood, but the defense mechanisms of ABA in plants are not fully understood. Instead of a linear and parallel defense pathway, a complex network of multiple signals is coordinated to enhance the plant's defense system. In biotrophic pathogens, such as wheat rust, the upregulation of *TaSTP6* expression can promote an increase in ABA, leading to elevated cytoplasmic sugar accumulation [21]. Additionally, the transcription factor *WRKY33* promotes ABA biosynthesis by upregulating the transcription of *NCED3* and *NCED5* in *Arabidopsis* after infection by necrotrophic pathogens [22]. The transcription factor *LeJA2* in tomato up-regulates the expression of *LeNCED1*, which in turn promotes ABA synthesis, thereby restricting pathogen invasion through stomata [2].

Pitaya is a climbing tropical and subtropical plant belonging to the *Hylocereus* or *Selenicereus*, which exhibits high tolerance to high temperatures, drought, and disease, among other stresses [23]. In 2021, pitaya genomic data have now been published, enabling a detailed analysis of the ABA signaling pathway gene families of pitaya [24, 25]. We identified 19 *YPLs*, 70 *PP2Cs*, and 14 *SnRK2s* in the pitaya genome and determined their phylogenetic relationships, protein motifs, and gene structures. In addition, we constructed protein interaction networks, determined subcellular localizations, and applied exogenous ABA sprays to observe pitaya resistance to pitaya canker. This systematic study will enable us to gain a better understanding of the potential transcriptional regulation of *PYL-PP2C-SnRK2*s under various conditions, and also offer new insights into pitaya disease resistance.

Results

Identification and characteristics of *PYL-PP2C-SnRK2s* in pitaya

Based on the annotation information of the pitaya genome, we identified a total of 19 *PYLs*, 70 *PP2Cs*, and 14 *SnRK2s* (Table S1). To explore the evolutionary relationships and functional diversity of the PYL, PP2C, and SnRK2 proteins, we constructed a maximum likelihood phylogenetic tree with 1,000 bootstraps using the complete PYL-PP2C-SnRK2 protein sequences of pitaya and *Arabidopsis thaliana* (Fig. 1).

Based on phylogenetic analysis, PYLs, PP2Cs, and SnRK2s were classified into 5 (PYL_Group1-4), 14 (A-N) and 4 (SnRK2_Group1-4) subfamilies, respectively. Among them, HpPYL was named based on homology with *Arabidopsis thaliana*. The analysis showed that the PYL_Group 1 subfamily was the largest. Within this subfamily, the PYL_Group 1-a subfamily contained 7 HpPYLs and 2 AtPYLs (2–3), which were

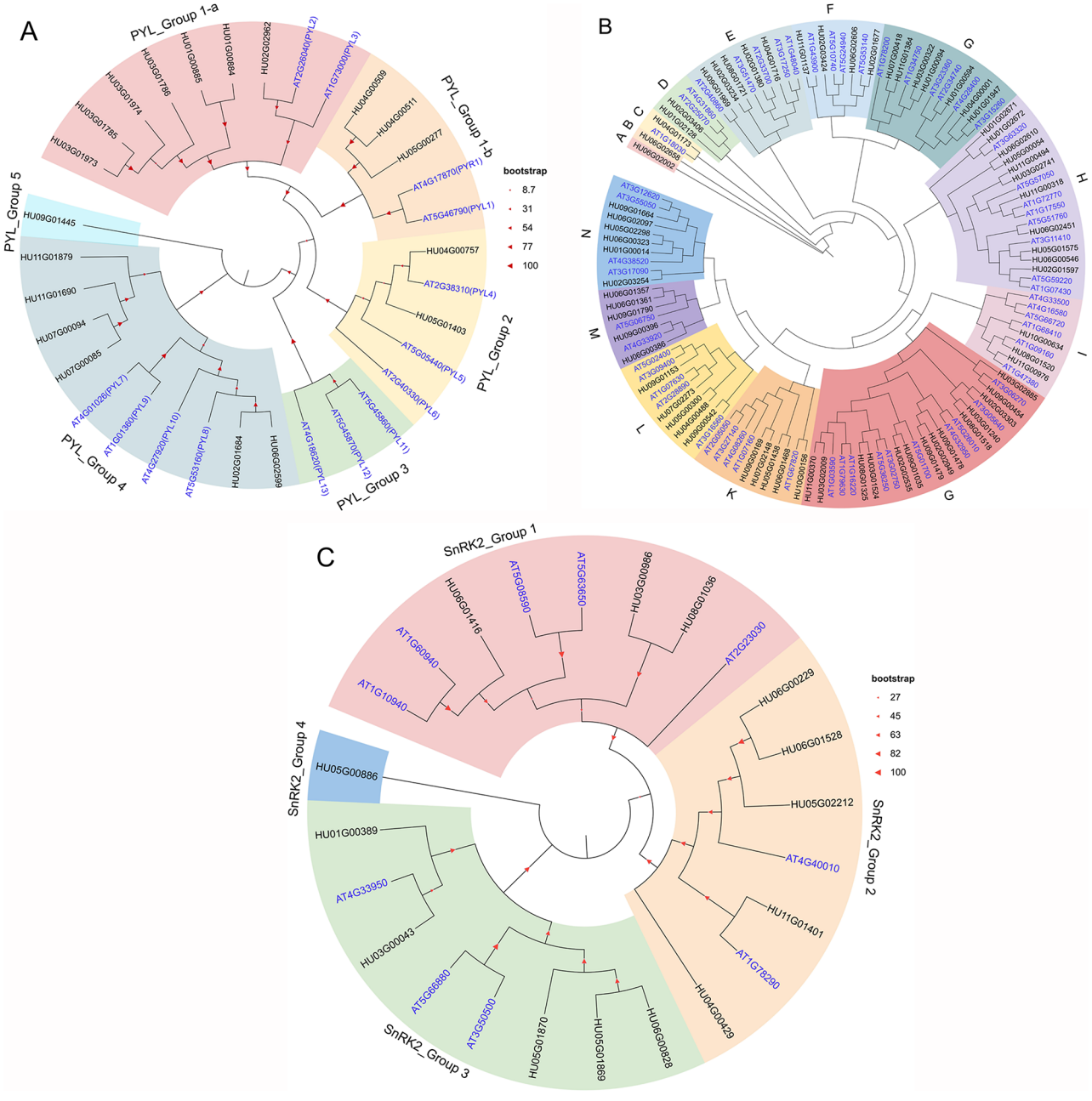

Fig. 1 Phylogenetic analysis of PYL **(A)**, SnRK2 **(B)**, and PP2C **(C)** proteins in pitaya. The blue font represents *Arabidopsis* proteins. The phylogenetic tree was constructed using the maximum likelihood method of MEGA software and by 1000 bootstrap. At the same time, subfamily numbers were established based on the *Arabidopsis* classification applicable to this analysis

clustered together. In contrast, the PYL_Group 1-b subfamily includes 3 HpPYLs and 2 AtPYLs (PYL1, PYR1). In PYL_Group 2, there are 2 HpPYLs tightly clustered with 3 AtPYLs (4–6). In PYL_Group 4, 6 HpPYL are clustered with 4 AtPYL (7–10). Finally, we found that HU09gG01445 protein in PYL_Group 5 did not homologize with *Arabidopsis* PYL family proteins (Fig. 1A).

Comparison of PP2C protein families in pitaya and *Arabidopsis* reveals the evolutionary relationships and functional diversity among protein family members in these two species. The analysis showed that the PP2C protein family was divided into 14 subfamilies, with subfamily G being the largest, containing 24 protein members (Fig. 1B).

For the SnRK2 protein family, we found that the subfamilies SnRK2_Group 1 and SnRK2_Group 3 were the largest, containing three and 5 proteins in pitaya, respectively. Notably, in subfamily SnRK2_Group 4, we found no proteins homologous to the *Arabidopsis* SnRK2 family, suggesting that there may be some degree of species specificity or functional differentiation in this particular subfamily (Fig. 1C).

Gene structure and conserved motif analysis of *PYL-PP2C-SnRK2s*

The diversity of exon/intron structures and protein structural domains plays a crucial role in the evolution of gene families. Therefore, we examined the exon-intron structural patterns of the *PYL-PP2C-SnRK2s* and conserved structural domains based on their phylogenetic relationships (Fig. 2). The analysis of exon/intron structures revealed that most members within the same subfamily exhibit similar numbers of exons or introns but varying lengths (Fig. 2A). The structures of the *PYL-PP2C-SnRK2s* were essentially similar across the groups, but there were differences in the exon/intron arrangement of some genes. This suggests that the *PYL-PP2C-SnRK2*s are relatively conserved during evolution, ensuring the integrity of their gene structures.

Based on the phylogenetic relationships, conserved motifs in pitaya were analyzed using MEME and CDD. Ten motifs were obtained for each gene family (Fig. 2). For the *PYLs* gene family, motifs 1, 2, and 3 belong to the SRPBCC domain superfamily, while the functions of motifs 4–10 are still unknown. Among these, motifs 1, 2, 3, and 5 are the most conserved motifs in the *PYL* family (Fig. 2A). For the *PP2Cs* gene family, motifs 1–5 belong to the PP2Cc domain superfamily, and motif 6 also belongs to the PP2Cc domain superfamily. For the *PP2Cs* gene family, motifs 1–5 belong to the PP2Cc domain, while motif 6 belongs to the PTZ00224 superfamily. The functions of motifs 7–10 are still unclear. Among them, motifs 1–4 are the most conserved motifs in the *PP2C* family (Fig. 2B). In *SnRK2s*, motifs 1–6 and 8 all belong to the PKc_like domain superfamily, of which motif 1–9 is the most conserved motif in the *SnRK2* family (Fig. 2C). This indicates a typical gene family feature in the *PYL-PP2C-SnRK2s* family. In addition, specific motifs were identified in different categories. For example, motif 4 was found exclusively in PYL_Group1 within the *PYLs* family, while motif 9 was only found in *HU04G00509* and *HU04G00511*. Motif 6 was present in all PYL_Groups except PYL_Group1, and motif 6 was found in all PYL_Groups except PYL_Group1. The sequence *HU09G01445* contains only motif 3 (Fig. 2A). In the *PP2Cs* gene family, motif 5 is predominantly found in group L, M and N (Fig. 2B). In the *SnRk2* gene family, motif 10 is only present in SnRK2_Group1, and *HU05G00886* does not contain motifs 9–10 (Fig. 2C).

Chromosomal distribution, gene duplication, and syntenic analysis of pitaya *PP2C-PYL-SnRK2s*

The distribution of *PYL-PP2C-SnRK2s* on chromosomes was found to be uneven (Fig. 3). The *PYLs* gene family has the highest number of genes located on chromosome 3, with four members. The *PP2C* family had the highest number of members on chromosome 6, with 12, and the lowest on chromosome 7, with seven. Chromosome 6 contained the highest number of *SnRk2* gene family members, with four. Furthermore, there were differences in the chromosomal distribution of *PYL-PP2C-SnRK2s*. Most of the *PYL-PP2C-SnRK2s* were located at either end of the chromosomes. However, on chromosomes 2, 8, and 11, the *PYL-PP2C-SnRK2s* were concentrated at one end of each chromosome. The distribution of this gene on chromosomes may be related to the function of the *PYL-PP2C-SnRK2* genes.

The *PYL-PP2C-SnRK2s* genes showed tandem repeats in pitaya (Fig. 3). The *PYLs* gene family contains four tandem duplication pairs, belonging to PYL_Groups 1, respectively. The *PP2Cs* family contains five pairs of tandem duplications, each belonging to groups F, G, H, I and M, respectively. The *SnRK2s* family contains a single tandem duplication pair, SnRK2s_Group 3. These duplications suggest that the *PYL-PP2C-SnRK2s* genes might have evolved through gene expansion events, potentially leading to functional diversification within the family. To further investigate the potential evolutionary relationship of the *PYL-PP2C-SnRK2s* family with *Arabidopsis* and *Beta vulgaris* L., we constructed a Co-linearity analysis (Fig. 4). In pitaya, each member of the *PYL-PP2C-SnRK2s* has one or more straight homologous genes in *Arabidopsis* and *Beta vulgaris* L. This co-occurrence happens within the same subclass, indicating that the replication events of *PYL-PP2C-SnRK2s* take place in plants before pitaya, *Arabidopsis*, and *Beta vulgaris L.* differentiate. Taken together, these results suggest that tandem

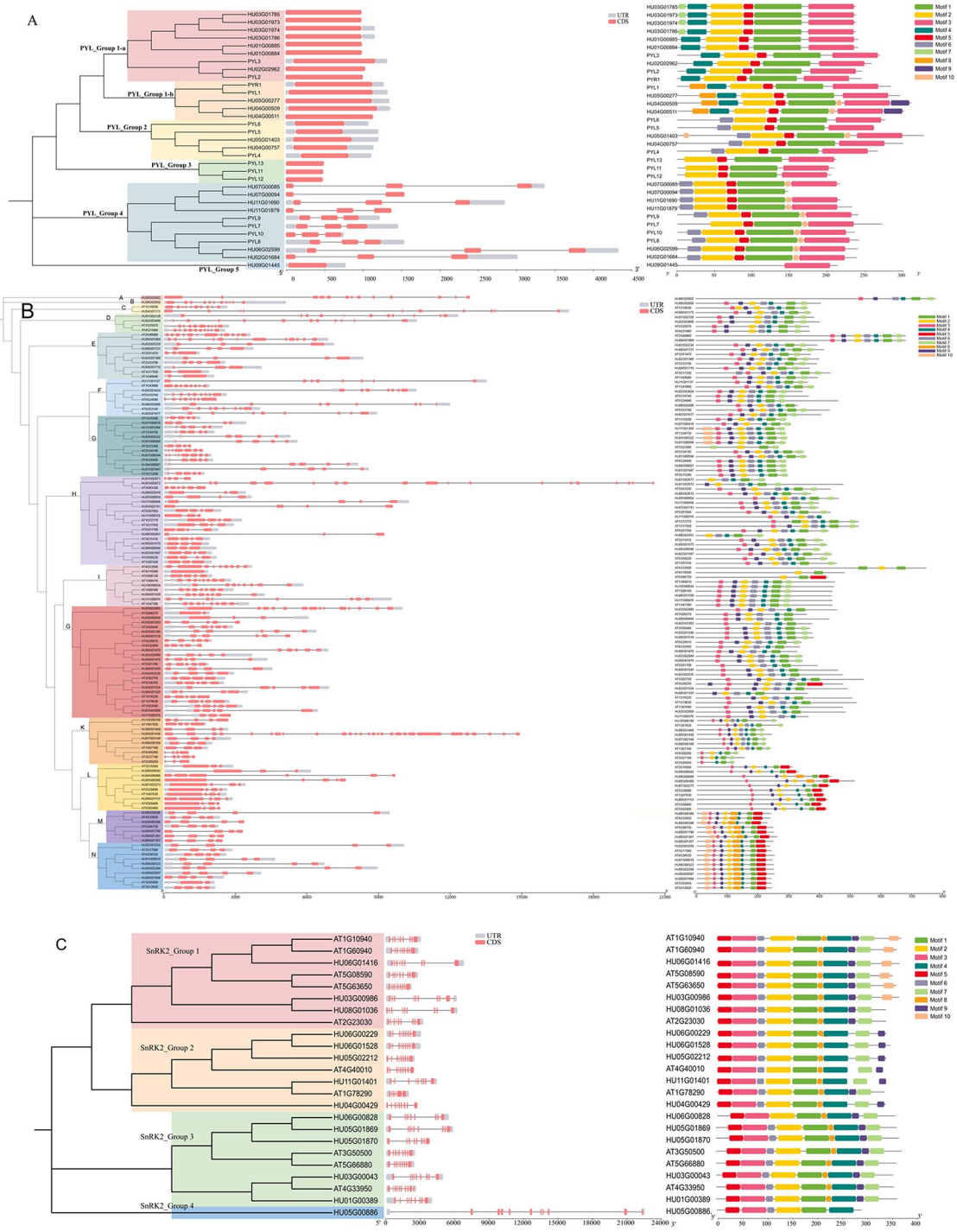

Fig. 2 Maximum likelihood phylogeny, exon-intron structure, and motif composition of *PYLs*(**A**), *PP2Cs*(**B**), and *SnRK2s*(**C**) proteins from pitaya and *Arabidopsis* using complete protein sequences. The reliability of the tree was assessed using 1,000 bootstrap replicates

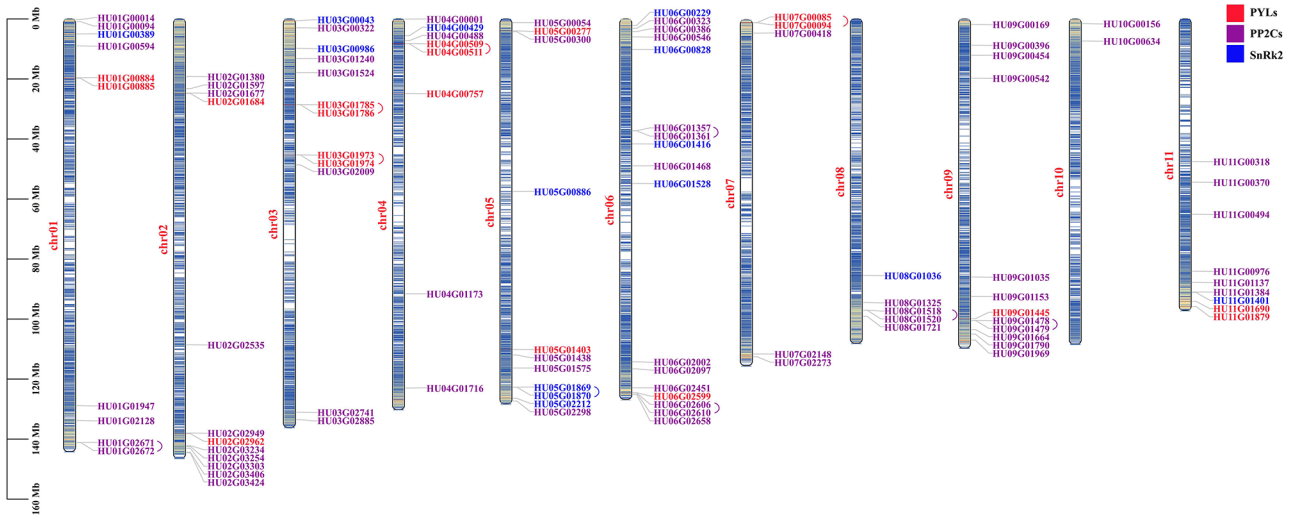

Fig. 3 Chromosomal (Chr) localization and duplication of 103 *PYL-PP2C-SnRK2s* on pitaya chromosomes. The various colors in chromosomes represent gene density, while the arcs depict tandem repeats

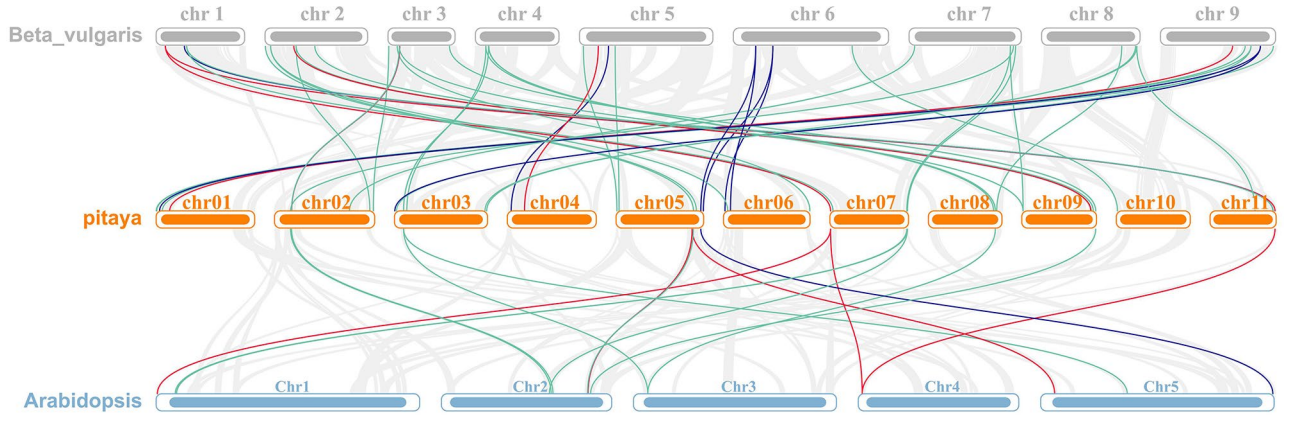

Fig. 4 Synteny analysis of the *PYL-PP2C-SnRK2s* family in pitaya, *Arabidopsis*, and *Beta vulgaris L.*. The red, blue, and green lines highlight the collinear blocks of *PYLs*, *PP2Cs*, and *SnRK2s* gene pairs separately, while gray lines indicate all the other collinear blocks in the pitaya genome. The chromosome number is shown at the top or bottom of each chromosome

duplication is the primary amplification mechanism of the *PYL-PP2C-SnRK2s* family.

The *PYL-PP2C-SnRK2s* is involved in pitaya's response to pitaya canker

To investigate the role of *PYL-PP2C-SnRK2s* in pitaya's response to biotic stress, we analyzed RNA-seq data during *N. dimidiatum* invasion of pitaya (Fig. 5). The results indicated that 21/70 *PP2Cs*, 9/19 *PYLs*, and 7/14 *SnRK2s* exhibited up-regulated expression on day 5 after inoculation. On day 8, 18/70 *PP2Cs*, 6/19 *PYLs*, and 6/14 genes showed up-regulated expression. On day 11, 15/70 *PP2Cs*, 4/19 *PYLs*, and 5/14 *SnRK2s* showed up-regulated expression. On day 15, 15/70 *PP2Cs*, 8/19 *PYLs*, and 3/14 *SnRK2s* showed up-regulated expression. These results indicate the potential involvement of *PYL-PP2C-SnRK2* genes in the response of pitaya to pitaya-canker stress. In addition, 18/ 19 PYLs genes, 14/ 70 PP2C genes, and 13/ 14 SnRK2s were involved in the ko04075 pathway to regulate stomatal closure as analyzed by KEGG enrichment (Fig. S1). This suggests that the *PYL-PP2C-SnRK2s* gene family is involved in ABA-induced guard cell closure (Table S2).

PYL-PP2C-SnRK2s response to ABA treatments

To evaluate the expression pattern response to hormone ABA treatment, 5 *PYLs*, 5 *PP2Cs*, and 5 *SnRK2s* were selected based on their expression in the transcriptome, as well as disease resistance-related genes such as LRR serine/threonine-protein kinases, endochitinase, Ca^{2+}, ROS, and stomatal-related transcription factors for qRT-PCR analysis (Fig. 6). The results showed that these genes had different expression levels under ABA treatment. For the PYLs gene family, *HU07G00085*, *HU07G00094*, and

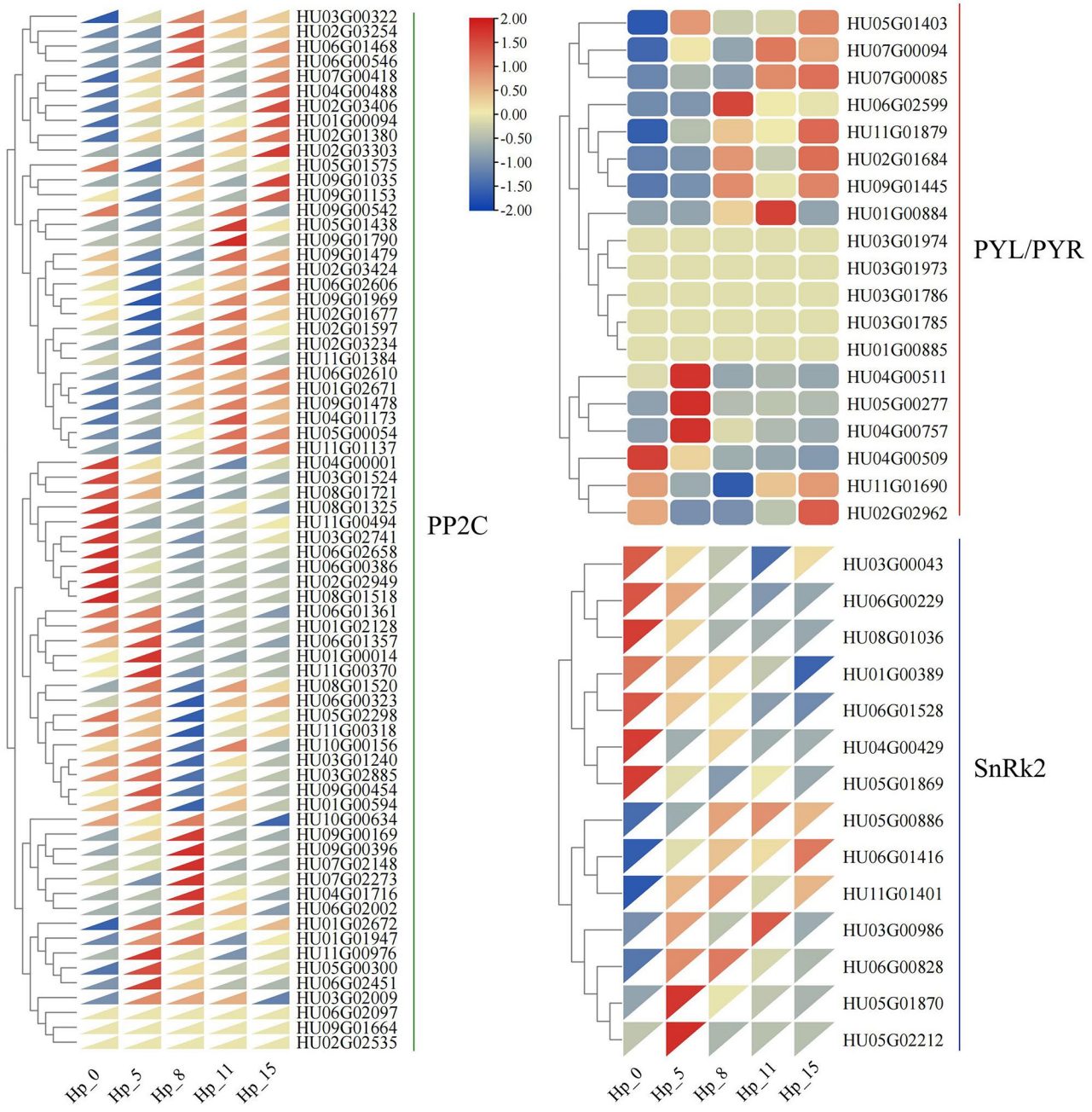

Fig. 5 Heatmap analysis of *PYL-PP2C-SnRK2s* differential expression genes (DEGs) in the interactions between pitaya and *N. dimidiatum* responding to ABA. The color bar represents the logarithm of gene expression fold change (log_2FC), with red indicating up-regulated genes and blue representing down-regulated genes

HU11G01879 were up-regulated by 3.3-fold, 2.1-fold, and 2.4-fold respectively. For the *SnRK2s* gene family, *HU05G02212*, *HU05G01870*, and *HU06G00828* were up-regulated by 5.3-fold, 2.2-fold, and 2-fold respectively. For the *PP2Cs* gene family, *HU01G02671* was the most up-regulated, with a 10-fold increase. In addition, after ABA treatment, chitinases *HU06G01804* and *HU11G01457* were up-regulated by 15.8-fold and 20.2-fold respectively, while LRR serine/threonine-protein kinases, Ca^{2+}, and ROS also showed varying degrees of upregulation. Interestingly, stomatal-related transcription factors were downregulated. These results indicate that an appropriate concentration of ABA treatment can induce the expression of *PYL-PP2C-SnRK2s* and activate the plant's defense mechanisms.

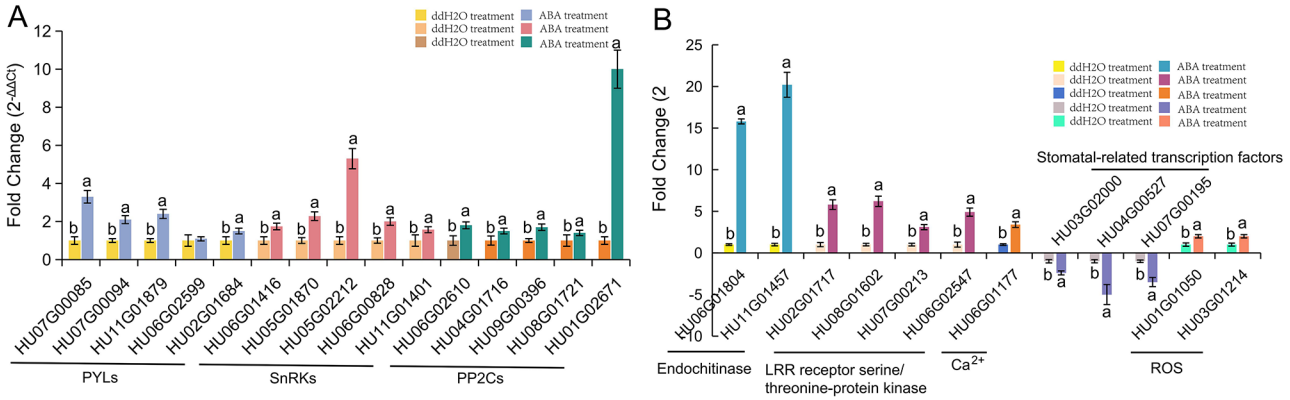

Fig. 6 The expression of the *PYL-PP2C-SnRK2s* gene family and disease resistance-related genes after ABA treatment was examined. The relative expression of *PYL-PP2C-SnRK2s* and disease resistance-related genes in two samples, including ddH_2O and ABA-treated pitaya, was validated using qRT-PCR (-ΔΔCT), with the *UBQ* gene as an internal control. Each set of data is derived from three replicates. Data are given as means ± SD. Significant differences were determined by t-tests using R (a and b indicate significant differences, $p < 0.05$)

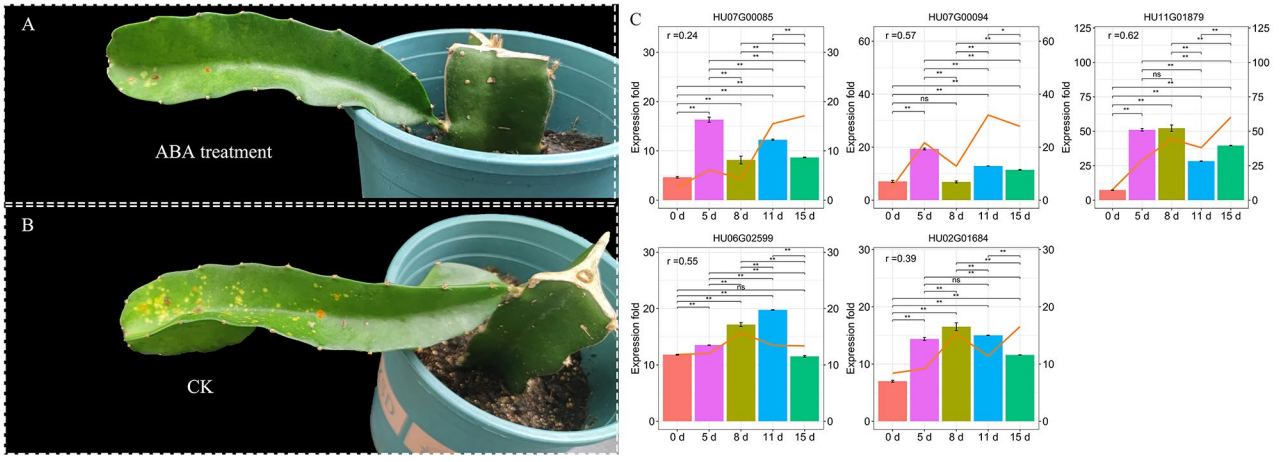

Fig. 7 Compared the disease in pitaya treated with ABA and inoculated with N. dimidiatum to validate RNA-seq results by qRT-PCR. **A**, **B**: Pitayas were pretreated with 2 mg/L ABA and, after one week, inoculated with a spore suspension of *N. dimidiatum*. Sterile water was used as a control. The disease phenotypes of the pitaya canker were observed and photographed after 15 days. **C**: 5 key genes were selected for qPCR analysis during the intercropping of pitaya and *N. dimidiatum*. Bar represents qRT-PCR, while the orange lines represent transcriptome data. Data are given as means ± SD. Significant differences were determined by t-tests using R (ns: no significant difference, * $p < 0.05$, ** $p < 0.01$). r represents the correlation coefficient between the gene expression results of qRT-PCR (bar) and RNA-seq (line) under *N. dimidiatum* treatment

Exogenously spraying ABA increases pitaya resistance to *N. dimidiatum*

Studies have shown that ABA can enhance plant resistance and inhibit the invasion of pathogenic fungi by regulating stomatal movement. We applied ABA through exogenous spraying, and the results demonstrated a significant increase in pitaya resistance to *N. dimidiatum* after the advance treatment with ABA (Fig. 7A-B, Fig. S2).

Interaction network analysis of *PYL-PP2C-SnRK2s*

To further explore the regulation pattern of gene expression during pitaya response to *N. dimidiatum*, we constructed a network map of 103 *PYL-PP2C-SnRK2s* using string. Protein-protein interactions (PPIs) during pitaya and *N. dimidiatum* interactions were analyzed using cytoscape software, based on the MCODE model. The results revealed that *HU11G01879*, *HU02G01684*, *HU07G00085*, *HU06G02599*, and *HU07G00094* were the key genes (Fig. 8). These genes were distributed in the PYL_Group 4 clusters and up-regulated their expression in response to *N. dimidiatum* infestation.

Expression analysis of five key genes during pitaya and *N. dimidiatum* interactions

To confirm the expression of five key genes (*HU11G01879*, *HU02G01684*, *HU06G02599*, *HU07G00085*, and *HU07G00094*), we conducted qRT-PCR analysis (Fig. 7C). The results revealed that the expression levels of *HU07G00085*, *HU07G00094*, *HU11G01879*, and *HU02G01684* were significantly up-regulated 5 days after inoculation with *N. dimidiatum*.

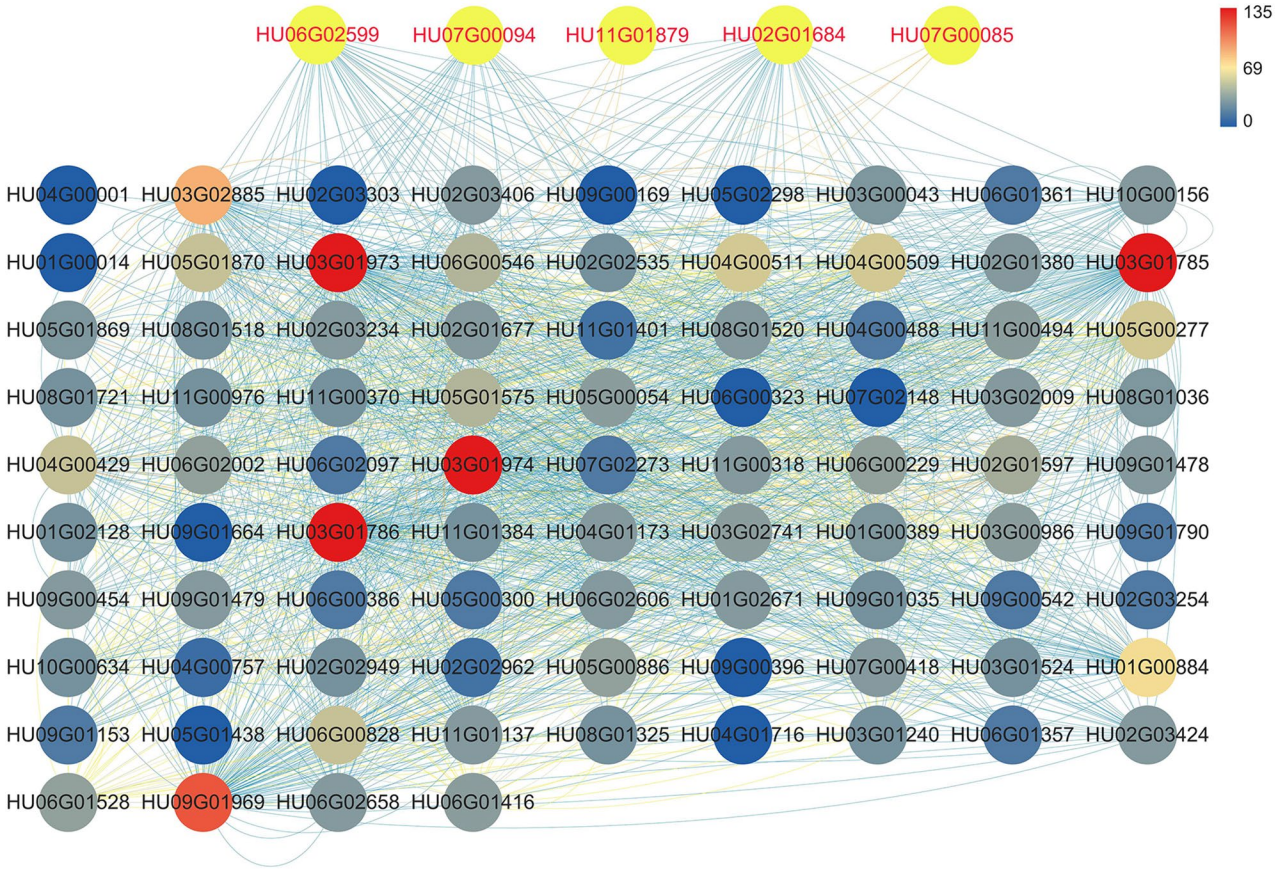

Fig. 8 Protein-protein interaction network among all the *PYL-PP2C-SnRK2s*, as analyzed by string. Denser lines between proteins represent tighter interactions than looser lines. Yellow indicates the core genes calculated using the MCODE model of cytoscape, while the blue to red gradient represents the strength of gene interactions individually

After 8 days, the expression levels of *HU07G00085*, *HU11G01879*, *HU06G02599*, and *HU02G01684* also showed significant up-regulation. Furthermore, *HU07G00085*, *HU07G00094*, *HU11G01879*, *HU06G02599*, and *HU02G01684* exhibited significant up-regulation 11–15 days after inoculation with *N. dimidiatum*. These results suggest that the five key genes are involved in the response to canker disease during the interaction between pitaya and *N. dimidiatum*.

Subcellular localization of *Hp1879*

The *HU11G01879* gene showed the most significant up-regulation during pitaya and *N. dimidiatum* interactions. To further validate the protein function of this gene, we cloned the *HU11G01879* gene and named it *Hp1879*. In order to determine the location of the *Hp1879* gene function, we constructed a vector expressing the GFP-Hp1879 translational fusion protein. This vector was used to infiltrate tobacco leaves with the *Agrobacterium tumefaciens* strain GV3101, causing infiltration into the tobacco leaves. The results showed that the GFP fluorescent signal containing the Hp1879 protein was detected in the cell membrane, cytoplasm, and nucleus (Fig. 9).

Discussion

Gene structure and evolution

The ABA signaling pathway is crucial for plants to respond to biotic and abiotic stresses, such as high temperature, low temperature, hyperosmolarity, and pathogenic microorganisms [8, 26]. The *PYL-PP2C-SnRK2s* family constitutes the central network of the ABA signaling pathway in plants and has been studied in various plant species, including tomato, *Arabidopsis*, rice, and wheat [7, 27, 28]. However, the regulatory mechanism of *PYL-PP2C-SnRK2s* in pitaya is not yet clear. In this study, we identified 19 *PYLs*, 70 *PP2Cs*, and 14 *SnRK2s* in the pitaya genome (Figs. 1 and 2). All of these proteins contain the PYR/PYL -like domain, the PP2Cc domain, and the protein kinase domain, which are consistent with the homologous genes in *Arabidopsis*, tomato, rubber, cotton and brachypodium [7, 29, 30]. This suggests that the *PYL-PP2C-SnRK2s* family genes are conserved in pitaya.

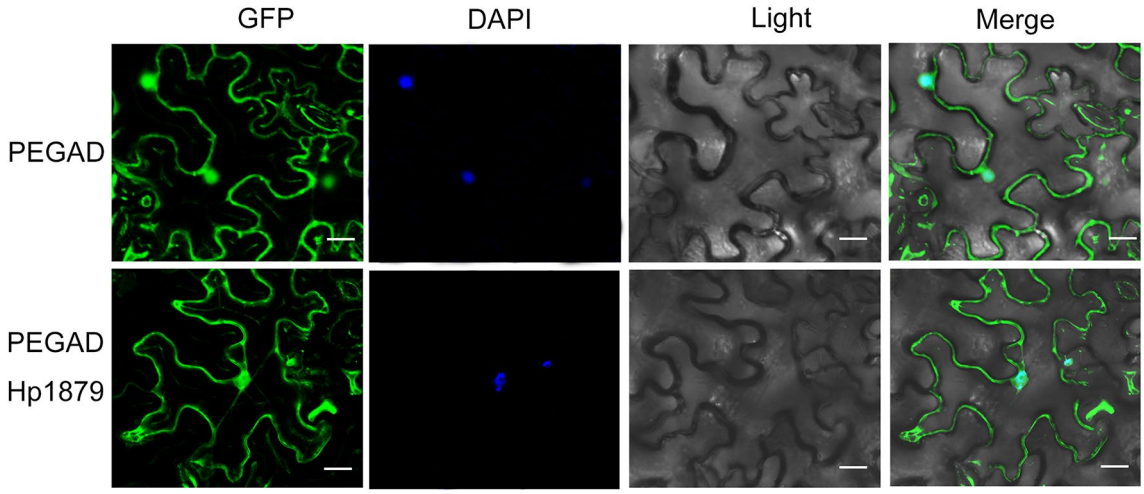

Fig. 9 Subcellular Localization of the Hp1879 protein. All fluorescence signals were detected using the confocal microscope. The PEGAD empty plasmid was used as the control. The scale bar was 10 μm

Tandem, segmental and whole-genome duplication events of *PYL-PP2C-SnRK2s* have been reported in different plants, and they can drive the expansion of plant gene families [7, 31]. In this study, tandem duplication was found to be the main driver of the *PYL-PP2C-SnRK2s* gene family expansion in pitaya (Fig. 4).

Regulation of gene expression

In recent years, the fundamental components of the ABA signaling pathway have been revisited and characterized [32]. The stimulation of ABA is recognized and transduced through ABA receptors, phosphatases, protein kinases, and transcription factors, which then trigger a series of physiological reactions mediated by ABA-regulated genes [26]. However, current data does not clearly indicate whether the components of the ABA signaling pathway function optimally under stress conditions. This is because the components involved in ABA signal transduction have not been fully analyzed to date. In this study, we analyzed the expression of *PYL-PP2C-SnRK2s* during the infection of pitaya by *N. dimidiatum*. The results showed that *PYL-PP2C-SnRK2s* were differentially expressed on days 5, 8, 11, and 15 post-infection (Fig. 5), with the most significant difference observed on day 5, indicating that ABA plays a role in resisting pathogens from the initial stage of infection. Studies have proven that the biosynthesis of ABA is essential for effective resistance against necrotrophic fungal pathogens [33–35]. Additionally, our analysis using qRT-PCR on pitaya branches sprayed with ABA showed that the *PYL-PP2C-SnRK2s* gene all responded to ABA (Fig. 6). Meanwhile, some disease resistance genes, such as Ca^{2+}, LRR serine/threonine-protein kinases, ROS, and particularly chitinase, exhibited high differential expression (Fig. 6), indicating that exogenous application of ABA can activate broad-spectrum resistance in pitaya. Notably, the down-regulation of bHLH transcription factors related to stomatal regulation may suggest that ABA treatment not only affects the expression of disease resistance-related genes but also possibly influences stomatal activity by adjusting the expression of bHLH transcription factors, thereby affecting the plant's adaptability to stress to some extent.

Pathogens must penetrate plant tissues to successfully infect their host. Fungi achieve this by secreting cutinases and cell wall-degrading enzymes to breach the plant's defenses, while others exploit mechanical injuries or natural openings, such as stomata, to invade [36, 37]. In response, plants have evolved complex mechanisms to mediate stomatal closure as an immune reaction against pathogens [38, 39]. Virulent pathogens like *Xanthomonas axonopodis* pv. *citri* counteract this natural defense by secreting effector proteins that prevent stomatal closure, thereby facilitating further invasion [40]. Thus, the ABA-dependent mechanism of stomatal closure becomes a crucial pre-invasion defense barrier. Through KEGG analysis of the *PYL-PP2C-SnRK2s* gene family, we found their involvement in the stomatal regulation pathway (Table S2), indicating their regulation by ABA and participation in the early defense against biological invasions. This discovery lays the foundation for studying the role of *PYL-PP2C-SnRK2s* in pitaya's resistance to biological stress.

Although ABA plays a role in the final outcome of immune responses, the specific components of the ABA signal and the mechanisms through which ABA positively influences immune reactions in specific plant-pathogen interactions remain largely unknown. Using the MCODE model of the protein interaction network, we analyzed data during the infection of pitaya by *N.*

dimidiatum and discovered that *PYLs* play a core role in the process of pitaya canker. Furthermore, five key genes were identified through qRT-PCR, all showing upregulated expression during the interaction (Figs. 7C and 8), with *Hp1879* expressed in the plant cell nucleus (Fig. 9). In *Arabidopsis*, it has been proven that ABA synthesis and pathogen-induced *PYR1* expression coincide spatially in the vascular system, supporting the vascular tissue as an integrative node to trigger stress signals and initiate local and systemic immune responses in plants [41–43]. Therefore, PYLs (*HU07G00085*, *HU07G00094*, *HU11G01879*, *HU06G02599*, and *HU02G01684*) play a vital role in the early detection of pathogen invasion and activation of the plant's immune function through ABA production.

Finally, by externally applying ABA followed by inoculation with *N. dimidiatum*, we observed a significant enhancement in pitaya's resistance to *N. dimidiatum*. Therefore, these genes could potentially be utilized to enhance pitaya's resistance to *N. dimidiatum*.

Conclusions

In pitaya, we identified 19 *PYLs*, 70 *PP2Cs*, and 14 *SnRK2*s genes. The phylogeny of these genes is consistent with the species phylogeny. Transcriptome analysis indicates that the *PYL-PP2C-SnRK2s* are involved in the biological stress response to *N. dimidiatum* infection. Furthermore, the exogenous application of ABA followed by inoculation with *N. dimidiatum* significantly enhanced pitaya's resistance to pitaya canker. Through a protein interaction network analysis, we identified five key *PYLs* genes upregulated during the interaction between pitaya and *N. dimidiatum*, and their expression was verified using qRT-PCR. We analyzed the subcellular localization of *Hp1879* and discovered it to be localized in the nucleus. Therefore, this study not only deepens our understanding of the *PYL-PP2C-SnRK2s* in response to ABA but also offers a new perspective on disease resistance in pitaya.

Materials and methods

Growth conditions and inoculation of pathogenic fungi in pitaya

For the pitaya inoculation fungus experiment, the healthy and consistently growing "Jindu No. 1" pitaya old stems were collected and transported to Hainan University in Haikou City, Hainan Province for treatment. The stems were cut into lengths of about 20 cm for potting, with watering scheduled once every two days, fertilization once a week, and exposure to natural light for irradiation. ABA treatment involves spraying the new shoots of pitaya with a 2 mg/L ABA solution once. One week later, the pitaya sprayed with the ABA solution was inoculated, while the pitaya branches not sprayed with ABA were used as the control group.

Treatment of pathogenic fungi involves using PDA medium on activated *N. dimidiatum* for 10 days. The spores are washed down with ddH_2O and the mycelium is filtered out using three layers of filter paper. The spores are then diluted to a concentration of 1×10^5 and uniformly sprayed on the surface of pitaya, followed by moisturization. Three to five replicates are made for each sample, and the pitaya branches are allowed to grow under natural light. Samples were taken 5, 8, 11, and 15 days after spraying *N. dimidiatum* spores, immediately frozen in liquid nitrogen, and stored at -80 °C.

Identification of pitaya *PYL, PP2C,* and *SnRK2* genes

The whole genome sequence, protein sequence, and annotated sequence of pitaya can be downloaded from the website http://www.pitayagenomic.com/download.php. *Beta vulgaris* L., *Arabidopsis* whole genome sequences, as well as protein sequences, were downloaded from https://plants.ensembl.org/index.html. The NCBI's Conserved Structural Domain Database (CCD) (https://www.ncbi.nlm.nih.gov/cdd) and MEME (http://meme-suite.org/tools/meme) were used to verify the conserved structure of the candidate *PYL-PP2C-SnRK2s*. The resulting structure was visualized and mapped using TBtools [44]. A maximum likelihood tree was constructed by selecting PYL-PP2C-SnRK2s proteins from pitaya and *Arabidopsis* using MEGA 7.0 software, with a bootstrap value of 1000 [45]. Using cytoscape software, we explored the protein interactions mediated by PYL-PP2C-SnRK2s in pitaya [46].

Chromosome location analysis and collinearity study

Chromosomal localization analysis of pitaya was conducted using TBtools to compare the genomes of pitaya, *Beta vulgaris L.*, and *Arabidopsis* through two-by-two blasts. Using MCScanX to search for homology, the protein-coding genes from the pitaya genome were compared against themselves and those from *Beta vulgaris L. and Arabidopsis* genomes using BLASTp, with the retrieval threshold set at E-value < 1e-5 [44, 47]. The whole-genome BLASTp results were utilized to compute collinear blocks for all possible pairs of chromosomes and scaffolds. Subsequently, TBtools was used to identify and highlight the collinear pairs of *PYL-PP2C-SnRK2s* [44].

Expression profile of pitaya *PYL-PP2C-SnRK2s* responding to biotic and abiotic stresses

The transcriptomic data of pitaya interactions with *N. dimidiatum* (accession number PRJNA1027117) were obtained from the publicly available NCBI-SRA database [23]. The number of mapped reads and transcript length were first normalized across the all samples. FPKM (Fragments Per Kilobase of transcript per Million fragments

mapped) was utilized for normalization as a metric of transcript or gene expression level. Difference analysis was conducted using DESeq2 software [48]. A fold change of ≥2 and a false discovery rate (FDR) of <0.01 were used as the screening criteria. The fold change represents the ratio of expression between two samples (groups).

RNA extraction and quantitative reverse transcription qRT-PCR

Total RNA was extracted using the CTAB method and used as templates for cDNA synthesis [23]. Reverse transcription was performed using HiScript II Q RT SuperMix for qPCR (gDNA wiper) (Vazyme Biotech Co., Ltd, China). qRT-PCR was performed using an ABI 7500 Fast Real-Time PCR system (Applied Biosystems Inc., Foster City, CA, USA). The reaction mixture (20 μL) contained 0.5 μL of cDNA, 10 μL of SYBR Premix Ex Taq (Vazyme), and 0.2 μM of forward and reverse primers were used. The UBQ serving as an endogenous control. The reaction mixture (20 μL) contained 0.5 μL of cDNA, 10 μL of SYBR Premix Ex Taq (Vazyme), and 0.2 μM of forward and reverse primers, and the primers listed in the Table S3. Data were analyzed using the $2^{\wedge-\Delta\Delta CT}$ method.

Plasmid construction of *Hp1879* gene

The *Hp1879* was amplified from pitaya cDNA for tobacco subcellular localization using 2× Rapid Taq Master Mix (Vazyme Biotech Co., Ltd, China) and the primers listed in the Table S1. For the transient expression assay, the *Hp1879* genes were cloned into the vector PEGAD. The resulting vectors were verified by sequencing and then individually transformed into the *Agrobacterium tumefaciens* strain GV3101 using chemical induction.

Agroinfiltration of *N. benthamiana*

Agrobacterium tumefaciens carrying the target gene was cultured overnight in LB medium. *A. tumefaciens* cultures were pelleted by centrifugation at 3,600 × g for 5 min, washed three times with buffer (10 mM $MgCl_2$, 10 mM MES, pH 5.7, 100 nM acetosyringone), and then incubated in the buffer for an additional 2 h. *A. tumefaciens* cultures were adjusted to appropriate concentrations, with an OD_{600} of 0.6, and were infiltrated into 5–6-week-old *N. benthamiana* leaves using a needleless syringe. Fluorescence was observed and captured using fluorescence confocal microscopy 24–48 h later.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10665-9.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Not applicable.

Author contributions
H.T. Conceptualization, Supervision, Project administration, M.W. Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. S.L.K., Z.W.W. provide transcriptome data. S.R.J., Z.J.Y., Z.X. Investigation, Validation.

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Data availability
The PYL, PP2C, and SnRK2 protein sequences from *Arabidopsis* are available from TAIR (https://www.arabidopsis.org/) and the accession numbers are listed in Table S1. The RNA-Seq raw data have been uploaded to the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/bioproject) with the accession numbers PRJNA1027117 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1027117).

Declarations

Ethics approval and consent to participate
No specific permits were required for the described field studies. All experiments were performed according to institutional guidelines of Hainan University, China. *H. polyrhizus* plant material is widely cultivated in China. The authors complied with international, national, and institutional guidelines. This study does not contain any research requiring ethical consent or approval.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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