Genome-wide survey of Calcium-Dependent Protein Kinases (CPKs) in five Brassica species and identification of CPKs induced by Plasmodiophora brassicae in B. rapa, B. oleracea, and B. napus

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Calcium-dependent protein kinase (CPK) is a class of Ser/Thr protein kinase that exists in plants and some protozoa, possessing Ca²⁺ sensing functions and kinase activity. To better reveal the roles that Brassica CPKs played during plant response to stresses, five Brassica species, namely Brassica rapa (B. rapa), Brassica nigra (B. nigra), Brassica oleracea (B. oleracea), Brassica juncea (B. juncea), and Brassica napus (B. napus) were selected and analyzed. In total, 51 BraCPK, 56 BniCPK, 56 BolCPK, 88 BjuCPK, and 107 BnaCPK genes were identified genome wide and phylogenetics, chromosomal mapping, collinearity, promoter analysis, and biological stress analysis were conducted. The results showed that a typical CPK gene was constituted by a long exon and tandem short exons. They were unevenly distributed on most chromosomes except chromosome A08 in B. napus and B. rapa, and almost all CPK genes were located on regions of high gene density as non-tandem form. The promoter regions of BraCPKs, BolCPKs, and BnaCPKs possessed at least three types of cis-elements, among which the abscisic acid responsive-related accounted for the largest proportion. In the phylogenetic tree, CPKs were clustered into four primary groups, among which group I contained the most CPK genes while group IV contained the fewest. Some clades, like AT5G23580.1 (CPK12) and AT2G31500.1 (CPK24) contained much more gene members than others, indicating a possibility that gene expansion occurred during evolution. Furthermore, 4 BraCPKs, 14 BolCPKs, and 31 BnaCPKs involved in the Plasmodiophora brassicae (P. brassicae) defense response in resistant (R) or susceptible (S) materials were derived from online databases, leading to the discovery that some R-specific induced CPks, such as BnaC02g08720D, BnaA03g03800D, and BolC04g18270.2J.m1 might be
ideal candidate genes for *P. brassicae* resistant research. Overall, these results provide valuable information for research on the function and evolution of CDK genes.

**KEYWORDS**

Brassica species, calcium-dependent protein kinase, evolution, expression pattern, Plasmodiophora brassicae

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**Introduction**

Ca²⁺ is a generic form of the second messenger to participate in numerous signaling pathways (Hetherington and Brownlee, 2004; Zhang et al., 2014b). The change of Ca²⁺ concentration in cells can be captured by calcium sensors. Based on whether or not they are adjusted by the combination with Ca²⁺, calcium sensors are divided into four classes in plants, namely calmodulin (CaM) or CaM-like proteins (CMLs), calcineurin B-like proteins (CBLs), CBL interacting protein kinases (CIPKs), and the calcium-dependent protein kinases (CPKs) and their relatives, CPK-related kinases (CRKs) (Luan et al., 2002; Yang et al., 2021). Among them, CPKs exist in plants and some protozoa, especially in plant extracts. Additionally, rich calcium-stimulated protein kinase activity is found to be related to CPKs (Harmon et al., 2000). Typical CPK proteins contain an N-terminal variable domain, a protein kinase domain, an autoinhibitory domain, and a calmodulin-like domain (Cheng et al., 2002). The protein kinase domain is the catalytic domain containing an adenosine triphosphate binding site adjacent to the inhibitory domain, and the successive calmodulin domains always contain a protein kinase domain and EF-hands to determine whether the CPK is calmodulin dependent (Cheng et al., 2002; Hrabak et al., 2003).

Numerous studies have reported that CPKs are involved in plant responses to abiotic stresses. For example, *Arabidopsis* CPK1 and CPK2 were rapidly expressed under drought and high salinity stress, and CPKs played a significant role in enhancing abiotic stress tolerance (Urao et al., 1994). In maize, 12 of 40 CPK genes responded to low temperature, drought, salt, ABA, and hydrogen peroxide (Kong et al., 2013). In wheat, 10 of 14 CPK genes were responsive to abiotic stress, including drought, NaCl, and ABA. In potatoes, *StCPKs* were responsive to biotic and abiotic stresses as well as hormone stimulation (Bi et al., 2021). Among them, 20 *StCPKs* showed differential expression patterns in drought-tolerant and drought-sensitive potato varieties under drought stress, which allowed for recognition of abiotic stress of potatoes. In grapes, the response of *VpCPKs* to NaCl treatment and transcriptional responses at low and high temperatures has been studied (Zhang et al., 2015). Almost all 19 CPKs were up or down-regulated under at least one abiotic stress, but some genes had a rather mild response, and some grape CPK genes, like *VpCPK6*, *VpCPK9*, *VpCPK14*, *VpCPK16*, and *VpCPK19* had positive effects in fighting against powdery mildew. Overall, most CPK-related studies focused on the role in abiotic stress and rarely mentioned biotic stressors.

*Brassica* is a member of the *Brassicaceae* family, which contains many important vegetables, oil crops, and forage crops and has high economic value. CPK gene is a candidate gene for modifying plant stress resistance cations. Although there are some studies on CPKs of *Brassica* plants, such as turnip and canola (Zhang et al., 2014a; Wang et al., 2017), there are few studies on *Brassica* CPKs as a whole. Additionally, most of the studies on CPKs are conducted in one type of plant, which cannot be accurately used for comparison between plants. In this study, five *Brassica* Species (*B. rapa*, *B. nigra*, *B. oleracea*, *B. juncea*, and *B. napus*) CPKs (*BraCPKs*, *BniBCPKs*, *BolCPKs*, *BjuCPKs*, *BnaCPKs*) were identified, for phylogenetic, chromosomal mapping, collinearity, promoter analysis, and biological stress analysis. This study specifically studied the *Brassica* CPK induced by clubroot, which is caused by the obligate biotrophic pathogen *P. brassicae*, one of the most devastating diseases affecting *Brassicae* (Zhang et al., 2016). Our results provide important information on the evolutionary history and biological function of the *Brassica* Species CPKs in biotic stress.

**Materials and methods**

**Identification of CPK genes**

To identify CPK genes in *Brassica*, the whole-genomes of five *Brassica* species were downloaded from the Brassica Database (BRAD) (Chen et al., 2022) (http://brassicadb.cn/). The HMMER profiles of the protein kinase domains (PF00069) and EF-hand domains (PF00036, PF13499, or PF13833) derived from Pfam...
dependent proteins, and calcium/calmodulin-dependent proteins. The conserved domains and EF-hand quantity were dependent protein kinases. The remaining proteins were regarded as CPK-related proteins, calcium/calmodulin-dependent protein kinases. The sequences that were CPK-related proteins, calcium/calmodulin-dependent proteins, and calcium and calcium/calmodulin-dependent protein kinases. The remaining proteins were regarded as CPKs. The conserved domains and EF-hand quantity were predicted using the NCBI-BLASTP query database.

The terminal acetylation sites were predicted using NMT (https://mendel.imp.ac.at/myristate/SUPLpredictor.htm), CSS-Palm4.0 (Ren et al., 2008; Chica, 2020) (http://csspalm.biocuckoo.org/index.php), and NetAcet-1.0 (Kiemer et al., 2005) (https://services.healthtech.dtu.dk/service.php?NetAcet-1.0), respectively.

To study the CPKs induced by P. brassicae infection, three RNA-seq datasets of clubroot-resistant (R) and clubroot-susceptible (S) lines of B. rapa (PRJNA298858), B. oleracea (PRJNA298858), and B. napus (PRJNA345072) were downloaded from NCBI (Chen et al., 2015; Zhang et al., 2016; Li et al., 2019). The time points for B. rapa dataset were 0, 12, 72, and 96 hours post-inoculation (hpi). The time points for B. oleracea were 0, 7, and 14 days post-inoculation (dpi), and for B. napus were 0, 12, 24, 60, and 96 hpi. The TPM (Transcripts Per Kilobase Million) value was used to display the gene expression levels, and the heat map was drawn by TBtools (Chen et al., 2020). Correlation analysis was carried out according to their expression levels in the relevant time after P. brassicae infection, so as to judge the influence of P. brassicae in Brassica species.

**Expression characteristics of CPK genes induced by Plasmodiophora brassicae**

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**Results**

**Identification and characterization of CPKs in Brassica**

To identify CPKs in Brassica, we performed a genome-wide analysis of the CPK gene family in the five Brassica species through a BLAST search and HMMER analysis. Proteins with protein kinase domain and EF-hand domain were identified, and those that might possess similar domains, such as CPK-related proteins, calcium/calmodulin-dependent proteins, and calcium and calcium/calmodulin-dependent protein kinases were carefully excluded. Altogether, we identified 51 BraCPKs, 56 BniCPKs, 56 BolCPKs, 88 BjuCPKs, and 107 BnaCPKs from B. rapa, B. nigra, B. oleracea, B. juncea, and B. napus, respectively. The typical CPK genes were constituted by a long exon and tandem short exons (Table S1; Figure S1). Three quarters of CPK genes in five species possessed six to eight exons, with detailed proportions of each species possessing this number of exons at 80.39% (B. rapa), 78.57% (B. nigra), 78.57% (B. oleracea), 80.68% (B. juncea), and 73.83% (B. napus). The exon number ranged from 2 to 21, such as BjuO0112843 having only two short exons and BraA02g042460.3 having 21 exons of varying lengths.

In general, CPK proteins contained one Protein kinase and four EF-hand domains, with some CPKs varying in the number of EF-hand domains, ranging from one to six (Table S1; Figure S2). Furthermore, the majority of CPK proteins possessed palmitoylation sites at their N-termini (B. rapa, 75.44%; B.
nigra, 76.79%; B. oleracea, 73.21%; B. juncea, 69.32%; B. napus, 70.09%). This is crucial for regulation, as palmitoylation plays important roles in modulating proteins’ trafficking, stability and sorting (Martin and Busconi, 2006; Draper et al., 2007; Greaves and Chamberlain, 2007; Linder and Deschenes, 2007). Moreover, half of CPK proteins contained myristoylation sites, and several contained N-terminal acetylation sites.

Chromosomal localization and gene expansion

To understand the genomic distribution of CPKs, gene positions were acquired from the genome database of Brassica species (Figure 1). BraCPK genes were located on 9 out of 10 chromosomes with an exception of Chromosome A08 (Figure 1A). Chromosome A03 carried 11 evenly distributed BraCPK genes, which was the maximum number on one chromosome. Meanwhile, chromosomes A01 and A04 possessed only three BraCPK genes. 55 BniCPK genes were spread out across eight chromosomes, and among them, 12 BniCPK genes were located on Chromosome B08, mostly in cluster manner (Figure 1B). The BniCPK gene number on other chromosomes varied, ranging from four to nine. As seen in Figure 1C, BolCPK genes distributed on all chromosomes, and most genes clustered on chromosome C03 which was followed by chromosome C07, C09, and C02. As shown in Figure 1D, BjuCPK genes were unevenly dispersed on chromosomes excluding A08 and B04 which carried no BjuCPK genes. The gene number on different chromosomes varied greatly. For example, there are nine genes on chromosome A03 and only 2 genes on chromosome A01. Similar to the distribution of BraCPKs and BolCPKs, BnaCPK genes were unevenly dispersed on chromosomes. This uneven distribution excluded ChrA08 which was the only one that carried no BnaCPK genes (Figure 1E). The quantity of BnaCPK genes on different chromosomes ranged from 2 to 10. ChrC09 had 10 BnaCPK genes which was the largest number found on a single chromosome, while ChrC06 contained only 2 BnaCPKs.

Considering that B. napus is derived from the recombination of B. rapa and B. oleracea, B. juncea is derived from B. rapa and B. nigra, the positions of BnaCPK genes on chromosomes ChrAs or ChrCs and BjuCPK genes on chromosomes ChrA or ChrB showed highly consistent with those of corresponding homologous BraCPK, BolCPK or BniCPK genes. Additionally, almost all CPK genes were located on regions of high gene density as non-tandem form. In total, only seven CPK gene clusters were found in five species, including one in B. rapa (BraA09g325600.3C and BraA09g325610.3C), two in B. rapa (BniB012674-TA and BniB012675-TA; BniB003258-TA; BniB003259-TA and BniB003260-TA), one in B. oleracea (BolC02g059690.2f.m1, BolC02g059700.2f.m1 and BolC02g059710.2f.m1), two in B. juncea (BjuA001247 and BjuA001248, BjuA026199 and BjuA026200), and one in B. nigra (BnaC02g41570, BnaC02g41580 and BnaC02g415790).

To better reveal the expansion of CPK genes in five Brassica genomes, the duplication patterns of CPK genes were predicted within and between each genome with the Arabidopsis thaliana genome added for comparison (Figure 2). The result showed that there were 15 CPK gene pairs in A. thaliana, and the gene pair number of Brassica species was tripled or even tenfold, namely, 47 pairs in B. rapa, 51 pairs in B. nigra, 52 pairs in B. oleracea, 154 pairs in B. juncea, and 200 pairs in B. napus (Figure 2A). Moreover, the possible syntenic relationship of CPK genes between Brassica genomes was also investigated. Subsequently, we obtained 234 orthologous gene pairs between B. rapa and B. napus, 177 between B. rapa and B. juncea, 158 between B. oleracea and B. napus, and 182 between B. nigra and B. juncea, which are shown in Figure 2B. Chromosome A03/A09, B03/B08, and C03/C07/ C09 possessed the higher orthologous regions in B. rapa, B. nigra and B. oleracea, respectively. Correspondingly, chrA03, chrA09, chrC03, and chrC09 subgenomes were classified as higher orthologous regions in B. napus. These higher orthologous regions were not present in chromosome A03, A09, B03, and B08 subgenomes in B. juncea, which may be due to the imperfect assembly of the B. juncea genome.

Additionally, the nonsynonymous/synonymous mutations (Ka/Ks) analysis was performed on orthologous CPK genes between A. thaliana and the other five Brassica species (Table S2). The Ka/Ks value of the predicted CPK paralogs that had average values of 0.087 (B. rapa), 0.091 (B. nigra), 0.083 (B. oleracea), 0.088 (B. juncea) and 0.083 (B. napus) were less than one, suggesting that all pairs of CPK proteins were under strong purifying selection pressure.

Phylogenetic relationships of CPKs

To explore the phylogenetic relationship of CPK genes, neighbor-joining trees were constructed by CPK protein sequence from five Brassica species (Figure 3). CPKs were clustered into four major groups based on tree topology (group I, group II, group III, group IV), which agreed with the classification of Arabidopsis CPKs (Cheng et al., 2002). In the phylogenetic tree of five species, the CPK genes of five Brassica species and their orthologs in Arabidopsis formed distinct clades. Among four groups, group I contained the most CPK genes, including 15 BraCPKs, 19 BniCPKs, 19 BolCPKs, 31 BjuCPKs, and 37 BnaCPKs. A clade including AT5G23580.1(CPK12) in group I contributed the largest number of orthologs from Brassica species, reaching up to 36. The second largest group was group III, including 105 CPK genes, in which the top two clades were AT2G31500.1 (CPK24) and AT3G51850.1 (CPK13), possessing 20 and 19 members from five species respectively. There were 99 CPK genes in group II, and four clades, namely AT5G12180.1 (CPK17), AT5G19360.1 (CPK34), and
AT4G04710.1 (CPK22), AT4G23650.1 (CPK3) occupied about 60% of orthologs from *Brassica* species in this group, reaching up to 59 genes. The group with a minimum number of members was group IV, including only three clades with a total of 32 genes. In group IV, clades AT5g66210.2 (CPK28), AT2G17890.1 (CPK16), and AT4G36070.2 (CPK18) possessed 14, 11, and 7 members respectively. In addition, some clades had very few members, such as AT4G04700.1 (CPK27), AT4G04695.1
(CPK31), AT2G35890.1 (CPK25), and AT1G50700.1 (CPK33) merely having one or two orthologs from Brassica species.

Analysis of conserved motifs in BraCPK, BolCPK, and BnaCPK

It was found that some CPKs duplicated or lacked certain types of motifs. Most CPKs consisted of 9 to 11 motifs, and covered all motif types (Table S1; Figures S3, S4). As shown in Figure 4, 39 of 51 CPKs in B. rapa, 45 of 56 in B. oleracea, and 78 of 107 in B. napus include ten types of motifs, the proportions of which were up to 76.5%, 80.4%, and 72.90%, respectively. The top three types of motifs were motif 4/1/7 in B. rapa, motif 8/6/4 in B. oleracea, and motif 8/4/1 in B. napus. Further analysis revealed that the composition and the number of conserved motifs or domains varied to a large extent in some CPKs (Figure S3). For example, BnaA02g32830D, BnaA03g46140D, BnaA05g11330D, and BraA09g025610 lacked three to five types of motifs, while BnaC09g22660D had the highest motif
number of 15. In addition, some motif types in BraA02g042460 were tripled. Compared to BraCPKs and BolCPKs, BnaCPK proteins showed more diverse combinations of the motifs.

**Analysis of cis-acting elements in the promoter region of BraCPKs, BolCPKs, and BnaCPKs**

Cis-acting elements distributed in the promoter region can help predict the function of candidate genes. As most CPK genes are related to stress response, we further analyzed the cis-acting elements involved in stress response. Specifically, cis-acting elements are classified as MeJA responsive, gibberellin responsive, abscisic acid responsive, salicylic acid responsive, and defense and stress responsive related (Table S1; Figure 5 and Figure S5). Among them, BnaCPKs, BraCPKs, and BolCPKs contained the largest number of cis-acting elements related to abscisic acid responsivity, followed by MeJA or gibberellin responsivity (Figure 5). Most genes had three or more promoter elements, but a few genes possessed only one type of cis-acting element, such as six BnaCPK genes (BnaA02g32840D, BnaA03g31330D, BnaA05g11330D, BnaA05g28200D, BnaC05g42370D and BnaC09g04790D), two BraCPK genes (BraA06g030040 and BraA09g009710), and three BolCPK genes (BolC03g031480, BolC06g047200 and BolC07g044910) (Figure S5), and this presence of single type may give them corresponding functional specificities. In addition, some CPK genes contained no promoter elements, which may have a certain impact on gene expressions, such as BnaA04g24150D, BnaA05g11330D, and BnaC09g047910D, which may not be involved in plants responding to stress.

**Expression pattern of CPK genes induced by Plasmodiophora brassicae**

*Plasmodiophora brassicae* Wor., an obligate and biotrophic pathogen Rhizaria (Schwelm et al., 2015), can infect over 3,700 species in *Brassicaceae* (Hwang et al., 2012), and lead to clubroot, causing significant economic losses every year (Dixon, 2015).
To further reveal the roles of CPKs in *P. brassicae* defense responses, we analyzed the corresponding expression profile data available online. Three RNA-seq datasets were downloaded and analyzed (Chen et al., 2015; Zhang et al., 2016; Li et al., 2019). In total, 43 of 51 BraCPK, 49 of 56 BolCPK, and 100 of 107 BnaCPK genes showed expression fluctuation after inoculation in resistant (R) and susceptible (S) plants (Figure S6). The expression of most CPK genes were down-regulated, and only 4 BraCPK, 14 BolCPK and 31 BnaCPK genes were induced by *P. brassicae* in resistant (R) or susceptible (S) materials at a certain time point (Figure 6). Among them, most CPKs presented expression specificity between R and S, such as 18 CPKs that were induced merely in R, including 13 BnaCPKs (BnaA02g0340D, BnaA03g0340D, BnaA03g0380D, BnaC04g19170D, BnaC05g1610D, BnaC06g52460D, Bna C07g47900D, BnaC02g08720D, BnaA09g32390D, BnaA06g13080D, BnaA02g32830D, BnaA07g45520D, BnaC09g5044D), one BraCPK (BraA05g01340.3C), and four BolCPKs (BolC02g005200.2J.m1, BolC04g018270.2J.m1, BolC03g018890.2J.m1, BolC08g03690.2J.m1). Additionally, 17 CPKs were only induced in S, including eight BnaCPKs (BnaA10g20490D, BnaA02g01160D, BnaA01g11790D, BnaA03g59670D, BnaA09g20300D, BnaC02g41580D, BnaA06g40890D, BnaA03g18230D), one BraCPK (BraA03g020560.3C), and eight BolCPKs (BolC07g024810.2J.m1, BolC09g060610.2J.m1, BolC03g06590.2J.m1, BolC01g015300.2J.m1, BolC02g047200.2J.m1, BolC08g014120.2J.m1, BolC05g036390.2J.m1). Some CPKs were highly induced reaching a level of 14.82 (BnaA03g18230D, 96 hpi in S), 11.02/10.31 (BnaC02g08720D, 12 hpi/60 hpi in R), and 10.31 (BolC09g060610.2J.m1, 14 dpi in S) times comparing to their initial concentrations. Apart from BnaC02g08720D, other R-specific induced genes, like BnaA03g03800D (6.06 folds in 12 hpi, 5.26 folds in 96 hpi) and BolC04g018270.2J.m1 (5.4 folds in 7 dpi, 5.9 folds in 14 dpi) might also be good candidates for *P. brassicae* resistance research. Moreover, in the phylogenetic tree built by CPKs from *Arabidopsis* and five *Brassica* species, up-regulated genes were distributed in all groups (Figure 7). It is remarkable that the majority of BraCPK, BolCPK, and BnaCPK genes in two families, orthologs of AT5G66210.2 (CPK28) and AT5G12180.1 (CPK17), were up-regulated after *P. brassicae* inoculation, underlining their functional conservation during evolution.

**Discussion**

*B. napus* (AACC, 2n = 38) and *B. juncea* (AABB, 2n = 36) are both allopolyploid species formed by the hybridization of two diploid species. *B. napus* is formed through the hybridization of *B. rapa* (AA = 20) and *B. oleracea* (CC = 18), while the formation of *B. juncea* occurs through the hybridization of *B. rapa* (AA = 20) and *B. nigra* (BB = 16) (Chalhoub et al., 2014; Lu et al., 2019). In our phylogenetic tree, the division of groups was consistent with that of *A. thaliana* (Cheng et al., 2002). The evolution of CPK genes was relatively conserved among the five *Brassica* species. In general, the gene number of BraCPKs was almost equal to that of BolCPKs and BniCPKs in the same clade, with duplicated number for BnaCPKs and BjuCPKs. Nevertheless, in terms of individual branches, the quantity of BjaCPK genes was less than two times of that of BraCPKs and BniCPKs, which might be due to inaccurate assembly occurring during genetic recombination or imperfect sequencing. When selecting *Arabidopsis* CPKs as a reference, there was no doubt that the gene expansion occurred among five species during evolution, especially in some clades, such as Clade AT5G23580.1(CPK12) and AT2G31500.1(CPK24). Of course, the possibility that certain *Arabidopsis* CPK genes have been lost during evolution cannot be ruled out. As the structure of exons/introns and the type and number of introns can account for the evolutionary
FIGURE 5
Distributions of cis-acting elements in BraCPKs (A), BolCPKs (B), and BnaCPKs (C) promoter. The Upset plot shows the distribution of cis-acting elements in CPKs promoter. The bar chart above represents the number of genes contained in each type of CPK. The bar chart at the bottom left represents the number of genes included in each type of cis-acting element. The dotted line shows the types of cis-acting elements contained in the group.
history of organisms, we analyzed the exon/introns substructure of all CPKs in this study. Genes in group IV had the most exons, from eleven to thirteen, not including BjuA026200. Most of the CPK genes in other groups contained less than ten exons. In general, CPK genes clustered in the same subfamily showed similar exon/intron structures, indicating a close evolutionary relationship between them.

At present, CPK genes have been identified in Arabidopsis (Cheng et al., 2002), rice (Asano et al., 2005), soybean (Liu et al., 2016), maize (Kong et al., 2013; Ma et al., 2013), cucumber (Xu et al., 2015), tomato (Hu et al., 2016) and many other plants (Yang et al., 2017; Wen et al., 2020; Zhang et al., 2020; Zhao et al., 2021), and their roles in biotic and abiotic stresses have been understood to some extent (Asano et al., 2012; Boudsocq and Sheen, 2013; Ranty et al., 2016; Bredow and Monaghan, 2019). Previous studies found that plant CPKs are involved in abscisic acid signaling pathways (Liu et al., 2006; Yu et al., 2006; Zhu et al., 2007; Jiang et al., 2013; Chen et al., 2019), jasmonic acid signaling pathway (Ulloa et al., 2002; Hettenhausen et al., 2013; Matschi et al., 2015), gibberellin signaling pathway (Abou-el-Saad and Wu, 1995; Abbasi et al., 2004; Ishida et al., 2008; Nakata et al., 2009), and also response to drought stress (Ma and Wu, 2007; Ciesla et al., 2016; Yang et al., 2017; Wen et al., 2020; Zhang et al., 2020; Zhao et al., 2021). In line with these results, a large number of cis-acting elements related to abscisic acid, jasmonic acid, gibberellin, and drought stress response were found in the promoter region of Brassica CPK genes in this study.

Plasmodiophora, as one class of obligate biotrophic pathogens (Ludwig-Müller et al., 2009), is highly destructive to many Brassica plants and exerts negative effect on their quality and yield (Dixon, 2009), which seriously hinders the development of agricultural industry. In this study, utilizing the expression data from the resistant (R) and susceptible (S) lines in three Brassica species (B. rapa, B. oleracea, and B. napus), we found 49 up-regulated genes induced by P. brassicae. These up-regulated genes would be helpful to identify positive regulators in the process of response to P. brassicae (Figure 6). Interestingly, the distribution of these 49 genes was extremely uneven among the three Brassica species. Among them, 31 genes were found in B. napus, accounting for 29% of BnaCPKs, while only 4 genes were found in B. rapa, with a proportion of 8%, suggesting that BnaCPKs played an important role in B. napus taking charge in response to P. brassicae infection, while BraCPKs might be mainly involved in other stresses. Moreover, of the 49 up-regulated genes, 18 genes were specifically up-regulated in R, and those genes identified from incompatible interactions might be good candidates for further functional assignment of CPK genes against P. brassicae infection. The up-regulated genes of the three species were distributed in the four groups in varying amounts (Figure 7). There were 9 CPK genes in group I, 23 in group I, 11 in group III, and 6 in group IV in B. napus, and 5 in group I, 11 in group II, 13 in group III, and 0 in group IV in B. rapa. In B. oleracea, 13 genes were found in group I, 11 in group II, 13 in group III, and 0 in group IV. This result is consistent with previous studies that BnaCPKs play a significant role in B. napus resistance to P. brassicae infection.
and only 6 in group IV, suggesting that CPK genes involved in *P. brassicae* response evolved independently in four groups along with the functional divergence in *Brassica* species. However, the genes in group I may play a broader role in response to *P. brassicae* infection. It is worth noting that more genes in clade AT5G12180.1 (CPK17) and clade AT5G6621.2 (CPK28) were up-regulated by *P. brassicae* induction. Evolutionarily, these two clades of genes were functionally conserved, and most likely to be active gene clusters during response to *P. brassicae* infection in *Brassica* species.

**Conclusion**

The gene and protein characterization, localization, evolution, and expression analysis of highly conserved CPK genes in *Brassica* showed that most of the CPK gene families in this study were relatively conserved during evolution. Genome-wide identification and expression analysis of CPK family members can provide an alternative strategy for enhancing resistance to *P. brassicae* in *Brassica* species. These identified genes may be good candidates for further identification of CPK genes of *P. brassicae* infection and provide theoretical basis and preliminary guidance for prevention of *P. brassicae* pathogen damage to *Brassica* plants.

**Data availability statement**

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

**Author contributions**

JL: formal analysis, writing the original draft, data curation, and validation. NY: investigation, resources, and visualization.
YZ: data curation. ZC: data validation. TZ: writing-review and editing, and supervision. WL: conceptualization, writing-review and editing, and supervision. All authors contributed to the article and approved the submitted version. All authors have read and agreed to the published version of the manuscript.

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**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**Supplementary material**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1067723/supplementary-material

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