Genome-wide identification of the *PWWP* gene family in blue-seed-coat *Brassica napus* and its response to salt stress

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Abstract

PWWP domain proteins are a class of epigenetic regulatory factors widely present in eukaryotes and play important roles in transcriptional regulation, DNA repair, and chromatin remodeling. Recent studies have shown that members of the PWWP family are closely associated with plant responses to salt stress. To investigate the sequence characteristics, evolutionary relationships, and salt-stress responses of the PWWP domain protein family in Brassica napus L., this study used bioinformatics methods to identify members of this family at the whole-genome level and analyze their chromosomal localization, protein physicochemical properties, phylogeny, conserved motifs, cis-acting elements, and collinearity. Quantitative real-time PCR (qRT-PCR) was used to detect changes in the expression levels of each member during seed germination after treatment with 120 $mmol \cdot L^{-1}$ NaCl. The results showed that: (1) A total of 43 PWWP family members were identified in Brassica napus, of which 22 were located on the A genome and designated BnaPWWP1-BnaPWWP22, while the other 21 were located on the C genome and designated BnaPWWP23-BnaPWWP43. (2) The encoded proteins contained 177-1383 amino acids, with molecular weights of 19688.44-149901.99 Da and isoelectric points (pI) of 4.7-10.01; all were hydrophilic proteins, and 88.27% were localized in the nucleus. (3) The phylogenetic tree showed that PWWP family members from Brassica napus, Arabidopsis thaliana, Brassica oleracea, and Brassica rapa could be divided into 7 subfamilies, and members of the same subfamily exhibited high similarity in conserved motifs, conserved domains, and gene structures. (4) Promoter cis-acting element analysis identified 29 types of cis-elements, of which 22 categories were associated with growth and development, phytohormones, and abiotic stress responses; ARE, ABRE, CGTCA-motif, and TGACG-motif were the core elements with the highest frequencies and broadest gene coverage. (5) Under salt stress, the expression levels of 5 PWWP family members increased significantly, among which BnaPWWP10, BnaPWWP11, and BnaPWWP24 were markedly upregulated, and their promoter regions were rich in salt-stress-related cis-acting elements. In summary, this study systematically analyzed the BnaPWWP gene family and identified several BnaPWWP genes involved in the salt-stress response during seed germination in Brassica napus, laying a foundation for further investigation of the functions of BnaPWWP genes in the response of rapeseed to salt stress.

Full Text

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Abstract: PWWP domain proteins are a class of epigenetic regulatory factors widely present in eukaryotes and play important roles in transcriptional regulation, DNA repair, and chromatin remodeling. Recent studies have shown that members of the *PWWP* family are closely related to plant responses to salt stress. To investigate the sequence characteristics, evolutionary relationships, and how this family responds to salt stress in the PWWP domain protein family of blue-seed-coat *Brassica napus* (*Brassica napus* L.), this study used bioinformatics methods to identify the members of this family at the whole-genome level, and analyzed their chromosomal locations, protein physicochemical properties, phylogeny, conserved motifs, *cis*-acting elements, and collinearity. Real-time fluorescence quantitative PCR (qRT-PCR) was used to detect changes in the expression levels of each member during seed germination after treatment with 120 mmol · L^{-1} NaCl. The results showed: (1) A total of 43 *PWWP* family members were identified in blue-seed-coat *Brassica napus*, of which 22 were located on the A subgenome and designated *BnaPWWP1*-*BnaPWWP22*, while the other 21 were located on the C subgenome and designated *BnaPWWP23*-*BnaPWWP43*. (2) The encoded proteins contained 177–1 383 amino acids, with molecular weights of 19 688.44–149 901.99 Da and isoelectric points (pI) of 4.7–10.01; all were hydrophilic proteins, and 88.27% were localized in the nucleus. (3) The phylogenetic tree showed that *PWWP* family members in blue-seed-coat *Brassica napus*, *Brassica rapa*, *Brassica napus*, and cabbage could be divided into seven subfamilies, and members of the same subfamily showed high similarity in conserved motifs, conserved domains, and gene structure. (4) Promoter *cis*-acting element analysis identified 29 types of *cis*-elements, among which 22 categories belonged to growth and development, phytohormone, and abiotic stress responses; ARE, ABRE, CGTCA-motif, and TGACG-motif were the core elements with the highest frequency and the broadest gene coverage. (5) Under salt stress, the expression levels of five *PWWP* family members increased significantly, among which *BnaPWWP10*, *BnaPWWP11*, and *BnaPWWP24* were markedly upregulated, and their promoter regions were rich in salt-stress-related *cis*-acting elements. In summary, this study systematically analyzed the *BnaPWWP* gene family and identified several *BnaPWWP* genes involved in salt-stress responses during seed germination in blue-seed-coat *Brassica napus*, laying a foundation for further study of the functions of *BnaPWWP* genes in the response of rapeseed to salt stress.

Keywords: blue-seed-coat *Brassica napus*; *PWWP* gene family; bioinformatics analysis; salt stress; expression analysis

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Abstract: PWWP domain proteins are epigenetic regulators widely present in eukaryotes. They play important roles in transcriptional regulation, DNA repair, and chromatin remodeling. Recent studies have shown that *PWWP* family members are closely associated with plant salt stress responses. To investigate the sequence characteristics, evolutionary relationships, and salt stress responses of the PWWP domain protein family in *Brassica napus*, this study used bioinformatics methods to identify the family members at the whole-genome level. The study analyzed their chromosomal locations, protein physicochemical properties, phylogeny, conserved motifs, *cis*-acting elements, and synteny. Real-time quantitative PCR (qRT-PCR) was used to detect the expression changes of all members during seed germination under 120 mmol · L^{-1} NaCl treatment. The results were as follows: (1) A total of 43 *PWWP*

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family members exist in *Brassica napus*. Among them, 22 members are located on the A genome and are designated *BnaPWWP1-BnaPWWP22*, while the other 21 members are located on the C genome and are designated *BnaPWWP23-BnaPWWP43*. (2) The encoded proteins range in length from 177 to 1 383 amino acids, with molecular weights from 19 688.44 to 149 901.99 Da and isoelectric points from 4.7 to 10.01. All proteins are hydrophilic, and 88.27% of them are predicted to localize in the nucleus. (3) A phylogenetic tree

showed that the *PWWP* family members from *Brassica napus*, *Arabidopsis thaliana*, *Brassica oleracea*, and *Brassica rapa* can be divided into seven subfamilies. Members within the same subfamily share highly similar conserved motifs, conserved domains, and gene structures. (4) Promoter *cis*-acting element analysis identified 29 types of elements. Among them, 22 types are associated with growth and development, plant hormones, and abiotic stress responses. ARE, ABRE, CGTCA-motif, and TGACG-motif are the core elements with the highest frequency and the widest gene coverage. (5) Under salt stress, the expression of five *PWWP* family members increased significantly. Among them, *BnaPWWP10*, *BnaPWWP11*, and *BnaPWWP24* are notably up-regulated, and their promoter regions are rich in salt stress-related *cis*-acting elements. In summary, this study systematically analyzes the *BnaPWWP* gene family and identifies several *BnaPWWP* genes that participate in the salt stress response during seed germination in *Brassica napus*. These findings lay a foundation for further study of the functions of *BnaPWWP* genes in rapeseed under salt stress.

Key words: *Brassica napus*, *PWWP* gene family, bioinformatics analysis, salt stress, expression analysis

The PWWP domain was first discovered in the histone methyltransferase WHSC1 (wolf-hirschhorn syndrome candidate 1, Wolf-Hirschhorn syndrome candidate protein 1) (Stec et al., 1998). Its name derives from the conserved proline-tryptophan-tryptophan-proline (Pro-Trp-Trp-Pro) motif in this domain. The PWWP domain contains approximately 100–150 amino acids. Its encoding genes are widely present in eukaryotic genomes, but have not yet been found in prokaryotes (Rona et al., 2016). Structural studies have shown that the PWWP domain possesses a conserved "aromatic cage" structure, which can specifically recognize methyl-lysine through cation-π interactions and can synergistically bind histones and DNA. This gives the PWWP domain nucleosome-binding ability and chromatin-localization functions (Qin & Min, 2014).

Proteins containing PWWP domains play important roles in chromatin-associated epigenetic regulation of gene expression (Scheid et al., 2021). In humans, mutations in this class of proteins are associated with multiple diseases (Min & Liu, 2021). For example, mutations in NSD3, MSH6, and ZMYND11 lead respectively to breast cancer, endometrial cancer, and abnormal transcriptional elongation by RNA polymerase II (He et al., 2013; Li et al., 2013; Wen et al., 2014). PWWP-domain proteins perform biological functions such as chromatin remodeling, DNA damage repair, and metabolic regulation in animals and fungi (Ponne et al., 2025; Yu et al., 2025). In contrast, there are relatively few reports on PWWP-domain proteins in plants, and current studies mainly focus on the regulation of growth and development and flowering time. Zhou et al. (2018) identified three PWWP-domain proteins in the model plant *Arabidopsis thaliana*, named PDP1, PDP2, and PDP3, respectively. They can accelerate flowering by binding FVE (*FVE gene*, Arabidopsis flowering regulator FVE) and MSI5 (MULTICOPY SUPPRESSOR OF IRA1 5, IRA1

multicopy suppressor 5) to repress *FLC* (FLOWERING LOCUS C) transcription. Under long-day or short-day conditions, the *pdp1*, *pdp2*, and *pdp3* mutants all exhibit extremely late flowering. The Arabidopsis PWWP domain-polycomb interacting factor (PWO) family plays a key role in regulating plant development. Studies have shown that PWO proteins can maintain the repressive state of H3K27me3 (H3 lysine 27 trimethylation) and determine its distribution in the nucleus, thereby affecting higher-order chromatin organization (Yang et al., 2024). Arabidopsis PWO1 can interact with all three histone methyltransferase subunits of polycomb repressive complex 2 (PRC2), thereby delaying flowering (Hohenstatt et al., 2018). Recent research found that the Arabidopsis PWO-domain protein HUA2, as an H3K36me3 reader, promotes histone acetylation during activation of *FLC* and *MAF1* transcription, thereby suppressing premature flowering in plants (Xie et al., 2025). Seo and Jang (2025) used a forward genetics approach to obtain a rice (*Oryza sativa* L.) salt-tolerance-enhanced line *sitl3*. In this line, there is a gene *OsPWWP4* encoding a protein with two PWWP domains; a single-base substitution from guanine (G) to thymine (T) occurs in one of its PWWP domains, so that the downstream sequence of this site cannot be translated into amino acids. This is the first report in plants that a PWWP domain is associated with salt stress. The *CsSDG36* gene cloned from tea encodes a protein containing domains such as PWWP and SET. Its overexpression weakens the plant' s osmotic-stress tolerance by promoting water loss, increasing reactive oxygen species accumulation, and raising stomatal density, and affects the expression of stomatal-development-related genes, indicating that *CsSDG36*

is a negative regulatory factor in the drought response of tea plants (Chen et al., 2021). In summary, PWWP domain proteins are a class of protein family closely related to epigenetic inheritance. In addition to playing important roles in the regulation of plant growth and development, their potential functions in abiotic-stress pathways have also gradually been discovered by researchers. Therefore, further in-depth and detailed research on them in the field of stress-response pathways is highly necessary.

Brassica napus L. is one of the major oil crops in China and is also a high-quality source of edible vegetable oil (Guan Zhilin et al., 2024). In Hunan, Jiangxi, and other regions of China, rapeseed cultivation follows the "rice–rice–rapeseed" three-crop rotation model; therefore, it is necessary to breed early-flowering and early-maturing varieties to ensure yield. In northern and western saline-alkali soil regions, salt-alkali-tolerant rapeseed varieties need to be developed, thereby increasing yield by expanding the planting area of rapeseed (Liu Zigang et al., 2017; Ding Fugong et al., 2020). Current studies show that genes encoding PWWP domain proteins play important roles in the regulation of flowering time and in responses to salt stress in plants; however, this gene family has not yet been identified, functionally analyzed, or validated in Brassica napus. Based on this, the present study takes Brassica napus as the research object. Relying on the genomic databases of Brassica napus and Arabidopsis, and using a combination of bioinformatics and real-time fluorescence quantitative PCR

(qRT-PCR), this study aims, through genome-wide identification, evolutionary analysis, structural prediction, and expression detection under salt stress, to explore the following scientific questions: (1) what functions the Brassica napus PWWP gene family may perform in salt-stress responses; and (2) how the structural characteristics of its members, promoter regulatory elements, and expression changes under salt stress are associated, and thereby participate in mechanisms regulating salt tolerance. Around these scientific questions, the specific research contents include: identifying the number of members of this family; analyzing their chromosomal distribution, protein physicochemical properties, conserved sequences, and gene structural characteristics; predicting cis-acting elements in promoter regions and evaluating their correlations with salt-stress responses, hormone responses, and growth and development; detecting expression changes of PWWP family members during seed germination under 120 mmol · L^-1 NaCl treatment; and screening candidate genes that may be involved in salt-tolerance regulation. The aim is to provide a theoretical basis and candidate genes for in-depth analysis of the biological functions of PWWP genes in Brassica napus and for genetic improvement of salt tolerance in rapeseed.

1 Materials and Methods

1.1 Experimental materials

The material used in this experiment was the Brassica napus variety 'Zhongshuang 11' (ZS 11), obtained from the Hunan Provincial Key Laboratory of Crop Performance Genetic Regulation and Development, Hunan Agricultural University. Plump, large seeds were selected, with 20 seeds in each group. Under room-temperature conditions of 26°C and a 16 h/8 h (light/dark) photoperiod, the experimental group was soaked in 120 mmol · L^-1 NaCl for 24 h, while the control group was treated with distilled water. The experiment was repeated three times. After liquid-nitrogen freezing, samples were stored in an ultra-low-temperature freezer at −80°C.

1.2 Identification of gene-family members and analysis of physicochemical properties

Whole-genome sequence, gene annotation, and protein sequence files of Brassica napus, Brassica oleracea, and Brassica rapa were downloaded from the BnIR database (https://yanglab.hzau.edu.cn/BnIR/download?module=genomics), and the protein sequence of Arabidopsis thaliana was downloaded from the TAIR database (https://www.arabidopsis.org/download/list?dir=Proteins). The Pfam-A.hmm file and the hidden Markov model (PF00855) for the conserved structural domain of PWWP family members were obtained from the InterPro database (https://www.ebi.ac.uk/interpro/download/Pfam/). Using the Simple HMM Search function of TBtools-II software, with E-value < e^-10 set as the threshold, preliminary screening was performed

(Liu Rong et al., 2024). Verification was carried out using the NCBI-CDD website (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) and the SMART website (https://smart.embl.de/). Finally, PWWP gene-family members in Brassica napus, Brassica oleracea, Brassica rapa, and Arabidopsis thaliana were identified, and Brassica napus PWWP gene-family members were named BnaPWWP1-BnaPWWP43 according to their chromosomal positions. TBtools-II software was used to calculate the amino acid number, molecular weight, isoelectric point, instability coefficient, aliphatic index, and hydrophobicity index of the above proteins. Subcellular localization was predicted using the WOLF PSORT website (https://wolfpsort.hgc.jp/) (Hu Guojin et al., 2026).

1.3 Construction of the phylogenetic tree and chromosomal localization

After aligning the PWWP protein sequences of Brassica napus, Brassica oleracea, Brassica rapa, and Arabidopsis thaliana using TBtools-II, a phylogenetic tree was constructed. The Bootstrap value was set to 1 000 (Qiao et al., 2024), and the tree was beautified using the ChiPlot website (https://www.chiplot.online/circleTree.html) (Xie et al., 2023). TBtools-II software was used to draw the chromosomal localization map of BnaPWWP genes (Yang Jie et al., 2024).

1.4 Conserved sequence, structural domain, and gene-structure analysis

The Simple MEME Wrapper tool in TBtools-II software was used, with the maximum number of conserved motifs set to 20, to complete annotation of conserved sequence results. The NCBI-CDD online conserved domain database (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) was used to complete prediction of conserved domains in genes. The Gene Structure View analysis tool in TBtools-II software was used to draw the integrated map (Yuan Yuan, 2026).

1.5 Analysis of Promoter Cis-Acting Elements and Collinearity Analysis

The 2,000 bp sequences upstream of the *BnaPWWP* family genes were extracted as promoters, and cis-acting element analysis was performed using the PlantCARE website, with visualization carried out using TBtools-II (Chen et al., 2024). The MCScanX program in TBtools-II was used to conduct collinearity analysis of *PWWP* genes in *Brassica napus*, *Brassica napus* and Chinese cabbage, and visualization was performed for both intra-species and inter-species relationships.

1.6 Protein Homology Modeling and Interaction Network Analysis

Homology modeling was performed using the SWISS-MODEL online tool (https://swissmodel.expasy.org/interactive) (Tiwari et al., 2021). The STRING database (https://cn.string-db.org/) was used to obtain the interaction map among members of the PWWP family in *Brassica napus* (Li et al., 2023).

1.7 RNA Extraction and Real-Time Fluorescence Quantitative PCR of *Brassica napus*

Total RNA was extracted from *Brassica napus* tissues using the Trizol method, after which its integrity was assessed by 1% agarose gel electrophoresis, and its concentration and purity were determined using a BioTek Epoch microplate spectrophotometer. Suitable high-quality RNA samples were selected, and reverse transcription was performed using the Vazyme HiScript II Q RT SuperMix for qPCR kit to obtain cDNA as the template. According to the *Brassica napus* database website (https://yanglab.hzau.edu.cn/BnIR/), qRT-PCR primers were designed (Table 1), and qRT-PCR reactions were performed using the Vazyme ChamQ Universal SYBR qPCR Master Mix kit. Each sample was set up with three biological replicates and three technical replicates. The relative expression level of each gene was calculated using the 2^(-ΔΔCT) method, and the results were summarized and visualized using Excel software (Jiang Ling et al., 2024).

Table 1 qRT-PCR primer sequences

Table 1 Primer sequences in qRT-PCR

Gene name	Accession number	Upstream primer	Downstream primer
BnaPWWP1	BnaA01T0410200ZS	TCAGGGACGGAAAGCTTCTG	ACCGGTATGCAGG
BnaPWWP2	BnaA02T0009900ZS	TCATCCGTTGCGTCTCTCAAGC	TCTCTCATAAGC
BnaPWWP3	BnaA02T0390500ZS	CCCAAAGACCTATCACTTACCT	TGTAAGTTACTGG
BnaPWWP4	BnaA03T0313600ZS	CTTGTTCTTGTGTCTCTTCTCC	GATCAGCTCATCAG
BnaPWWP5	BnaA03T0402700ZS	AGTACCGTCCGGTCTCTTCC	AAAGAAATTGTCTG
BnaPWWP6	BnaA03T0500900ZS	AGGAGTGAAGAAGCTTCTTCC	GACATGGCACC
BnaPWWP7	BnaA04T0003100ZS	ATCAAAGCATGCATCTCTCC	TGATATCGTGG
BnaPWWP8	BnaA04T0204300ZS	TCCGAAAGGACCTCTCTTCC	TCACTCTAAACAGAGG
BnaPWWP9	BnaA05T0170800ZS	AGGATTTGAGAAGCTTCTTCC	AAACCAGG
BnaPWWP10	BnaA05T0344400ZS	TCAAGATGGATCGTCTCTCC	TCGCTTCGTTTATCC
BnaPWWP11	BnaA05T0488900ZS	TGTTCATTCTCCTCTCTTCC	CTGTGTGCTTGT
BnaPWWP12	BnaA06T0022000ZS	TAGAGCCCGATGAAGCTTCC	TGGCTCTTAAGT
BnaPWWP13	BnaA06T0040800ZS	ATGAAACCACGCTCTCTTCC	ATCTGGTTCC TCTG
BnaPWWP14	BnaA06T0169500ZS	AAAGAGGAGATGCTCTCTTCC	AAGAAGAGCTAGG
BnaPWWP15	BnaA06T0380400ZS	TTAGCTTGGGAGCTCTCTTCC	TCCAAATCCGTC
BnaPWWP16	BnaA07T0172900ZS	GGTTAAGAACGCTCTCTTCC	ATCGACTCTTC

Gene name	Accession number	Upstream primer	Downstream primer
BnaPWWP17	BnaA07T0217700ZS	TGAGTCCTTTGCCGAATCG	TGTCAGGAAAGC
BnaPWWP18	BnaA07T0222400ZS	ATTACACGCCGGATTTTCG	GCTACTGGAGC
BnaPWWP19	BnaA09T0057300ZS	TGCCACCAATTCTACCATCG	CCGTCACATTAGC
BnaPWWP20	BnaA09T0679400ZS	TTATGCAACTTGTCGTCCG	ACTTCGCAG
BnaPWWP21	BnaA10T0099000ZS	TGGGGTAAATCTCGCATCG	CGGCTGGTTACC
BnaPWWP22	BnaA10T0255100ZS	AGATATCTACACATCGTCG	TAAGAGAGCCTG
BnaPWWP23	BnaC01T0505200ZS	ATCCGGTTGAAGTACGTCG	CCGGTATGCAGG
BnaPWWP24	BnaC02T0007300ZS	CTCAAGTTTGCTACGTCG	AGAAGAAGGTCC

Gene name	Gene ID	Forward primer	Reverse primer
BnaPWWP25	BnaC02T0017600ZS	ACTGAGAAGGTTCGAGTCG	TGAACGGTTTTGG
BnaPWWP26	BnaC02T052200ZS	CCCAAAGACCTATCACTCG	AGTAAGTTACTGG
BnaPWWP27	BnaC03T0376200ZS	GAGAGTAGCAAACCATCG	GTCCCTCGACTC
BnaPWWP28	BnaC04T0257500ZS	TCCGGACAACCATCATCG	ACTCCACCTGTG
BnaPWWP29	BnaC04T0510300ZS	AGGTGTCACAGATCGATCG	AGGTGGGCAATG
BnaPWWP30	BnaC05T0370400ZS	GAGTAGTGATGATCGATCG	TCCAACAAGGAGG
BnaPWWP31	BnaC05T0547200ZS	ATGACTGCATTGACGCTCG	ACAACCATCTC
BnaPWWP32	BnaC06T0059700ZS	CCTGTAAGTGGCACGATCG	TTTGTCTTGCTG
BnaPWWP33	BnaC06T0114400ZS	TTAAAGGGAAGACGATCG	GTATACAAGC
BnaPWWP34	BnaC06T0164900ZS	GAAGCAGGAATTCTTCGTCG	TGAAGACTCGAAGG
BnaPWWP35	BnaC06T0232500ZS	GCTGCAGAGCAACATACGTCG	ACCTTTCAGTC
BnaPWWP36	BnaC06T0239300ZS	ATTACACGCCGGATTTTCG	GCTACTGGAGC
BnaPWWP37	BnaC07T0310300ZS	TTAGCTTGGGAGTCTACGTCG	CCAAATCCGTC
BnaPWWP38	BnaC07T0374400ZS	CACTGGCGATCAGATTTCG	AGCTCATTGTCACTG
BnaPWWP39	BnaC07T0479300ZS	ATACC GAGTTTGAAACG-GTGC	ACTAGTAGCTTTCTCCTTCCTG
BnaPWWP40	BnaC08T0286100ZS	CTCAAAAGAGTCGAGGTCG	TCTGAAACTCAC
BnaPWWP41	BnaC09T0044600ZS	GTTGGACGGTGATTCGATCG	ATGTCTCCTG
BnaPWWP42	BnaC09T0356100ZS	GAATGAAAGGGACTATCGTCG	TGGTTACCAGC
BnaPWWP43	BnaC09T0568900ZS	CCATATCCAGTATTAGTCG	ATGAGGAAAGG

2 Results and Analysis

2.1 Chromosomal localization and collinearity analysis of the *PWWP* gene family in blue-seeded rapeseed

A total of 43 *PWWP* genes were identified in blue-seeded rapeseed and, according to their chromosomal locations, were named *BnaPWWP1*–*BnaPWWP43*. They were unevenly distributed across 18 chromosomes. Twenty-two genes were located in the A subgenome, and 21 genes were located in the C subgenome.

Among them, chromosome C06 contained the largest number of *PWWP* genes, up to 6; whereas chromosomes A01, C01, C03, and C08 each contained only one *PWWP* gene, and no *PWWP* genes were distributed on chromosome A08. Most genes were distributed at the two ends and in the middle regions of chromosomes. On chromosome A07, *BnaPWWP17* and *BnaPWWP18* showed tandem duplication (Fig. 1A).

To further understand the expansion mode of the *PWWP* gene family and whether it has been conserved during evolution, we conducted collinearity analyses within the species and between species. Intraspecific analysis showed that, in blue-seeded rapeseed, 30 pairs of *PWWP* genes were collinear with each other (Fig. 1B). Collinearity analysis of *PWWP* family members between blue-seeded rapeseed, Chinese cabbage, and cabbage showed that there were 62 pairs of collinear genes between blue-seeded rapeseed and cabbage, and 58 pairs of collinear genes between blue-seeded rapeseed and Chinese cabbage (Fig. 1C). This indicates that the gene groups of the three species have retained a high degree of gene-order consistency during evolution, and also confirms the collinear relationship between the A and C subgenomes of rapeseed and the ancestral species Chinese cabbage and cabbage.

A. Chromosomal localization of members in the *BnaPWWP* family; B. Intraspecific collinearity analysis of *BnaPWWP*; C. Interspecific collinearity analysis of *PWWP* gene family members among *Brassica napus*, *B. oleracea*, and *B. rapa*.

Fig. 1 Chromosomal localization analysis and interspecies collinearity analysis of *PWWP* gene family members in *Brassica napus*

2.2 Analysis of the physicochemical properties and subcellular localization of PWWP family proteins in *Brassica napus*

Physicochemical-property analysis showed that the proteins encoded by members of the *PWWP* family in *Brassica napus* ranged in size from 177 amino acids (*BnaPWWP39*) to 1,383 amino acids (*BnaPWWP26*), indicating considerable variation in the molecular size of proteins in this family. Their molecular weights ranged from 19,688.44 Da (*BnaPWWP39*) to 149,901.99 Da (*BnaPWWP26*). The predicted pI ranged from 4.7 to 10.01, among which 19 were acidic proteins (pI $<$ 7) and 24 were basic proteins (pI $>$ 7). The instability index ranged from 44.92 (*BnaPWWP6*) to 66.8 (*BnaPWWP13*), and the aliphatic index ranged from 52.2 (*BnaPWWP1*) to 87.02 (*PWWP33*). In hydrophilicity prediction, all BnaPWWP member proteins showed hydrophilicity. Subcellular-localization prediction indicated that 38 proteins were localized in the nucleus, 3 in the chloroplast, 1 in the cytoplasm, and 1 in the endoplasmic reticulum. This indicates that proteins of this family are mainly localized in the nucleus, accounting for 88.37% (Table 2).

Table 2 Prediction of physicochemical properties and subcellular lo-

calization of representative BnaPWWP family proteins

Protein	Number of amino acids	Molecular weight (Da)	Theoretical pI	Instability index	Aliphatic index	Grand average of hydro-pathic-ity	Subcellular local-iza-tion
BnaPWWP1	568	64 104.63	6.94	55.96	52.2	-1.09	Nucleus
BnaPWWP6	920	103 470.85	9.03	44.92	70.36	-0.486	Chloroplast
BnaPWWP9	528	57 876.18	9.78	53.93	78.26	-0.605	Cytoplasm
BnaPWWP16	604	64 891.08	8.83	49.35	72.78	-0.597	Nucleus
BnaPWWP17	741	83 302.34	7.15	59.22	56.6	-0.992	Nucleus
BnaPWWP13	399	45 899.24	4.7	66.89	70.05	-0.738	Chloroplast
BnaPWWP20	1025	116 235.35	8.3	45	73.82	-0.513	Nucleus
BnaPWWP24	408	45 314.76	6.1	47.09	60.74	-0.57	Nucleus
BnaPWWP26	1383	149 901.99	8	62.22	61.55	-0.802	Nucleus
BnaPWWP29	1040	117 264.46	8.2	46.63	74.78	-0.483	Nucleus
BnaPWWP36	613	66 170.66	9.21	52.02	71.26	-0.649	Nucleus
BnaPWWP33	905	101 121.55	9.01	48.12	87.02	-0.453	Endoplasmic Retic-ulum
BnaPWWP36	1338	147 922.99	5.4	55.22	60.79	-0.833	Nucleus
BnaPWWP37	522	58 939.35	6.07	45.53	74.06	-0.659	Chloroplast
BnaPWWP38	1276	137 683.99	8.48	61.53	61.2	-0.806	Nucleus
BnaPWWP39	177	19 698.44	10.01	45.93	69.38	-0.702	Nucleus
BnaPWWP43	1361	146 668.64	8.96	57.9	61.22	-0.791	Nucleus

Fig. 2 Phylogenetic analysis of the *PWWP* gene in *Brassica napus*, *B. rapa*, *B. oleracea*, and *Arabidopsis thaliana*

Figure 1: Fig. 2 Phylogenetic analysis of the *PWWP* gene in *Brassica napus*, *B. rapa*, *B. oleracea*, and *Arabidopsis thaliana*

2.3 Phylogenetic analysis of the *PWWP* family members in *Brassica napus*

To investigate the phylogenetic relationships of the *PWWP* family in *Brassica napus*, this study identified 43, 21, 20, and 19 *PWWP* family members in *Brassica napus*, *Brassica rapa*, *Brassica oleracea*, and *Arabidopsis thaliana*, respectively (Fig. 2). Based on the protein sequences of the above species, a phylogenetic tree was constructed, and the genes were divided into seven subfamilies. Among them, the Family5 subfamily contained the largest number of *BnaPWWP* members, with 16 members, accounting for 55.17% of the family; the Family1, Family2, Family3, Family4, and Family6 subfamilies contained the fewest *BnaPWWP* family members, with an average of 3 each. In addition, the Family7 subfamily contained 11 *BnaPWWP* family members, accounting for 45.83%. The *PWWP* genes of *B. oleracea* and *B. rapa* were distributed in all Family1–Family7 subfamilies, while those of *A. thaliana* were distributed in all subfamilies except Family3. These results support the hypothesis that the origin of *B. napus* arose from allopolyploidization through hybridization between *B. oleracea* and *B. rapa*. Members within each subfamily showed obvious species-specific clustering, suggesting that gene functions were conserved after duplication.

Fig. 2 Phylogenetic analysis of the *PWWP* gene in *Brassica napus*, *B. rapa*, *B. oleracea*, and *Arabidopsis thaliana*

2.4 Analysis of conserved motifs, conserved structural domains, and gene structures of *PWWP* family members in *Brassica napus*

To analyze the sequence characteristics of the *PWWP* family members in *Brassica napus*, 20 conserved motifs were identified. Except for *BnaPWWP1*, all other members contained Motif11, indicating that Motif11 is highly conserved. Some members (*BnaPWWP2* and 11 other members) simultaneously contained Motif7, Motif11, and Motif16, as well as 1–2 conserved structural domains. Another group (*BnaPWWP9* and 6 other members) contained Motif4, Motif8, Motif9, Motif11, and Motif15, whereas *BnaPWWP31* lacked Motif8; this was predicted to be due to fragment loss during evolution, resulting in the loss of the DNA sequence encoding Motif8. Ten members, including *BnaPWWP3*, contained Motif1, Motif2, Motif3, Motif5, Motif14, and PWWP_HULK.

conserved structural domains, among which 6 also contained the RPR conserved

structural domain, and 4 contained the VHS_ENTH_ANTH_superfamily (Fig. 3). Subfamily members with close phylogenetic relationships were highly conserved in terms of conserved motifs and structural-domain composition, while differences among different motifs may be involved in species evolutionary divergence. Gene-structure analysis found that all *BnaPWWP* genes contained exons, and some members contained 2–5 introns, suggesting that their core functions are conserved and that introns participate in expression regulation.

A. Conserved motif; B. Conserved structural domain; C. Gene structure.

A. Conserved Motif; B. Conserved structure domain; C. Gene structure.

Fig. 3 Analysis of conserved motifs, conserved domains, and gene structure of the *PWWP* gene family in *Brassica napus*

2.5 Analysis of promoter cis-acting elements of members of the *PWWP* family in *Brassica napus*

The results of promoter cis-acting element analysis showed that a total of 29 types of cis-acting elements were identified (Fig. 4). After removing elements with uncertain function and light-responsive elements, the remaining 22 types could be divided into three major categories, namely growth- and development-responsive elements, phytohormone-responsive elements, and abiotic-stress-responsive elements (Fig. 5). Development-related elements (AACA motif, CAT-box, CCAAT-box, GCN4 motif, O2-site, HD-Zip 1, circadian, MBSI) are responsible for regulating photosynthesis, embryonic development, meristem differentiation, tissue-specific expression, and other related functions. Phytohormone-responsive elements include abscisic acid (ABA)-responsive elements (ABRE), auxin-responsive elements (AuxRR-core, TGA-element), methyl jasmonate (MeJA)-responsive elements (CGTCA-motif and TGACG-motif), gibberellin-responsive elements (GARE-motif, P-box and TATC-box), and salicylic acid-responsive elements (TCA-element). Abiotic-stress-responsive elements include low-temperature-responsive elements (LTR), drought-responsive elements (MBS), defense- and stress-responsive elements (TC-rich repeats), anaerobic-induction-responsive elements (ARE), and heat-stress-responsive elements (AT-rich element). Among them, ABRE, MBS, TC-rich repeats, and LTR are elements associated with salt stress (Wang et al., 2021; Du Guoning et al., 2022; Ling et al., 2023). Based on the element number distribution map, ARE, ABRE, CGTCA-motif, and TGACG-motif had the highest overall frequencies and covered the largest number of genes, indicating that expression regulation of *BnaPWWP* family genes is centered on abiotic-stress responses and hormone (abscisic acid and methyl jasmonate) signal regulation; meanwhile, some genes participate in growth and development regulation. These results provide important cis-acting element evidence for subsequent analysis of the functions and regulatory network of *BnaPWWP* family genes.

Fig. 4 Analysis of cis-acting elements in the promoters of *BnaPWWP*

Fig. 4 Analysis of cis-acting elements in the promoters of *BnaPWWP* gene family members

Figure 2: Fig. 4 Analysis of cis-acting elements in the promoters of *BnaPWWP* gene family members

Fig. 5 Total frequency of cis-acting elements related to development, hormone response, and abiotic stress

Figure 3: Fig. 5 Total frequency of cis-acting elements related to development, hormone response, and abiotic stress

gene family members

Fig. 5 Total frequency of cis-acting elements related to development, hormone response, and abiotic stress

response in *BnaPWWP* family genes

2.6 Analysis of the Protein Interaction Network and Homology Modeling of PWWP Family Members in *Brassica napus*

The protein interaction network of PWWP family members in *Brassica napus* was constructed using the STRING database. The results (Fig. 6) showed that some members of the BnaPWWP family formed a highly connected network module centered on six core proteins. Among them, BnaPWWP8, BnaPWWP20, BnaPWWP29, BnaPWWP36, BnaPWWP38, and BnaPWWP43 were densely connected, with a high degree of interconnection among nodes, forming the core framework of the network. This suggests that extensive coordinated regulatory relationships exist among these proteins, and they may represent core nodes in the family' s function. In contrast, BnaPWWP33 had only a single interaction connection with BnaPWWP43 and was located at the periphery of the network, indicating that its interaction association with the core members is relatively weak and that its function is comparatively independent. In addition, most members of this family were not incorporated into the interaction network under this confidence threshold, reflecting a lack of sufficient evidence supporting their interactions with the core proteins, and their functions may be relatively singular.

Fig. 6 Protein interaction network among PWWP family members in *Brassica napus*
Fig. 6 Interaction network of BnaPWWP family proteins

Fig. 6 Interaction network of BnaPWWP family proteins

Figure 4: Fig. 6 Interaction network of BnaPWWP family proteins

Fig. 7 Tertiary structure prediction of BnaPWWP proteins

Figure 5: Fig. 7 Tertiary structure prediction of BnaPWWP proteins

Using the SWISS-MODEL online tool, we obtained a high-quality predicted three-dimensional structural model of the PWWP protein in *Brassica napus*. The most prominent feature of this model is that its main body is a typical PWWP domain and contains a very clear "aromatic cage"composed of conserved aromatic amino acids, as shown in Fig. 7. The successful modeling of this structure not only verifies that the target protein belongs to the PWWP-domain protein family, but also provides an important basis for further studying how it affects chromatin structure and gene transcription by binding specific histone modifications.

Fig. 7 Tertiary structure prediction of BnaPWWP proteins

2.7 Expression analysis of *PWWP* family genes in salt-stressed canola

The qRT-PCR results showed that, in seeds of canola under salt stress, the expression levels of five *PWWP* genes were significantly upregulated, namely *BnaPWWP6*, *BnaPWWP10*, *BnaPWWP11*, *BnaPWWP24*, and *BnaPWWP30*; seven genes showed downregulated expression, namely *BnaPWWP8*, *BnaPWWP9*, *BnaPWWP14*, *BnaPWWP17*, *BnaPWWP27*, *BnaPWWP29*, and *BnaPWWP32*. We speculate that such genes are repressed to reduce energy consumption and allocate resources to salt-tolerance-related genes, thus showing a downward trend. Gene expression levels also differed among different subfamilies. Family1, Family4, and Family7 each contained one gene with significantly increased expression; Family5 contained two, whereas expression differences in Family2, Family3, and Family6 were not obvious (Fig. 8, Fig. 9, and Fig. 10). Overall, canola

The oilseed rape *PWWP* gene family significantly responded to the salt pathway, among which *BnaPWWP6*, *BnaPWWP10*, *BnaPWWP11*, *BnaPWWP24*, and *BnaPWWP30*, as upregulated genes, may be key links connecting salt-pathway signaling with the seed-germination process.

BnaPWWP31 and *BnaPWWP6* belong to subfamily 1; *BnaPWWP26* and *BnaPWWP14* belong to subfamily 2; *BnaPWWP17*, *BnaPWWP20*, and *BnaPWWP22* belong to subfamily 3; *BnaPWWP5* and *BnaPWWP10* belong to subfamily 4; *BnaPWWP27*, *BnaPWWP33*, and *BnaPWWP41* belong to subfamily 6.

BnaPWWP31 and *BnaPWWP6* belong to subfamily 1; *BnaPWWP26* and *BnaPWWP14* belong to subfamily 2; *BnaPWWP17*, *BnaPWWP20*, and *BnaPWWP22* belong to subfamily 3; *BnaPWWP5* and *BnaPWWP10* belong to subfamily 4; *BnaPWWP27*, *BnaPWWP33*, and *BnaPWWP41* belong to

Figure 10. Expression levels of PWWP subfamily 7 in *Brassica napus* during seed germination under salt stress

Figure 6: Figure 10. Expression levels of PWWP subfamily 7 in *Brassica napus* during seed germination under salt stress

subfamily 6.

Fig. 8 Expression levels of *PWWP* subfamilies 1, 2, 3, 4, and 6 in *Brassica napus* during seed germination under salt stress

Fig. 9 Expression levels of *PWWP* subfamily 5 in *Brassica napus* during seed germination under salt stress

Fig. 10 Expression levels of *PWWP* subfamily 7 in *Brassica napus* during seed germination under salt stress

3 Discussion and Conclusion

As one of the important modes of epigenetic regulation, histone modification plays an important role in plant responses to stress (Yu et al., 2025). The PWWP domain is a highly conserved chromatin-binding module in eukaryotes and acts as a molecular reader that specifically recognizes histone modification signals during epigenetic regulation (Huang et al., 2024). Research on plant PWWP-domain proteins has long focused on the regulation of plant growth and development. Recently, studies in tea plant and rice have shown that PWWP-domain proteins are highly involved in biological processes by which plants respond to abiotic stress (Chen et al., 2021; Seo and Jang 2025). These findings provide inspiration for further exploration of the functions of PWWP-domain proteins related to plant epigenetic regulation, and their research results have potential application value in crop stress resistance. Nevertheless, current research on PWWP-domain proteins in crops remains scarce, except in rice.

Rapeseed is the oilseed crop with the largest planting area and the highest total output in China, playing an important role in ensuring the supply of edible oil (Li Gucheng et al., 2024). Planting rapeseed in saline-alkali land and expanding the cultivation area of rapeseed are of great significance for ensuring the security of China' s edible oil supply. However, high salt stress in saline-alkali land is a key adverse environmental factor that restricts rapeseed growth and yield. Discovering and elucidating the response mechanisms of salt-tolerance-related genes is an important prerequisite for molecular breeding of salt-tolerant rapeseed. Therefore, this study systematically analyzed and validated the PWWP-domain protein family in the widely cultivated *Brassica napus* in China. First, at the whole-genome level, we identified a total of 43 PWWP family members in *Brassica napus*, of which 22 were located in subgenome A and 21 in subgenome C, indicating that this family did not undergo large-scale asymmetric loss during heterologous tetraploidization. Analysis of physicochemical protein properties

showed that these proteins differed greatly in amino acid number, molecular weight, and isoelectric point; all were hydrophilic proteins, and 88.27% were localized in the nucleus, consistent with their predicted function as intranuclear chromatin-binding factors. Systematic evolutionary analysis divided the 43 members into seven subfamilies. Members within the same subfamily were highly conserved in conserved motifs, domain composition, and gene structure, suggesting that they may have functional similarities. In addition, homology modeling successfully constructed the three-dimensional structure of one BnaPWWP protein, and the model clearly presented an "aromatic cage" composed of conserved aromatic amino acids, which is the core structural basis for the specific recognition of methylated histones by the PWWP domain (Qin & Min, 2016). These results systematically elucidate the basic characteristics of the *PWWP* gene family in *Brassica napus*.

Cis-acting elements in promoter regions are an important molecular basis for gene transcriptional regulation (Fueyo et al., 2022). Accordingly, this study systematically analyzed the cis-element composition in the promoter regions of the *PWWP* gene family in *Brassica napus*. The results showed that ARE (anaerobic stress-responsive element), ABRE (abscisic acid-responsive element), CGTCA-motif, and TGACG-motif (methyl jasmonate-responsive elements) were the core elements with the highest total frequencies and the broadest gene coverage. This suggests that transcription of the *PWWP* family genes in *Brassica napus* is very likely to be coordinately regulated by multiple abiotic stresses such as salt stress, drought, and low temperature, as well as stress-related endogenous hormones such as ABA and MeJA. As a key signal in the plant salt-stress response pathway, ABA ···

signal substance and can maintain intracellular Na+/K+ ion balance by regulating the expression of downstream transcription factors and SOS pathway ion-transport proteins (Yang & Guo, 2018). The promoter of the *BnaPWWP* gene is generally enriched in ABRE cis-elements, suggesting that members of this family may participate in plant salt-stress responses through ABA-dependent signaling pathways. Meanwhile, the high-frequency distribution of CGTCA-motif and TGACG-motif in the promoter region also implies that genes of the *PWWP* family may be involved in salicylic-acid-signal-mediated stress-regulation processes.

To further explore which *BnaPWWP* genes truly respond to salt stress, this study analyzed the expression changes of the *BnaPWWP* gene family during seed germination under salt stress (120 $mmol \cdot L^{-1}$ NaCl). The qRT-PCR results showed that, among the 43 *BnaPWWP* genes, 5 genes (*BnaPWWP6*, *BnaPWWP10*, *BnaPWWP11*, *BnaPWWP24*, and *BnaPWWP30*) were significantly upregulated by more than twofold, whereas 7 genes were significantly downregulated. This result suggests that members of the *PWWP* family exhibit functional differentiation in response to salt stress and may respectively perform roles in activating or repressing downstream salt-tolerance genes. To deeply elucidate the response mechanisms of *PWWP* family members to salt stress, this study further analyzed the composition of cis-acting elements in the promoter

regions of the above five upregulated genes. The results showed that the promoter regions of *BnaPWWP10*, *BnaPWWP11*, and *BnaPWWP24* were rich in multiple salt-stress-related elements (ABRE, MBS, TC-rich repeats, and LTR). Specifically, *BnaPWWP10* and *BnaPWWP11* each contained 2 ABREs, 2 TC-rich repeats, and 2 LTRs; *BnaPWWP24* contained 5 ABREs and 2 TC-rich repeats. The presence of these elements is consistent with their significant upregulation under salt stress, indicating that they may participate in salt-alkali stress responses through abscisic-acid-dependent or -independent pathways. Among them, the ABRE element is the core cis-acting element of the ABA signaling pathway, and its number is positively correlated with the response intensity of genes to ABA and salt stress (Nakashima & Yamaguchi-Shinozaki, 2013). The presence of as many as 5 ABRE elements in the promoter of *BnaPWWP24* suggests that this gene may be particularly sensitive to ABA signals. In addition, *BnaPWWP6* and *BnaPWWP30* were also significantly upregulated by salt-stress induction, but no typical salt-stress-related elements were detected in their promoter regions, implying that they may respond to salt stress through other indirect pathways.

Finally, based on promoter-element analysis and qRT-PCR results, we screened a total of 3 family members highly correlated with the salt-stress response, namely *BnaPWWP10*, *BnaPWWP11*, and *BnaPWWP24*, providing potential target genes at the transcriptional-regulation level for breeding salt-alkali-tolerant rapeseed. This study provides an important foundation for subsequent in-depth analysis of the functions and regulatory mechanisms of the *BnaPWWP* gene family in salt tolerance in rapeseed. Nevertheless, the mechanisms of action of the above genes in the salt-tolerance process of rapeseed still need to be further explored through molecular biology and genetic-engineering approaches.

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