

UNE Gene Family Analysis and Overexpression of UNE12 Enhances Salt Resistance in Brassica napus

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Abstract

Salt stress inhibits the growth, development, yield, and quality formation of the oilseed crop (rapeseed). This study identifies salt tolerance determinants within the UNE gene family of *Brassica napus* through integrated genomic and functional analyses. Genome-wide characterization revealed 21 BnUNE genes exhibiting structural diversification and parallel evolution with orthologs in *Brassica rapa* and *Brassica oleracea*, while core functional domains remained conserved. Phylogenetic analysis classified BnUNEs into 6 subclades with distinct motif architectures, intron/exon patterns, and predicted subcellular localization. Notably, subclade I members showed unique intron insertions and peroxisomal targeting, suggesting specialized roles in reactive oxygen species (ROS) metabolism and detoxification. Functional validation demonstrated that under 300 mM NaCl stress, transgenic lines BnUNE12-OE exhibited significantly enhanced salt tolerance compared to wild-type (WT) plants. The results that increased root length and fresh weight (37.2% and 29.8%), reduced the malondialdehyde (MDA) content (31.5%), elevated proline accumulation (2.3-fold), upregulated antioxidant genes (SOD1, CAT2). The study demonstrates that BnUNE12 significantly enhances salt stress tolerance in *B. napus* by synergistically regulating osmotic balance (promoting proline accumulation) and alleviating oxidative stress (enhancing ROS detoxification capacity). This work elucidated the adaptive evolution of the UNE gene family within the *Brassica* genus, and established BnUNE12 as a key molecular target for breeding salt tolerant rapeseed cultivars, providing a valuable genetic resource for molecular breeding of stress-resistant crops.

Key Message

Genome-wide analysis identified 21 *UNE* members in *Brassica napus*. The overexpression of *UNE12* alleviate membrane oxidative damage and regulate osmotic homeostasis, thereby enhancing the salt tolerance of plants.

1. Introduction

Brassica napus (rapeseed), an allopolyploids species ($2n=38$, AACC) within the *Brassicaceae* family, serves as the second-largest source of edible oil globally, accounting for approximately 13-16% of the total production (Song et al. 2021). Its applications extend beyond edible oil production to include use as vegetables, animal feed, nectar sources, ornamental plants, and fertilizers (Raboanatahiry et al. 2021). Crucially, it contributes significantly to soil remediation, arable land expansion, and food security (Kniuiptyé et al. 2023). *B. napus* originates from the interspecific hybridization between *Brassica rapa* ($2n=20$, AA) and *Brassica oleracea* ($2n=18$, CC), thereby emerging as a pivotal model organism for investigating interspecific evolutionary trajectories (Chalhoub et al. 2014). However, *B. napus* faces substantial yield and quality constraints due to its susceptibility to diverse abiotic and biotic stresses, including drought, salinity, insect pests, and heavy metal contamination (Neik et al. 2017; Hammac et al. 2017), which exert profound adverse effects on its growth, development, yield, and quality, thereby ultimately impeding the sustainable advancement of the *B. napus* industry. Accordingly, investigating the stress-specific gene expression profiles of *B. napus* under adverse conditions, enhancing its stress tolerance via biotechnological approaches, and further exploiting the utilization potential of *B. napus* are of crucial significance for meeting the global food production demands.

Salinized land is expanding, posing a significant threat to agricultural productivity, with fragile and water-scarce ecosystems being disproportionately affected and a substantial portion of such salinized areas holding considerable potential for agricultural development (Wang et al. 2024). Salinity induces physiological drought, impairs soil structure (causing compaction and poor aeration), inhibits microbial activity, reduces fertility, and ultimately leads to significant crop yield losses, jeopardizing food security (Zhang, K et al. 2019; Zhang, W et al. 2019). While tens of millions of hectares of saline-alkali land potentially suitable for *B. napus* cultivation, overwintering challenges persist in these cold, arid regions (Wu et al. 2019; Dai et al. 2024). Improving salt tolerance of *B. napus* for large-scale cultivation on such marginal land would not only utilize critical reserve arable resources but also significantly boost domestic rapeseed oil production, enhancing edible oil security and reducing import dependence.

The basic helix-loop-helix (bHLH) transcription factor superfamily plays critical roles in plant biological processes. Characterized by a conserved DNA-binding basic region and a helix-loop-helix (HLH) dimerization domain, bHLH proteins regulate diverse processes including growth, development, stress responses, and metabolism (Zimmermann et al. 2004; Hao et al. 2021). Examples include *Triticum aestivum* bHLH39 (TabHLH39), which significantly enhances the salt tolerance by modulating stress-responsive genes (Zhai et al. 2016), and *Arabidopsis thaliana* bHLH112, which binds to E-box and GCG-box motifs to regulate the expression of genes associated with proline, peroxidase (POD), and superoxide dismutase (SOD), and consequently improving plant stress resistance (Sun et al. 2018). The haploid female gametophyte, alternatively termed the embryo sac, represents the fundamental reproductive unit in angiosperms, it differentiates within the ovule of the ovary and comprises an egg cell and a central cell (Skinner and Sundaresan 2018). As a pivotal tissue indispensable to plant reproduction, the embryo sac mediates the entire sexual reproduction whereby mature pollen grains discharged from the anther

deposits on the pistil's stigma, germinate, upon successful recognition, form a pollen tube that penetrates the ovule's micropyle to reach the embryo sac, after whose rupture two male gametes are released, one fusing with the egg cell to generate a zygote that further develops into the embryo while the other unites with the two polar nuclei of the central cell to yield the primary endosperm nucleus that differentiates into endosperm, thus culminating in the completion of pollen fertilization (Johnson et al. 2019; Shivanna and Tandon 2020; Shin et al. 2021). Research on plant embryo sacs has predominantly focused on cell development, breeding, fertilization, and subsequent embryonic development processes. In contrast, studies integrating unfertilized embryo sacs (*UNEs*) with abiotic stresses remain exceedingly scarce. The *A. thaliana* *UNE12* (*AtUNE12*), a member of the bHLH superfamily, exhibits structural and functional features that not only adhere to familial commonalities but also display distinct regulatory modalities. It exerts an irreplaceable role in the growth regulation and environmental adaptation of *A. thaliana*. Related research facilitates the elucidation of intricate regulatory networks underlying plant life activities and offers a theoretical foundation for crop genetic improvement. While substantial advances have been achieved in research on *AtUNE*, investigations into the genetic evolution and functional characterization of *B. napus* *UNE* genes (*BnUNE*) remain relatively limited. In the present study, bioinformatics approaches were utilized to identify members of the *UNE* gene family within the *B. napus* genome and its ancestral species (*B. rapa* and *B. oleracea*). The experiment of overexpressing *UNE* family member *UNE12* under salt stress furnishes critical insights for further investigating the functions of *UNE* genes in *B. napus* growth and for the identification of salt-tolerant rapeseed germplasm.

2. Materials and Methods

2.1 Identification of members within the *UNE* gene family

Protein sequence data and genome annotation files for *B. napus* (ZS11.v0) and *B. rapa* (ECD04.v0) were retrieved from the *Brassica napus* Information Resource (BnIR) website, whereas the corresponding files for *B. oleracea* and *A. thaliana* were acquired from the Ensembl database and TAIR, respectively (Yates et al. 2022; Yang et al. 2023; Reiser et al. 2024). Building on prior studies, the Hidden Markov Model (HMM) file (PF00010) corresponding to the conserved domain of *UNE* genes was downloaded from the InterPro database (Blum et al. 2025), and the HMM file (PTHR16223) specific to the *UNE* gene family was retrieved from the PANTHER database (Thomas et al. 2022).

2.2 Physicochemical properties analysis and phylogenetic tree construction

The physicochemical properties of *BnUNEs* were predicted via ProtParam, an online tool within the ExPASy suite (Wilkins et al. 1999). Sequence alignment of *UNE* gene family members was performed using the MUSCLE algorithm integrated in MEGA software. Subsequently, a phylogenetic tree was constructed based on the alignment results using the neighbor-joining (N-J) method, with bootstrap analysis configured for 1000 replications (Tamura et al. 2021). The generated phylogenetic tree was subjected to optimization and visualization using the online platform Chiplot (Xie et al. 2023).

2.3 Chromosome mapping and intragenomic collinearity analysis

Chromosomal mapping and intragenomic collinearity analysis of *B. napus* were conducted using TBtools software, with gene distribution subjected to visualization (Chen et al. 2023). Furthermore, the Ka/Ks (nonsynonymous/synonymous substitution rate) ratios of *BnUNEs* were computed to assess the selection pressure exerted during the evolutionary process.

2.4 Analysis of conserved motifs, domains, and gene structures

Protein sequences of *UNE* family members were submitted to the online MEME suite, with the number of motifs set to 20 for prediction (other parameters retained default values), yielding files in XML format (Bailey et al. 2015). Conserved domains of *UNE* family members were predicted via the CD-Search Tool within NCBI (Marchler-Bauer et al. 2011; Lu et al. 2022). Ultimately, an integrated visualization of motifs, domains, and gene structures was generated using TBtools.

2.5 Analysis of cis-acting regulatory elements in the promoter region

In the present study, sequences 2000 bp upstream of *BnUNE* genes—corresponding to potential promoter regions—were retrieved from the *B. napus* genome using the Gff3 sequence extraction and Fasta extraction functions integrated in TBtools. Promoter sequences 2000 bp upstream of *BnUNEs* were extracted from genome annotation files. Subsequently, cis-acting element analysis of these sequences was performed using the online tool PlantCARE to predict cis-acting regulatory elements (CAREs) (Lescot et al. 2002). The results were visualized using TBtools. Identified CAREs were categorized based on their functional roles, with visualization of the outcomes conducted

via the Advanced Gene Structure View function in TBtools. This comprehensive analysis advanced our understanding of the regulatory mechanisms governing *BnUNE* gene expression.

2.6 Interspecies collinearity analysis and subcellular localization prediction

Prepared genome and genome annotation files were imported into TBtools, with analysis conducted using the One Step McScanX program. Following simplification processing, the results were visualized using the Multiple Synteny Plot program. Protein sequences of the *UNE* gene family were submitted to the WoLF PSORT server for prediction of subcellular localization (Horton et al. 2007).

2.7 Quantitative real-time PCR (qRT-PCR) analysis of *BnUNE12* expression under salt stress conditions

B. napus cultivar J9707 (seeds provided by the Crop Research Institute, Hunan Academy of Agricultural Sciences) was used as the test material in this study. All hydroponic experiments were conducted in a greenhouse under the following growth conditions: temperature 23/25°C (day/night), photoperiod 16 h light/8 h dark, and relative humidity 50-60%. The housekeeping gene for qRT-PCR is BnaA01G0324400ZS. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative gene expression levels (Livak and Schmittgen 2001).

2.8 Investigation of Salt Tolerance through *BnUNE12* Overexpression under Salt Stress

To explore the association between the *BnUNE12* gene (BnaA03G0270300ZS) and salt tolerance in *B. napus*, the pC2306 vector was linearized using KpnI and Sall as restriction sites, with the PCR product subjected to recovery (Fig. 8 A). The *BnUNE12* gene was cloned using cDNA from J9707 as a template (Fig. 8 B), followed by ligation of the gene fragment into the linearized pC2306 vector. Subsequent PCR validation and sequencing confirmed the successful construction of the overexpression vector. Agrobacterium-mediated transformation of *B. napus* explants was performed to generate *BnUNE12* overexpression plants. Total RNA was extracted from each *BnUNE12*-OE line and reverse-transcribed into cDNA, and qRT-PCR was conducted to quantify the expression level of each line, thereby verifying the overexpression efficiency.

3. Results

3.1 Delineation of the phylogenetic trajectory of *BnUNE* family

The hmmsearch program was employed to query both HMM profiles concurrently for the identification of candidate *UNE* genes within the *B. napus* proteome. An E-value cutoff of <0.01 was applied to intersect the search results, yielding 410 candidate members. Subsequently, using previously identified *UNE* protein sequences from *A. thaliana* as query sequences, a local BLASTP analysis was performed against these candidate genes, confirming 21 high-confidence *UNE* genes in *B. napus*. Application of the same methodology, identified 5, 10, and 9 *UNE* genes in *A. thaliana*, *B. rapa*, and *B. oleracea*, respectively. To elucidate the evolutionary relationships within the *UNE* gene family between *B. napus* and its ancestral species, *A. thaliana* was used as an outgroup to systematically identify orthologous *UNE* family members across the four genomes. A phylogenetic tree was subsequently constructed using the Chiot platform (Fig. 1). Among the analyzed species, *AtUNE* homologs exhibited the earliest evolutionary divergence, consistent with established species phylogeny. According to the U's triangle theory, which posits that *B. napus* originated from the natural hybridization between *B. oleracea* and *B. rapa* with genomic inheritance from both progenitors, *BnUNE* genes clustered phylogenetically with their counterparts in *B. rapa* and *B. oleracea*. Furthermore, these genes occupied identical phylogenetic positions with comparable branch lengths, indicating a pattern of parallel evolution. This observation suggests that *UNE* genes underwent species-specific diversification: following the divergence of ancestral species into distinct lineages, accumulation of structural variations lead to partial functional specialization. Consequently, while core functions remain conserved, lineage-specific functions have emerged. Collectively, these analyses provide tangible insights into the evolutionary trajectories of the *UNE* gene family across species and lay the foundation for further investigations into the relationship between gene function and species adaptability.

3.2 Interpretation of protein conserved domains and gene structure

Using the *A. thaliana*-containing clade as a reference, the *UNE* family was categorized into six subclades through the integration of phylogenetic tree topology, gene motifs, conserved domains, and gene structures (Fig. 1, 2). Subclade I comprises the largest number of members (13), which lack lineage-specific motifs. However, *Bna_UNE12.4* and *Bra_UNE12.4* acquired 4 and 2 introns, respectively, during evolution, leading to their structural homology with TRAPPC5_Tr31. TRAPP (transport protein particle) is a large, highly conserved multisubunit complex, with four types (I, II, III, and IV) identified in *Saccharomyces cerevisiae*, and Trs31, a conserved subunit of TRAPP complexes, plays a pivotal role in vesicle trafficking and autophagy (Zou et al. 2015). All members except *Bna_UNE12.6* and *Bo_UNE12.1* harbor Motifs 1, 2, 3, 4, and 5, implying that the bHLH domain is composed of one or more of these motifs—an assertion that requires

further validation via investigations into the three-dimensional structures of these proteins. Notably, *At_UNE12* is clustered in subclade VI, which contains 9 members. Following divergence from Arabidopsis, genes from the three *Brassica* species evolved to possess Motifs 12 and 16, whereas *Bo_UNE12.1* exclusively acquired Motif 16 while losing Motifs 1, 5, and 13.

3.3 Protein physicochemical properties and predicting subcellular localization

Data derived from ProtParam and WoLFPSORT were subjected to comprehensive summary and analysis (Table 1). The molecular weights of members across the four species ranged from 19.056 to 47.851 kDa. Subclade I exhibited the most pronounced variation in molecular weight, with the smallest member weighing 24.107 kDa and the largest reaching 47.851 kDa. In contrast, subclade III showed the least variation, with a maximum difference of merely 2.651 kDa. The overall variation in subclade VI did not exceed 1 kDa; however, *Bo_UNE12.1* underwent a significant reduction in molecular weight (to 23.014 kDa) due to the loss of motifs 5, 13, and 1 during evolution. Regarding pI, 10 *UNE* family members were basic, while the remainder were acidic. Within subclade VI, all members except *Bo_UNE12.1* exhibited acidic properties. This suggests that the C-terminus of *UNE12* is enriched in acidic amino acids, whereas the N-terminus contains a higher proportion of basic amino acids—an observation that explains the elevation in isoelectric point following the loss of C-terminal motifs. The aliphatic index of *UNE* family consistently exceeded 60, with some members reaching 80, indicating a high proportion of aliphatic amino acids and suggesting robust thermal stability. The Grand Average of Hydropathy (GRAVY) values reflected strong hydrophilicity. Collectively, these properties are consistent with the presence of a hydrophobic core within *UNE* proteins. Additionally, the instability index indicates that all *UNE* family are unstable protein, potentially due to the presence of specific degradation mechanisms or chaperone proteins that modulate their functional activity. WoLF PSORT analysis predicted that the majority of *UNE* gene family in *B. napus* are localized to the nucleus, with a subset residing in peroxisomes. The *UNE* gene family exerts primary functions in the nucleus, while peroxisomal members may participate in processes such as reactive oxygen species (ROS) metabolism and detoxification, fatty acid β -oxidation, and stress responses. Notably, all peroxisome-localized members belong to subclade I, signifying functional divergence within this subclade, with specialized roles in peroxisomes. *Bo_UNE12.4* was uniquely predicted to be predominantly localized to chloroplasts. The presence of a signal sequence upstream of its start codon supports this prediction, and implies potential core functions may be associated with chloroplast-related physiological processes. However, the specific functions of *Bo_UNE12.4* require experimental validation, including confirmation of its chloroplast localization and characterization of interacting protein networks.

Table 1 Physicochemical properties and subcellular localization across *A. thaliana*, *B. napus*, and their ancestral species

Sequence ID	Rename	Subclade	AA Number	MW/kDa	Theoretical pI	Instability Index	Aliphatic Index	GRAVY	PSLP
Bo2g028920.1	Bo_UNE12.9	☒	229	24.107	9.39	46.57	75.9	-0.459	nucl
BnaA02T0114000ZS	Bna_UNE12.2	☒	280	29.723	6.98	51.44	70.79	-0.524	nucl
Bo3g018990.1	Bo_UNE12.6	☒	286	30.196	8.76	51.6	72.03	-0.452	pero
BnaA10T0136900ZS	Bna_UNE12.10	☒	289	30.289	5.88	48.92	72.35	-0.436	pero
BnaC03T0125500ZS	Bna_UNE12.15	☒	286	30.367	8.76	51.2	72.03	-0.477	pero
BraA02t029661E	Bra_UNE12.2	☒	293	31.179	6.32	49.77	67.99	-0.561	nucl
AT5G58010.1	AT_UNE12.1	☒	297	31.470	5.79	50.15	70.37	-0.423	pero
BnaC02T0139100ZS	Bna_UNE12.13	☒	296	31.758	6.16	53.52	68.61	-0.62	nucl
BraA10t046360E	Bra_UNE12.9	☒	302	31.795	5.73	47.43	70.53	-0.46	pero
Bo9g133640.1	Bo_UNE12.7	☒	331	35.229	6.41	46.94	66.71	-0.598	pero
BnaC09T0412800ZS	Bna_UNE12.21	☒	355	38.177	6.2	48.12	75.92	-0.391	pero
BnaA03T0108600ZS	Bna_UNE12.4	☒	418	45.263	8.64	43.59	86.32	-0.258	pero
BraA03t008376E	Bra_UNE12.4	☒	441	47.851	8.19	42.93	87.78	-0.197	pero
BnaC01T0077700ZS	Bna_UNE12.12	☒	327	34.579	6.3	52.93	67.77	-0.432	nucl
Bo1g011740.1	Bo_UNE12.2	☒	327	34.579	6.3	52.93	67.77	-0.43	nucl
BnaA01T0064700ZS	Bna_UNE12.1	☒	327	34.591	6.3	52.35	68.96	-0.418	nucl
BraA01t024441E	Bra_UNE12.1	☒	327	34.591	6.3	52.09	68.96	-0.418	nucl
AT4G30980.2	AT_UNE12.3	☒	358	38.582	9.02	57.56	63.55	-0.59	nucl
BnaA04T0160400ZS	Bna_UNE12.6	☒	326	34.232	6.45	50.17	59.94	-0.555	nucl
BraA09t006007E	Bra_UNE12.8	☒	339	35.743	6.45	57.2	66.81	-0.428	nucl
Bo8g099910.1	Bo_UNE12.3	☒	340	35.786	6.3	55.43	67.74	-0.424	nucl
BnaC08T0433700ZS	Bna_UNE12.19	☒	340	35.831	6.16	53.32	68	-0.415	nucl
BnaA09T0581100ZS	Bna_UNE12.8	☒	344	36.229	6.45	57.62	68.37	-0.403	nucl
AT2G24260.1	AT_UNE12.4	☒	350	36.522	6.36	46.94	68.89	-0.356	pero
BnaC04T0455600ZS	Bna_UNE12.17	☒	348	36.745	6.67	51.09	63.42	-0.509	nucl
BraA04t043266E	Bra_UNE12.6	☒	348	36.812	6.3	49.43	63.71	-0.52	nucl
Bo4g154370.1	Bo_UNE12.8	☒	348	36.883	6.9	49.59	62.59	-0.541	nucl
BnaC01T0310400ZS	Bna_UNE12.11	☒	170	19.056	7.8	57.63	83.76	-0.414	nucl
BnaC05T0017800ZS	Bna_UNE12.18	☒	307	32.873	6.07	54.46	71.14	-0.432	nucl
BnaA10T0015800ZS	Bna_UNE12.9	☒	311	33.238	6.07	53.01	71.48	-0.423	nucl
AT1G03040.3	AT_UNE12.2	☒	333	36.364	6.32	50.84	72.91	-0.459	nucl
Bo5g003580.1	Bo_UNE12.4	☒	355	38.322	6.91	56.32	77.72	-0.379	chlo
BnaA02T0255600ZS	Bna_UNE12.3	☒	254	27.486	7.92	54.97	71.42	-0.485	nucl
BraA02t031288E	Bra_UNE12.3	☒	254	27.495	9.47	56.76	70.28	-0.475	nucl
Bo2g100750.1	Bo_UNE12.5	☒	298	31.725	5.91	60.06	70.74	-0.413	nucl
BnaC02T0344300ZS	Bna_UNE12.14	☒	299	31.824	5.91	59.89	71.47	-0.398	nucl

Bo9g005930.1	Bo_UNE12.1	☒	215	23.014	8.18	50.84	69.91	-0.493	nucl
BnaC09T0011500ZS	Bna_UNE12.20	☒	301	32.163	5.83	56.04	68.74	-0.492	nucl
BnaA09T0026700ZS	Bna_UNE12.7	☒	304	32.391	5.83	54.99	69.01	-0.475	nucl
BraA09t000195E	Bra_UNE12.7	☒	304	32.407	5.83	54.32	68.68	-0.484	nucl
BnaC03T0324600ZS	Bna_UNE12.16	☒	304	32.446	6.25	57.76	69.97	-0.445	nucl
Bo01031s030.1	Bo_UNE12.10	☒	307	32.747	6.31	58.55	69.28	-0.454	nucl
BraA03t010069E	Bra_UNE12.5	☒	308	32.747	6.07	57.69	71.27	-0.412	nucl
BnaA03T0270300ZS	Bna_UNE12.5	☒	310	32.861	6.07	59.02	69.55	-0.43	nucl
AT4G02590.1	AT_UNE12	☒	310	33.105	5.92	49.09	68.29	-0.488	nucl

Abbreviation AA:Animo Acid; MW:Molecular Weight; GRAVY: Grand Average of Hydropathy; PSLP: Protein Subcellular Localization Prediction

3.4 Analysis of the CAREs in the promoter regions of *B. napus*

16 major CAREs were identified in the *UNE* family of *B. napus*, encompassing a substantial number of light-responsive elements (247) and other cis-elements, including anaerobic induction (66), MeJA-responsiveness (60), Absciscic acid responsive element (ABRE, 44), Low-temperature responsiveness (LTR, 29), Gibberellin responsive element (GRE, 21), and Salicylic acid responsive element (SARE, 16) (Fig. 3). These elements suggest that *UNE* genes are pivotal in plant growth and development, stress responses, and hormone regulation.

The abundance of light-responsive CAREs such as G-box, TCT-motif implies a potential role for the *UNE* gene family in regulating photosynthesis-related pathways in *B. napus*. The G-box element can interact with light-induced transcriptional activators to modulate genes involved in chloroplast development and light energy utilization. This functional association aligns with its requirement for photosynthate accumulation in *B. napus* as a key oilseed crop (Toledo-Ortiz et al. 2014). Stress-related CAREs indicate that the *UNE* gene family employs diverse regulatory mechanisms in response to complex environmental stresses. Specifically, 66 anaerobic induction elements suggest that activation of certain *UNE* genes under root hypoxia stress, potentially maintaining cellular viability through regulation of glucose metabolism or energy metabolism. Additionally, 29 LTR elements implicate potential involvement of *UNE* genes in regulating cold adaptation, possibly acting synergistically with genes responsible for the synthesis of antifreeze proteins or osmotic. Notably, 44 ABREs suggest that ABA—a central stress signaling molecule—may drive *UNE* gene expression through the activation of its regulatory pathway. This ABA-mediated response could enhance cellular osmotic adjustment capacity (e.g., proline accumulation) and antioxidant enzyme activities (e.g., SOD, POD). This mechanism appears evolutionarily conserved, mirroring observations in *A. thaliana* where *AtUNE12* enhances salt tolerance via activation of the SOS pathway.

The identification of 60 MeJA-responsive elements and 21 GRE elements indicates potential integration of jasmonic acid (JA) and auxin (IAA) signaling pathways by the *UNE* gene family. Such integration may contribute to the balancing defense responses with growth and development in *B. napus*. JA signaling is frequently associated with plant insect and disease resistance (Campos et al. 2014), while, auxin-responsive elements may implicate *UNE* genes in embryonic development or organ morphogenesis. Furthermore, 16 SARE elements expand the potential regulatory network of *UNE* genes. As Salicylic acid (SA) is a pivotal signaling molecule in systemic acquired resistance (SAR), these elements suggest involvement of *UNE* genes in *B. napus* immune defense. Specifically, they may modulate the expression of pathogenesis-related protein genes during pathogen infection, thereby enhancing resistance (Tripathi et al. 2019; Han et al. 2022). Collectively, the co-occurrence of these multi-hormone responsive elements indicates that the *UNE* gene family likely integrates multiple signaling pathways to form a sophisticated gene regulatory network. This integrated system likely enables *B. napus* to address diverse physiological demands across different growth stages and environmental conditions.

3.5 Explanation of the chromosomal mapping and collinearity of the *BnUNEs*

Deciphering the *B. napus* genome annotation file revealed that *UNE* gene family members are distributed across merely 6 chromosomes in the A subgenome and 7 chromosomes in the C subgenome (Fig. 4). Intraspecific synteny analysis demonstrated that these genes undergo segmental duplications on chromosomes, with no tandem duplications observed. Brassicaceae plants have experienced multiple whole-genome duplication (WGD) events, including γ , α , and β triplications. The segmental duplications within the *UNE* gene family are highly likely to have originated from these ancient WGD events, thereby resulting in the formation of interchromosomal syntenic copies (Ferreira de Carvalho et al. 2019; Das Laha et al. 2020)(Fig. 5). Chromosomal segmental duplications may also contribute to the

amplification of *UNE* genes, resulting in the formation of syntenic homologous genes between non-homologous chromosomes. Ka/Ks data reveal that the *UNE* gene family has undergone purifying selection during evolution (SI Table 1). Synteny analysis between *B. napus* and its ancestral species indicates that chromosomes of the three species contain numerous syntenic blocks of the *UNE* gene family. This suggests that the *UNE* gene family in *B. napus* and its homologous genes in *B. rapa* and *B. oleracea* had established stable genomic distributions in their ancestral species, which were not subjected to large-scale rearrangements during allopolyploidization (Fig. 6).

3.6 Interpret the expression of *BnUNE12* under salt stress.

Based on BLAST results, the gene with the highest hit scores (*Bna_UNE12.5*, hereafter renamed *BnUNE12*) was selected for interpreting expression under salt stress. To investigate whether *BnUNE12* functions similarly, leaf samples were collected from *B. napus* plants treated with 300 mM NaCl at seven time points within a 24-hour period for RNA extraction and subsequent quantification of *BnUNE12* expression levels. The expression level displayed minimal variation from 0h to 1 h of salt stress, began to increase at 3 h, peaked at 6 h, decreased at 12 h but remained higher than that at 3 h, and continued to decline at 24 h to a level comparable to that at 3 h. Consequently, *BnUNE12* expression was time-dependent following NaCl treatment, with the maximal induction occurring at 6 h (Fig. 7). These results demonstrate that treatment with 300 mM NaCl induces *BnUNE12* expression, characterized by dynamic changes in expression levels over time. The 6 h time point represents the critical for peak salt stress-induced expression of this gene. Although transcript levels subsequently declined, they remained relatively elevated. Collectively, these findings indicate that a potential role for *BnUNE12* in the *B. napus* salt stress response pathway.

3.7 Preliminary functional validation of *BnUNE12* in salt tolerance

3.7.1 Construct overexpression *BnUNE12* lines

To further validate the involvement of *BnUNE12* in plant responses to salt stress, dual restriction enzyme sites (KpnI and SalI) were used to introduce the designed primers into the vector pC2306, thereby constructing *BnUNE12* overexpression lines (Fig. 8A, B). The forward primer sequence is ATGGCTAGTAACAACAACCCGC, and the reverse primer sequence is CTGTGGAGGAGGATTAGTCTCTGG. Quantification of *BnUNE12* expression levels in transgenic lines (Fig. 8C) revealed significantly elevated expression in three independent overexpression lines (*BnUNE12*-OE1, -OE2, and -OE3) compared to the wild-type (WT) J9707. The expression levels reached approximately 2.8–3.9 times that of WT. These results confirm successful generation of *BnUNE12* overexpression lines, providing essential materials for subsequent functional characterization.

3.7.2 Regulatory effects of *BnUNE12* on root length and fresh weight

Salt stress primarily impacts plants at the root level, where morphological alterations reflect stress responses. Root length and root fresh weight were quantified in WT and *BnUNE12*-OE lines pre- and post- salt treatment. Under normal conditions, *BnUNE12*-OE lines exhibited significantly reduced root length and fresh weight compared to WT (Fig. 9A, D, E), indicating that *BnUNE12* overexpression constrains root development in *B. napus*. Following 300 mM NaCl treatment, root length difference between WT and transgenic lines were eliminated. This convergence suggests severe salt-induced inhibition of root apical meristem activity and elongation zone expansion in WT plants, arresting primary root growth and suppressed lateral root development. *BnUNE12*-OE overexpression substantially mitigated these pathological manifestations. Furthermore, the significant difference ($P < 0.05$) in the fresh weight of the underground part between WT and *BnUNE12*-OE lines vanished after salt treatment. This indicated that *BnUNE12*-OE lines exhibit a certain tolerance to NaCl, and overexpression of *BnUNE12* enhances salt tolerance in *B. napus* roots.

Shoot phenotypes provided complementary evidence that NaCl treatment induced leaf yellowing and wilting across all lines, with symptoms markedly more severe in WT (Fig. 9A). Transgenic lines *BnUNE12*-OE1 and -OE3 maintained significantly higher ($P < 0.05$) shoot fresh weight than WT under stress, despite *BnUNE12*-OE2 showing reduced biomass under normal conditions (Fig. 9C). Whole-plant fresh weight analysis confirmed enhanced salt tolerance in transgenics, with no genotype difference under control conditions but significantly greater ($P < 0.05$) biomass in *BnUNE12*-OE lines following salt stress (Fig. 9B). These phenotypic analyses demonstrate that *BnUNE12* overexpression confers NaCl tolerance in *B. napus*. Notably, the observed root growth reduction and biomass decrease under normal conditions reflect a trade-off between growth and stress resistance. Analysis of CAREs revealed that the promoter region of *BnUNE12* harbors abundant growth- and development-related elements as well as stress-responsive elements, collectively underscoring its pivotal role in mediating plant growth, development, and stress responses. Overexpression of *BnUNE12*—characterized by a significant reduction in root length and a concomitant decrease in plant biomass—reflects a prioritization of stress resistance over growth in balancing these two physiological processes. This phenotypic observation, integrated with functional insights derived from CAREs analysis, indicates that utilizing the *UNE12* gene for crop stress resistance breeding requires careful consideration of its dual impacts on growth performance

and stress tolerance. The ultimate objective is to achieve a dynamic equilibrium between these traits through optimization of gene expression regulatory strategies.

3.7.3 *BnUNE12*-mediated protection of membrane integrity

Unsaturated fatty acids within cellular membrane systems incur lipid peroxidation upon exposure to stimuli such as oxidative stress, whereas malondialdehyde (MDA)—a key end product of lipid peroxidation—displays a positive correlation between its production levels and the extent of lipid peroxidation (Tsikas 2017). Upon membrane system damage, lipid peroxidation is exacerbated, leading to the excessive production of MDA. Consequently, MDA content quantification can indirectly reflect the extent of lipid peroxidation-induced damage in membrane systems and further assess impairments to membrane structure and function. Under normal growth conditions, MDA levels in WT plants were ~56.7% higher than in *BnUNE12*-OE1 and ~87.3% higher than in *BnUNE12*-OE3, with no significant difference relative to *BnUNE12*-OE2 (Fig. 10A). The highest MDA levels in WT indicated a relatively elevated lipid peroxidation status in J9707 cells under basal physiological conditions, likely due to inherent genetic traits. In contrast, MDA levels in *BnUNE12*-OE1 and *BnUNE12*-OE3 were significantly lower than in WT. Following exposure to 300 mM NaCl, MDA levels increased across all lines: WT exhibited a 249% increase, while *BnUNE12*-OE1, *BnUNE12*-OE2, and *BnUNE12*-OE3 showed increases of ~145%, 77.5%, and ~148.7%, respectively (Fig. 10A). The drastic elevation of MDA in WT upon salt stress indicated that salt stress severely disrupted intracellular redox homeostasis, triggering extensive lipid peroxidation. Although NaCl stress induced membrane damage in *BnUNE12*-overexpressing lines, the impairment was significantly attenuated ($P < 0.001$) relative to WT. These results demonstrate that *BnUNE12* overexpression enhances cellular tolerance to salt stress by mitigating membrane lipid peroxidation in *B. napus*. This protective mechanism may be evolutionarily conserved with *AtUNE12* in *A. thaliana*, which alleviates salt stress through regulating ROS-related gene expression. Further investigation is warranted to characterize the interaction between *BnUNE12* and its target gene promoters.

3.7.4 Role of *BnUNE12* in regulating proline accumulation

Salt stress disrupts the osmotic homeostasis between the intracellular and extracellular compartments in plant cells, impairing physiological processes. As a key cellular osmolyte, proline mitigates salt-induced damage. When WT and *BnUNE12*-OE plants were cultivated under control conditions, the proline content in WT was marginally higher than that in *BnUNE12*-OE lines, though this difference did not reach statistical significance (Fig. 10B). Following 300 mM NaCl treatment, both genotypes displayed characteristic accumulation in response to abiotic stress. However, significant variations emerged in both accumulation magnitude and final proline concentrations: salt-stressed WT accumulates approximately 1.69- to 1.87-fold more proline than *BnUNE12*-OE lines (Fig. 10B). While proline accumulation facilitates the modulation of osmotic potential in plants, its magnitude depends on stress intensity and duration, with higher levels not necessarily indicating superior tolerance. The elevated proline in WT may reflect compensatory biosynthesis to counteract severe cellular damage, whereas the significantly reduced accumulation in *BnUNE12*-OE lines suggests alternative protective mechanisms. This pattern indicates that *BnUNE12* overexpression alleviates salt stress through regulatory pathways that reduce dependence on massive proline accumulation for osmotic homeostasis.

3.7.5 Expression Analysis of *BnUNE12*-OE and ROS-related Genes

To identify the potential targets of *BnUNE12* regulation, two canonical ROS-associated genes, Superoxide Dismutase 1 (*SOD1*) and Catalase 2 (*CAT2*), were selected for expression analysis. Under control conditions, *SOD1* expression remained low across all genotypes, with no significant differences observed despite marginally higher levels in *BnUNE12*-OE1 (Fig. 11A). Following exposure to 300 mM NaCl, *SOD1* expression was upregulated in all lines. Notably, the relative *SOD1* expression in *BnUNE12*-OE3 was significantly higher than that in the J9707 ($P < 0.05$), while *BnUNE12*-OE1 showed elevated but non-significant induction. This salt-dependent modulation indicates *BnUNE12* influences *SOD1* expression, with particularly pronounced effect in *BnUNE12*-OE3. For *CAT2*, basal expression was uniformly low across genotypes under normal conditions (Fig. 11B). Salt stress significantly induced *CAT2* expression in both WT and *BnUNE12*-OE lines. Although no statistically significant inter-genotypic differences were detected despite subtle variations among individual lines. These findings suggest that *SOD1* and *CAT2* are likely not primary transcriptional targets of *BnUNE12*. Their regulation may involve cooperative interactions with other transcription factors (e.g., MYB family proteins). Future investigation of *BnUNE12*'s interplay with additional ROS-responsive genes is warranted to elucidate its precise mechanisms in mitigating membrane damage.

4. Discussion

The bHLH transcription factor superfamily represents the most structurally diverse class of regulatory proteins, characterized by a conserved bHLH domain that enables homo/heterodimerization and sequence-specific binding to E-box motifs (CANNTG) and other

CAREs, allowing spatiotemporal control of gene expression profiles critical for developmental patterning and stress adaptation (Michael et al. 2023; Schneider et al. 2023; Ning et al. 2025). The extraordinary diversity within the bHLH family enables its members to orchestrate a vast array of biological processes. In plants, this structurally versatile family has expanded into hundreds of members, forming intricate transcriptional regulatory networks—such as the canonical MYB-bHLH complex—that coordinate cellular differentiation and environmental responses (Qian et al. 2021). Leveraging this regulatory complexity, bHLH has emerged as key targets for improving stress resilience and optimize agronomic traits through precision breeding. Soil salinization impairs soil aeration and elevates osmotic potential, which in turn inhibits root growth, reduces the capacity of plants to absorb water and nutrients, and may even lead to physiological drought and subsequent wilting or death due to excessive osmotic stress (Munns et al. 2020; Abdalla et al. 2022; Liu et al. 2023). Soil salinization-induced accumulation of high-concentration salt ions can be taken up by plants, perturbing cellular metabolic homeostasis, inhibiting growth, and reducing crop yield and quality (Singh et al. 2022; Zhou et al. 2024). These constraints significantly challenge breeding by increasing costs and hindering sustainable agriculture. The bHLH family is integral to the orchestration of salt-stress tolerance in plants. A bHLH, designated *NtbHLH123*, occupies a critical cis-regulatory position immediately upstream of the respiratory burst oxidase homolog (Rboh) NtRbohE in *Nicotiana tabacum*, acting as a molecular rheostat to modulates the Rboh-dependent signaling circuitry that underpins systemic salinity responses, ultimately potentiating cellular resilience and organismal fitness under high-salt conditions (Liu et al. 2021), *CabHLH035* integrates ionic homeostasis and proline metabolism in *Capsicum annuum* (Zhang et al. 2022), *AhbHLH21* directly occupies canonical G/E-box motifs within the promoters of *AhPOD*, *AhCAT*, and *AhSOD*, transcriptionally amplifying the antioxidant enzymatic repertoire and potentiating cellular redox buffering in *Arachis hypogaea* under salt stress (Zhao et al. 2024). Despite these advances, most bHLH members remain functionally uncharacterized due to the superfamily's extraordinary and diversity. Within the bHLH superfamily, the *UNE* clade has emerged as a pivotal regulator of both developmental programming and environmental adaptation. Functional studies of *AtUNE12* and *Populus alba* × *Populus glandulosa* *PagUNE12* revealing distinct molecular paradigms, *AtUNE12* undergoes stimulus-dependent phosphorylation, enabling its high-affinity binding to G-box (CACGTG) and LTR15 (CCGAC) cis-motifs, activating a suite of ion-transport genes that restrict Na⁺ influx while sustaining K⁺ homeostasis, concomitantly, *AtUNE12* directly up-regulates *AtMYB61*, a key determinant of stomatal aperture, thereby reducing transpirational water loss and consolidating salt tolerance (He et al. 2022). During secondary growth, *PagUNE12* orchestrates lignin polymerization by trans-activating laccase genes *LAC40* and *LAC49* together with class III peroxidases *PER3* and *PER6*, promoting guaiacyl-type lignin deposition, thereby dictating the mechanical architecture and physicochemical properties of wood. (Zhao et al. 2013; Song et al. 2023).

B. napus holds a pivotal position in global food security and agricultural development, but its growth and yield are increasingly constrained by abiotic stresses, particularly soil salinity. Phylogenomic analysis positioned *BnUNE* paralogues as sister lineages to orthologs in progenitor species *B. rapa* and *B. oleracea*, consistent with *B. napus*' allopolyploid origin. Topological relationships indicate lineage-specific structural rearrangements and functional specialization following speciation, with retention of ancestral functions alongside species-specific innovations. These findings not only illuminate the evolutionary history of *UNE* genes during *Brassica* divergence but also underscore the imperative of functionally dissecting this family in *B. napus*. Integrative analyses of the *UNE* gene family reveal pronounced diversification and intricate regulatory circuitry. The family resolves into six well-supported subclades (I–VI) that differ markedly in motif assemblages and intronic landscapes. Subclade I members exhibit intron insertions that engender pronounced structural variants. PSLP indicate a peroxisomal bias for several subclade I proteins, implying that these splice variants may modulate ROS metabolism and detoxification (Corpas 2019). Promoter scanning further expands our understanding of the *UNE* transcriptional network. An abundance of light-responsive elements (e.g., G-box, I-box) suggests that *UNE* genes are deeply integrated into photosynthetic regulation, facilitating efficient solar-energy capture and its subsequent conversion into storage lipids in *B. napus*. Stress-responsive motifs, including (LTR) and ABRE elements, implicate *UNE* genes in the mitigation of salinity, cold and other abiotic constraints. The presence of ABRE motifs, hallmarks of ABA-dependent signaling, points to enhanced osmotic adjustment and antioxidant capacity (Fujita et al. 2005; Yu et al. 2020). Additionally, hormone-responsive elements such as MeJA-responsiveness and GRE motifs reveal that *UNE* genes are capable of integrating jasmonate, auxin and other phytohormonal pathways to balance defense responses with developmental programs. Collectively, the convergence of multiple signaling cascades within *UNE* promoters underscores a sophisticated regulatory architecture that enables *B. napus* to simultaneously optimize growth and withstand diverse environmental challenges. *BnUNE12* exhibited the highest sequence similarity to *AtUNE12*, therefore hypothesized to perform comparable salinity-tolerance. Time-course quantitative PCR revealed that *BnUNE12* may trigger a resource reallocation in which growth is temporarily sacrificed to fortify protective mechanisms, thereby enhancing salinity tolerance. Salt stress perturbs cellular redox homeostasis, resulting in the excessive accumulation of ROS that subsequently compromise membrane integrity and cellular function (Hasanuzzaman et al. 2021; Wani et al. 2021). Moreover, salinity disrupts cellular osmotic equilibrium, thereby impairing metabolic fluxes and physiological processes, cells accumulate large amounts of the compatible solute proline to re-establish homeostasis, which enhances water uptake from the apoplast and sustains turgor pressure, preserving cellular integrity and normal function under hyperosmotic stress (de Freitas et al. 2019; Hosseinifard et al. 2022). Accordingly, the relationship between *BnUNE12* and salinity tolerance can be interrogated through two

complementary physiological lenses: membrane integrity and osmolyte accumulation. Under non-stress conditions, transgenic lines over-expressing *BnUNE12* consistently exhibit lower MDA contents than WT plants, indicating that *BnUNE12* attenuates basal lipid peroxidation and fortifies membrane stability even in the absence of external stress. Following exposure to salt stress, MDA levels rise in all genotypes. The WT registers the sharpest increase, whereas *BnUNE12*-overexpressing lines show a significantly moderated increment ($P<0.001$). This provides compelling evidence that *BnUNE12* mitigates salt-induced membrane oxidative damage, thereby preserving cellular integrity under hyperosmotic challenge. Salt stress experiments demonstrated that *BnUNE12*-overexpressing lines significantly reduced ($P<0.001$) proline accumulation, which indirectly reflects that salt stress was notably alleviated ($P<0.001$). In the current study, no significant differences were observed in the expression levels of ROS-associated genes *SOD1* and *CAT2* between WT and *BnUNE12*-OE lines following salt treatment, implying that the impact of *BnUNE12* overexpression on *SOD1* and *CAT2* expression may involve intricate intrinsic mechanisms. A partial regulatory network involving *UNE* genes and the potential regulatory mechanism by which *BnUNE12* alleviates salt stress, which vividly delineates the rationale underlying this study (Fig. 12). Numerous CAREs distributed in the upstream regions of promoters within the *UNE* gene family are subject to modulation by hormone and environment, while sub-clade I members potentially mitigate ROS accumulation which can induce excessive peroxidation of membranes, impairing its barrier integrity, and in turn trigger the SOS signaling as well as elicit the de novo synthesis of large amounts of proline to maintain osmotic homeostasis, ultimately enhancing cellular tolerance to salt stress (Mahajan et al. 2008). Rboh, belonging to the NADPH oxidase family, function in concert with the ROS scavenging system to regulate ROS homeostasis and thus attenuate oxidative damage (Chapman et al. 2019). Among these, RbohE is predominantly expressed in plant roots, and *AtUNE12* has been demonstrated to enhance plant salt tolerance through the RbohE-mediated signaling, a mechanism supported by phylogenetic analyses revealing a high degree of structural conservation between *BnUNE12* and *AtUNE12* and by overexpression assays further corroborating their functional orthology, collectively suggesting that the RbohE signaling represents one of the conserved mechanisms by which *UNE12* confers salt stress alleviation. The ABA regulatory network, which is indispensable for plant adaptive responses to environmental stresses, not only activates the Rboh pathway and modulates stomatal dynamics to mitigate salt stress but also facilitates the transcription of target genes (e.g., *UNE*) via CAREs, thereby orchestrating multiple regulatory cascades that collectively counteract salt stress.

5. Conclusion

This study characterized the *UNE* gene family in *B. napus*. Through in-depth bioinformatic analyses, 21 *BnUNE* genes were identified from the *B. napus* genome, which exhibit a close evolutionary relationship with their orthologs in the ancestral species *B. rapa* and *B. oleracea*, displaying a pattern of parallel evolution. During speciation, these genes underwent structural variations and functional specialization, retaining core functionalities while acquiring species-specific adaptations. The *UNE* family was classified into 6 subclades, showing significant divergence in motif composition, intron distribution, and subcellular localization; notably, certain members of subclade I, due to unique intron insertion events, tend to localize in peroxisomes, implying their potential critical roles in ROS metabolism and detoxification. The *BnUNE12* gene was found to be significantly upregulated under salt stress; overexpression lines of *BnUNE12*, when subjected to 300 mM NaCl treatment, exhibited superior performance compared to controls in biomass traits (root length, fresh weight), membrane lipid peroxidation, proline accumulation, and expression of antioxidant-related genes. These findings demonstrate that *BnUNE12* enhances salt tolerance in *B. napus* by precisely modulating osmotic adjustment and oxidative stress responses, providing valuable genetic resources and a robust theoretical foundation for improving salt tolerance in *B. napus* and advancing stress-resistant molecular breeding. Future investigations into other *UNE* genes and integrated multi-omics approaches will further elucidate the role of this gene family in *B. napus* growth, development, and stress responses, contributing to the sustainable development of the rapeseed industry.

Declarations

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human participants performed by any of the authors.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, X.Z. and L.L.; methodology, F.X. and L.L.; software, F.X. and X.Z.; validation, X.Z., F.X. and Y.Z.; formal analysis, F.X. and Y.Z.; investigation, X.Z., F.X., Y.Z. and S.W. ; resources, L.L. and S.H.; data curation, Y.Z.; writing—original draft preparation, X.Z. and F.X.; writing—review and editing, L.L. and R.H.; visualization, X.Z. and S.H.; supervision, L.L.; project administration, X.Z. and S.H.; funding acquisition, X.Z. L.L. and S.H. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

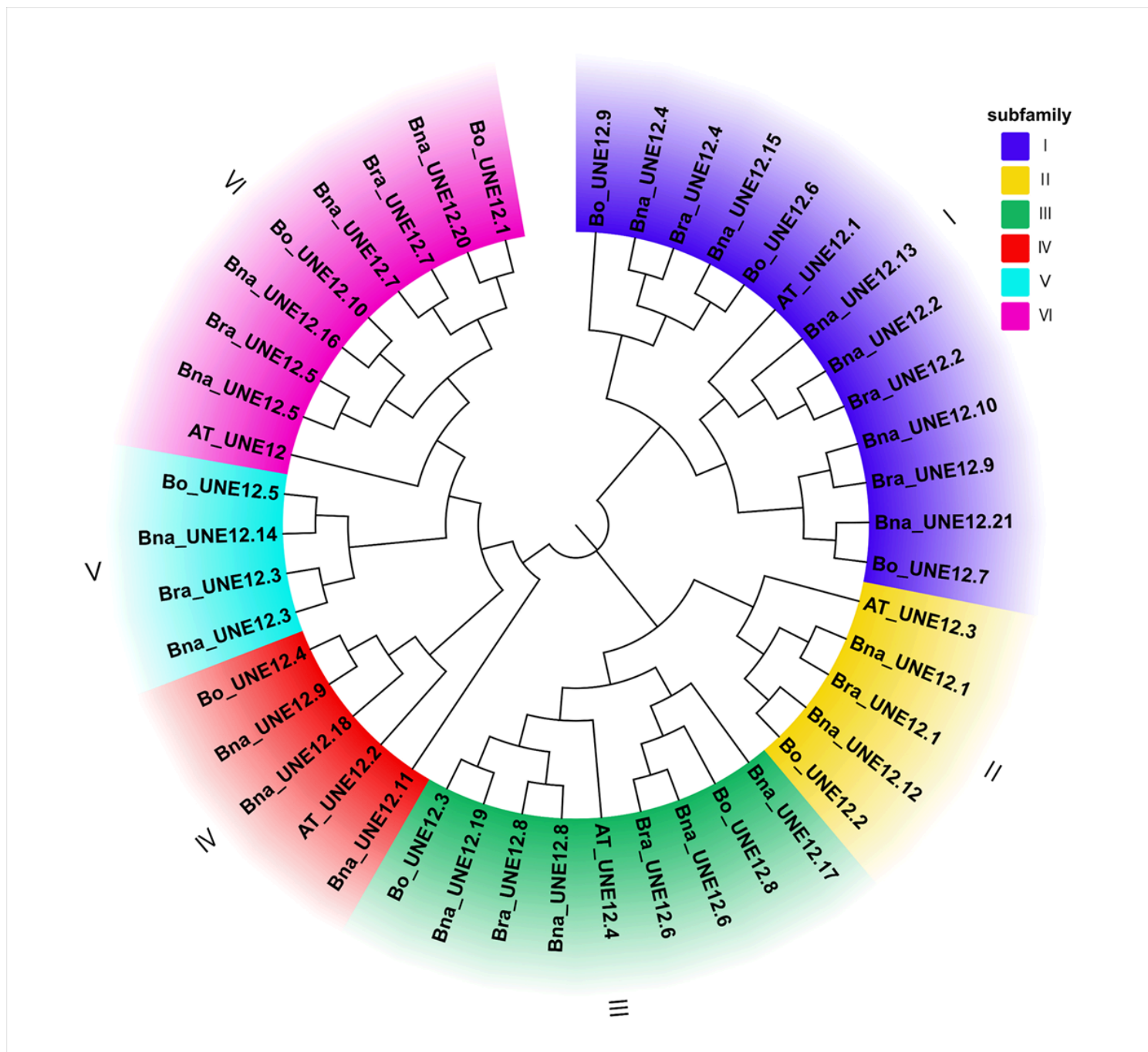


Figure 1

Phylogenetic tree of *A. thaliana*, *B. napus*, and its ancestral species. The four species were clustered into 6 subclades, each of which was assigned a distinct color for differentiation: subclade I (blue), subclade II (yellow), subclade III (green), subclade IV (red), subclade V (cyan), and subclade VI (purple).

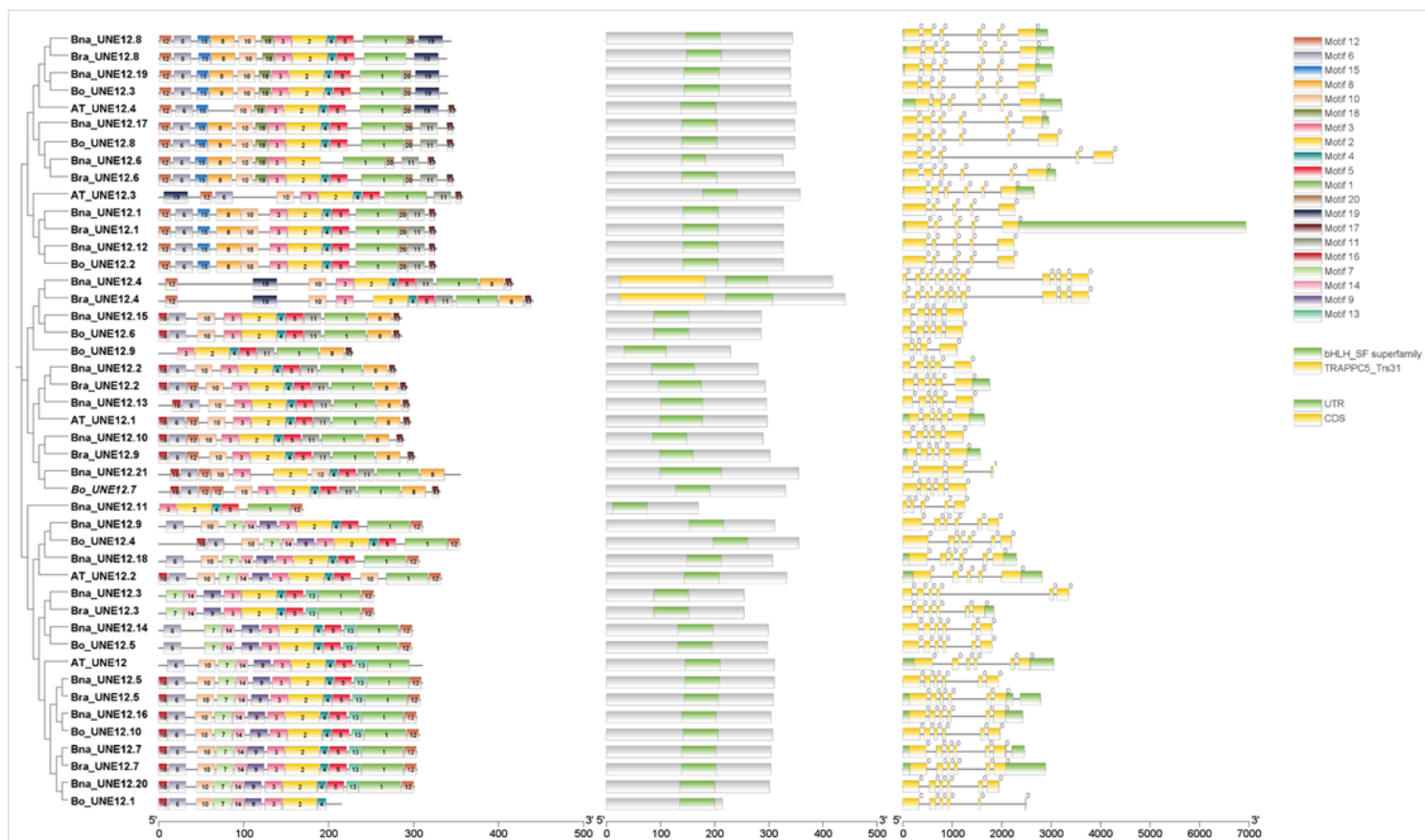


Figure 2

Integrated visualization of the phylogenetic tree, conserved motifs, protein domains, and gene structures of *A. thaliana*, *B. napus*, and its ancestral species. The right-hand legend is partitioned into three sections: the uppermost section differentiates conserved motifs of the *UNE* family; the middle section denotes distinct domains of the family's proteins; the lowermost section represents discrete components of the family's gene structures



Figure 3

CAREs of the *UNE* family in *B. napus*. Each tile denotes the 20 bp region at the C-terminus of the CARE, serving to indicate the termination position of the element; Tile colors are used to differentiate between distinct CAREs; Definitions of elements are as follows: ARE: Auxin responsive element; LTR: Low-temperature responsiveness; SARE: Salicylic acid responsive element; ABRE: Absciscic acid responsive element; GRE: Gibberellin responsive element; MRE: MYB binding site involved in light responsiveness; MBS: MYB binding site associated with drought inducibility. MBSI: MYB binding site involved in flavonoid biosynthetic genes regulation

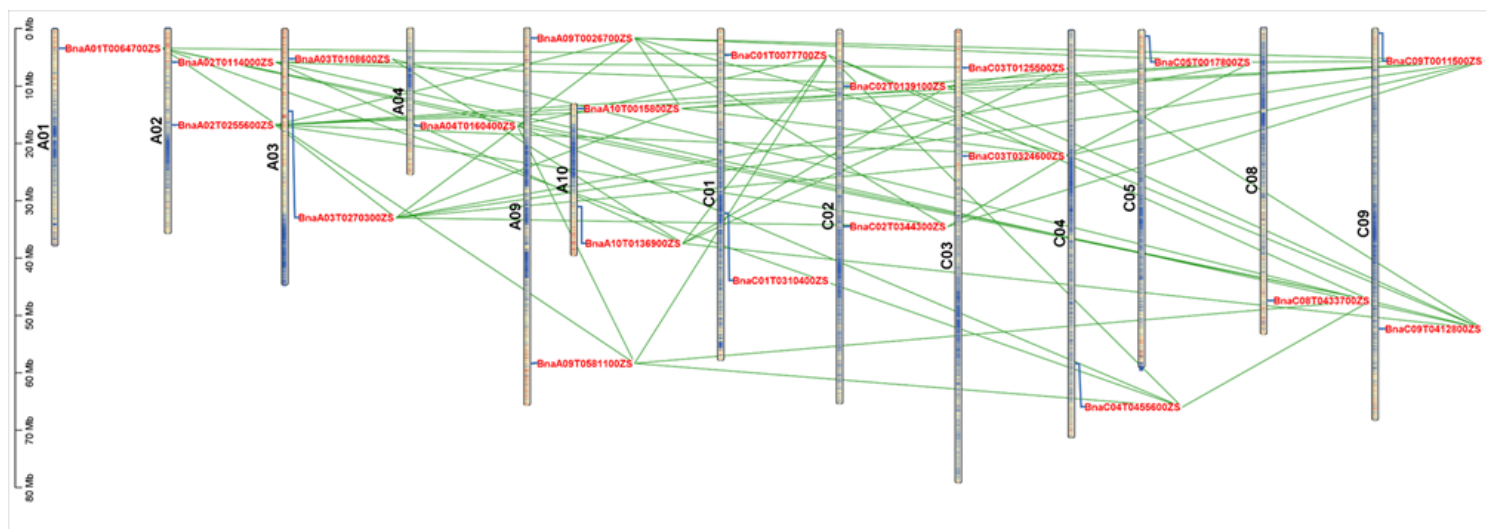


Figure 4

Chromosomal localization and syntenic genes of *BnUNE* family. *UNE* genes are not present in all chromosomes of *B. napus*; furthermore, these genes exclusively exhibit segmental duplications, with no tandem duplications observed

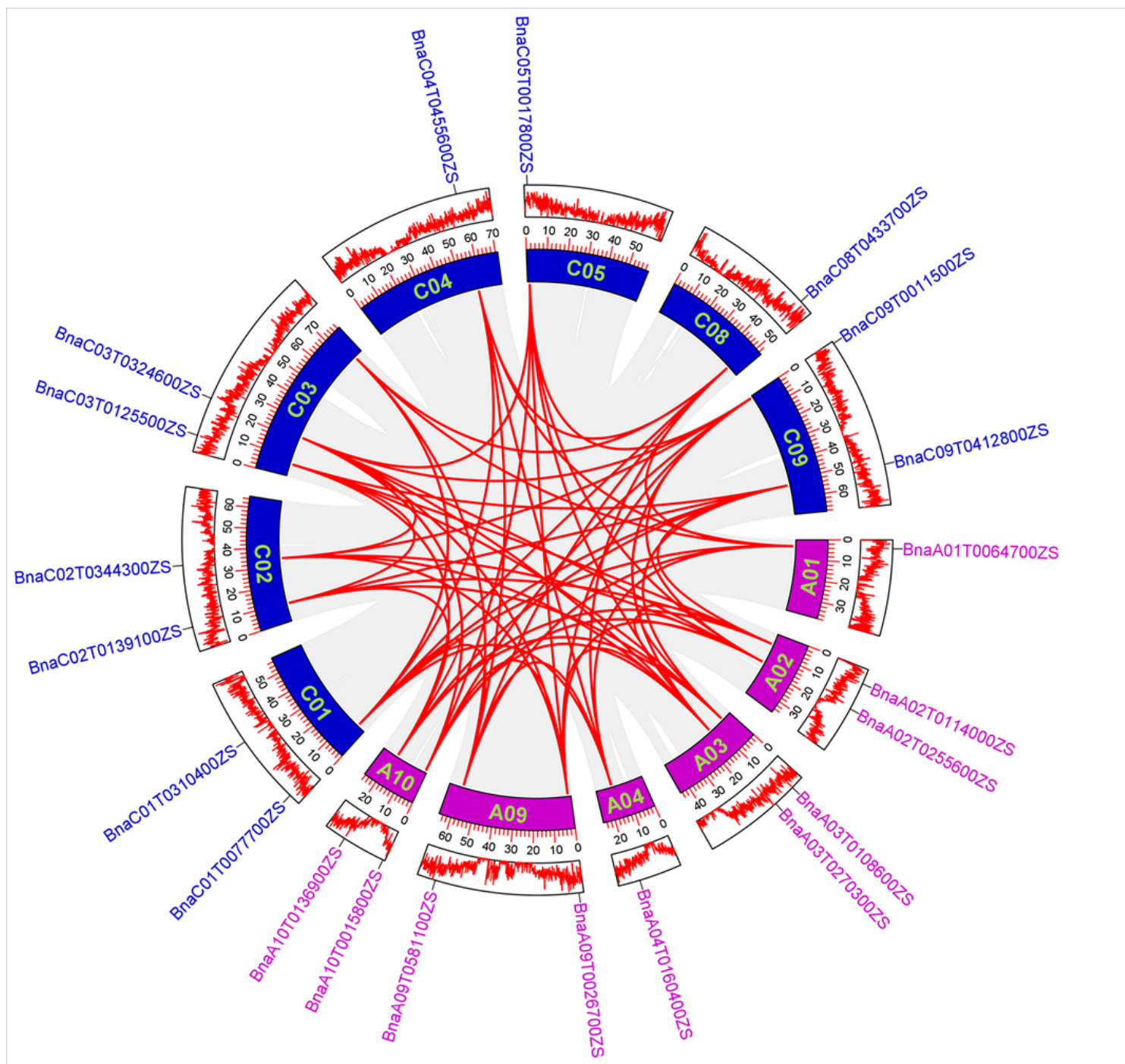


Figure 5

Intraspecific synteny analysis of *B. napus*. A01–A10 denote chromosome designations of the A subgenome, and C01–C09 denote those of the C subgenome. Red wavy lines represent gene density of the corresponding chromosomes, with lower gene density observed closer to the inner circle. Colors of gene IDs correspond to those of chromosomal blocks: purple represents the A subgenome, and blue indicates the C subgenome

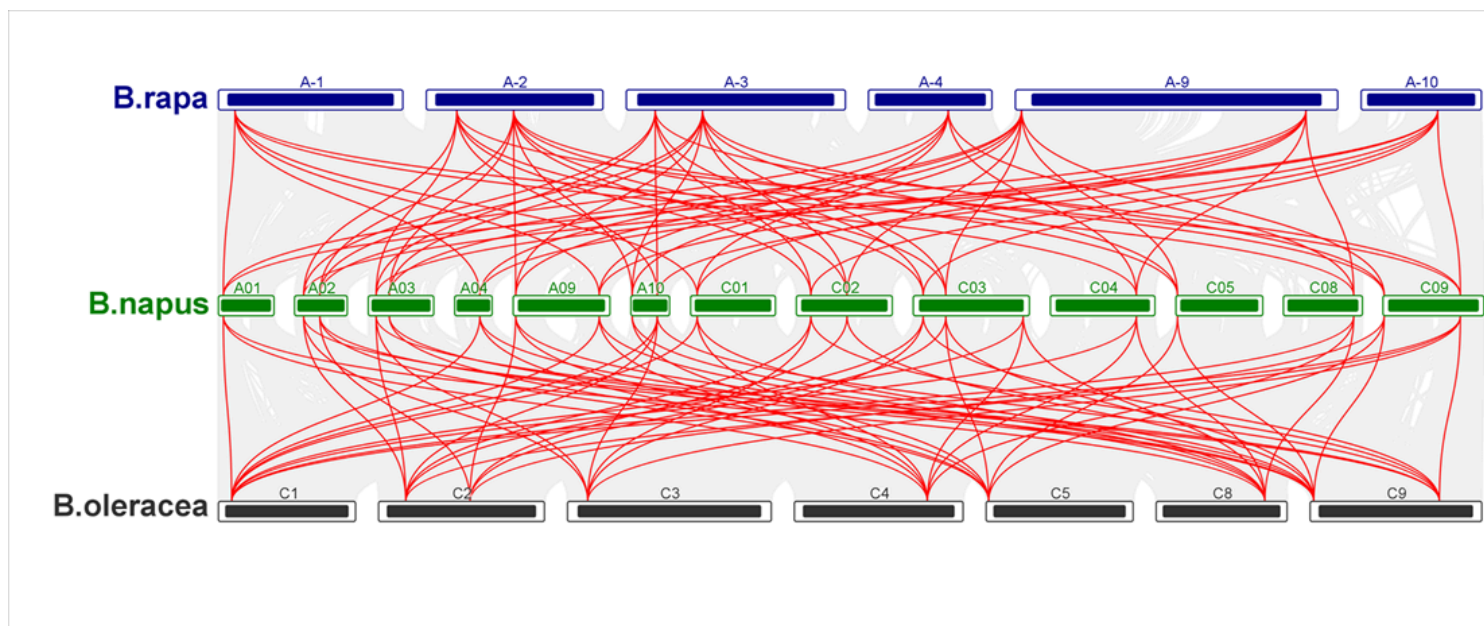


Figure 6

Chromosomal synteny analysis between *B. napus* and its ancestral species. Red lines represent syntenic gene pairs; *B. rapa* chromosomes are depicted in blue, *B. napus* chromosomes in green, and *B. oleracea* chromosomes in black

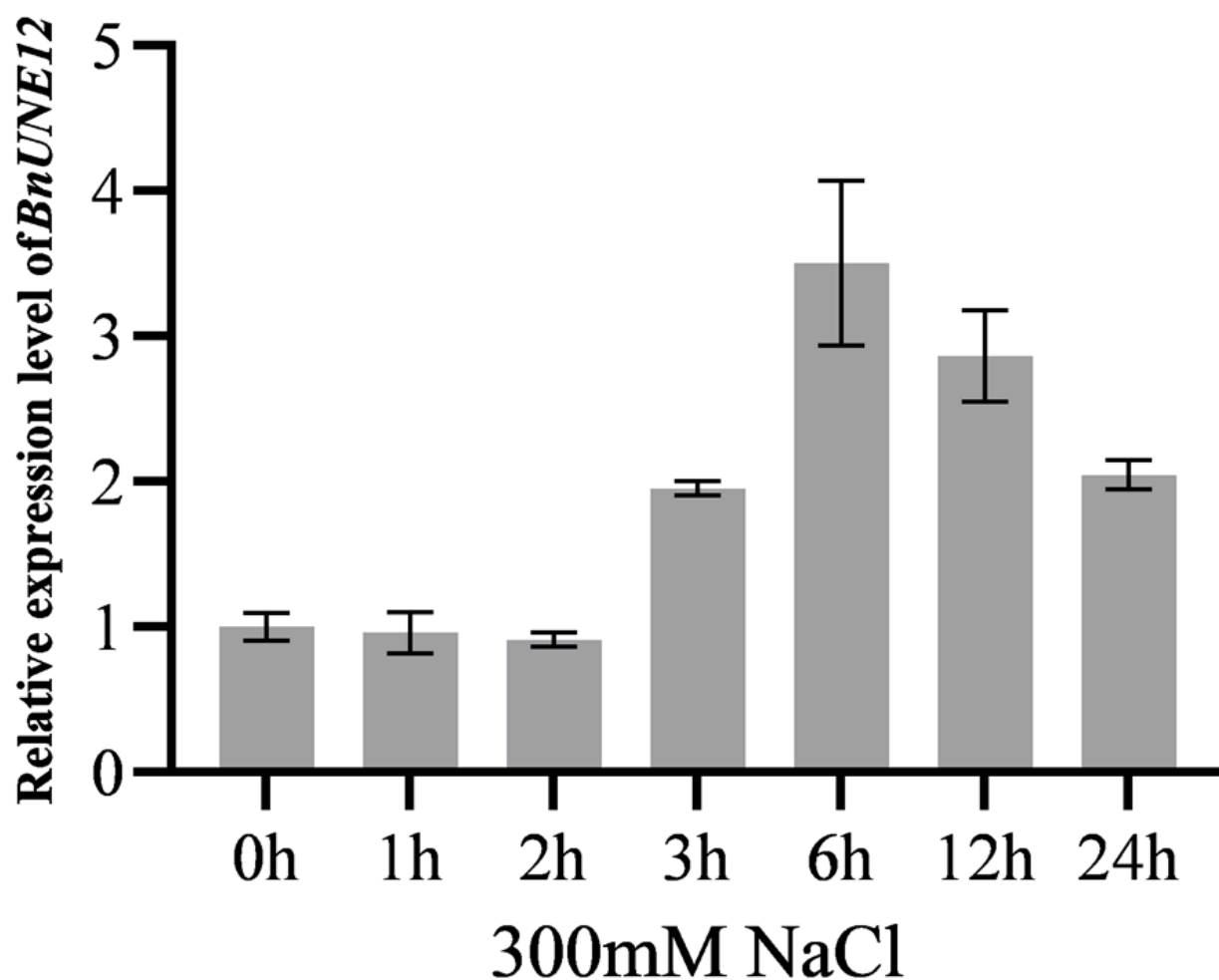


Figure 7

Time-course *BnUNE12* expression level in *B. napus* under salt stress

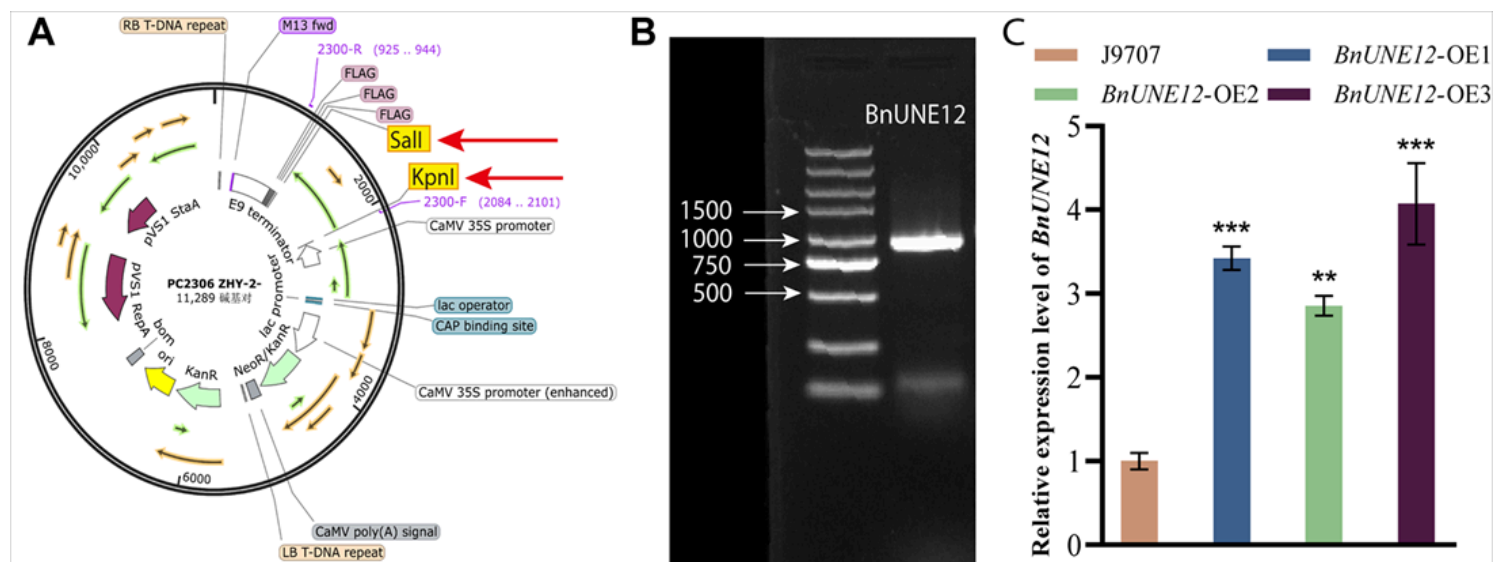


Figure 8

Construction of *BnUNE12* overexpression vector. A: pCAMBIA2306 vector map and electrophoretic map of restriction site; B: *BnUNE12* gene clone; C: The overexpression effect of *BnUNE12*-OE. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; unlabeled * indicates no significant difference, $P > 0.05$

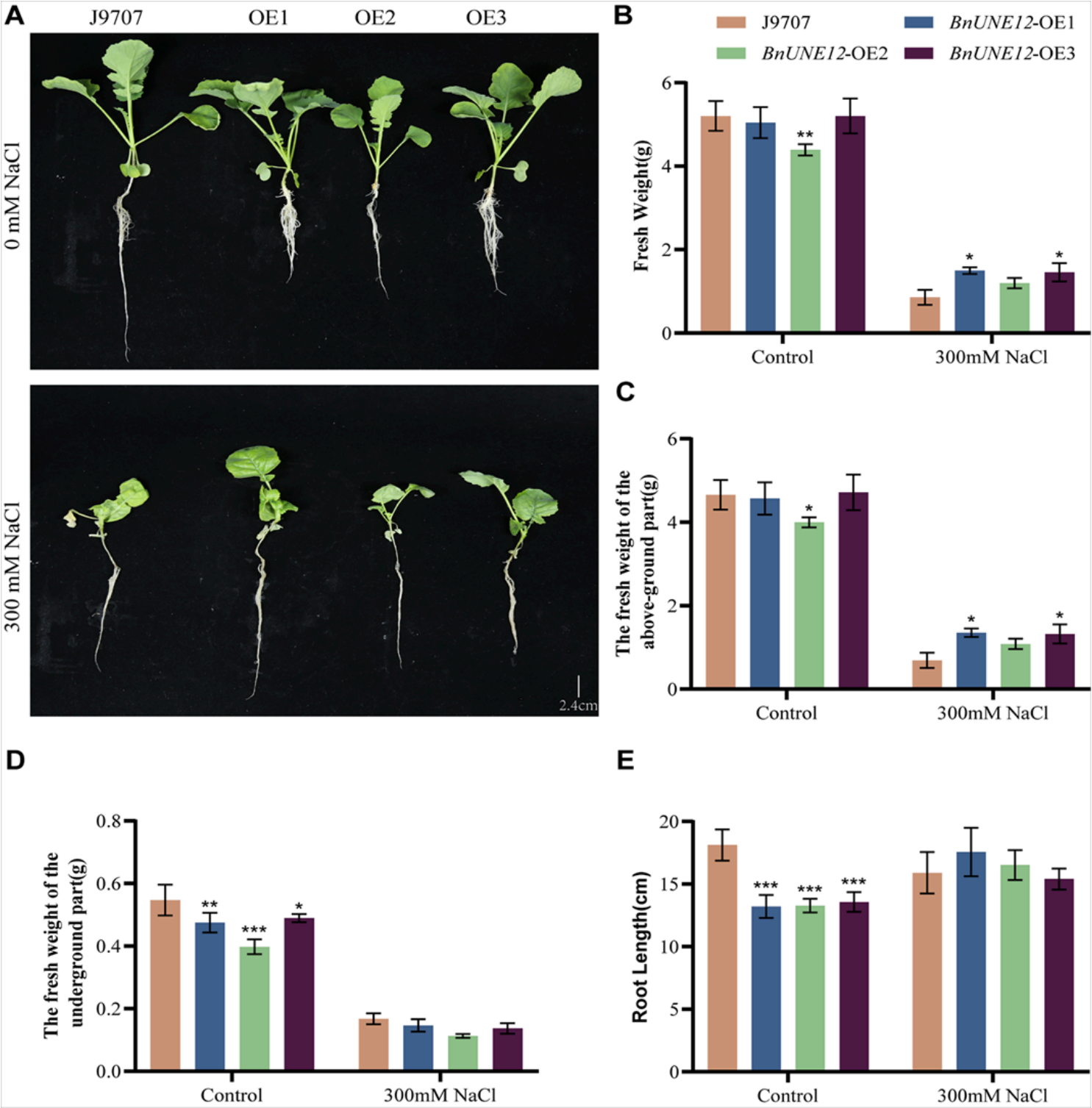


Figure 9

Overexpression of *BnUNE12* enhanced salt tolerance in *B. napus*. A: Phenotypic comparison between WT and *BnUNE12*-overexpressing lines under salt stress conditions; B: Total fresh weight of WT and *BnUNE12*-OE lines; C: Shoot fresh weight of WT and *BnUNE12*-OE lines; D: Root fresh weight of WT and *BnUNE12*-OE lines; E: Root length of WT and *BnUNE12*-OE lines. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; unlabeled * indicates no significant difference, $P > 0.05$

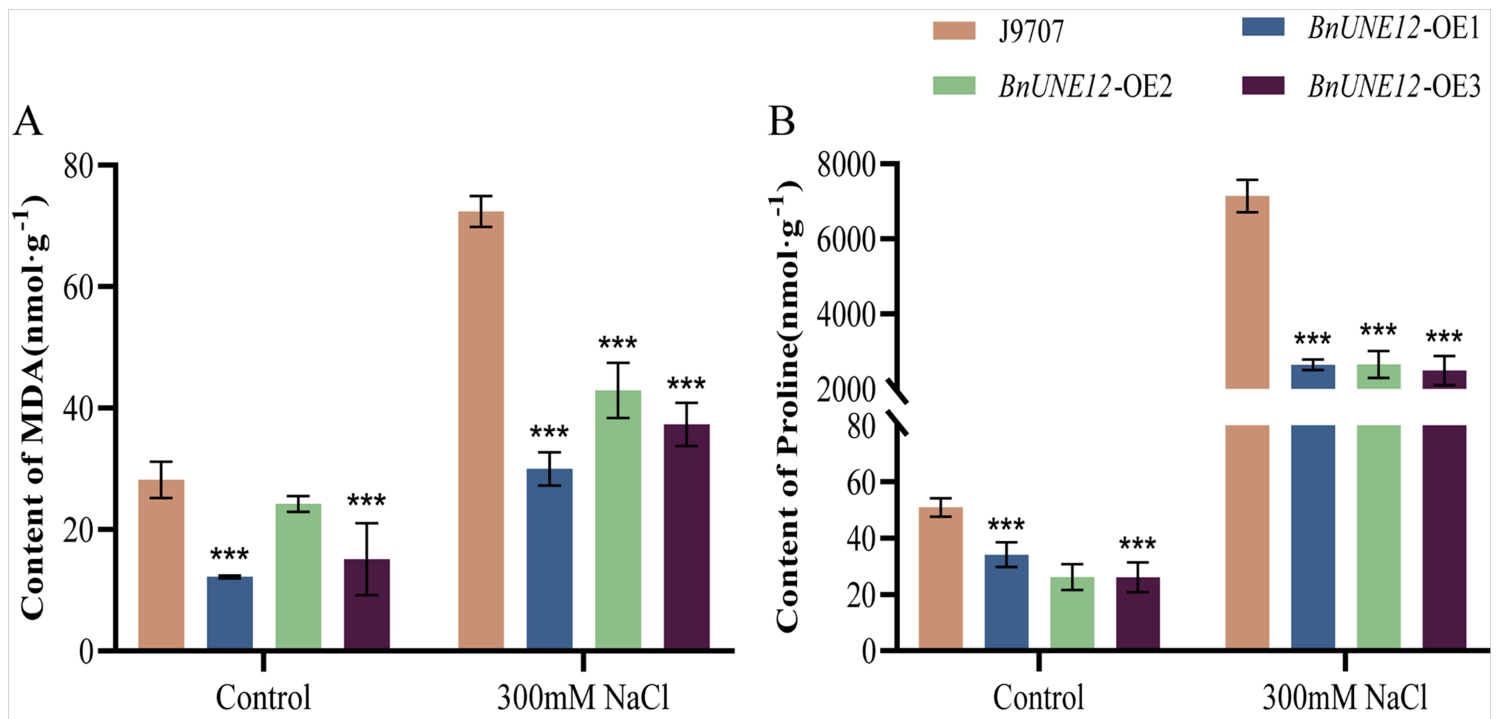


Figure 10

Organic osmotic regulation of *BnUNE12*-OE lines under salt stress. A: The effect of *BnUNE12* on MDA content under salt stress; B: The effect of *BnUNE12* on proline content under salt stress. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; unlabeled * indicates no significant difference, $P > 0.05$

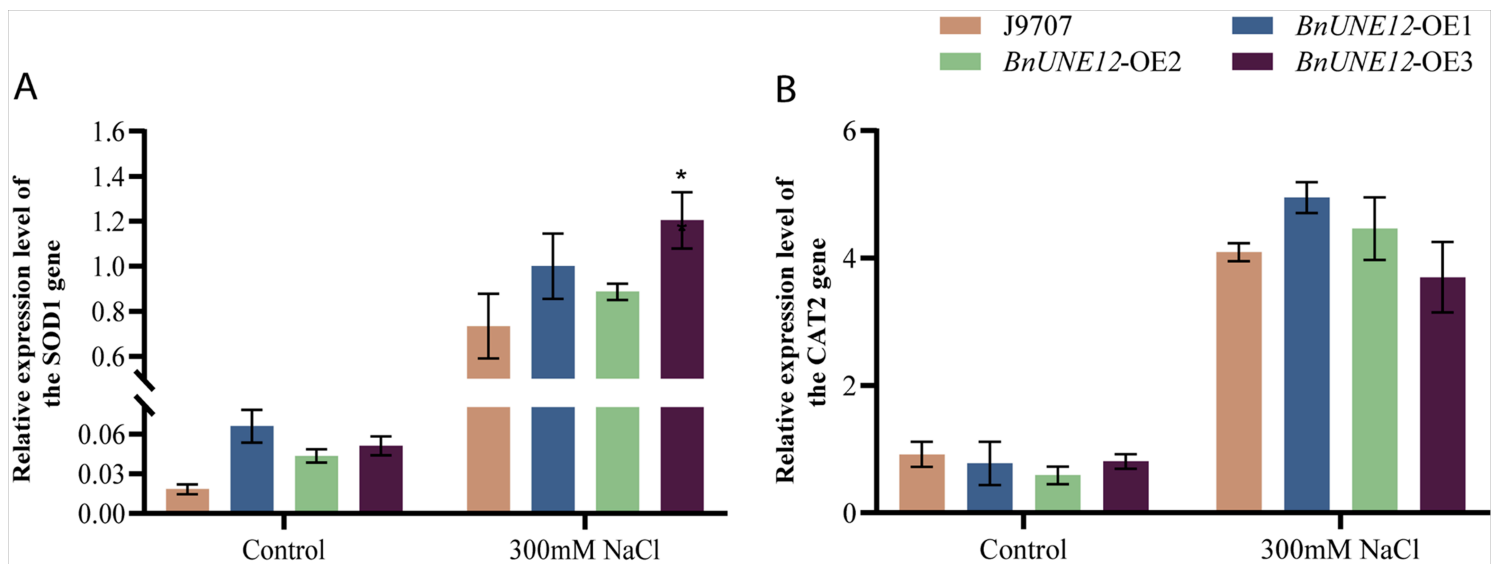


Figure 11

ROS-related genes expression level in *BnUNE12*-OE line. A: Relative expression level of *SOD1* in WT and *BnUNE12*-OE; B: Relative expression level of *CAT2* in WT and *BnUNE12*-OE. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; unlabeled * indicates no significant difference, $P > 0.05$

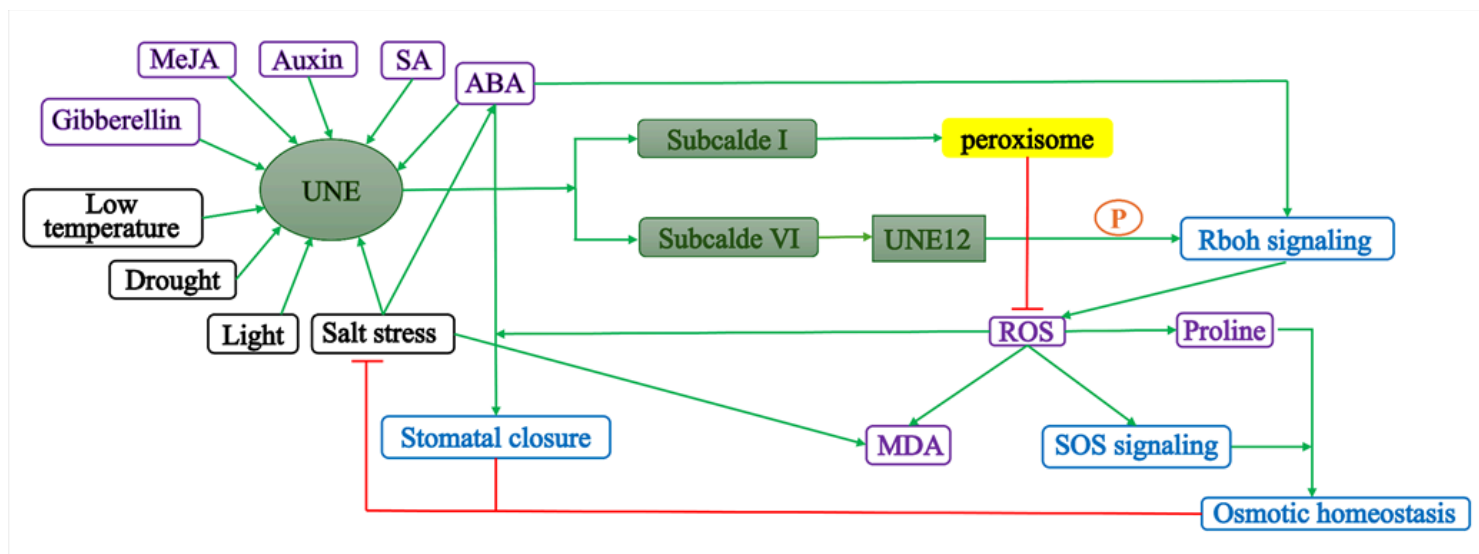


Figure 12

Schematic illustration of factors regulating the *UNE* family and potential mechanisms by which this family participates in salt stress responses. Dark green blocks indicate the *UNE* family; green arrows denote promotion or progression to the next step; red lines signify inhibition of the subsequent process; purple boxes refer to specific intracellular substances; black boxes correspond to exogenous signals perceived by cells; blue boxes represent intracellular physiological processes; the letter "P" in the orange ellipse stands for phosphorylation; and peroxisomes are indicated by yellow boxes (Mahajan et al. 2008; Tsikas 2017; Chapman et al. 2019; Postiglione and Muday 2020; Liu et al. 2021; He et al. 2022; Sharipova et al. 2022; Zhang et al. 2022; Zhao et al. 2024)