

RESEARCH

Open Access



Genome-wide identification and analysis of the *Delay Of Germination 1 (DOG1)* gene family in *Brassica napus* and its potential role in Manganese (Mn) stress response

Yan Hu^{1,2*}, Hui Ling^{1,2}, Xinyue Song^{1,2} and Weishe Hu³

Abstract

Background Manganese (Mn), a heavy metal, induces oxidative stress when present in excess, thereby inhibiting plant growth. The *Delay of Germination 1 (DOG1)* gene family plays a crucial role in seed dormancy and germination. However, the genome-wide organization and functional roles of the *DOG1* gene family under Mn stress remain uncharacterized in *Brassica napus*.

Results In this study, we identified 42 *BnDOG1* family members and elucidated their regulatory roles under Mn stress using whole-genome and differential transcriptomic analyses. The *BnDOG1* genes were evenly distributed across 16 chromosome scaffolds, and all members contained the conserved DOG1 domain. Phylogenetic analysis classified the *BnDOG1* genes into five subfamilies. Covariance analysis indicated that segmental duplication was the primary mechanism driving gene expansion. Gene Ontology (GO) enrichment analysis revealed the highest enrichment in biological process categories. Notably, *BnDOG1* genes were induced by Mn stress.

Conclusions In this study, we demonstrate that the *BnDOG1* gene plays a critical role in mediating physiological responses to environmental stress, thereby establishing a foundation for the development of heavy metal-tolerant crops.

Keywords *Brassica napus*, Manganese, *BnDOG1* genes, Transcriptome sequencing, Expression patterns

Background

Brassica napus is a globally important oilseed crop, and breeding high-quality varieties is essential. Identifying key genes and favorable genetic variations will accelerate varietal improvement [1]. Although abiotic stresses such as drought and lead induce cellular damage in *B. napus* [2, 3], the species exhibits strong tolerance to salinity and cadmium [4, 5]. Interestingly, while drought stress impairs maternal plants, it enhances the vigor of their offspring [3].

The *DELAY OF GERMINATION 1 (DOG1)* gene plays a critical role in seed dormancy adaptation to diverse

*Correspondence:

Yan Hu
ynwcyh@126.com

¹College of Agriculture and Biotechnology, Hunan University of Humanities, Science and Technology, Loudi, Hunan 417000, China

²Key Laboratory of Development, Utilization, Quality and Safety Control of Characteristic Agricultural Resources in Central Hunan Province, Loudi, Hunan 417000, China

³College of Agriculture and Biology, Liaocheng University, Liaocheng, Shandong 252059, China



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

environments. *DOG1* suppresses gibberellin (GA)-regulated gene expression and modulates endosperm properties [6, 7]. Moreover, the expression levels of *DOG1* regulators correlate with altitude, temperature, and climate [8, 9]. As a central dormancy regulator, *DOG1* likely interacts with plant hormones to control seed dormancy [10]. Other transcription factors also participate in dormancy regulation; for example, Basic LEUCINE ZIPPER TRANSCRIPTION FACTOR67 activates *DOG1* to establish primary dormancy in *Arabidopsis* [11], and *Arabidopsis thaliana* SEED DORMANCY 4-LIKE regulates dormancy by mediating both *DOG1* and GA pathways [12]. *DOG1* forms a regulatory network by interacting with ABA HYPERSENSITIVE GERMINATION1 and heme [13]. The *DOG1* protein can self-associate—a property not required for protein accumulation but essential for its function [14]. Natural variation in seed dormancy is primarily governed by two loci—REDUCED DORMANCY 5 and *DOG1*—which interact with the abscisic acid (ABA) signaling pathway to regulate germination [15]. Studies indicate that two type 2 C protein phosphatases, ABA-Hypersensitive Germination 1 (AHG1) and AHG3, play critical roles in releasing *DOG1*-mediated dormancy signals [16]. Additionally, *Arabidopsis* histone demethylases, LYSINE-SPECIFIC DEMETHYLASE LIKE 1 and 2, are crucial for establishing seed dormancy, although they repress the expression of dormancy-related genes such as *DOG1* [17]. Dormant seeds are typically smaller than non-dormant ones [18], and environmental perception by the endosperm is vital [19]. Ethylene also regulates seed dormancy, and cross-talk between ethylene and *DOG1* pathways has been reported [20]. Interestingly, nitric oxide release shows a negative correlation with dormancy [21]. Seed dormancy determines germination timing, and the amount of *DOG1* protein in harvested seeds dictates the duration of dormancy release [22, 23]. Three effective methods to break seed dormancy include cold stratification, gibberellin application, and ultrasound pretreatment, all of which significantly enhance germination rate and speed [24].

Manganese (Mn) is both an essential micronutrient and a heavy metal, and *Brassica napus* plays a role in responding to heavy metal stress. Previous studies have shown that *DOG1* genes are involved in various hormone signaling pathways. This study aims to investigate the role of *DOG1* genes in *Brassica napus* under manganese (Mn) stress. Excessive Mn causes oxidative stress and cellular damage [25]. Mn toxicity has been studied in several crops: in peanut (*Arachis hypogaea*), it leads to leaf spotting, inhibited root growth, and increased proline content [26]; in rice (*Oryza sativa*), the *OsZIP* gene family may modulate Mn toxicity [27]; in tea (*Camellia sinensis*), overexpression of *CsMetal Tolerance Protein 8* enhances stress tolerance under Mn stress [28].

Interestingly, selenium (Se) supplementation can alleviate Mn-induced stress in plants [25].

DOG1 is a key gene family involved in seed dormancy regulation. Previous studies have shown that *DOG1* expression can be induced by exogenous gibberellin and methyl jasmonate [29, 30]. However, the molecular mechanisms underlying *DOG1* family functions under abiotic stress remain unclear. In this study, we conducted comprehensive analyses of the *BnDOG1* gene family using bioinformatics and qRT-PCR, focusing on gene structure, phylogeny, physicochemical properties, selective pressure, homology, promoter cis-elements, protein interaction networks, GO enrichment, and transcriptional expression patterns. To better understand the role of the *DOG1* gene family in seed dormancy regulation and stress response, the specific scientific question addressed in this study is whether *DOG1* genes participate in the response of *Brassica napus* to Mn stress.

Methods

Identification of *BnDOG1* gene family members

Protein sequences and annotation files for *Arabidopsis thaliana* and *Brassica napus* were downloaded from Ensembl Plants (<http://plants.ensembl.org/index.html>) [31] and BRAD (<http://brassicadb.cn/#/>) [32]. The *Brassica napus* genome version used was Brana_ZS_HZAU_V1.0. Candidate *BnDOG1* genes were identified using the Hidden Markov Model (HMM) profile of the *DOG1* domain (PF14144) from PFAM (<https://www.ebi.ac.uk/interpro/entry/pfam/#table>) [33]. Additionally, known At*DOG1* protein sequences from UniProt (<https://www.uniprot.org/>) [34] were used as queries in BLAST searches against the *B. napus* proteome with an E-value cutoff of 1e-5. The initial HMM screening yielded 73 candidates, while BLAST identified 44. The intersection of these two sets was taken, and redundant sequences were removed, resulting in 42 non-redundant *BnDOG1* genes.

Multiple sequence alignment and phylogenetic analysis

To investigate evolutionary relationships, *BnDOG1* and At*DOG1* protein sequences were combined. Multiple sequence alignment was performed using MUSCLE in MEGA11. A maximum likelihood (ML) [35] phylogenetic tree was constructed with 1000 bootstrap replicates and visualized in circular format using iTOL (<https://itol.embl.de/>) [36].

Gene structure and conserved motif prediction

Exon-intron structures were analyzed using TBtools based on genome annotations. Conserved motifs were identified using the MEME suite (<https://meme-suite.org/meme/tools/meme>) [37] with a setting of 10 motifs. Results for motifs, domains, gene structures, and phylogeny were integrated and visualized using TBtools.

Chromosomal localization and synteny analysis

Chromosomal positions of *BnDOG1* genes were mapped using TBtools [38]. Within-species synteny analysis was conducted to detect gene duplication events. Cross-species synteny analyses were performed between *B. napus* and *(A) thaliana*, and among *(B) oleracea*, *B. rapa*, and *B. napus* to infer orthologous relationships. Protein and annotation data for *B. oleracea* and *B. rapa* were obtained from BRAD.

Physicochemical properties and subcellular localization

Protein characteristics—including amino acid count, molecular weight, and isoelectric point—were analyzed using ExPASy (<https://web.expasy.org/protparam/>) [39]. Subcellular localization was predicted using WoLF PSORT (<https://wolfpsort.hgc.jp/>) [40].

Selective pressure analysis

Ka (non-synonymous substitution rate), Ks (synonymous substitution rate), and Ka/Ks ratios were calculated using TBtools, with *AtDOG1* genes as references. Genes with fewer than one effective codon after alignment were excluded.

Promoter cis-regulatory element analysis

Upstream 2000 bp sequences from translation start sites were extracted using TBtools. Cis-elements were identified via PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) [41] and visualized with TBtools.

Protein-protein interaction network prediction

Interacting partners of *BnDOG1* proteins were predicted using STRING (<https://cn.string-db.org/>) [42] based on Arabidopsis orthologs. Networks were visualized and refined using Cytoscape.

GO enrichment analysis

Functional annotations were performed using EggNOG-mapper (<http://eggno-mapper.embl.de/>) [43]. GO enrichment analyses were conducted and visualized using TBtools.

Tissue-specific expression analysis

Expression profiles of *BnDOG1* genes across tissues and stress conditions were retrieved from BnIR (<https://yanglab.hzau.edu.cn/BnIR/>) [44]. Heatmaps of Log₂(FPKM + 1) values were generated using ChiPlot.

Plant materials and qRT-PCR analysis

Based on the expression patterns of *BnDOG1* gene members, the *Brassica napus* cultivar “Zhongshuang 11” was selected for cultivation. Rapeseed plants were grown hydroponically, and manganese (Mn) stress was applied

using a solution of 100 µg/L MnSO₄. The stress treatment was administered at the bolting stage via root immersion, and plant samples were collected 24 h after treatment. Lateral roots and leaf samples were, immediately ground in liquid nitrogen and stored at -80 °C or used for RNA extraction with TRIzol reagent. First-strand cDNA was synthesized using the BeyoRT™ II cDNA Synthesis Kit (Beyotime, Shanghai). Quantitative real-time PCR (qRT-PCR) was performed on a CFX96™ Real-Time System (Bio-Rad) with SYBR Green Master Mix (Sangon Biotech). Three independent biological replicates were established for the plant materials, and each was analyzed in triplicate during qRT-PCR assays. Relative gene expression levels were calculated using the 2^{-ΔΔCt} method [45]. Primers were designed using Primer5.0, and Actin served as the reference gene (Table S5).

Results

Identification and characterization of *BnDOG1* members

We identified 42 *BnDOG1* genes, each containing at least one DOG1 domain. The protein sequences of these members are provided in Table S6. Physicochemical analysis (Table 1) revealed coding sequences ranging from 230 (*BnaC05G007530*) to 1296 (*BnaC08G032460*) amino acids, molecular weights from 25.92 kDa (*BnaA09G059590*) to 146.67 kDa (*BnaC08G032460*), and isoelectric points from 4.89 (*BnaC01G012400*) to 9.82 (*BnaC05G006110*). Half of the proteins were acidic and half were basic. Instability indices ranged from 38.66 (Basic. Instability indices ranged from 38.66 (*BnaC02G066230*)) to 67.33 (*BnaC05G006110*); only one protein (*BnaC02G066230*) was stable (index < 40), suggesting dynamic regulatory rather than structural functions. Grand Average of Hydropathy (GRAVY) scores ranged from -0.75 to -0.166, indicating a hydrophilic nature and potential roles in metabolism, defense, and signaling. Subcellular localization predictions indicated that 73.81% of *BnDOG1* proteins localize to the nucleus, 14.29% to the cytosol, and 11.9% to chloroplasts.

Phylogenetic analysis of *BnDOG1* gene family

A phylogenetic tree was constructed using *BnDOG1* and *AtDOG1* sequences (Fig. 1). The 58 total members clustered into five clades, with Clade III containing the most members [17] and Clade II the fewest [5]. *BnDOG1* genes were interspersed within all five clades alongside *AtDOG1* genes, indicating deep evolutionary conservation.

Gene structure, conserved motifs, and domains

A phylogenetic tree of the 42 *BnDOG1* genes was used to analyze gene structure, motifs, and domains (Fig. 2). MEME identified 10 conserved motifs. All proteins contained Motif 1 and Motif 8. Members of Clades IV and

Table 1 Characterization of DOG1 proteins

Gene name	Number of Amino Acid	Molecular Weight(KDa)	Isoelectric point	Instability Index	Grand Average of Hydropathicity	Subcellular localization
BnaA09G059590	231	25.92	5.65	49.09	-0.166	Chloroplast
BnaC01G012400	245	27.49	4.89	58.76	-0.38	Nucleus
BnaA10G026400	364	41.82	6.61	42.59	-0.569	Nucleus
BnaA03G046270	285	31.50	5.51	51.3	-0.314	Cytosol
BnaA09G020560	280	31.03	5.18	62.2	-0.499	Nucleus
BnaC03G041570	341	37.89	8.64	54.9	-0.57	Nucleus
BnaC02G066230	370	42.23	7.81	38.66	-0.534	Nucleus
BnaA08G012500	287	32.80	5.35	62.9	-0.509	Nucleus
BnaA03G033600	331	36.83	8.99	54.67	-0.606	Nucleus
BnaC05G007530	230	26.32	6.23	56.6	-0.311	Chloroplast
BnaC02G002880	326	36.64	8.87	61.73	-0.735	Nucleus
BnaC03G078500	296	33.76	5.45	64.6	-0.472	Cytosol
BnaC01G054550	334	37.10	7.89	52.3	-0.535	Nucleus
BnaA07G026610	587	67.38	6.23	61.6	-0.729	Nucleus
BnaC07G051720	285	31.62	5.42	47.47	-0.368	Cytosol
BnaC05G053500	244	28.16	6.27	54.04	-0.494	Nucleus
BnaA06G005230	823	92.35	9.57	61.38	-0.567	Nucleus
BnaA05G033250	550	61.28	9.43	44.47	-0.503	Nucleus
BnaC02G004030	377	43.32	6.68	41.5	-0.515	Nucleus
BnaC05G006110	930	104.68	9.82	67.33	-0.536	Nucleus
BnaA01G009980	239	27.64	9.03	43.5	-0.193	Cytosol
BnaC01G012380	478	55.35	9.1	44.78	-0.194	Chloroplast
BnaA01G040870	334	37.12	7.89	50.39	-0.54	Nucleus
BnaC08G057890	232	26.08	5.43	45.32	-0.166	Chloroplast
BnaC09G027120	280	31.30	5.12	65.32	-0.531	Nucleus
BnaA05G030950	244	28.10	6.61	54.59	-0.488	Nucleus
BnaC09G069250	651	73.09	9.77	60.32	-0.596	Nucleus
BnaC03G056670	367	42.01	8.33	41.06	-0.555	Nucleus
BnaA09G039330	716	81.51	5.87	58.8	-0.645	Nucleus
BnaA09G008090	750	86.51	8.93	47.39	-0.644	Nucleus
BnaA02G044580	644	73.28	8.55	43.47	-0.576	Nucleus
BnaA06G027740	367	41.95	7.3	41.11	-0.545	Nucleus
BnaA02G005450	362	41.62	7.22	41.96	-0.538	Nucleus
BnaA06G006500	230	26.26	6.32	55.44	-0.293	Chloroplast
BnaA01G009990	279	31.49	4.99	59.3	-0.518	Nucleus
BnaC08G032460	1296	146.67	8.17	52.52	-0.53	Nucleus
BnaC07G031110	280	31.15	5.5	57.29	-0.428	Cytosol
BnaA07G040760	376	43.1	5.81	65.94	-0.602	Nucleus
BnaC09G009680	308	35.39	9.46	40.13	-0.614	Nucleus
BnaA06G040810	280	31.05	5.62	58.74	-0.42	Cytosol
BnaC08G057140	484	53.74	7.04	59.07	-0.498	Nucleus
BnaC05G056590	311	34.9	9.41	58.46	-0.554	Nucleus

V shared Motifs 1, 4, 5, and 8. All proteins possessed the DOG1 domain (Fig. 2b). Clades I–III predominantly encoded proteins with multiple domains, including bZIP in addition to DOG1, whereas Clades IV and V primarily encoded single-domain proteins. Exon numbers correlated with clade membership: Clades I–III had 6–14 exons, while Clades IV–V had 1–3 exons. Eight genes

lacked introns, suggesting evolutionary optimization for efficiency and functional conservation.

Chromosomal distribution, homology, and selection pressure analyses

BnDOG1 genes were evenly distributed across 16 chromosomes, with 1–4 genes per chromosome (Fig. 3). No genes were found on chromosomes A04, C04, or C06.

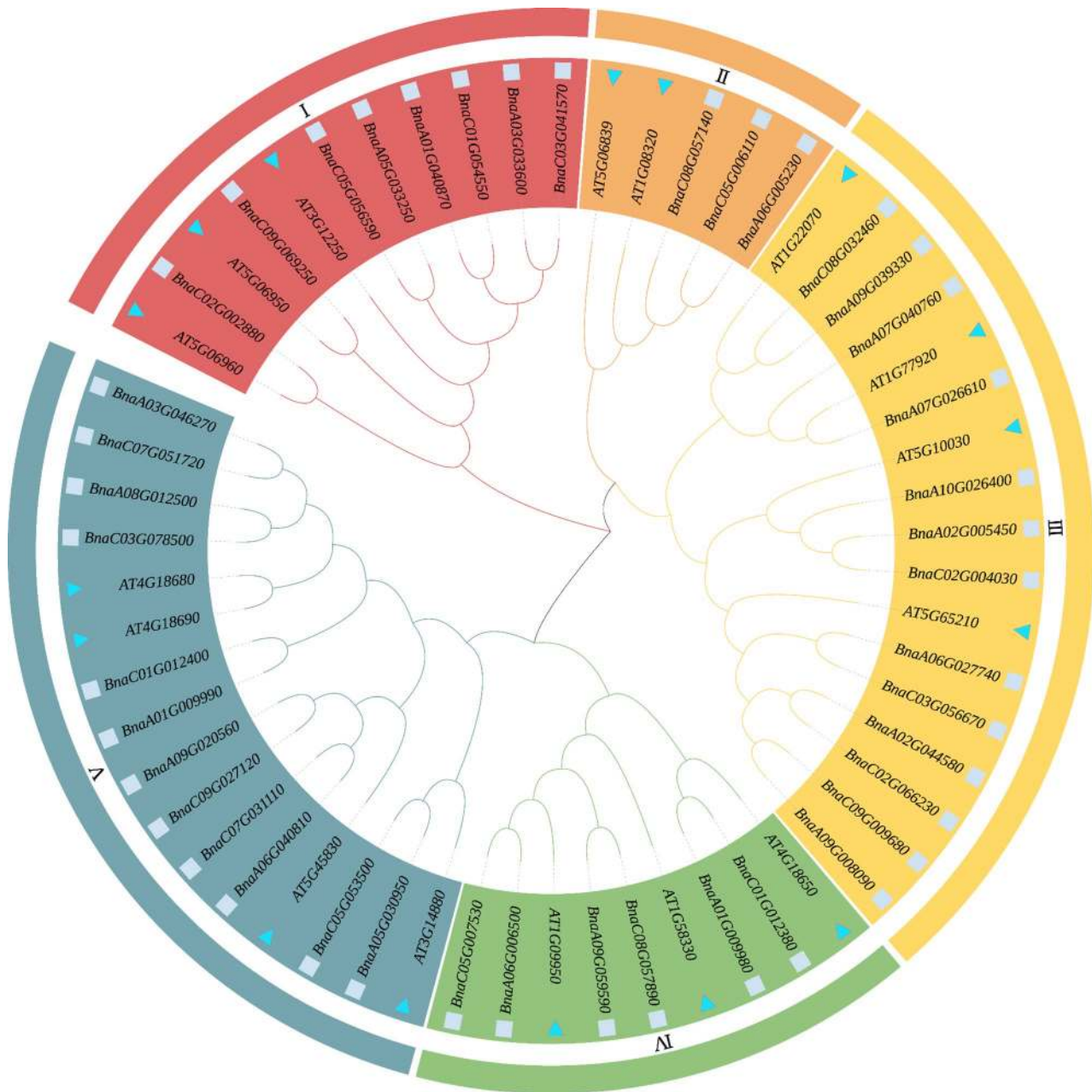


Fig. 1 Phylogenetic tree of *DOG1* gene family in *Arabidopsis thaliana* and *Brassica napus*. Light gray squares represent *BnDOG1* members; light blue triangles represent *AtDOG1* members

Syntenic analysis revealed 146 paralogous gene pairs, all derived from segmental duplications (Fig. 4), indicating segmental duplication as the dominant expansion mechanism (Figure S1). Comparative synteny with *(A) thaliana*, *(B) oleracea*, and *B. rapa* identified 72, 130, and 149 orthologous pairs, respectively (Fig. 5), supporting functional conservation of *BnDOG1* genes.

All Ka/Ks ratios of syntenic gene pairs were less than 1 (Table S1), indicating strong purifying selection acting on the *BnDOG1* family.

Promoter cis-regulatory elements

Cis-elements in the 2000 bp upstream regions were classified into four categories: light-responsive, hormone-responsive, development-related, and abiotic stress-related (Fig. 6). Light-responsive elements were the most abundant, whereas development-related elements were the least common. Hormone-responsive elements included those responsive to ABA, auxin, methyl jasmonate, GA, and salicylic acid. Development-related elements were associated with endosperm expression,

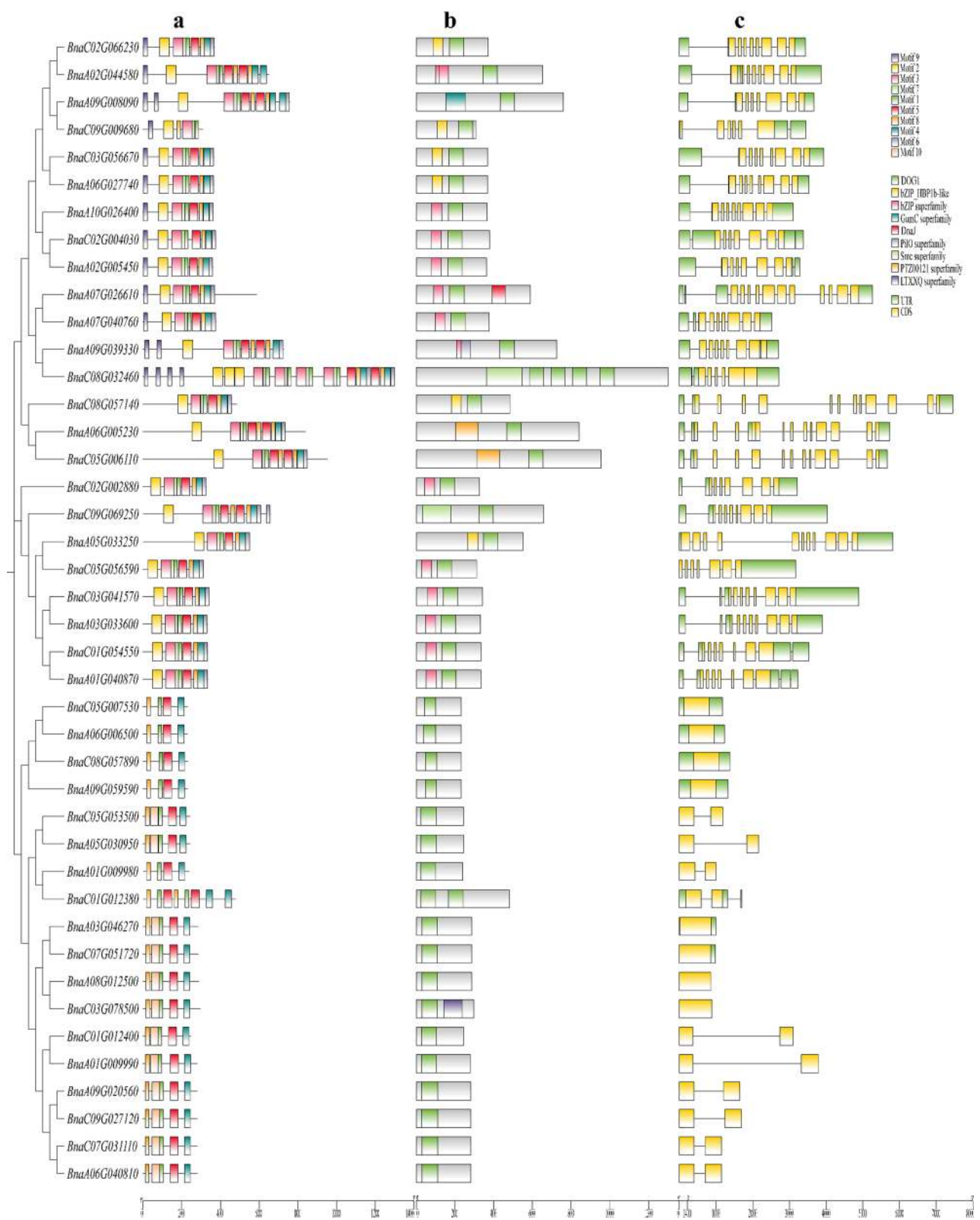


Fig. 2 Phylogenetic tree of *BnDOG1* genes and analysis of conserved motifs (a), domains (b), and gene structures (c)

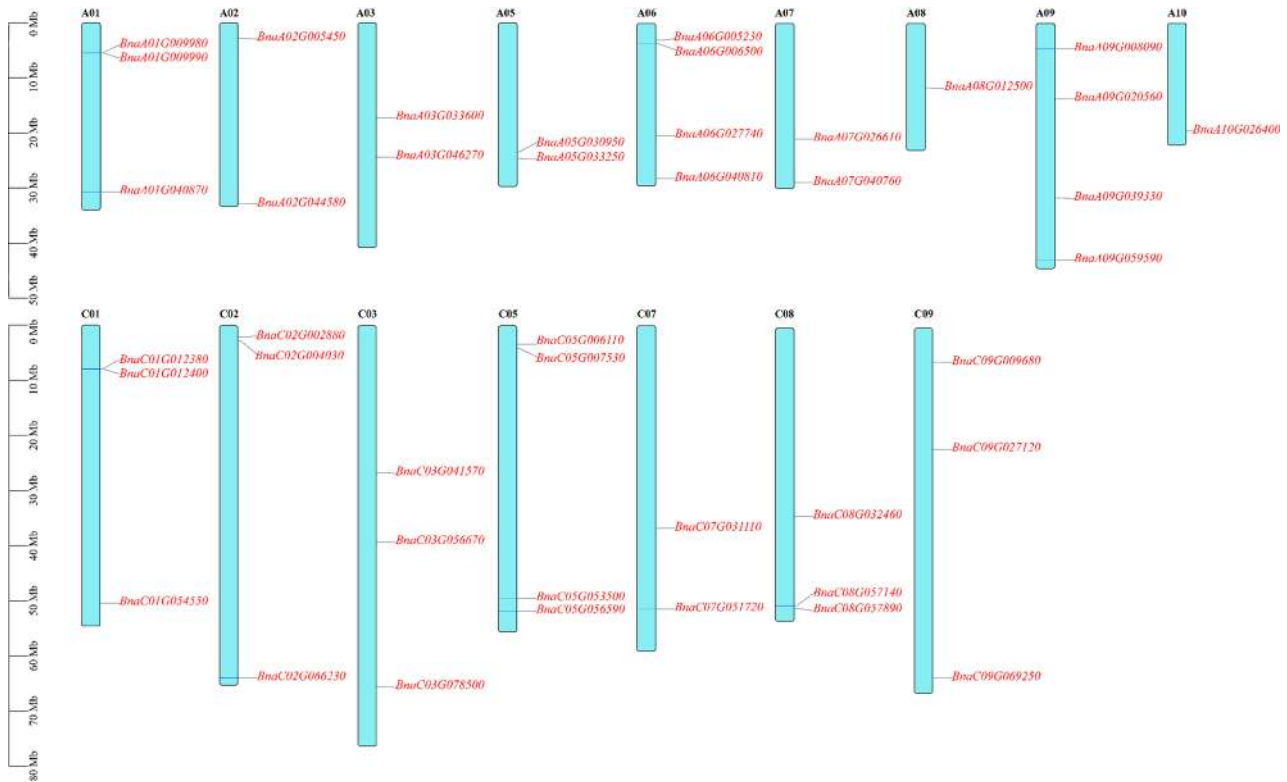


Fig. 3 Chromosomal distribution of *DOG1* genes in *Brassica napus*

palisade mesophyll differentiation, circadian rhythm, and meristem expression. Abiotic stress-related elements included those involved in anaerobic induction, low-temperature response, and drought induction. The abundance of cis-elements suggests complex regulatory functions for *BnDOG1* genes. Notably, promoter regions of multiple *DOG1* family members contain three cis-acting elements directly responsive to abiotic stresses—anaerobiosis, cold, and drought—strongly implicating the *DOG1* family in plant physiological responses to abiotic stress.

Protein interaction network

Thirteen *BnDOG1* proteins (Table S2) interacted with GRXC7, GRXC8, NIMIN-1, NIMIN-3, and SCL14 (Fig. 7). GRXC7 and GRXC8 regulate redox balance and stress responses. NIMIN-1 and NIMIN-3 fine-tune defense and development by suppressing NPR1 complex formation. SCL14 modulates detoxification gene networks under biotic and abiotic stress. This interaction network implies diverse regulatory roles for *BnDOG1* in stress adaptation. Collectively, these findings strongly indicate that the *DOG1* family plays a pivotal role in integrating environmental stress signals, maintaining redox homeostasis, and activating defensive metabolic

pathways to cope with diverse abiotic stresses through multiple regulatory strategies.

GO enrichment analysis

GO analysis categorized *BnDOG1* genes into molecular function, cellular component, and biological process (Table S3). Biological processes exhibited the highest number of enriched terms. The top 20 enriched GO terms are shown in Fig. 8. Molecular functions included nucleic acid binding (GO:0003676), heterocyclic compound binding (GO:1901363), and organic cyclic compound binding (GO:0097159). Cellular components were enriched in the nucleus (GO:0005634), intracellular membrane-bounded organelle (GO:0043231), and membrane-bounded organelle (GO:0043227). Biological processes highlighted regulation of cellular process (GO:0050794), regulation of biological process (GO:0050789), and biological regulation (GO:0065007). These results suggest roles in metabolic and stress-responsive pathways.

Tissue-specific expression patterns

Transcriptomic data revealed distinct expression patterns across tissues (bud, pollen, sepal, cotyledon, leaf, root, seed, silique, stem) and under stress conditions

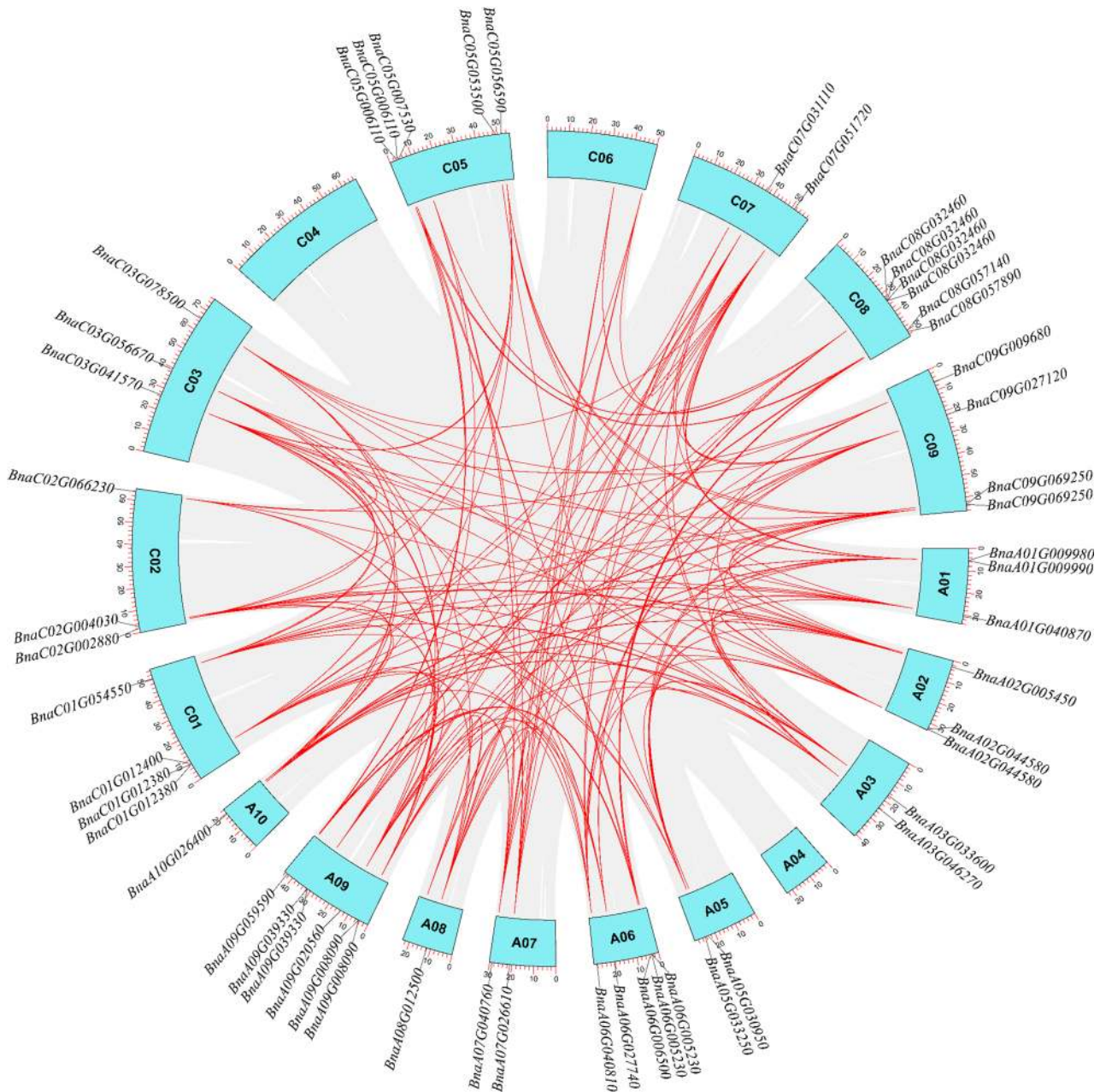


Fig. 4 Segmental duplication events of *BnDOG1* genes

(salt, cold, heat) (Fig. 9, Table S4). *BnDOG1* genes were predominantly expressed in roots and pollen. Under stress, expression remained high in roots but low in leaves. *BnaA06G027740* and *BnaC03G056670* exhibited high root expression, particularly under cold stress. *BnaA07G040760* was highly pollen-specific, whereas *BnaC07G031110* was seed-specific with minimal stress responsiveness. *BnaC01G012400* showed the lowest overall expression. These patterns indicate tissue-specific functions during development and stress responses. Collectively, these results demonstrate that *BnDOG1*

exhibits distinct tissue-specific expression, performs specialized roles during *Brassica napus* growth and development, and contributes to abiotic stress responses.

qRT-PCR validation of expression patterns

qRT-PCR confirmed expression changes under Mn stress (Fig. 10). *BnaA06G027740*, *BnaC03G056670*, *BnaA06G005230*, and *BnaC05G006110* were upregulated in roots but downregulated in leaves. *BnaC09G009680* was significantly downregulated in both tissues, while *BnaA09G008090* was significantly upregulated,

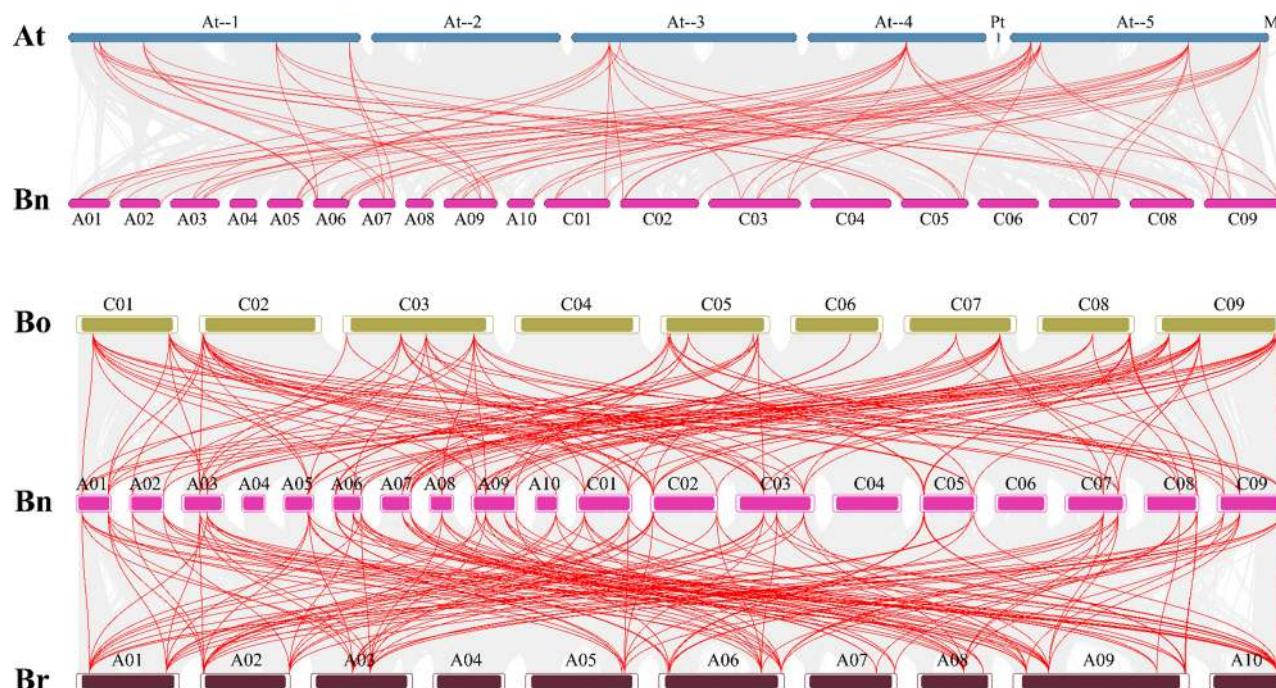


Fig. 5 Synteny analysis of *DOG1* genes among *Brassica napus*, *Arabidopsis thaliana*, *Brassica oleracea*, and *Brassica rapa*. Gray lines represent syntenic blocks; red lines highlight orthologous *DOG1* pairs

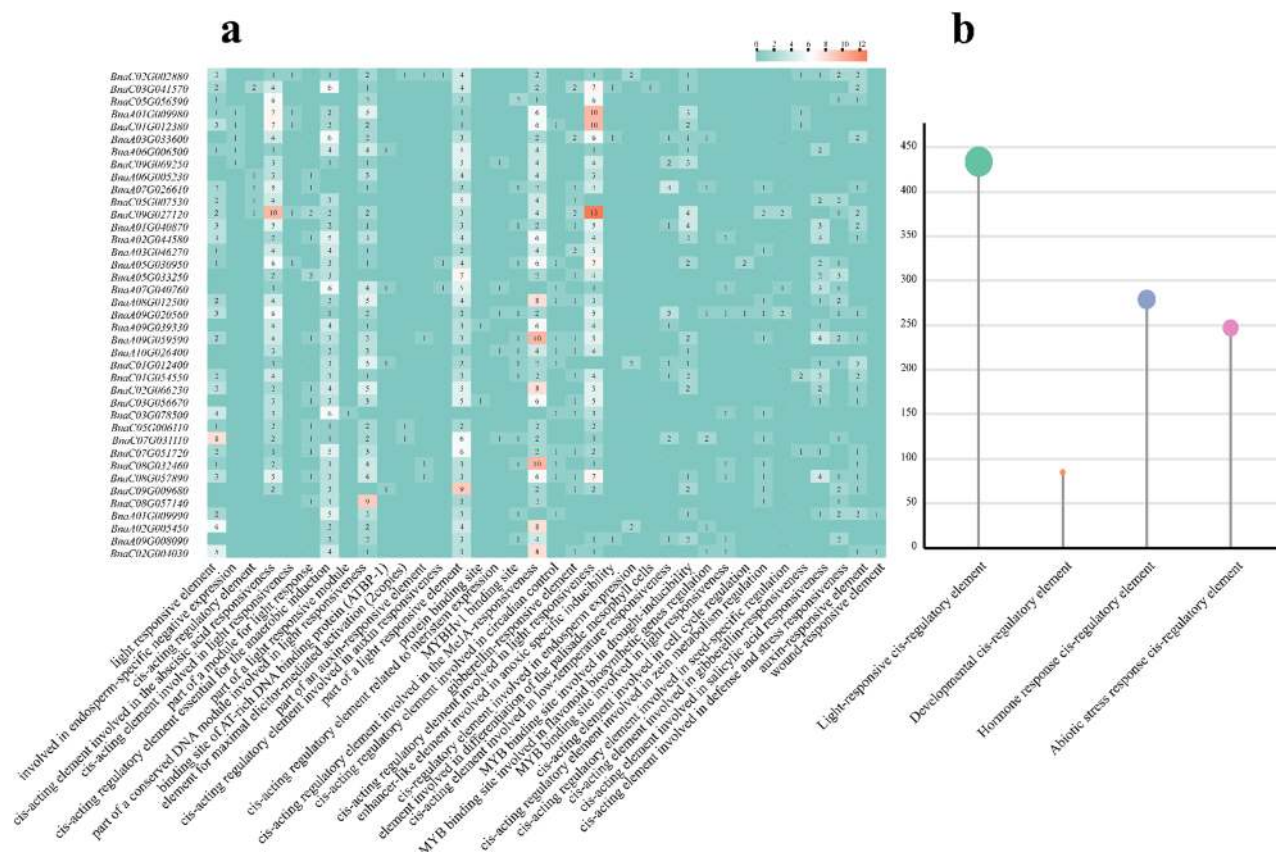


Fig. 6 **a** Predicted cis-regulatory elements in *BnDOG1* promoters. **b** Classification of cis-elements

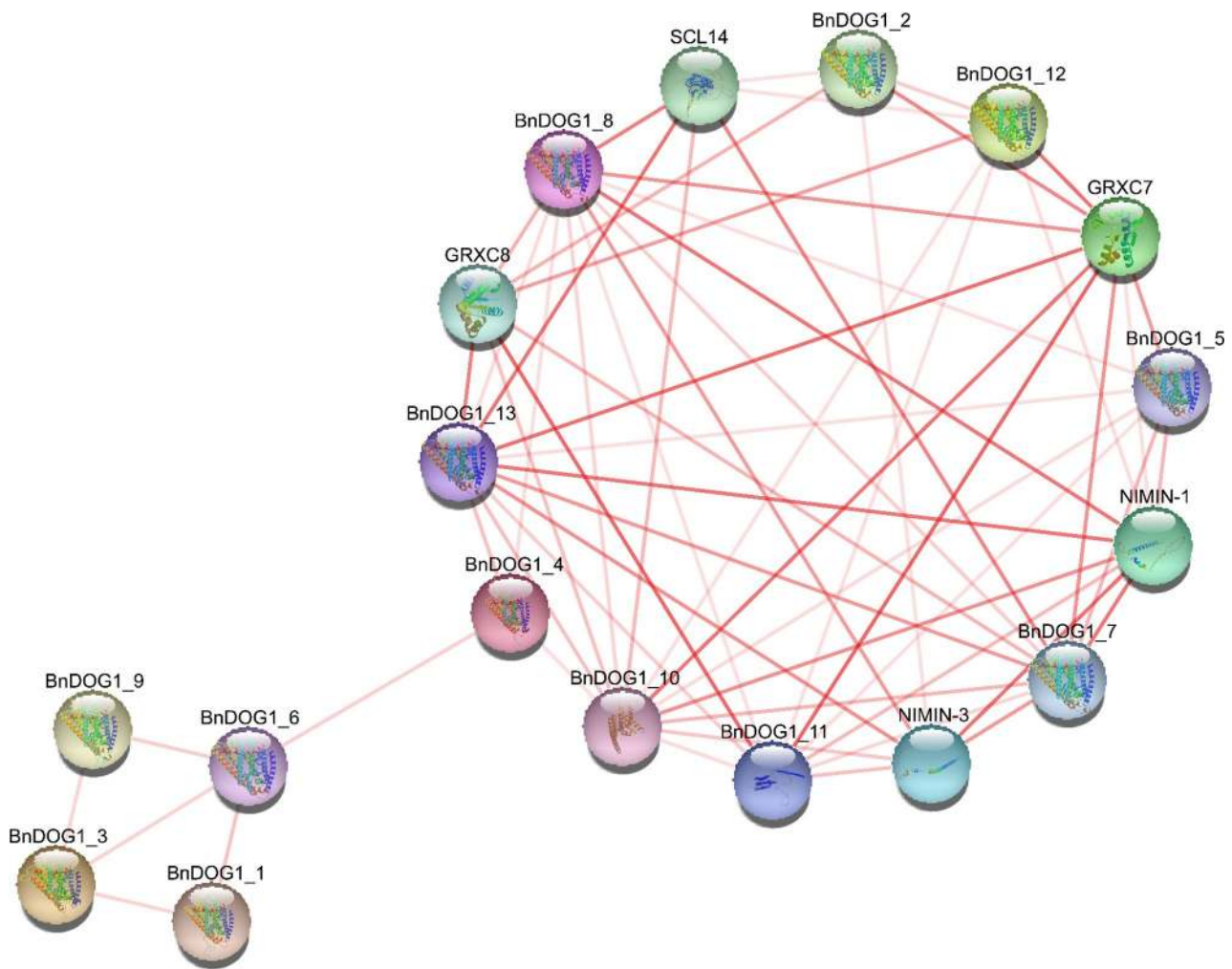


Fig. 7 Protein-protein interaction network of *BnDOG1* members

validating Mn-responsive regulation. Notably, *BnDOG1* genes show higher expression levels in roots than in leaves and are inducible under Mn stress.

Discussion

The *DOG1* gene family primarily regulates seed dormancy and germination. This study reveals an additional role for *DOG1* in mitigating Mn stress in *B. napus*. The dual functionality may stem from the presence of both *DOG1* and *bZIP* domains in many *BnDOG1* proteins (Fig. 2b). The *bZIP* transcription factor family is well known for its roles in abiotic stress response, development, and secondary metabolism [46–49]. Previous studies suggest a close relationship between the *DOG1* and *bZIP* gene families [50]. In the *Brassica napus* *bZIP* family, subgroup I is associated with heavy metal stress [51], implying that *DOG1* genes may not only regulate seed dormancy but also contribute to abiotic stress responses.

Our qRT-PCR results provide partial support for this hypothesis, showing significant expression changes under Mn stress. Notably, *DOG1* is not absolutely required for dormancy [52], and its functions extend beyond primary dormancy to other aspects of seed maturation [53].

DOG1 controls primary and secondary dormancy in *Arabidopsis*, regulated by chromatin retention and mRNA stability [54]. The chromatin remodeler *PICKLE* and the evening complex of the circadian clock directly regulate *DOG1* expression [55]. *DOG1* homologs have been identified in other species: 24 in moso bamboo (*Phyllostachys edulis*) [50] and 53 in wheat (*Triticum aestivum*) [56]. In moso bamboo, the *DOG1* gene is highly expressed in roots and leaves and can be induced by GA.

In wheat, *DOG1* gene expression is associated with ABA and methyl jasmonate (MeJA) signaling pathways. In *B. napus*, we identified 42 members, equally split between acidic and basic proteins, possibly reflecting

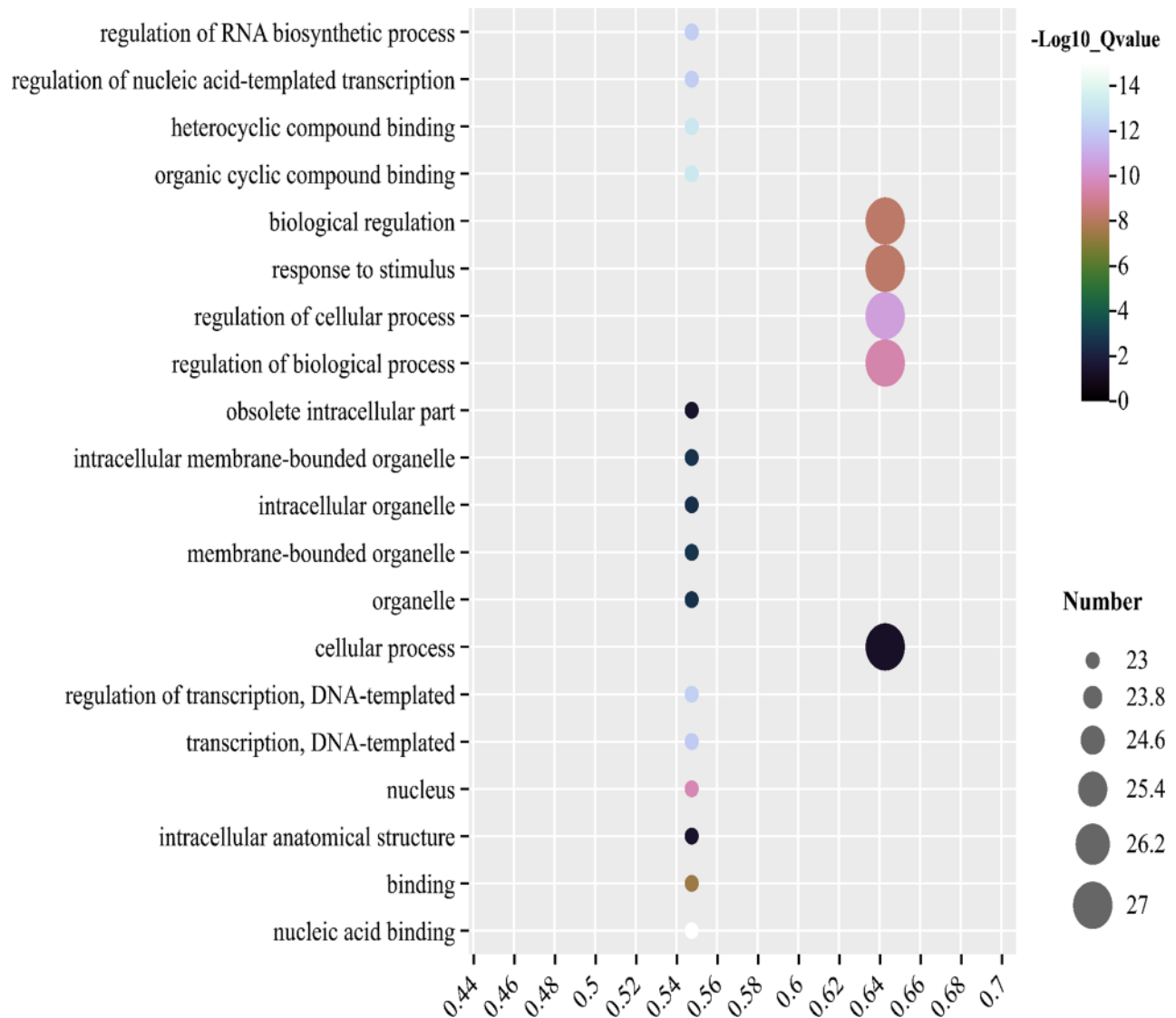


Fig. 8 Top 20 enriched GO terms for *BnDOG1* genes. Y-axis: GO terms; X-axis: gene ratio; dot size: number of genes

charge balance requirements. Most contain bZIP domains, similar to bamboo, though proportionally more so in bamboo [50]. Nuclear localization of most *BnDOG1* proteins aligns with their regulatory roles.

Phylogenetically, *BnDOG1* genes cluster into five clades, intermixed with *AtDOG1* homologs, unlike the three-clade classification observed in bamboo and wheat. Motifs 1 and 8 are universally conserved, suggesting core functional significance. Even chromosomal distribution and absence on A04, C04, and C06 may reflect genomic constraints. Segmental duplication dominates gene expansion. High synteny with *B. oleracea* and *B. rapa* (130 and 149 pairs, respectively) versus (*A. thaliana* (72 pairs) supports the hybrid origin of (*B. napus*. $Ka/Ks < 1$ indicates strong purifying selection. Promoter

cis-elements related to development (e.g., endosperm, circadian control) align with known dormancy mechanisms [6, 55], while stress-related elements (e.g., anaerobic, cold) may underlie Mn responsiveness. Interaction with stress-linked TFs (GRX, NIMIN, SCL14) further supports multifaceted stress regulation. GO enrichment in biological processes underscores the dynamic regulatory roles of *BnDOG1* genes.

Transcriptome data analysis revealed substantial differences in the expression patterns of *BnDOG1* family members across various tissues and under stress conditions. Roots are the primary site of Mn uptake. qRT-PCR results showed that *BnDOG1* genes were predominantly upregulated in roots but downregulated in leaves. Additionally, the promoters of *BnDOG1* genes contain

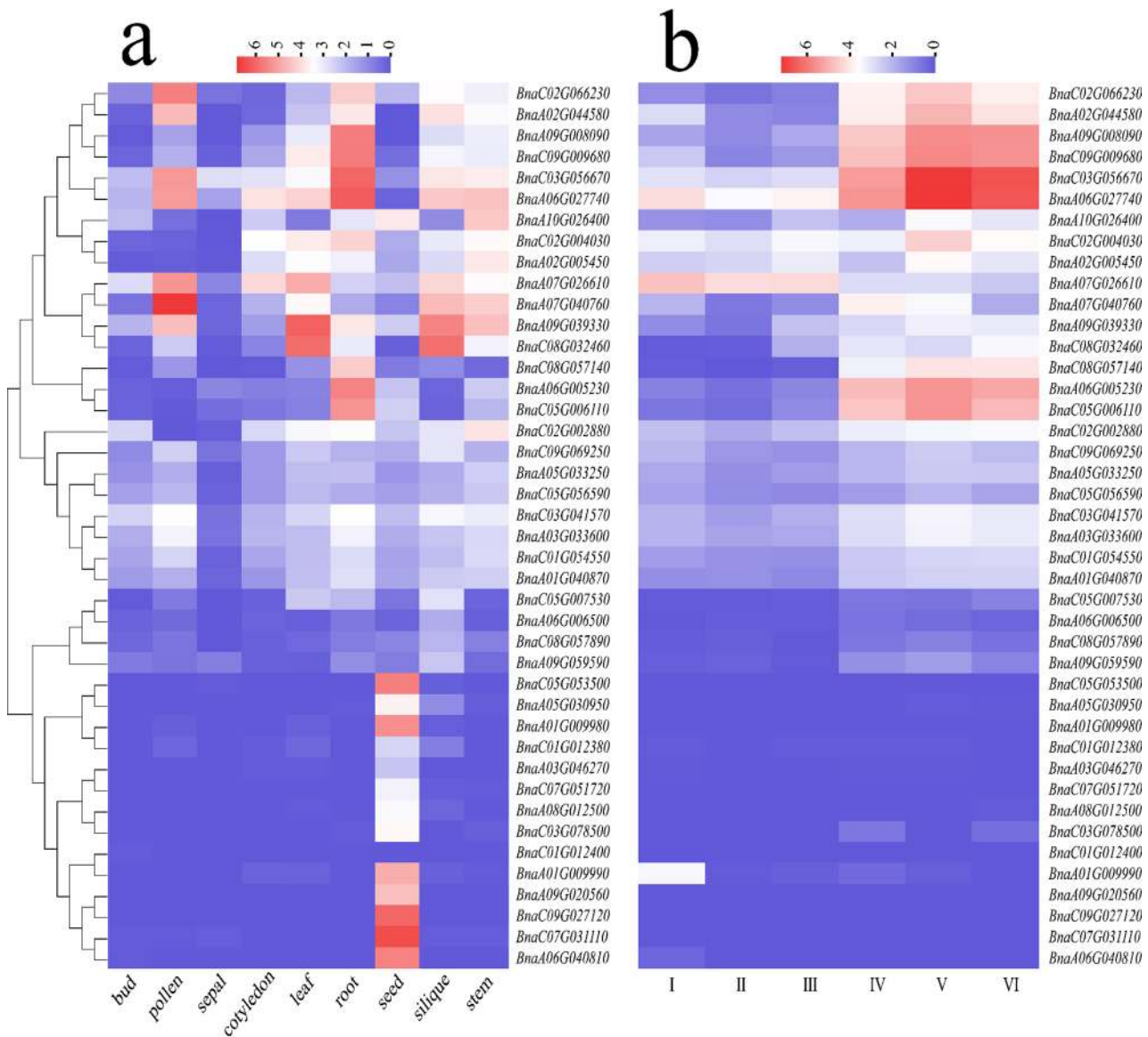


Fig. 9 **a** Tissue-specific expression of *BnDOG1* genes. **b** Expression under stress: I/IV=salt-stressed leaf/root; II/V=cold-stressed leaf/root; III/VI=heat-stressed leaf/root

regulatory elements associated with palisade mesophyll cell differentiation and meristematic expression, suggesting a potential role in regulating Mn transporter gene expression. The downregulation in leaves may serve to prevent excessive stress responses that could damage the photosynthetic system. These findings indicate significant differential expression of *BnDOG1* genes, suggesting that *DOG1* genes play a functional role in response to Mn stress.

Conclusions

This study performed a comprehensive bioinformatics analysis of the *DOG1* gene family in *Brassica napus* and specifically examined its response to Mn heavy metal stress. Bioinformatics analyses indicated that *BnDOG1* genes are functionally involved in abiotic stress responses. From cis-element identification to protein–protein interaction predictions, our work extended insights into stress regulation to the level of

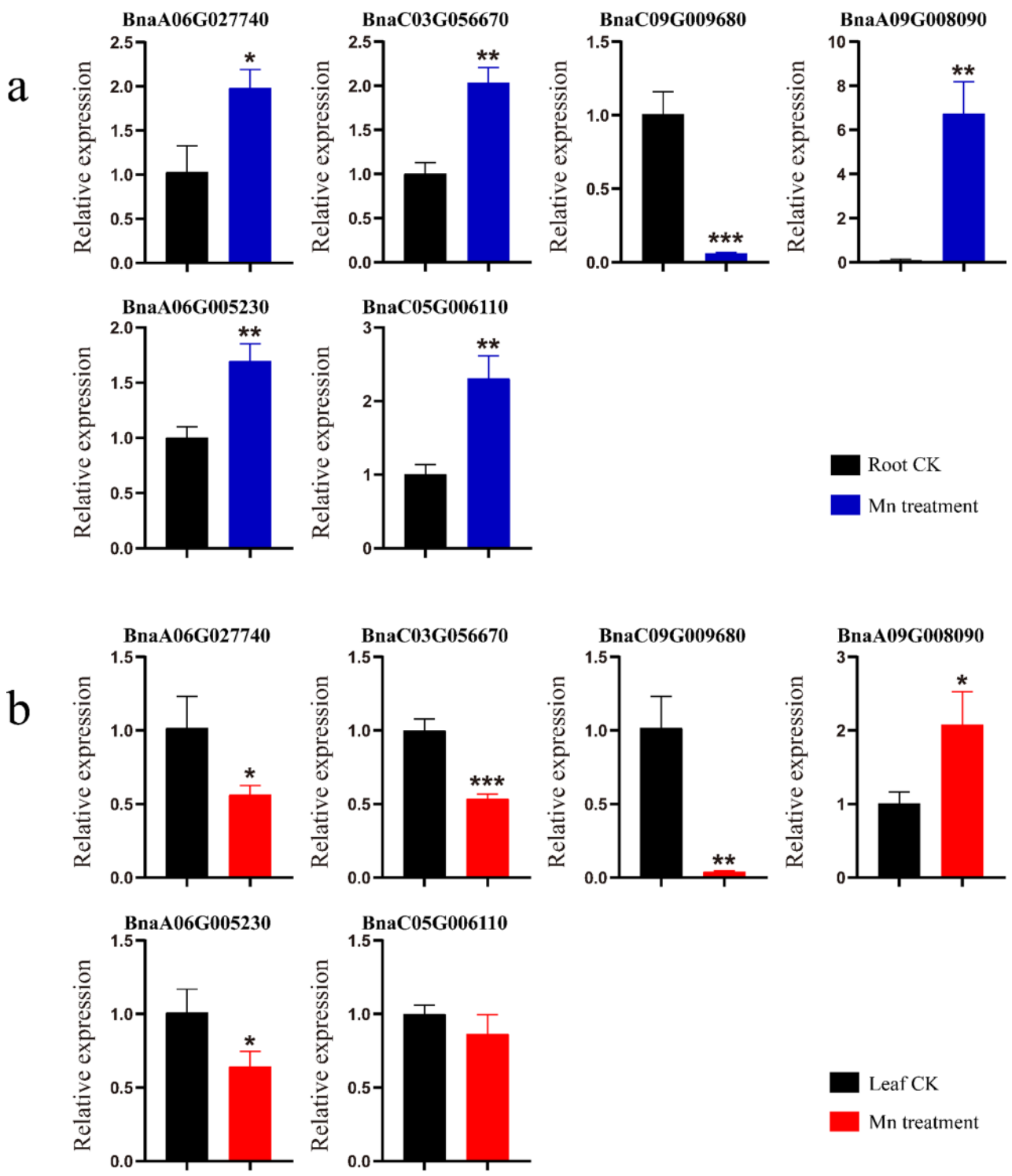


Fig. 10 **a** Expression of *BnDOG1* genes in roots under Mn stress. **b** Expression in leaves under Mn stress

functional molecular interactions. GO enrichment and transcriptome analyses further corroborated the role of this gene family in abiotic stress adaptation. Finally, qRT-PCR validation confirmed that *BnDOG1* genes are inducible under Mn stress. Future work will focus on transgenic functional validation of key *BnDOG1* genes to assess their potential for breeding heavy metal-tolerant crops.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-026-08296-1>.

Supplementary Material 1.

Acknowledgements

Not applicable.

Authors' contributions

YH designed the article structure and wrote the initial draft. YH and HL participated in some experiments and data collection. YH, HL, and YYS collected plant materials and conducted qRT-PCR experiments. YH and WSH performed data analysis. YH and HL revised the initial draft and finalized the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by Hunan Natural Science Foundation Project(2025JJ70318) and Graduate Research Innovation Project of Hunan University of Humanities, Science and Technology(ZSCX2025Y48).

Data availability

All data generated or analyzed in this study are included in this article or supplementary materials. Sequences of *Brassica napus*, *Brassica rapa*, and *Brassica oleracea* were obtained from BRAD database (<http://brassicadb.cn/#/>). *Arabidopsis thaliana* sequences were obtained from Ensembl Plants website (<http://plants.ensembl.org/index.html>), and *Arabidopsis thaliana* DOG1 protein sequences were obtained from The Arabidopsis Information Resource (<http://www.arabidopsis.org>). The HMM model was obtained from PFAM (<https://www.ebi.ac.uk/interpro/entry/pfam/#table>) using number "PF14144". Transcriptome data used in this study were obtained from BnIR database(<https://yanglab.hzau.edu.cn/BnIR>).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 7 December 2025 / Accepted: 29 January 2026

Published online: 11 February 2026

References

- Tan Z, Han X, Dai C, Lu S, He H, Yao X, et al. Functional genomics of *brassica napus*: Progress, challenges, and perspectives. *J Integr Plant Biol*. 2024;66(3):484–509.
- Ferreyroa GV, Lagorio MG, Trinelli MA, Lavado RS, Molina FV. Lead effects on *brassica napus* photosynthetic organs. *Ecotoxicol Environ Saf*. 2017;140:123–30.
- Hatzig SV, Nuppenau J-N, Snowdon RJ, Schießl SV. Drought stress has trans-generational effects on seeds and seedlings in winter oilseed rape (*Brassica napus* L). *BMC Plant Biol*. 2018;18(1):297.
- Fu Y, Mason AS, Zhang Y, Lin B, Xiao M, Fu D, et al. MicroRNA-mRNA expression profiles and their potential role in cadmium stress response in *brassica napus*. *BMC Plant Biol*. 2019;19(1):570.
- Naheed R, Aslam H, Kanwal H, Farhat F, Abo Gamar MI, Al-Mushhin AAM, et al. Growth attributes, biochemical modulations, antioxidant enzymatic metabolism and yield in *brassica napus* varieties for salinity tolerance. *Saudi J Biol Sci*. 2021;28(10):5469–79.
- Graeber K, Linkies A, Steinbrecher T, Mummenhoff K, Tarkowska D, Turečková V, et al. *Delay Of Germination 1* mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. *Proc Natl Acad*. 2014;111(34):E3571–E80.
- Kendall SL, Hellwege A, Marriot P, Whalley C, Graham IA, Penfield S. Induction of dormancy in *Arabidopsis* summer annuals requires parallel regulation of DOG1 and hormone metabolism by low temperature and CBF transcription factors. *Plant Cell*. 2011;23(7):2568–80.
- Vidigal DS, Marques ACSS, Willems LAJ, Buijs G, Méndez-Vigo B, Hilhorst HWM, et al. Altitudinal and Climatic associations of seed dormancy and flowering traits evidence adaptation of annual life cycle timing in *Arabidopsis thaliana*. *Plant Cell Environ*. 2016;39(8):1737–48.
- Footitt S, Müller K, Kermod AR, Finch-Savage WE. Seed dormancy cycling in *arabidopsis*: chromatin remodelling and regulation of DOG1 in response to seasonal environmental signals. *Plant J*. 2014;81(3):413–25.
- Li Q, Chen X, Zhang S, Shan S, Xiang Y. DELAY OF GERMINATION 1, the master regulator of seed dormancy, integrates the regulatory network of phytohormones at the transcriptional level to control seed dormancy. *Curr Issues Mol Biol*. 2022;44(12):6205–17.
- Bryant FM, Hughes D, Hassani-Pak K, Eastmond PJ. Basic LEUCINE ZIP-
PER TRANSCRIPTION FACTOR67 transactivates DELAY OF GERMINATION1 to Establish primary seed dormancy in *Arabidopsis*. *Plant Cell*. 2019;31(6):1276–88.
- Cao H, Han Y, Li J, Ding M, Li Y, Li X, et al. *Arabidopsis thaliana* SEED DORMANCY 4-LIKE regulates dormancy and germination by mediating the Gibberellin pathway. *J Exp Bot*. 2020;71(3):919–33.
- Nishimura N, Tsuchiya W, Moresco JJ, Hayashi Y, Satoh K, Kaiwa N, et al. Control of seed dormancy and germination by *DOG1-AHG1* PP2C phosphatase complex via binding to Heme. *Nat Commun*. 2018;9(1):2132.
- Nakabayashi K, Bartsch M, Ding J, Soppe WJJ. Seed dormancy in *Arabidopsis* requires self-binding ability of *DOG1* protein and the presence of multiple isoforms generated by alternative splicing. *PLoS Genet*. 2015;11(12):e1005737.
- Penfield S. Seed dormancy and germination. *Curr Biol*. 2017;27(17):R874–8.
- Née G, Kramer K, Nakabayashi K, Yuan B, Xiang Y, Miatton E, et al. *Delay Of Germination 1* requires PP2C phosphatases of the ABA signalling pathway to control seed dormancy. *Nat Commun*. 2017;8(1):72.
- Zhao M, Yang S, Liu X, Wu K. *Arabidopsis* histone demethylases LDL1 and LLD2 control primary seed dormancy by regulating *Delay Of Germination 1* and ABA signaling-related genes. *Front Plant Sci*. 2015;6:159.
- Wyse SV, Dickie JB. Ecological correlates of seed dormancy differ among dormancy types: a case study in the legumes. *New Phytol*. 2017;217(2):477–9.
- Buijs G, Vogelzang A, Nijveen H, Bentsink L. Dormancy cycling: translation-related transcripts are the main difference between dormant and non-dormant seeds in the field. *Plant J*. 2020;102(2):327–39.
- Li X, Chen T, Li Y, Wang Z, Cao H, Chen F, et al. ETR1/RDO3 regulates seed dormancy by relieving the inhibitory effect of the ERF12-TPL complex on DELAY OF GERMINATION1 expression. *Plant Cell*. 2019;31(4):832–47.
- Nagel M, Alqudah AM, Bailly M, Rajjou L, Pistrick S, Matzig G, et al. Novel loci and a role for nitric oxide for seed dormancy and preharvest sprouting in barley. *Plant Cell Environ*. 2019;42(4):1318–27.
- Nakabayashi K, Bartsch M, Xiang Y, Miatton E, Pellengahr S, Yano R, et al. The time required for dormancy release in *Arabidopsis* is determined by DELAY OF GERMINATION1 protein levels in freshly harvested seeds. *Plant Cell*. 2012;24(7):2826–38.
- Née G, Xiang Y, Soppe WJJ. The release of dormancy, a wake-up call for seeds to germinate. *Curr Opin Plant Biol*. 2017;35:8–14.
- Ahmadnia F, Alebrahim MT, Nabati Souza L, MacGregor DR. Evaluation of techniques to break seed dormancy in redroot Pigweed (*Amaranthus retroflexus*). *Food Sci Nutr*. 2023;12(4):2334–45.
- Kleiber T, Krzesiński W, Przygocka-Cyna K, Spizewski T. Alleviation effect of selenium on manganese stress of plants. *Ecol Chem Eng S*. 2018;25(1):143–52.
- Liu Y, Zhao M, Chen J, Yang S, Chen J, Xue Y. Comparative transcriptome analysis reveals complex physiological response and gene regulation in peanut roots and leaves under manganese toxicity stress. *Int J Mol Sci*. 2023;24(2):1161.
- Zeng X, Yang S, Li F, Yao Y, Wu Z, Xue Y, et al. Genome-wide identification of oszips in rice and gene expression analysis under manganese and selenium stress. *Genes*. 2024;15(6):696.
- Li Q, Li Y, Wu X, Zhou L, Zhu X, Fang W. Metal transport protein 8 in *camellia sinensis* confers superior manganese tolerance when expressed in yeast and *Arabidopsis thaliana*. *Sci Rep*. 2017;7(1):39915.

29. Ni Y, Guo C, Zhang X, Ma M, Liu X, Li Y, et al. Evolutionary and functional diversification of the *DOG* gene family in wheat (*Triticum aestivum* L.) and their roles in seed dormancy. *BMC Genomics*. 2025;26(1):861.
30. Zhijun Z, Peiyao Y, Bing H, Ruifang M, Vinod KK, Ramakrishnan M. Genome-wide identification and expression characterization of the dog gene family of Moso bamboo (*Phyllostachys edulis*). *BMC Genomics*. 2022;23(1):357.
31. Dyer SC, Austine-Orimoloye O, Azov AG, Barba M, Barnes I, Barrera-Enriquez VP, et al. Ensembl 2025. *Nucleic Acids Res*. 2025;53(D1):D948–57.
32. Chen H, Wang T, He X, Cai X, Lin R, Liang J, et al. BRAD V3.0: an upgraded brassicaceae database. *Nucleic Acids Res*. 2022;50(D1):D1432–41.
33. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar Gustavo A, Sonnhammer ELL, et al. Pfam: the protein families database in 2021. *Nucleic Acids Res*. 2021;49(D1):D412–9.
34. UniProt Consortium T. UniProt: the universal protein knowledgebase. *Nucleic Acids Res*. 2018;46(5):2699.
35. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol*. 2021;38(7):3022–7.
36. Letunic I, Bork P. Interactive tree of life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Res*. 2024;52(W1):W78–82.
37. Bailey TL, Williams N, Misleh C, Li WW. MEME: discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Res*. 2006;34(suppl2):W369–73.
38. Chen C, Wu Y, Li J, Wang X, Zeng Z, Xu J, et al. TBtools-II: A one for all, all for one bioinformatics platform for biological big-data mining. *Mol Plant*. 2023;16(11):1733–42.
39. Duvaud S, Gabella C, Lisacek F, Stockinger H, Ioannidis V, Durinx C. Expassy, the Swiss bioinformatics resource Portal, as designed by its users. *Nucleic Acids Res*. 2021;49(W1):W216–27.
40. Horton P, Park K-J, Obayashi T, Fujita N, Harada H, Adams-Collier CJ, et al. WoLF PSORT: protein localization predictor. *Nucleic Acids Res*. 2007;35(suppl2):W585–7.
41. Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, et al. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in Silico analysis of promoter sequences. *Nucleic Acids Res*. 2002;30(1):325–7.
42. Szklarczyk D, Nastou K, Koutrouli M, Kirsch R, Mehryary F, Hachilif R, et al. The STRING database in 2025: protein networks with directionality of regulation. *Nucleic Acids Res*. 2025;53(D1):D730–7.
43. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, von Mering C, et al. Fast Genome-Wide functional annotation through orthology assignment by eggNOG-Mapper. *Mol Biol Evol*. 2017;34(8):2115–22.
44. Yang Z, Wang S, Wei L, Huang Y, Liu D, Jia Y, et al. BnIR: A multi-omics database with various tools for brassica *Napus* research and breeding. *Mol Plant*. 2023;16(4):775–89.
45. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using Real-Time quantitative PCR and the 2 – $\Delta\Delta$ CT method. *Methods*. 2001;25(4):402–8.
46. Hu W, Wang L, Tie W, Yan Y, Ding Z, Liu J, et al. Genome-wide analyses of the bZIP family reveal their involvement in the development, ripening and abiotic stress response in banana. *Sci Rep*. 2016;6(1):30203.
47. Yang X, Li X, Wang X, Chen C, Wu D, Cheng Y, et al. Genome-wide identification and characterization of bZIP gene family explore the responses of PsebZIP44 and PsebZIP46 in *Pseudoroegneria libanotica* under drought stress. *BMC Plant Biol*. 2024;24(1):1085.
48. Zhu Q, Wang W, Chen T, Yan F, Chen J, Jiang Z, et al. Identification of bZIP gene family in *Bergenia purpurascens* and functional characterization of BpbZIP37 under heat stress. *Int J Mol Sci [Internet]*. 2025;26(21):10262.
49. Zhu X, Gao T, Bian K, Meng C, Tang X, Mao Y. Genome-wide analysis and expression profile of the bZIP gene family in *Neopyropia yezoensis*. *Front Plant Sci*. 2024;15:1461922.
50. Zhijun Z, Peiyao Y, Bing H, Ruifang M, Vinod KK, Ramakrishnan M. Genome-wide identification and expression characterization of the dog gene family of Moso bamboo (*Phyllostachys edulis*). *BMC Genomics*. 2022;23(1):357.
51. Peng X, Wu D, Zhang X, Liu Q, Lu Q, Song M. Identification and characterization of the HD-Zip gene family and dimerization analysis of HB7 and HB12 in *brassica napus* L. *Genes [Internet]*. 2022;13(11):2139.
52. Footitt S, Ölçer-Footitt H, Hambidge AJ, Finch-Savage WE. *Plant. Cell Environ*. 2017;40(8):1474–86.
53. Carrillo-Barral N, Rodríguez-Gacio MD, Matilla AJ. Delay of Germination-1 (DOG1): A key to Understanding seed dormancy. *Plants*. 2020;9(4):480.
54. Wang C, Brzeźniak L, Sacharowski S, Krzyszton M, Manjunath VH, Olechowski MJ, et al. UBA2A regulates seed dormancy and the stability of chromatin-retained DOG1 messenger RNA. *J Integr Plant Biol*. 2025;67(12):3109–22.
55. Zha P, Liu S, Li Y, Ma T, Yang L, Jing Y, et al. The evening complex and the Chromatin-Remodeling factor PICKLE coordinately control seed dormancy by directly repressing DOG1 in *Arabidopsis*. *Plant Commun*. 2020;1(2):100011.
56. Ni Y, Guo C, Zhang X, Ma M, Liu X, Li Y, et al. Evolutionary and functional diversification of the *DOG* gene family in wheat (*Triticum aestivum* L.) and their roles in seed dormancy. *BMC Genomics*. 2025;26(1):861.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.