



Regulatory roles of *ABF/AREB/ABI5* transcription factors in nutrient stress and hormonal signaling in oilseed rape

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Abstract

The balance between nutrients and hormones is critical for optimal plant growth and development, as imbalances can trigger severe physiological and developmental disorders. ABRE Binding Factors (*ABFs*), ABA-Responsive Element Binding Factors (*AREBs*), and ABA INSENSITIVE 5 (*ABI5*) are key regulators of stress-responsive gene expression, particularly under abiotic stresses. However, their roles in oilseed rape (*Brassica napus* L.) under nutrient stress and phytohormone regulation remain largely unexplored. In this study, we identified and characterized 30 putative *ABFs/AREBs/ABI5s* in the *B. napus* genome through phylogenetic and structural analysis, grouping them into five distinct clades. Sequence alignment revealed strong conservation among family members, and subcellular localization confirmed their nuclear presence. Expression profiling showed that many *BnaABFs/AREBs/ABI5s* genes respond dynamically to nutrient deprivation, salt stress, and exogenous phytohormone treatments (abscisic acid, gibberellic acid, and salicylic acid), with both up- and downregulation observed. Among them, *BnaABF1-a*, *BnaABF3-c*, and *BnaABI5-b* exhibited the most pronounced changes under nutrient and hormonal stress conditions. These findings provide valuable insights into the evolution, regulation, and functional potential of *BnaABFs/AREBs/ABI5s* genes in oilseed rape. By elucidating their roles in nutrient stress responses, this work lays the foundation for targeted functional studies and breeding strategies to develop nutrient-efficient, stress-resilient oilseed rape cultivars, contributing to sustainable agriculture and climate-resilient crop improvement.

Keywords Nutrient stress · Phytohormones · Gene expression · Stress resilience · Subcellular localization · Phylogenetic analysis · Sustainable agriculture

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Introduction

Oilseed rape (*Brassica napus* L.) is one of the most important oilseed crops globally, originating from the natural hybridization of *Brassica oleracea* (CC, 2n=18) and *Brassica rapa* (AA, 2n=20). It serves as a key source of edible oil, food products, and raw materials for various industries (Moggré et al. 2022). Despite its economic significance, oilseed rape cultivation faces persistent challenges related to nutrient-use efficiency and adaptation to environmental stresses.

Higher plants have evolved complex regulatory systems to cope with abiotic stresses such as nutrient deficiency, drought, salinity, extreme temperatures, and osmotic stress. These stresses significantly affect plant growth and yield, posing threats to global food security (Ayub et al. 2025; Raza et al. 2023; Farooq et al. 2022; Wang et al. 2023). A

major limitation in oilseed rape production is its inherently low nitrogen use efficiency (NUE). To compensate, excessive fertilizer applications are common, which contributes to environmental pollution and disrupts beneficial soil microbial communities, particularly nitrogen-fixing bacteria (Muncer et al. 2024; Sun et al. 2025; Xin et al. 2025).

Among essential macronutrients, nitrogen (N), phosphorus (P), and potassium (K) play central roles in plant metabolism, including nucleic acid, proteins and chlorophyll synthesis, and overall physiological growth. Deficiencies in these nutrients result in stunted growth, chlorosis, and significant yield losses (Xie et al. 2025; Ren et al. 2025; Liu et al. 2019a).

Transcription factors (TFs) are central to plant responses against environmental stresses, as they regulate gene expression and coordinate developmental and metabolic pathways (Zhang et al. 2025; Fiallos-Salguero et al. 2023; Liu et al. 2019b). In particular, ABRE Binding Factors (*ABFs*), ABA-Responsive Element Binding Factors (*AREBs*), and ABA INSENSITIVE 5 (*ABI5*) belong to the basic leucine zipper (bZIP) transcription factor family. In *Arabidopsis thaliana*, at least nine homologous bZIP proteins, including *ABF1*, *AREB1/ABF2*, *ABF3*, *AREB2/ABF4*, *ABI5/DPBF1*, have been identified. These TFs regulate diverse abiotic stress responses, including drought, salinity, cold, and osmotic stress (Yoshida et al. 2010; Fiallos-Salguero et al. 2023; Zhao et al. 2016; Wang et al. 2016; Linden et al. 2021). They function as core regulators of the abscisic acid (ABA) signaling pathway, influencing processes such as seed germination, ABA–glucose signaling, anther development, nitrate signaling in the endodermis, and root development (Zhao et al. 2020; Contreras-López et al. 2022; Hossain et al. 2010; Ma et al. 2018; Fernando et al. 2018).

Phytohormones, including ABA, gibberellic acid (GA), cytokinin (CK), auxin (IAA), and ethylene (Eth), act as master regulators of plant growth and stress adaptation. They modulate signaling cascades that govern molecular, biochemical, and physiological responses to environmental stress (Raza et al. 2023; Mubarik et al. 2021; Altaf et al. 2023; Kumari et al. 2023). These hormones influence critical processes such as cell elongation, root system architecture, nutrient uptake, stomatal regulation, seed development, and germination (Ma et al. 2018; Jing and Strader 2019). However, while extensive work has been conducted on stress-responsive TFs and hormone-related pathways in model plants, the roles of *BnaABFs/AREBs/ABI5s* genes in *B. napus* under nutrient deficiency and phytohormone treatments remain poorly understood. stress and phytohormonal treatments remains largely unexplored.

Previous studies have characterized *ABFs/AREBs/ABI5s* TFs in *Arabidopsis* and other crops (Wang et al. 2016; Kerr et al. 2018), yet their specific regulatory functions in oilseed

rape are largely unknown. specific roles in oilseed rape under nutrient stress and phytohormone regulation remain unclear. Understanding how these genes respond to key macronutrient deficiencies (N, P, K) and phytohormone signaling is critical for improving NUE and stress tolerance in oilseed rape. Furthermore, unraveling transcription factor-mediated regulatory networks offers promising opportunities for developing climate-resilient and nutrient-efficient cultivars.

This study aims to systematically identify and characterize the *BnaABFs/AREBs/ABI5s* subgene family in oilseed rape using phylogenetic and structural analyses. It further explores their expression profiles under nine nutrient stress conditions, multiple abiotic stresses, and phytohormone treatments, with an emphasis on their roles in nutrient uptake and stress adaptation via ABA signaling.

By filling a key knowledge gap, this research provides novel insights into the evolution, regulation, and functional dynamics of *BnaABF/AREB/ABI5* genes in oilseed rape. The findings will support breeding strategies aimed at enhancing crop productivity, resource-use efficiency, and environmental sustainability under changing climatic conditions.

Materials and methods

Identification of *ABFs/AREBs/ABI5s* gene subfamily members

Protein sequences of *AtABFs/AREBs/ABI5s* from *Arabidopsis thaliana* (Swarbreck et al. 2007) were retrieved from the TAIR database and used as queries. Thirty protein sequences were obtained through BLASTp searches against the *Brassica* genome database (BRAD; Chen et al. 2022), with an E-value cutoff of $\leq 1 \times 10^{-20}$. Homologous sequences from *B. oleracea*, *B. rapa*, and *B. napus* were retained.

To validate the identified genes, Hidden Markov Model (HMM) profiles of *ABFs/AREBs/ABI5s* gene family were downloaded from the HMM database. Local HMMER searches ($E\text{-value} \leq 1 \times 10^{-20}$) were conducted, and domain integrity was confirmed using the Pfam database. Sequences lacking conserved *ABFs/AREBs/ABI5s* domains or with an $E\text{-value} > 1 \times 10^{-20}$ were excluded. This procedure was consistently applied to *B. oleracea* and *B. rapa* (Nan et al. 2021).

Phylogenetic and synteny analysis

Phylogenetic relationships were reconstructed using the Neighbor-Joining method in MEGA 11 (Tamura et al. 2013) with 1,000 bootstrap replicates. The analysis incorporated *ABFs/AREBs/ABI5s* protein sequences from *B. napus*,

(*A. thaliana*, *Oryza sativa*, *Solanum lycopersicum*, (*B. oleracea*, and *B. rapa*.

Synten analysis was conducted using the latest *B. napus* genome assembly (Brana_Dar-V5) from BRAD (Chen et al. 2022). Non-synonymous (Ka) and synonymous substitution rate (Ks) substitution rates were calculated using DNA Sequence Polymorphism (DnaSP) v6 (Librado and Rozas 2009) to evaluate selective pressure. MCScanX was employed for synten block detection and whole-genome duplication (WGD) analysis (Nan et al. 2021). Gene duplication events were categorized as segmental, dispersed, proximal, or tandem, and results were visualized in TBtools (Nan et al. 2021).

Gene and protein analysis

Physiochemical characteristics of *ABFs/AREBs/ABI5s* proteins were computed using ProtParam. Gene structures were examined via GSDS (Hu et al. 2015), while transmembrane domains were predicted using SCAMPI (Bernsel et al. 2008). Subcellular localization predictions was inferred using WoLF PSORT (Nan et al. 2021).

Conserved motifs were identified using the MEME suite (Bailey et al. 2009), specifying a maximum of 15 motifs, a minimum of 9 motifs, and a motif width of 50. A phylogenetic tree of *B. napus ABFs/AREBs/ABI5* proteins was also generated using MEGA 11 (Tamura et al. 2013).

Three-dimensional protein structure modeling homology

FASTA sequence of *ABFs/AREBs/ABI5s* proteins were submitted to SwissModel for structural modeling. Structural templates were identified from the Protein Data Bank (PDB). Model quality was assessed using QMEAN and GMQE scores, along with Ramachandran plots. Further refinement and molecular dynamics simulations were conducted carried out with iMOD.

Chromosomal localization, gene duplication, and evolutionary analysis

Chromosomal positions of *ABFs/AREBs/ABI5s* genes were obtained from the NCBI database and mapped using MapChart. Orthologous and paralogous relationships between (*A. thaliana* and (*B. napus* were detected through BLAST. Segmental and tandem duplication events were further analyzed using the Plant Genome Duplication Database (PGDD). Divergence time were estimated using the formula $T = Ks/2\lambda \times 10 - 6 \text{ Mya}$ (where $\lambda = 1.5 \times 10^{-8}$), and selection pressures were inferred from Ka/Ks ratios (Carbone et al. 2007).

Gene ontology, promoter analysis, and protein interaction networks

Gene Ontology (GO) annotations were assigned using Blast2GO, classifying genes into biological processes, molecular functions, and cellular components ($p < 0.05$). Data visualization was performed using TBtools (Chen et al. 2023). Promoter regions (2000 bp upstream of the start codon) were retrieved from NCBI and analyzed via the PlantCARE database. Protein-protein interaction (PPI) networks were inferred via the STRING.

Transcriptome analysis under abiotic stress and phytohormone treatments

Expression profiles of *ABFs/AREBs/ABI5s* genes in shoots and roots under abiotic stresses (salt, cold, osmotic, drought, heat and freezing) and phytohormone treatments (ABA, ethylene (ACC), Auxin (IAA), jasmonate (JA), brassinosteroid (BL), gibberellic acid (GA), cytokinin (TZ) were extracted from the BnTIR database. Transcript abundance was normalized and expressed as transcripts per million (TPM).

Plant material and growth conditions

The rapeseed cultivar Zhongshuang 11 (*ZS11*) was used in this study. Seeds were germinated in Petri plates and transferred to a controlled growth chamber maintained at 25 °C/18°C (16 h light/8 h dark), 55–70% relative humidity, and 300 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ light intensity from full-spectrum LEDs. Seedlings were cultivated hydroponically in 10 L containers with aerated nutrient solution containing Na_2SiO_3 (0.2 mM), K_2SO_4 (0.1 mM), CaCl_2 (0.5 mM), MgSO_4 (0.4 mM), KH_2PO_4 (0.2 mM), $\text{Ca}(\text{NO}_3)_2$ (1.25 mM), KNO_3 (1 mM), and micronutrients, adjusted to pH 6.8 with CaCO_3 . Nitrate levels were monitored with test strips, and the solution was replenished when concentrations dropped below 30%.

After 24 days, plants were exposed to individual nutrient deprivation treatments (–Ca, –K, –S, –P, –Mg, –N, –Co, –Cl), while controls remained under standard conditions. Root samples were harvested after 10 days, flash-frozen in liquid nitrogen, and stored at –80 °C. Three independent biological replicates were collected for transcriptomic analyses.

Quantitative real-time PCR validation

Total RNA was extracted from root samples using a Biotek kit (Biotek, Beijing, China) and assessed by gel electrophoresis. cDNA synthesis was performed using PrimeScript

RT reagent Kit (TransGen, Beijing, China). RNA-seq data were normalized as $\text{Log}_2(\text{FPKM} + 1)$.

qRT-PCR was conducted on an ABI QuantStudio 5 system (Applied Biosystems, CA, USA) under the following conditions: 94°C for 2 min, 45 cycles of 94°C for 15 s, 60°C for 15 s, and 72°C for 30 s. Gene-specific primers were designed with Primer Premier 6 and validated using NCBI BLAST. Relative expression was quantified using the $2^{-\Delta\Delta\text{CT}}$ method (Liu et al. 2021).

Data visualization was performed using TBtools, and statistical analyses were conducted in SPSS v17.0. Significance was determined by Student's t-test ($p < 0.05$). RNA-seq data are available under NCBI-SRA BioProject IDs PRJNA394926, PRJNA358784, and PRJNA748031.

Results

Identification and classification of ABFs/AREBs/ABI5s genes

Thirty *ABFs/AREBs/ABI5s* genes were identified in group A of the *bZIP* gene family in *B. napus* and named based on their chromosomal position and closest orthologues of *A. thaliana* (Table 1). All identified genes contain the characteristic bZIP domain, which defines the bZIPs TFs family. To explore their evolutionary relationships, a phylogenetic tree was constructed, including (*A. thaliana*, (*B. rapa*, *B. oleracea*, *B. napus* and *Solanum. lycopersicum* (Fig. 1). Most of these proteins were predicted to localize in the nucleus (Table S5; Fig. 4A). The genes were classified into nine subgroups. The *B. napus ABFs/AREBs/ABI5s* genes encode proteins ranging from 237 to 410 amino acid residues, with 2 to 8 exons, and molecular weights between 27133.92 and 45525.63 kDa, and Isoelectricpoint (pI) varying from 7.08 to 9.87.

Phylogenetic analysis and evolutionary relationship of *BnaABFs/AREBs/ABI5s* genes

Phylogenetic analysis placed the *ABFs/AREBs/ABI5s* genes into five groups: *ABFs*, *AREBs*, *ABI5s*, and *DREBs*, across the five species (Fig. 1).

The largest cluster, Group C, contained 11 genes from *B. oleracea*, 11 from *B. napus*, 3 from (*A. thaliana*, 7 from (*B. rapa*, and 3 from *S. lycopersicum*. To assess evolutionary selection pressure, orthologous gene pairs between (*A. thaliana* and (*B. napus* were analyzed for synonymous (Ks) and non-synonymous (Ka) substitution rates (Ka) (Table S2).

The Ka values ranged from from 0.0407 (*BnaAREB3-c*) to 0.3304 (*BnbZIP15a*) with an average of 0.1719 (*BnaABF1-a*)

and 0.1726 (*BnaABF2/AREB1-a*). Ks values ranged from 0.3256 (*BnaDPBF4/EEL-b*) to 0.8093 (*BnaAREB3/DPBF3-a*), with an average of 0.5114 (*BnaABF3-c*). Ka/Ks values where ratios were consistently below 1.0, suggesting purifying selection maintained their functions (Table S1). Paralogous gene pairs within *B. napus* exhibited Ka values ranging 0.0029 (*BnaAREB3/DPBF3-f_BnaAREB3/DPBF3-c*) to 0.034 (*BnaABF4/AREB2-d_BnaABF4/AREB2-b*) and Ks values from 0.014 (*BnaDPBF4/EEL-b_BnaDPBF4/EEL-a*) to 0.0588 (*BnaABF1-b_BnaABF1-a*), with an average of 0.0412 (*BnaABF3-c_BnaABF3-b*). These findings further support that *BnaABFs/AREBs/ABI5s* genes have undergone purifying selection (Table S1,2).

Chromosomal location, duplication patterns, gene structure, and conserved motif analysis

The *BnaABFs/AREBs/ABI5s* genes were unevenly distributed across the *B. napus* genome (Figure S1). Twelve genes were located on the A subgenome, while 13 were mapped to the C subgenome. Chromosomes A05, C04, and Cnn_random contained the highest number of *BnaABFs/AREBs/ABI5s* genes, whereas C06 and C04 contained only two. Other chromosomes had single members.

Gene duplication analysis revealed large-scale whole genome duplications (WGD) and small-scale duplications. Segmental duplication was detected across different chromosomes, whereas tandem duplication was observed in *BnaDPBF4/EEL-a* and *BnaDPBF4/EEL-b* on the same chromosome (Figure S1). The segmental duplications indicate that.

Motif analysis showed that highly similar motifs were present among closely related genes (Fig. 2C). The phylogenetic tree classified these genes into five subfamilies based on motif distribution, suggesting that these motifs may play crucial structural and functional roles in protein activity.

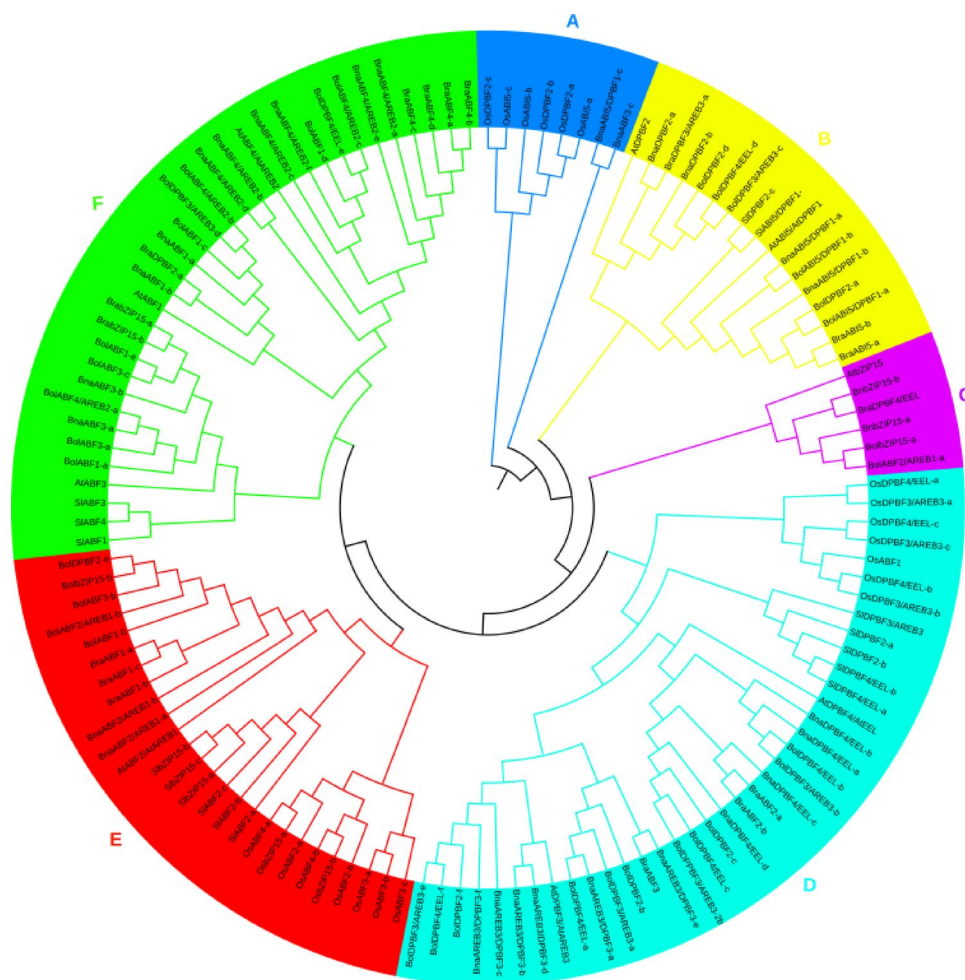
Promoter cis-Acting element analysis

To understand transcriptional regulation, cis-regulatory elements were analyzed in the *BnaABFs/AREBs/ABI5s* genes using the PlantCARE database. A total of 51 cis-elements were identified, including light-responsive (23), phytohormone-responsive (9), stress-responsive (8), and growth regulation elements (9) (Fig. 3). To understand transcriptional regulation, cis-regulatory elements were analyzed in the 2 kb upstream regions of the *BnaABFs/AREBs/ABI5s* genes using the PlantCARE database. A total of 51 cis-elements were identified, including light-responsive (23), phytohormone-responsive (9), stress-responsive (8), and growth regulation elements (9) (Fig. 3).

Table 1 Characterization of 30 *ABFs/AREBs/ABFs* transcription factors identified in *B. napus*

Gene Name	Direction	Subfamilies	Chr.	start	end	CDS(bp)	Protein length(aa)	MW(kDa)	PI	GRAVY	Exon	Intron
BnaABF1-a	Reverse	bZIP-A	C6	3,442,534	3,443,805	714	237	27133.92	9.87	-0.932	7	6
BnaABF1-b	Forward	bZIP-A	A6	1,843,059	1,845,065	993	330	36277.61	9.36	-0.768	5	4
BnaABF2/AREB1-a	Forward	bZIP-A	A10	1,246,991	1,248,976	909	302	32948.16	9.32	-0.66	8	7
BnaABF2/AREB1-b	Forward	bZIP-A	C6	667,000	668,818	1008	335	36020.63	9.23	-0.563	5	4
BnaABF3-a	Forward	bZIP-A	C7	43,127,915	43,130,504	1233	410	45525.63	8.82	-0.638	5	4
BnaABF3-b	Forward	bZIP-A	C1	2,267,066	2,269,501	1053	350	39043.31	9.26	-0.665	7	6
BnaABF3-c	Forward	bZIP-A	Ann-R	30,456,414	30,458,977	1050	349	39028.35	9.45	-0.681	7	6
BnaABF4/AREB2-a	Forward	bZIP-A	A5	16,192,619	16,195,405	1068	355	38173.55	9.45	-0.726	5	4
BnaABF4/AREB2-b	Forward	bZIP-A	Cnn-R	40,108,198	40,110,395	1065	354	39263.98	9.28	-0.83	4	3
BnaABF4/AREB2-c	Forward	bZIP-A	A1	18,271,993	18,274,689	1059	352	37978.42	9.56	-0.766	5	4
BnaABF4/AREB2-d	Reverse	bZIP-A	A3	17,201,034	17,203,488	1095	364	39799.51	9.26	-0.712	4	3
BnaABF4/AREB2-e	Reverse	bZIP-A	C5	33,061,771	33,064,585	1065	364	38468.09	9.23	-0.718	5	4
BnaABF4/AREB2-f	Reverse	bZIP-A	C1	3,458,625	3,461,309	1176	391	42655.68	9.42	-0.806	4	3
BnaAB15/DPBF1-a	Forward	bZIP-A	Ann-R	7,324,416	7,326,100	1215	404	43330.22	9.64	-0.816	4	3
BnaAB15/DPBF1-b	Forward	bZIP-A	C4	6,774,985	6,777,669	1185	394	42392.31	9.45	-0.811	6	5
BnaAB15/DPBF1-c	Forward	bZIP-A	A5	4,413,679	4,416,365	1200	399	42802.59	9.26	-0.818	6	5
BnaDPBF2-a	Reverse	bZIP-A	Cnn-R	3,101,021	3,103,208	1041	346	39238.27	7.72	-0.809	4	3
BnaDPBF2-b	Reverse	bZIP-A	C3	42,826,053	42,828,247	1041	346	39477.58	7.76	-0.849	4	3
BnaAREB3/DPBF3-a	Reverse	bZIP-A	C4	25,708,019	25,709,771	873	290	31290.12	9.38	-0.772	3	2
BnaAREB3/DPBF3-b	Reverse	bZIP-A	Cnn-R	19,153,299	19,154,756	753	250	28088.1	9.8	-0.781	5	4
BnaAREB3/DPBF3-c	Forward	bZIP-A	C8	28,592,332	28,593,802	756	251	28317.09	9.49	-0.885	5	4
BnaAREB3/DPBF3-d	Reverse	bZIP-A	A7	14,327,414	14,328,525	780	259	28760.62	9.18	-0.701	3	2
BnaAREB3/DPBF3-e	Reverse	bZIP-A	A4	1,865,066	1,866,354	816	271	29980.76	9.23	-0.845	3	2
BnaAREB3/DPBF3-f	Forward	bZIP-A	A9	26,219,385	26,221,415	744	247	27780.48	9.27	-0.858	5	4
BnaDPBF4/EEL-a	Forward	bZIP-A	C4	320,757	322,244	825	274	30782.82	8.89	-0.861	4	3
BnaDPBF4/EEL-b	Reverse	bZIP-A	A5	1,106,190	1,107,659	849	282	31,694	9.06	-0.82	3	2
BnaDPBF4/EEL-c	Reverse	bZIP-A	A4	17,514,725	17,516,695	831	276	31021.15	7.08	-0.769	2	1
BnaDPBF4/EEL-d	Forward	bZIP-A	C4	4,365,415	4,366,904	819	272	30762.92	7.08	-0.775	2	1
BnbZIP1 5a	Forward	bZIP-A	C4	16,147,885	16,149,515	1176	391	43116.39	9.42	-0.707	3	2
BnbZIP1 5b	Reverse	bZIP-A	A6	24,141,291	24,143,218	1188	395	43828.23	9.53	-0.74	3	2

Fig. 1 Phylogenetic tree of *ABFs/AREBs/ABI5s* genes among *B. napus*, (A) *thaliana*, (B) *oleracea*, *B. rapa*, and *S. lycopersicum*. The neighbor-joining method used to generate the phylogenetic tree with MEGA 11.0. by using the neighbor-joining (NJ) method with 1000 bootstrap replicates. The proteins were distributed into five subgroups. A, B, C, D, E sections indicate the five subfamilies of the *ABFs/AREBs/ABI5s* proteins



TATA and CAAT boxes were commonly found across all genes. Light-responsive elements (G-box, Box4, GTI motif) were present in over 70% of the genes. Abscisic acid (ABA)-responsive elements (ABRE) were detected in all genes except *BnaABF3-a*, *BnaAREB/DPBF3-a*, *BnaAREB/DPBF3-b*, *BnaAREB/DPBF3-c*, and *BnaDPBF4/EEL-c*. Additionally, MeJA-responsive elements (CGTCA and TGACG) were found in 18 genes, while salicylic acid-responsive (TCA), drought stress (MBS), low-temperature responsive (LTR), and defense-related (TC-rich) elements were also identified (Fig. 3).

Sub cellular localization, gene, protein interaction prediction, GO annotation in the *B. napus*

All the *BnaABFs/AREBs/ABI5s* genes were predicted using an online tool (nctu.edu.tw) named as Subcellular Localization Predictive System: (CELLO) (Yu et al. 2006) mostly all *ABFs/AREBs/ABI5s* genes members expressed in the nucleus and cytoplasm (Fig. 4A).

Furthermore, protein protein interaction revealed the relationships between *ABFs/AREBs/ABI5s* proteins, these

17 nodes among 22 showed high interactions as these take part in biological processes (Abscisic acid-activated signaling pathway and regulation, positive regulation of transcription-DNA template, and biosynthetic process) molecular function (DNA binding, transcription cis-regulatory region binding and DNA binding transcription factor activity) and cellular component as these all are present in the nucleus. According to the gene ontology, these genes are highly active in the biological process, molecular functions, and cellular activity. Moreover, some gene members such as *BnaC07g31800D*, *BnaAnng41460D*, *BnaC07g16480D*, *BnaA01g34560D*, and *BnaAnng26730D* predicted to be interlinked with *ABFs/AREBs/ABI5s* as novel functional partners proteins which belong to protein kinase superfamily members. All gene members are found in the nucleus (Fig. 4C). On the basis of gene ontology annotation, the gene functions was classified into cellular components, molecular process and biological process (Fig. 4B-C). GO annotation of *ABFs/AREBs/ABI5s* members details are provided in supplementary data (Table S5). During the biological process, *BnaABFs/AREBs/ABI5s* gene members involved in the abscisic acid-activated signaling pathways, intracellular

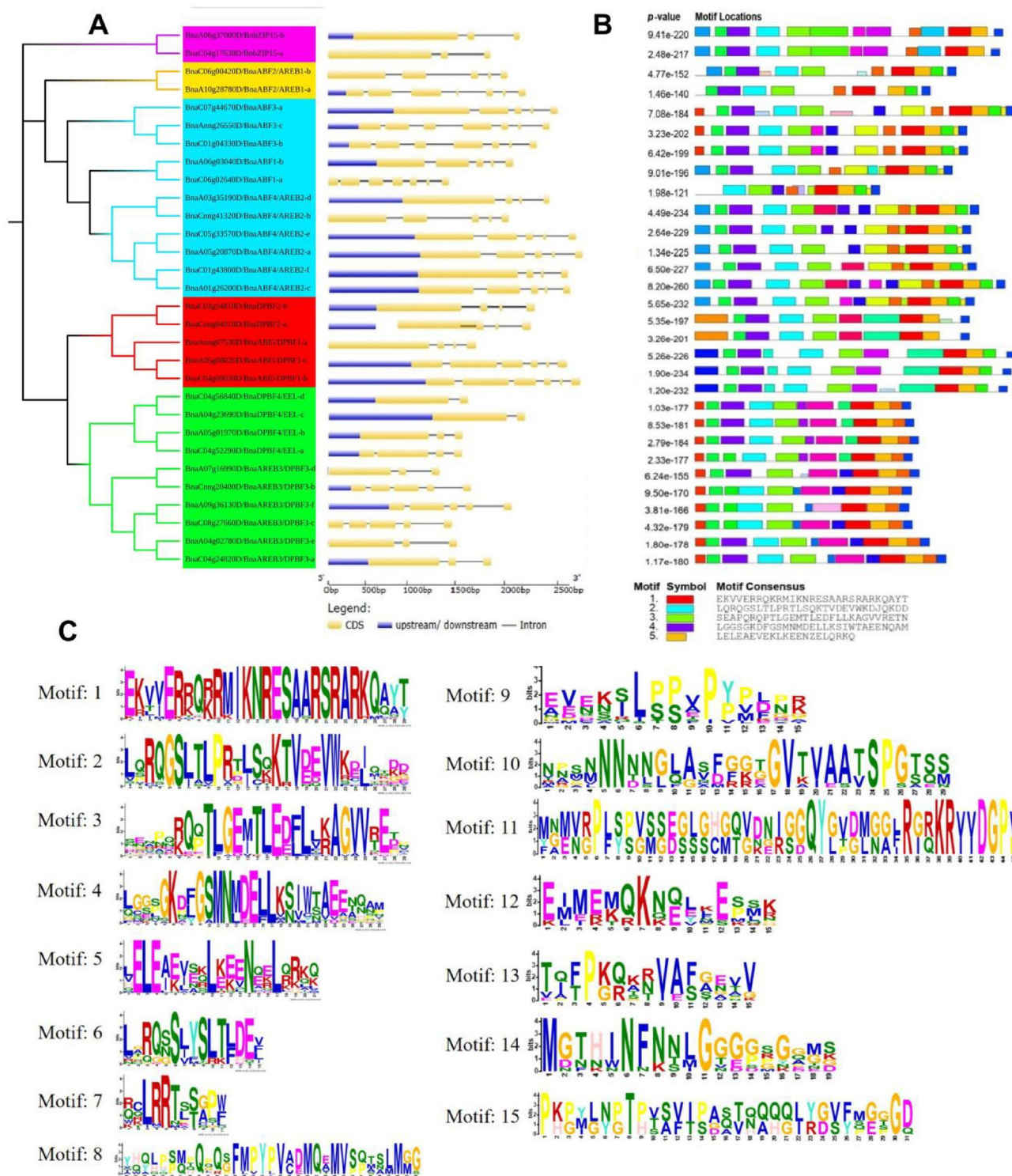


Fig. 2 The *BnaABFs/AREBs/ABI5s* phylogenetic tree, structure of genes (Fig. 2A), motif location (Fig. 2B)

signal transduction, involved in the cellular process, signal transduction, transcription-DNA templated biosynthetic, positive regulation of the abscisic acid process and regulation of the cellular metabolic process. With regard to molecular functions, *BnaABFs/AREBs/ABI5s* genes participated

in transcription cis-regulatory region binding, DNA binding transcription activator activity and DNA binding. For cellular components, all *ABFs/AREBs/ABI5s* proteins were localized in the nucleus. These results provide strong evidence that *BnaABFs/AREBs/ABI5s* genes involved in plants

Cis Element	Putative Function	<i>BnaABF1-a</i>	<i>BnaABF1-b</i>	<i>BnaABF2/AREB1-a</i>	<i>BnaABF2/AREB1-b</i>	<i>BnaABF3-a</i>	<i>BnaABF3-b</i>	<i>BnaABF3-c</i>	<i>BnaABF4/AREB2-a</i>	<i>BnaABF4/AREB2-b</i>	<i>BnaABF4/AREB2-c</i>	<i>BnaABF4/AREB2-d</i>	<i>BnaABF4/AREB2-e</i>	<i>BnaABF4/AREB2-f</i>	<i>BnaABI5/DPBF1-a</i>	<i>BnaABI5/DPBF1-b</i>	<i>BnaABI5/DPBF1-c</i>	<i>BnaDPBF2-a</i>	<i>BnaDPBF2-b</i>	<i>BnaAREB3/DPBF3-a</i>	<i>BnaAREB3/DPBF3-b</i>	<i>BnaAREB3/DPBF3-c</i>	<i>BnaAREB3/DPBF3-d</i>	<i>BnaAREB3/DPBF3-e</i>	<i>BnaAREB3/DPBF3-f</i>	<i>BnaDPBF4/EEL-a</i>	<i>BnaDPBF4/EEL-b</i>	<i>BnaDPBF4/EEL-c</i>	<i>BnaDPBF4/EEL-d</i>	<i>BnaZPI15a</i>	<i>BnaZPI15b</i>
CAAT-box	Common cis-acting element																														
TATA-box	Core promoter element																														
ACE	Light responsive element																														
Box-II	Light responsive element																														
AE-box	Light responsive element																														
AT1-motif	Light responsive element																														
ATCT-motif	Light responsive element																														
ATC-motif	Light responsive element																														
Box-4	Light responsive element																														
G-box	Light responsive element																														
GATA-motif	Light responsive element																														
GT1-motif	Light responsive element																														
I-box	Light responsive element																														
LAMP-element	Light responsive element																														
Gap-box	Light responsive element																														
TCCC-motif	Light responsive element																														
Sp1	Light responsive element																														
MRE	Light responsive element																														
G-Box	Light responsive element																														
TCT-motif	Light responsive element																														
GA-motif	Light responsive element																														
GTGGC-motif	Light responsive element																														
3-AF1	Light responsive element																														
AAAC-motif	Light responsive element																														
4c-CMA1a	Light responsive element																														
ABRE	Absciscic acid responsive element																														
AuxRR-core	Auxin responsive element																														
CGTCA	MeJA responsive element																														
GARE-motif	Gibberlin responsive element																														
P-box	Gibberlin responsive element																														
TATC-box	Gibberlin responsive element																														
TCA-element	Salicylic acid responsive element																														
TGA-element	Auxin responsive element																														
TGACG-motif	MeJA responsive element																														
ARE	Essential for anaerobic induction																														
GC-motif	Involved in anoxic specific inducibility																														
MBS	Involved in drought inducibility																														
MSA	Involved in cell cycle regulation																														
LTR	Low temperature responsiveness																														
TC-rich repeats	Defense and stress responsiveness																														
WUN-motif	Wound Responsive element																														
AT-rich	Maximal elicitor-mediated activation																														
3-AF3	Conserved DNA module array																														
CCAAT-box	MYBHv1 binding site																														
Box-II like	Cis acting regulatory element																														
CAT-box	Related to meristem expression																														
GCN4-motif	Involved in endosperm expression																														
MBS1	Flavonoid biosynthesis genes regulation																														
O2-site	Involved in zein metabolism regulation																														
AACA-motif	Involved in endosperms expression																														
Circadian	Involved in circadian control																														

Fig. 3 The *BnaABFs/AREBs/ABI5s* members initiation codon of 2 kb upstream region in *B. napus*. On the left, Cis elements including light, phytohormone, abiotic stress and growth regulation responsive elements are represented by using five different types of colors like

(brown, blue, yellow, purple and orange). On the right, Green square shows the absence of cis-element while the red indicates the presence of cis-element in the promoter region

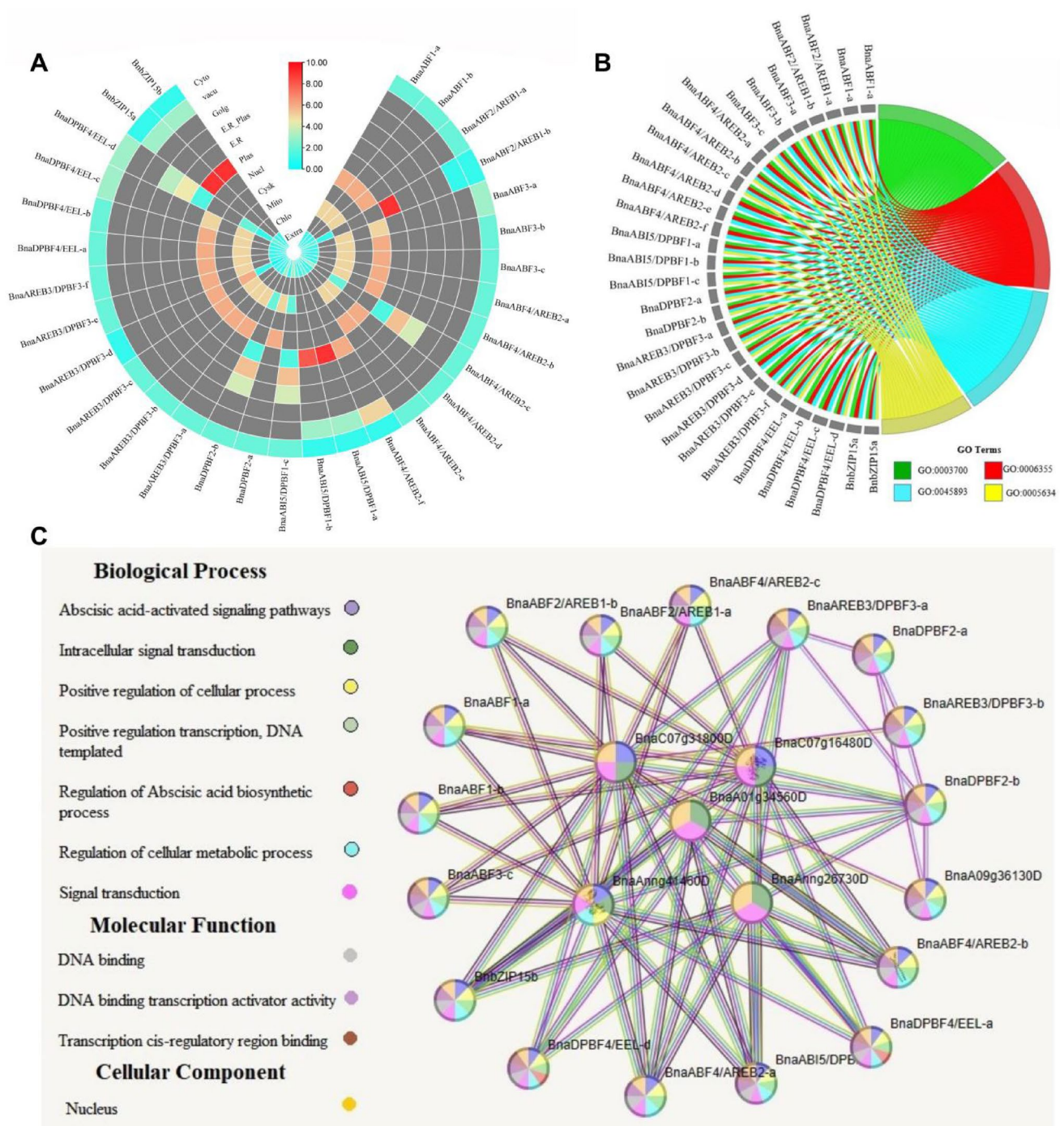


Fig. 4 Cellular Localization, Protein Protein Interaction and GO annotation of the *BnaABFs/AREBs/ABI5s* genes in *B. napus* (Fig. 4). Subcellular Localization of *BnaABFs/AREBs/ABI5s* genes in *B. napus* (Fig. 4A), Biological process including abscisic acid activated signaling pathways, intracellular signal transduction, positive regulation of cellular process, positive regulation transcription-DNA templated, regulation of abscisic acid biosynthetic process, regulation of cellular metabolic process, signal transduction, Molecular

functions are DNA binding, DNA binding transcription activator activity, Transcription cis-regulatory region binding, highlighted with different colors in *BnaABFs/AREBs/ABI5s* gene members localized in nucleus (Fig. 4A/C). Gene ontology of *ABFs/AREBs/ABI5s* genes (GO:0006355) regulation of DNA-templated transcription, (GO:0005634) nucleus and (GO:0045893) positive regulation of DNA-templated transcription, (Fig. 4B)

growth and development under unfavourable environmental conditions. Further GO annotation described under stress regulation pathways (Figure S4).

Synteny analysis and tertiary structures of *ABFs/AREBs/ABI5s* proteins

ABFs/AREBs/ABI5s genes were identified in genomes of *B. rapa*, *B. oleracea*, *(A) thaliana*, and *S. lycopersicum* to further verify the phylogenetic mechanism and we constructed the phylogenetic map among these species including (*B. oleracea*, (*B. rapa* and *Arabidopsis*)). The strong ancestral relationships of *ABFs/AREBs/ABI5s* genes between *B. oleracea*, *B. rapa*, *B. napus*, *S. lycopersicum* and *A. thaliana* were explored by collinearity analysis (Fig. 5). For more details, genes pairwise syntenic relationships among (*A. thaliana* and (*B. napus* (Figure S2A), *B. rapa* and *B. napus* (Figure S2B), *B. Oleracea* and *B. napus* (Figure S2C), *S. lycopersicum* and *B. napus* (Figure S2D) were showed to evaluate the driving force for *ABFs/AREBs* evolution.

The Swiss model was used to predict the protein structures by selecting the appropriate templates from the Protein Data Bank (PDB) based on sequence similarities. Tertiary structures showed various parallel α -helixes resented on a three-dimensional frame. These comparisons between *ABFs/AREBs/ABI5s* protein structures showed the differences and conserved structures similarities. The results of *BnaABFs/AREBs/ABI5s* protein members showed that there is high differences in their structures, as all members have different structures and diversities which may indicate the obligations in expression and functions of proteins. Ramachandran plots of *BnaABFs/AREBs/ABI5s* revealed the structure prediction was highly reliable as well as most of the amino acids dihedral angles particularly found in preferred regions. *BnaABF3-a* showed the most complex and different structure among all members.

Sequence analysis of *B. napus ABFs/AREBs/ABI5s* proteins

Sequence analysis of *ABFs/AREBs/ABI5s* proteins was performed among each other and showed the high-level sequence similarity between them (Figure S3). Identical amino acids showed with green, pink, and brown, majorities of amino acid sequences of *ABFs/AREBs/ABI5s* are highly similar to each other and have specific binding site differences which may be results of evolution. Multiple sequence alignment *ABFs/AREBs/ABI5s* proteins. The alignment among *ABFs/AREBs/ABI5s* protein sequences, green, pink and brown color indicate the high sequence similarity (Figure S3).

ABFs/AREBs/ABI5s genes expression level in different organs of *B. napus*

The *ABFs/AREBs/ABI5s* genes expressions were analyzed in different organs based on their biological functions (Fig. 6). According to the results, *ABFs/AREBs/ABI5s* genes showed higher expression levels in most of the tissues. Mostly genes showed maximum expression in all parts of plants including root, the significant expression was observed in *BnaABF1-a*, *BnaABF2/AREB-a*, *BnaABF3-a*, *BnaABF3-c*, *BnaABF4/AREB2-b*, *BnaABF4/AREB2-c*, *BnaABF4/AREB2-e*, *BnaABI5/DPBF1-b*, *BnaABI5/DPBF1-c*, *BnaAREB3/DPBF3-c*, *BnaAREB3/DPBF3-f* and *BnaDPBF4/EEL-d*, while in leaf following genes were expressed; *BnaABF2/AREB1-a*, *BnaABF2/AREB1-b*, *BnaABF3-a*, *BnaABF3-b*, and *BnaABF3-c*, *BnaABF4/AREB2-a*, *BnaABF4/AREB2-e*, *BnaABF4/AREB2-f*, *BnaAREB3/DPBF3-f*, in silique; *BnaABF1-a*, *BnaABF2/AREB1-a*, *BnaABF3-a*, *BnaABF3-c*, *BnaABF4/AREB2-a*, *BnaABF4/AREB2-e*, *BnaABI5/DPBF1-a*, *BnaAREB3/DPBF3-c* and *BnaAREB3/DPBF3-f*, in stem; *BnaABF2/AREB1-a*, *BnaABF3-b*, *BnaABF3-c*, *BnaABF4/AREB2-c*, *BnaABF4/AREB2-f*, *BnaAREB3/DPBF3-a*, *BnaAREB3/DPBF3-c* and *BnaAREB3/DPBF3-f* were significantly expressed excepts some genes members didn't showed their expression in different parts of plant, such as *ABF1/2* genes members didn't showed their expression in blossomy pistil, *ABI5/DPBF1-a* gene in leaf and wilting pistil, *BnaDPBF2-a* expression in silique, flower, ovule, and pericarp while *BnaDPBF2-b* expression in silique, flower, root, ovule, stamen and pistil. Moreover, *BnaAREB3/DPBF3-d* and *BnaAREB3/DPBF3-e* expression only in ovule and pericarp (Fig. 6A) as well as showed significant expression under three developmental stages of 17 tissues (Fig. 6B), *BnaDPBF4/EEL-a* showed weak expressions in pericarp and pistil, *BnaDPBF4/EEL-b* did not show its expression in 12 tissues but showed significant expression under developing stages of tissues. While *BnaZIP15a* and *BnaZIP15b* showed expression in silique, leaf, ovule, and pistil respectively (Fig. 6A) and at different developmental stages showed its significant expression in 17 tissues of *B. napus* (Supplementary Table S. 16; Fig. 6B).

BnaABFs/AREBs/ABI5s transcriptome expression pattern under exogenous phytohormones applications and abiotic stresses

Gene expression patterns of *BnaABFs/AREBs/ABI5s* genes were investigated under exogenously applied phytohormones hormones (Indole acetic acid (IAA), ethylene (ACC), t-zeatin (TZ), Brassinosteroid (BL), Gibberellic acid 3 (GA), Absciscic acid (ABA), jasmonic acid (JA), cytokinin (Ck), and abiotic stresses including heat, salt, cold

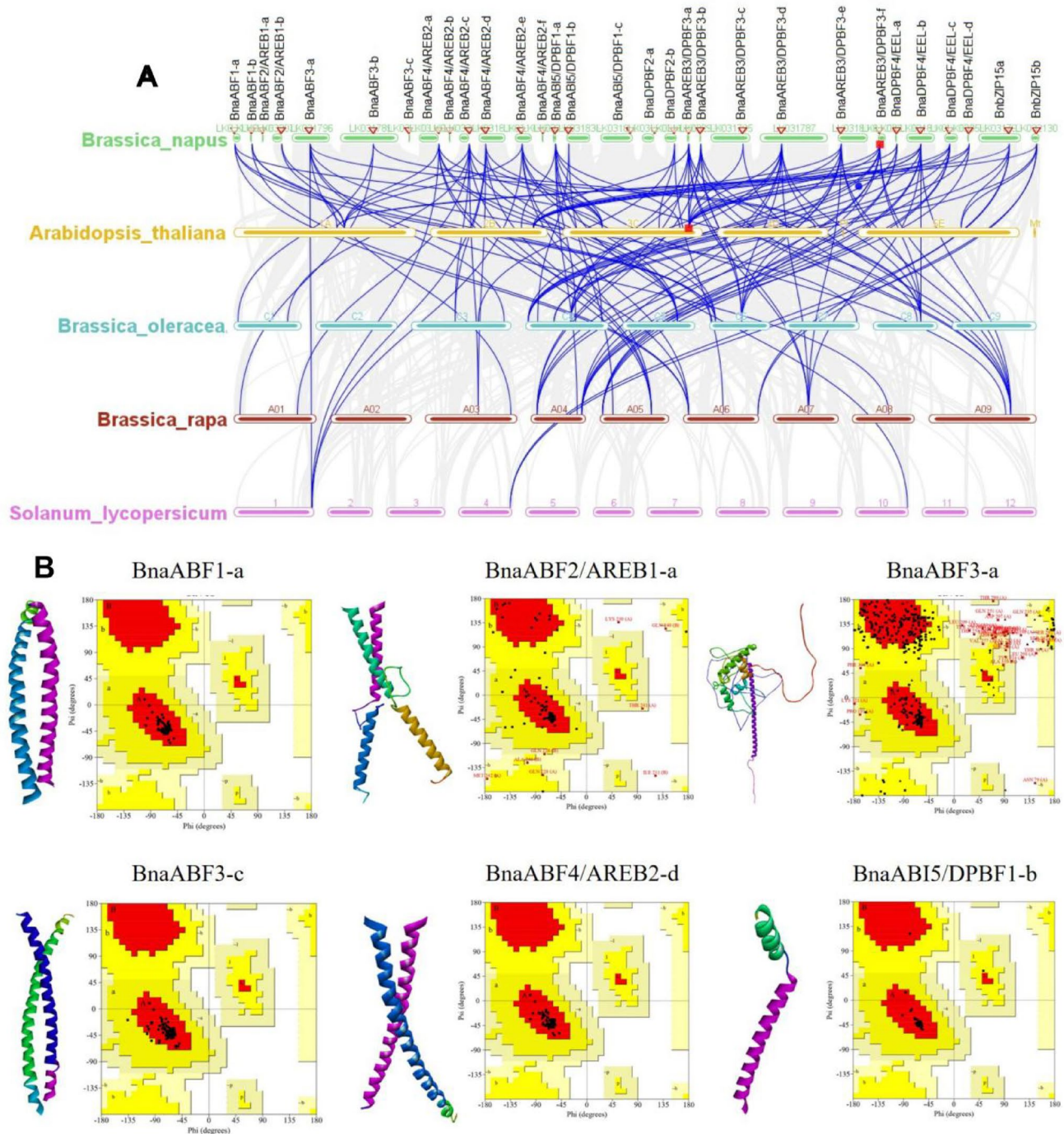


Fig. 5 Synteny relationship of *ABFs/AREBs/ABI5s* genes among *B. napus*, *B. oleracea*, *B. rapa*, *S. lycopersicum* and *Arabidopsis*, chromosomes are labeled with different colors and genes pair relationships between (A) *thaliana* and (B) *napus*, *B. napus* and *B. rapa*, *B. napus* and *B. oleracea*, *B. napus* and *S. lycopersicum* shown in supplementary Figs. 2 A, 2B, 2 C and 2D respectively. To express the colinearity,

E-value 'score/max' ratio colouring with blue ≤ 0.25 , green ≤ 0.50 , orange ≤ 0.75 , red > 0.75 pattern was used. The 3D structures of *BnaABFs/AREBs/ABI5s* proteins and Ramachandran plot analysis. The brown, yellow, and red represent the generally permitted areas, permitted, and preferred areas respectively. The white area or lightest areas structures indicated the impossible

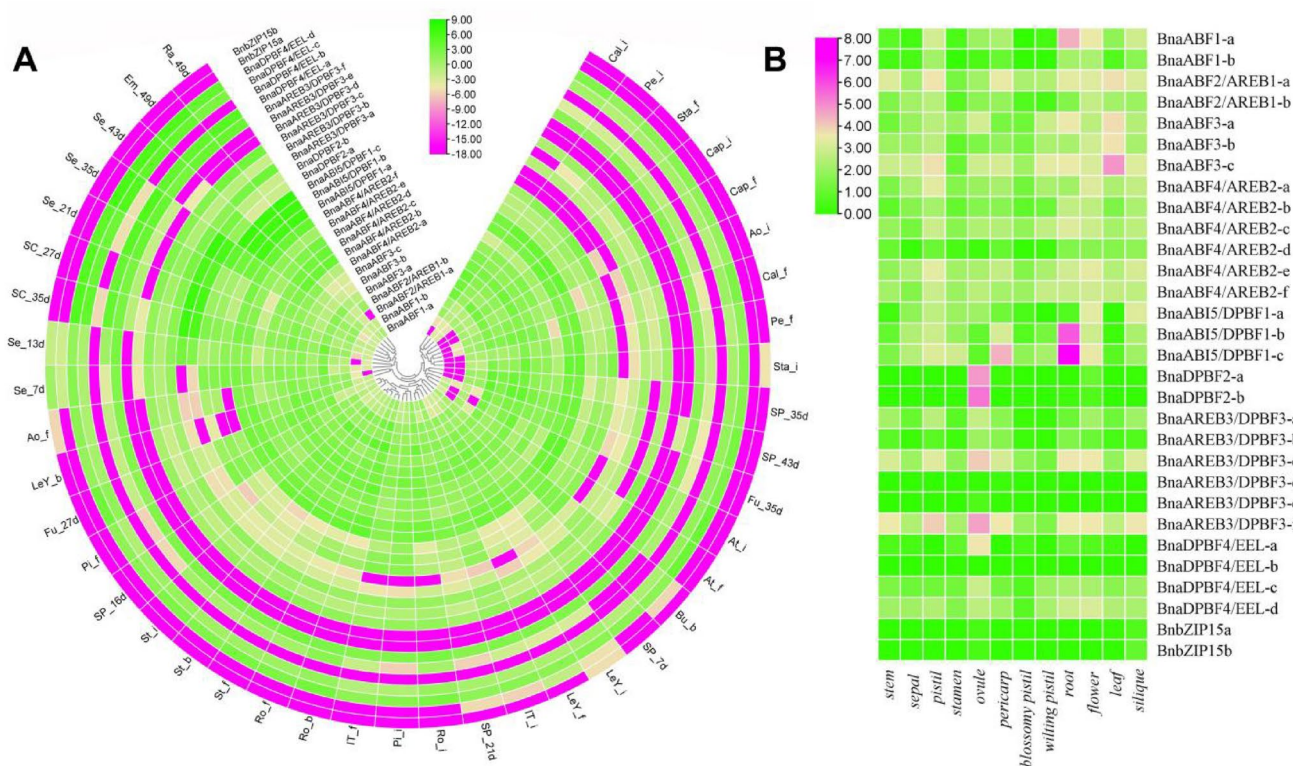


Fig. 6 The *BnaABFs/AREBs/ABI5s* genes expression patterns in twelve tissues (Stem, blossomy pistil, silique, leaf, flower, root, wilting pistil, pericarp, ovule, stamen, pistil and sepal) (Fig. 7A) and in different tissues (the top of main inflorescence; filaments, anthers, stamens, embryos, seedcoat, funiculus, silique pods, cotyledons, hypocrites, roots, stems, leaves, buds, seeds, flowers) at various developmental stages (Bud stage (b), Initial bloom stage (i), Full-bloom stage(f)(Fig. 7B)). At different development stages and tissue, genes

freezing, drought, and osmotic stress under 4-time intervals (6 h, 3 h, 1 h, 0.5 h) (Fig. 7B) and for growth hormones (10 $\mu\text{mol}\cdot\text{L}^{-1}$) (IAA, ABA, BL, GA, TZ, MeJA and ETH) at 5-time intervals (6 h, 3 h, 1 h, 0.5 h, 0) of control in root and leaves (Fig. 7A).

Furthermore, under abiotic stresses, 5-time intervals for stresses like drought (1 h exposure with airflow), freezing (-4°C temperature for 3 h and 25°C with normal recovery), heat (38°C for 3 h stress temperature by 25°C recovery temperature), cold (4°C), salinity stress ($200\text{ mmol}\cdot\text{L}^{-1}$ NaCl) and osmosis stress ($300\text{ mmol}\cdot\text{L}^{-1}$ mannitol) treatments were applied to plants for 24 h and 8-time intervals for the control treatment (24 h, 12 h, 6 h, 3 h, 1 h, 0.5 h, 0.25 h). Genes expression was calculated by $\text{Log}_{10}(\text{TPM}+1)$ and visualized through TBTools software (Fig. 7B).

Under exogenous hormone treatments, various genes were expressed at low level either didn't express in roots and leaves. *BnaABF3-c*, and *BnaABF3-b*, were highly expressed in all time points and hormones. *BnaABF3-a*, *BnaABF1-b*, *BnaABF1-a*, *BnaABF2/AREB1-a*, *BnaABF2/AREB1-b*, were highly expressed with maximum time intervals under

expression were calculated by fragments per kilobase of exon model per million (FPKM) values obtained from the RNA-seq data. Tissue abbreviation file attached in S8. Heatmap showed all 30 genes presents in twelve tissues of *B. napus* Cv *ZS11*. Color bar showed the log2 FPKM values. Significantly, some genes showed higher expression in five tissues such as stem, root, flower, leaf and silique represented in graph bar with S.E value

CK, ABA, ACC, IAA, BL, and GAs treatments, while *BnaABI5/DPBF1-c*, *BnaABI5/DPBF1-b* expressed in 0.5 h time point with Ga, JA, TZ, BL, ACC and IAA (Fig. 7A). Moreover, *BnaABF1s* showed significant response in root and shoot at 6-hour time intervals with all phytohormones (Figure S8).

Expression analysis of *BnaABFs/AREBs/ABI5s* under nitrogen deficiency and other nutrients

To further investigate the *BnaABFs/AREBs/ABI5s* genes expression patterns under nitrogen and other 8 individual nutrient deficiency in *B. napus*, plants were grown in the normal nutrients media, and after 24 h of growing, seedlings were separated into seven sub-groups including a control solution which consists of complete nutrients and other six groups exposed to specific single macronutrient (–P, –Mg, –S, –Ca, –K, or –N,) and other 3 nutrients S, Cl and Co deprivation. After ten days of nutrient stress, control plants, and a specific single nutrient-deprived plant were harvested. The relative expressions of *BnaABFs/AREBs/ABI5s* genes

A



were determined under nutrient deprivation relative to the control treatment as shown in (Fig. 8).

BnaABF1-a and *BnaABF1-b* genes were down regulated under all nutrient deprivation and did not show a significant difference, while the *BnaABF2/AREB1-a* gene was also down regulated among all treatments comparative to control but showed significance under Mg deprivation with compared to control treatment. *BnaABF2/AREB1-b* gene had 2-fold up-regulation under S, Cl, N deprivation and down-regulation under P, Mg, K, Ca, Mn as well as the gene was highly significant under S, Cl, Ca, and N deprivation relative to control. *BnaABF3-a* gene was 1–2-fold up regulated under Cl, Co, Mg, N and was down regulated under P, K, Ca, Mn, S. *BnaABF3-a* gene was significantly regulated under N, Mg, Mn, Co, Cl with comparative to control treatment. *BnaABF3-b* gene was 1–3-fold significantly up regulated while 2.8-fold under N, 3-fold under Co, and 2-fold under Mn deprivation (Fig. 8).

The expression of *BnaABFs/AREBs/ABI5s* genes displayed distinct and stress-specific patterns under nutrient deprivation (Fig. 8). Among them, *BnaABF3-c* was strongly induced, showing a 3.4-fold increase under nitrogen (N) and cobalt (Co) deficiency, a 3-fold increase under magnesium (Mg) and chlorine (Cl) deficiency, and a 2.5-fold increase under manganese (Mn) deficiency. In contrast, *BnaABF4/AREB2-a* was consistently repressed, with a 2-fold down-regulation under Mg, calcium (Ca), and Cl deprivation.

Members of the *BnaABF4/AREB2* subfamily exhibited divergent expression patterns. *BnaABF4/AREB2-b*, *BnaABF4/AREB2-c*, and *BnaABF4/AREB2-d* were generally downregulated across most stresses; however, *BnaABF4/AREB2-b* was induced by N deprivation, while *BnaABF4/AREB2-d* was upregulated under Mg deficiency. Similarly, *BnaABF4/AREB2-e* and *BnaABF4/AREB2-f* were broadly repressed under all nutrient stresses, except that *BnaABF4/AREB2-f* was uniquely upregulated twofold under Ca deficiency.

The *BnaABI5/DPBF1* subfamily showed a stress-specific response to Cl deficiency, with *BnaABI5/DPBF1-b* and *BnaABI5/DPBF1-c* upregulated 2.6-fold and 2.2-fold, respectively, but both were repressed under other nutrient stresses. Most *BnaAREB3/DPBF3* members (*BnaAREB3/DPBF3-a*, *-b*, *-c*, and *-f*) were slightly downregulated, though changes were not significant compared to the control. Finally, *BnaDPBF4/EEL-c* and *BnaDPBF4/EEL-d* were consistently repressed under nutrient deprivation, with *BnaDPBF4/EEL-c* showing particularly strong reductions of 1.7-fold and 1.4-fold under Mg deficiency.

Discussion

In this study, we systematically identified and characterized the *ABFs/AREBs/ABI5s* TF family in oilseed rape. A genome-wide survey revealed 30 *BnaABFs/AREBs/ABI5s* genes, which clustered into five conserved groups alongside their *Arabidopsis* orthologs in phylogenetic and synteny analyses (Zhao et al. 2016). This strong evolutionary conservation indicates that the core regulatory functions of these TFs in stress signaling have been largely maintained following genome duplication events in *B. napus*.

Consistent with their role as transcriptional regulators, subcellular localization analysis showed that most *BnaABFs/AREBs/ABI5s* proteins are targeted to the nucleus. Promoter analysis further revealed a high abundance of cis-elements associated with abiotic stress and phytohormone responses, supporting their involvement in diverse regulatory networks. These in silico predictions were validated by expression profiling, which demonstrated that *BnaABFs/AREBs/ABI5s* genes are dynamically regulated by tissue type, developmental stage, and environmental stimuli.

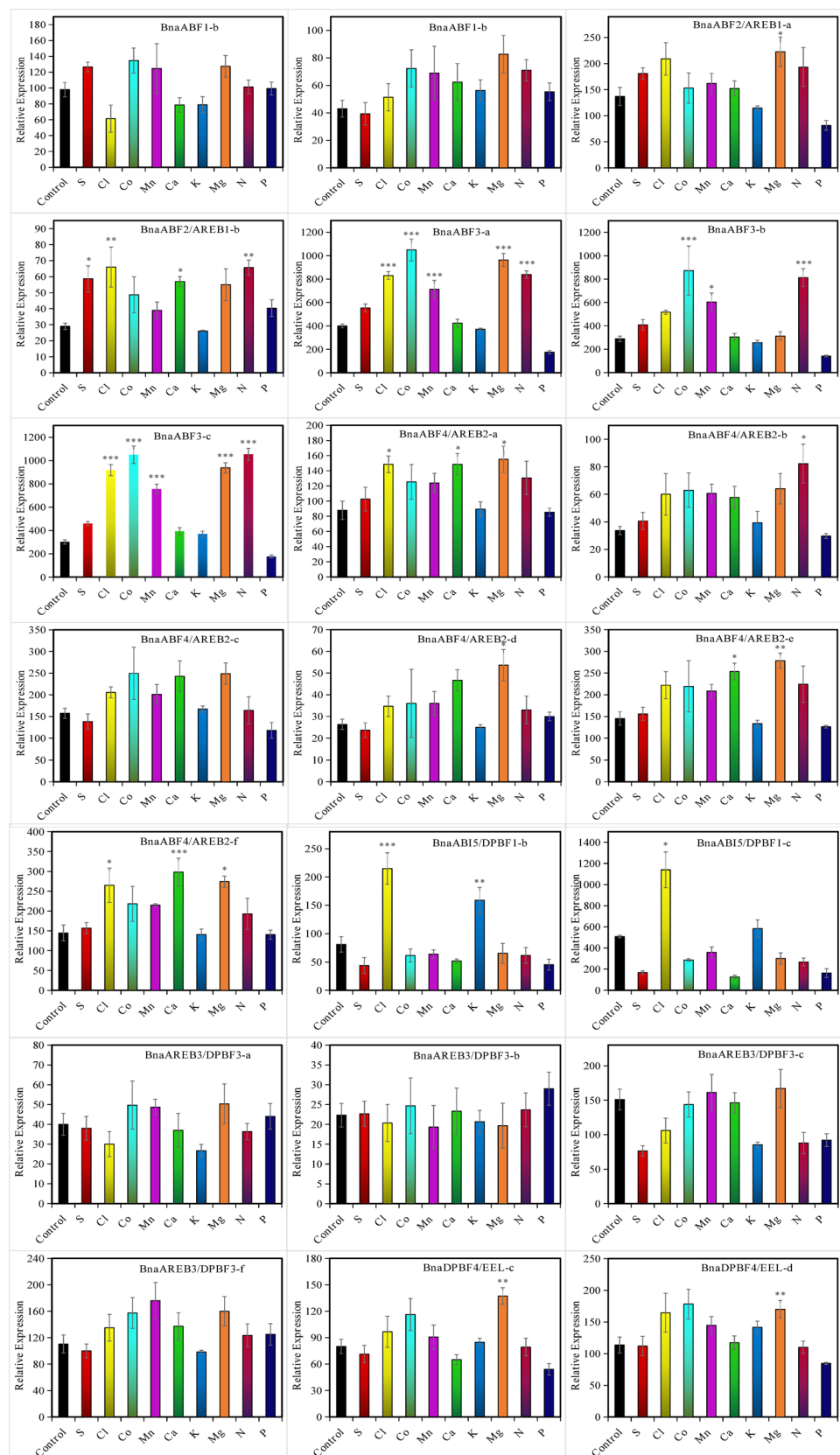
Tissue-specific expression analysis indicated functional specialization: that *BnaABF1-a*, *BnaABI5/DPBF1-b* and *BnaABI5/DPBF1-c* were highly expressed in roots, while *BnaABF3-c* exhibited significant expression in shoots. Moreover, *BnaDPBF2-a*, *BnaDPBF2-b*, *BnaAREB3/DPBF3-c*, *BnaAREB3/DPBF3-f* genes were predominantly expressed in ovule (Kim et al. 2004; Muñiz García et al. 2012).

Our most significant finding is the distinct and highly specific expression patterns of *BnaABFs/AREBs/ABI5s* genes under diverse stresses. While these TFs are established mediators of ABA signaling and drought stress, their complex regulation by both nutrient deprivation and a broad spectrum of phytohormones is less characterized in polyploid crops.

The response to exogenous hormone application was complex and time-dependent. Notably, *BnaABF3-b* and *BnaABF3-c* emerged as highly responsive hubs, showing significant expression across multiple time points for all applied hormones. Other genes, such as *BnaABF1-a*, *BnaABF1-b*, and *BnaABF2/AREB1-a*, *BnaABF2/AREB1-b*, exhibited maximum expression at later intervals (6 h) under CK, ABA, ACC, IAA, BL, and GA treatments. This suggests a hierarchy in the hormonal response network, with certain TFs acting as early responders and others as late effectors, fine-tuning the plant's adaptation strategy.

This complex hormonal regulation directly complements our findings under nutrient stress. For instance, the strong upregulation of *BnaABF3-c* under multiple nutrient deficiencies (N, Co, Mg, Cl, Mn) positions this paralog as a master integrator of both nutrient and hormonal status. The

Fig. 8 The expression patterns and regulatory responses of *BnaABFs/AREBs/ABI5s* genes to nine individual nutrient deprivation treatments were analyzed in roots and compared to a control (Figure 8). A heatmap of Log2 fold changes shows that *BnaABF/AREB/ABI5* genes clustered based on their expression patterns, with red indicating up-regulation and blue indicating down-regulation (Figure 8B). Statistical significance, determined by a Student's *t*-test comparing the control to the 10-day nutrient stress groups, is denoted by asterisks (**p* < 0.05, ***p* < 0.01, ****p* < 0.001). Data are presented as mean values ± standard error (SE) (**n* = 3 biological replicates)



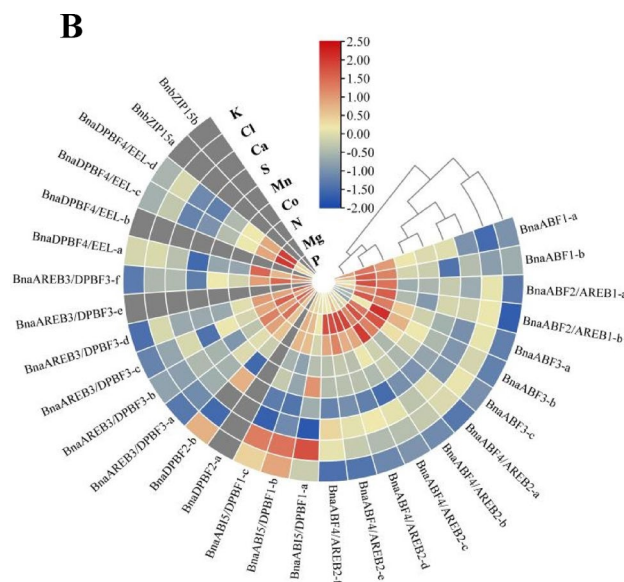


Fig. 8 (continued)

fact that the same gene is highly responsive to hormones like ABA, JA, and ACC suggests a mechanism where nutrient imbalances are perceived and signaled through classic hormone pathways, with *BnaABFs/AREBs/ABI5s* TFs acting as central processors of this information.

Furthermore, the divergent expression of paralogous genes such as the specific downregulation of *BnaABF4/AREB2-a* under Mg, Ca, and Cl stress versus its response to hormones highlights the functional diversification that has occurred following the whole-genome duplication in *B. napus*. This subfunctionalization provides *B. napus* with a sophisticated and nuanced regulatory network to prioritize responses to a multitude of simultaneous environmental cues.

Furthermore, temporal expression profiling under hormonal treatments (ABA, IAA, BR, MeJA, GA, SA) at multiple time points indicated that these TFs are dynamic early responders, integral to signaling pathways that regulate root system architecture, seed maturation, and germination (Jing and Strader 2019; Ma et al. 2018; Wei et al. 2022).

Notably, *BnaABF2/AREB1-a* and *BnaABF1-b* emerged as highly responsive nodes under phytohormonal induction. Under abiotic stress conditions, *BnaABF3-c*, *BnaABF3-b* and *BnaABF3-a* were significantly upregulated across all time points, underscoring their pivotal role in stress regulation. The functional characterization of *ABF1*, *ABF2*, *ABF3* genes highlighted their involvement in heat, drought, osmotic and salt stress tolerance by modulating ABA signaling pathways to reduce water loss under high-temperature conditions (Yoshida et al. 2010; Fujita et al. 2013; Mubarik et al. 2021; Muñiz García et al. 2014; Yang et al. 2020; Wang et al. 2016). Furthermore, *ABI5* played a crucial role

in seed maturation, germination, and ABA-mediated stress responses, exhibiting strong crosstalk with other phytohormones (Skubacz et al. 2016; Linden et al. 2021).

Beyond phytohormonal and stress responses, nutrient deprivation, particularly nitrogen deficiency, significantly influenced *ABFs/AREBs/ABI5s* gene expression. Nutrient imbalances (Mg, N, S, K, Ca, P) are known to cause phenotypic symptoms such as leaf chlorosis, root ramification, ultimately impacting seed yield, plant growth and development (O'Rourke et al. 2020; Abubakar et al. 2023). The deficiency of a single macronutrient can disrupt the uptake of others, exacerbating nutrient stress (Courbet et al. 2021).

Our results demonstrated that *BnaABF3s*, *BnaABF2s*, *BnaABF1s* were significantly upregulated under nitrogen stress, with *BnaABF3-c* showing a remarkable 3.5-fold induction, while *BnaABF2/AREB1-b*, *BnaABF3-b* and *BnaABF3-c* exhibited 1- to 2-fold fold increases in expres-

sion. Nitrate a crucial macronutrient, plays a fundamental role in plant growth and metabolism, operating through ABA signaling in the endoplasmic reticulum in the endoplasmic reticulum (Ondzighi-Assoume et al. 2016). The strong induction of *ABF2* and *ABF3* in response to nutrient deprivation suggests their involvement in key biological processes including transport mechanism, hormone response, root development (Nan et al. 2021; Xie et al. 2022). Particularly in *Arabidopsis thaliana*, *ABF2/ABF3* genes were implicated in nitrate response regulation within the endodermis and epidermis, modulating the expression of downstream genes to mitigate the nitrogen stress (Contreras-López et al. 2022).

These findings demonstrate that *BnaABFs/AREBs/ABI5s* TFs act as central regulators of hormonal, abiotic, and nutrient stress responses in *B. napus*. Given that rapeseed is the world's third-largest oilseed crop yet suffers from inherently low NUE, our study has strong agronomic significance. Excessive fertilizer application is often required to sustain yields, leading to nutrient leaching, soil degradation, and economic inefficiency (Ayub et al. 2025; Farooq et al. 2022).

Future research should prioritize the functional characterization of key candidates such as *BnaABF2s* and *BnaABF3s* to clarify their roles in nitrate uptake, utilization, and downstream signaling. Ultimately, integrating this knowledge into breeding programs could accelerate the development of nutrient-efficient and climate-resilient oilseed rape cultivars, thereby enhancing productivity while reducing environmental costs.

Conclusions

In this study,, we identified 30 *BnaABFs/AREBs/ABI5s* genes in *B. napus* and classified them into five phylogenetically conserved groups. Integrative analyses of their evolutionary relationships, chromosomal distribution, duplication events, subcellular localization, and cis-regulatory elements provided valuable insights into their potential regulatory roles.

profiling demonstrated that *BnaABFs/AREBs/ABI5s* genes are dynamically regulated by nutrient deprivation, phytohormone treatments, and abiotic stresses across different tissues. Among them, *BnaABF1-a*, *BnaABF1-b*, *BnaABF2-a*, *BnaABF2-b*, *BnaABF3-a*, *BnaABF3-b*, *BnaABF3-c*, *BnaABI5-a*, and *BnaABI5-b* exhibited strong transcriptional responses. Notably, *BnaABF2s* and *BnaABF3s* genes were highly induced under nitrogen deficiency, underscoring their pivotal roles in nutrient signaling and adaptive stress responses in *B. napus*.

Collectively, these findings advance our understanding of the *BnaABFs/AREBs/ABI5s* sub-family as key regulators of stress resilience and hormone-mediated signaling in *B. napus*. Importantly, given the crop's inherently low nitrogen use efficiency and dependence on high fertilizer inputs, this work provides a foundation for targeted functional studies of *BnaABF2s* and *BnaABF3s*. Such efforts may ultimately enable the development of improved rapeseed cultivars with enhanced nitrogen uptake and utilization, greater stress tolerance, and reduced environmental impact contributing to more sustainable agricultural practices.

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Data availability The RNA-Seq data used in this study are available in the Sequence Read Archive (SRA) at NCBI (SRA accession: PRJNA394926; PRJNA748031) repository.

Declarations

Competing interests The authors declare no competing interests.

Supplementary Information Table S1: paralogous genes Ka/Ks ratio. Table S2: Orthologous genes Ka/Ks ratio. Table S3: Cis-element annotation. Table S4: Motif details. Table S5: Gene Subcellular localization.

Table S6: Gene location on chromosome. Table S7: Gene ontology. Table S8: Genes expression in different organs. Table S8A: The abbreviation of organs and Tissues. Table S9: Genes expression under Different Hormones. Table S10: Genes under various Abiotic stresses. Table S11: Genes expression under macronutrient deprivation. Table S12: Genes Primers. Figure S1: Gene localization and duplication. Figure S2: Collinearity analysis. Figure S3: Protein sequence analysis. Figure S4: GO annotation pathways.

Author contributions Author Contributions: Conceptualization, A.A. and Y.G.; writing—original draft preparation, A.A.; software, A.N., K.A and D.S; formal analysis, Y.N., M.F., H.A and X.Y; writing—review and editing, H.T., J.H and Y.G. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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