




Article

Genome-Wide Identification of Brassicaceae Hormone-Related Transcription Factors and Their Roles in Stress Adaptation and Plant Height Regulation in Allotetraploid Rapeseed

Shengjie Ma ^{1,2,†}, Liwei Zheng ^{1,2,†}, Xiaohan Liu ³, Kaiyan Zhang ^{1,2}, Linlin Hu ^{1,2}, Yingpeng Hua ¹ and Jinyong Huang ^{1,2,3,*} 

¹ School of Agricultural Sciences, Zhengzhou University, Zhengzhou 450001, China

² Zhengzhou Key Laboratory of Quality Improvement and Efficient Nutrient Use for Main Economic Crops, Zhengzhou 450001, China

³ School of Life Sciences, Zhengzhou University, Zhengzhou 450001, China

* Correspondence: jinyhuang@zzu.edu.cn

† These authors contributed equally to this work.



Citation: Ma, S.; Zheng, L.; Liu, X.; Zhang, K.; Hu, L.; Hua, Y.; Huang, J. Genome-Wide Identification of Brassicaceae Hormone-Related Transcription Factors and Their Roles in Stress Adaptation and Plant Height Regulation in Allotetraploid Rapeseed. *Int. J. Mol. Sci.* **2022**, *23*, 8762. <https://doi.org/10.3390/ijms23158762>

Academic Editor: Karen Skriver

Received: 7 July 2022

Accepted: 3 August 2022

Published: 6 August 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Phytohormone-related transcription factors (TFs) are involved in regulating stress responses and plant growth. However, systematic analysis of these TFs in Brassicaceae is limited, and their functions in stress adaptation and plant height (PH) regulation remain unclear. In this study, 2115 hormone-related TFs were identified in nine Brassicaceae species. Specific domains were found in several Brassicaceae hormone-related TFs, which may be associated with diverse functions. Syntenic analysis indicated that expansion of these genes was mainly caused by segmental duplication, with whole-genome duplication occurring in some species. Differential expression analysis and gene co-expression network analysis identified seven phytohormone-related TFs (*BnaWRKY7*, *21*, *32*, *38*, *52*, *BnaGL3-4*, and *BnaAREB2-5*) as possible key genes for cadmium (Cd) toxicity, salinity stress, and potassium (K) and nitrogen (N) deficiencies. Furthermore, *BnaWRKY42* and *BnaARR21* may play essential roles in plant height. Weighted gene co-expression network analysis (WGCNA) identified 15 phytohormone-related TFs and their potential target genes regulating stress adaptation and plant height. Among the above genes, *BnaWRKY56* and *BnaWRKY60* responded to four different stresses simultaneously, and *BnaWRKY42* was identified in two dwarf rapeseeds. In summary, several candidate genes for stress resistance (*BnaWRKY56* and *BnaWRKY60*) and plant height (*BnaWRKY42*) were identified. These findings should help elucidate the biological roles of Brassicaceae hormone-related TFs, and the identified candidate genes should provide a genetic resource for the potential development of stress-tolerant and dwarf oilseed plants.

Keywords: Brassicaceae; phytohormone; transcription factors; abiotic stress; plant height

1. Introduction

Plant hormones (phytohormones) are chemical messengers that coordinate plant growth and stress adaptation [1]. Phytohormones include auxin (IAA), cytokinin (CTK), abscisic acid (ABA), gibberellin (GA), ethylene (ET), brassinosteroid (BR), jasmonic acid (JA), salicylic acid (SA), and strigolactones (SL) [2]. Phytohormone-related transcription factors (TFs) [e.g., auxin response factors (ARFs) for IAA [3]; response regulators (RRs) for CTK [4]; ABA-insensitive (ABIs) and ABA-responsive element-binding (AREBs) for ABA [5]; gibberellin-insensitive (GAIs), rapid geriatric assessment (RGAs), and RGA-LIKE (RGLs) for GA [6]; ethylene-insensitive (EINs) and ethylene-insensitive-like (EILs) for ET [7]; brassinazole-resistant (BZR) and BES1/BZR1 HOMOLOG (BEHs) for BR [8]; MYB21, MYB24, MYB75, MYC2, MYC3, MYC4, and GLABRA3 (GL3) for JA [9]; WRKY18, WRKY38, WRKY53, WRKY54, WRKY58, WRKY59, WRKY66, and WRKY70 for SA [10];

and TCP12 and TCP18 for SL [11] play essential roles in phytohormone signal transduction, plant growth, and stress adaptation.

ARFs regulate auxin response genes during plant growth and defense. For example, *arf2* mutants contain large seeds and are drought-tolerant [12], while overexpression of *TaARF4* in *Arabidopsis* causes shortened primary root length and plant height [13]. RRs for CTK can be categorized into types A and B according to their structure, expression pattern, and function [14]. Type-A RR genes, such as *ARR4* and *ARR5*, can be up-regulated by drought, salinity, and cold temperatures [15]. Overexpression of type-B *OsORR2* results in dwarfism in rice [16]. There are two types of ABA-related TFs (ABIs and AREBs) in *Arabidopsis*. Lateral root growth and photosynthesis are under the control of *ABI5* [17], while *AREB1* participates in the regulation of drought tolerance [18]. Five DELLA proteins, including *GAI*, *RGA*, *RGL1*, *RGL2*, and *RGL3*, mediate GA signal transduction [19], and play essential roles in promoting internode elongation [20]. In addition, *EIN3* and *EIL1* mediate ethylene responses, including stem elongation, maturation, senescence, and stress adaptation [21,22].

As a class of phytohormone, BR controls growth and stress tolerance. *BZR1* and *BES1* are critical components of the BR signaling pathway [8] and participate in drought resistance positively and negatively [23]. *BES1* and *BZR1* regulate plant architecture and their mutants are dwarfs [24]. JAs are well-recognized stress-related hormones and are involved in plant growth and development [25]. *MYB21*, *MYB24*, *MYB75*, *GL3*, and *MYC2-4* are reported to influence JA signals, cell differentiation, and plant defense reactions [26]. SA affects seed germination, cell growth, stomatal closure, stress response, nitrogen fixation, senescence, and seed setting rate [27]. Six WRKY TFs (*WRKY18*, *WRKY38*, *WRKY53-54*, *WRKY58-59*, *WRKY66* and *WRKY70*) mediate SA signals [10,28]. As a class of carotenoid-derived plant hormones, SLs affect branching, plant height (PH), drought adaptation, and phosphate starvation. Furthermore, *TCP12* and *TCP18* in the SL signaling pathway regulate plant architecture [29].

Abiotic stresses, including salinity, drought, nutrient deficiencies, and metal toxicity, severely affect agricultural crop productivity [30], and Brassicaceae is no exception. For instance, drought and high salinity can result in reduced biomass, seed germination, and shoot and root growth in *Brassica napus* and *Brassica oleracea* [31]. Plants have developed multiple mechanisms to respond to damage caused by stress, including transcription and regulation. For example, inducible expression of *ARR2* enhances drought and freezing tolerance in *Arabidopsis* [32]; *BnaWRKY47* contributes to the adaptation of rapeseed to B deficiency [33]; and *BnaRGA* proteins play important roles in *B. napus* adaptation to water-deficit stress [34].

Brassicaceae plants are important vegetable and oil crops [35]. Phytohormone-related TFs play crucial roles in plant growth and stress adaptation. However, the systemic study of TFs in Brassicaceae remains scarce. In this study, comprehensive analyses of TFs were performed in nine Brassicaceae species, including *Arabidopsis thaliana* (*A. thaliana*), *Brassica napus* (*B. napus*), *Brassica carinata* (*B. carinata*), *Brassica juncea* (*B. juncea*), *Brassica nigra* (*B. nigra*), *Brassica oleracea* (*B. oleracea*), *Brassica rapa* (*B. rapa*), *Capsella rubella* (*C. rubella*) and *Camelina sativa* (*C. sativa*). Chromosome locations, gene/protein structures, and phylogenetic and syntenic analyses were determined. Their potential involvement in regulating Cd, salt, K and N stress, and PH were investigated in allotetraploid rapeseed. The findings of this study should provide a valuable resource for Brassicaceae hormone-related TFs.

2. Results

2.1. Identification, Conserved Domain, and Gene Structure Analyses of Phytohormone-Related TF Genes in Brassicaceae

In total, 2115 phytohormone-related TFs were identified, including 82, 318, 314, 308, 223, 216, 203, 289, and 162 in *A. thaliana*, *B. napus*, *B. carinata*, *B. juncea*, *B. nigra*, *B. oleracea*, *B. rapa*, *C. sativa*, and *C. rubella*, respectively (Figure S1a and Table S1). Furthermore, we identified 363 IAA-, 473 CTK-, 123 ABA-, 70 GA-, 106 ET-, 143 BR-, 288 JA-, 454 SA-,

and 96 SL-related TFs in the above species. These genes were named based on their chromosomal location (Figure S2).

In general, each type of Brassicaceae hormone-related TF contained conserved domains (Figure S3), with some exceptions. For example, nine additional domains (i.e., atrophin-1, HSF, COG5099) were found in IAA-related TFs, and 12 specific domains were found in CTK-related TFs, e.g., Med15 in BcaARR18 and A_thal in BjuARR40. Each class of phytohormone-related TF shared a similar gene structure. Of note, several TFs [e.g., BcaTCP18-2 (12 kb, Figure S3-2) and BcaARF10 (24 kb, Figure S3-4)] contained long introns.

2.2. Phylogenetic and Syntenic Analyses of Brassicaceae Hormone-Related TFs

Phylogenetic trees showed that most *B. napus*, *B. carinata*, *B. juncea*, *B. nigra*, *B. oleracea*, *B. rapa*, *C. sativa*, and *C. rubella* hormone-related TFs were highly homologous to the *Arabidopsis* TFs (Figure S4). In addition, the TFs were divided into distinct groups depending on phylogenetic analysis. For example, RGL3s were clustered in subgroup a, RGL2s in sub-group b, RGL1s in sub-group c, GAIs in sub-groups d and e, and RGAs in sub-group f (Figure S4-4).

To determine the expansion patterns of phytohormone-related TFs, duplication events in the nine Brassicaceae species were investigated (Figure S5). In total, 2030 duplicated gene pairs were found, including five, 359, 393, 386, 176, 165, 154, 357, and 40 pairs in *A. thaliana*, *B. napus*, *B. carinata*, *B. juncea*, *B. nigra*, *B. oleracea*, *B. rapa*, *C. sativa*, and *C. rubella*, respectively (Figure S1b). To further understand the potential roles of unknown Brassicaceae hormone-related TFs, syntenic analysis between eight non-model Brassicaceae species and *Arabidopsis* was performed. In total, 120, 215, 223, 157, 145, 135, 206, and 90 gene pairs were identified in *A. thaliana*-*B. napus*, *A. thaliana*-*B. carinata*, *A. thaliana*-*B. juncea*, *A. thaliana*-*B. nigra*, *A. thaliana*-*B. oleracea*, *A. thaliana*-*B. rapa*, *A. thaliana*-*C. sativa* and *A. thaliana*-*C. rubella* (Figures S1c and S6).

The ratio of non-synonymous to synonymous substitutions (Ka/Ks) was calculated to clarify divergence among orthologous gene pairs. The Ka/Ks ratios in most gene pairs were less than 1, except for *CsaWRKY48_CsaWRKY27*, *CsaARR14_CsaARR22*, *CsaMYB75-10_CsaMYB75-1*, *CsaWRKY48_CsaWRKY18*, *BcaMYB21/24-2_BcaGL3-6*, *BjuARR7_BjuARR69*, *BnaARR11_BnaARR52*, *BnaARR13_BnaARR56*, and *BnaTCP12-1_BnaTCP12-2* (Table S2). The Ka/Ks ratios between gene pairs in Brassicaceae and *Arabidopsis* were all less than 1 (Table S3).

2.3. Expression Profiles of *B. napus* Hormone-Related TFs in Response to Abiotic Stress

To identify the potential roles of rapeseed hormone-related TFs in adapting to abiotic stress, their responses to four stresses were investigated. FPKM (fragments per kilobase of transcript sequence per million mapped reads) was calculated to assess gene expression levels, and $p < 0.05$ and $|\log_2(\text{fold-change})| \geq 1$ were set as the criteria for identifying differentially expressed genes (DEGs). The DEGs were grouped into two clusters (1 and 2) according to their expression level. We then identified hub genes using Cytoscape (v3.8.2). Deleted genes with FPKM values < 1 and correlation value > 0.95 were used as the threshold for screening interactions between genes.

In shoots, 46 and 28 hormone-related TFs were increased and decreased, respectively, after Cd treatment (Figure 1a). Among these DEGs, *BnaWRKY7* was a core gene based on gene co-expression network analysis (GCNA) (Figure 1b). In roots, *BnaWRKY49*, *BnaWRKY38*, *BnaBZR11*, and *BnaBZR23* in cluster 1 were up-regulated by Cd, while *BnaARF33* and *BnaARR75* in cluster 2 were significantly reduced after Cd treatment (Figure 1c). *BnaWRKY38* may be a hub gene responding to Cd in roots (Figure 1d). Salinity severely inhibited rapeseed growth and yield [36]. In shoots, *BnaWRKY12* in cluster 1 was inhibited by salt, whereas *BnaWRKY49* in cluster 2 was induced (Figure 1e). Among them, *BnaAREB2-5* was identified as a hub gene in the gene co-expression network (Figure 1f). In roots, 86 phytohormone-related TFs were regulated by salt, with down-regulated TFs

found in cluster 1 and up-regulated TFs found in cluster 2 (Figure 1g). Based on GCNA, *BnaWRKY52* played an essential role in salt adaptation (Figure 1h).

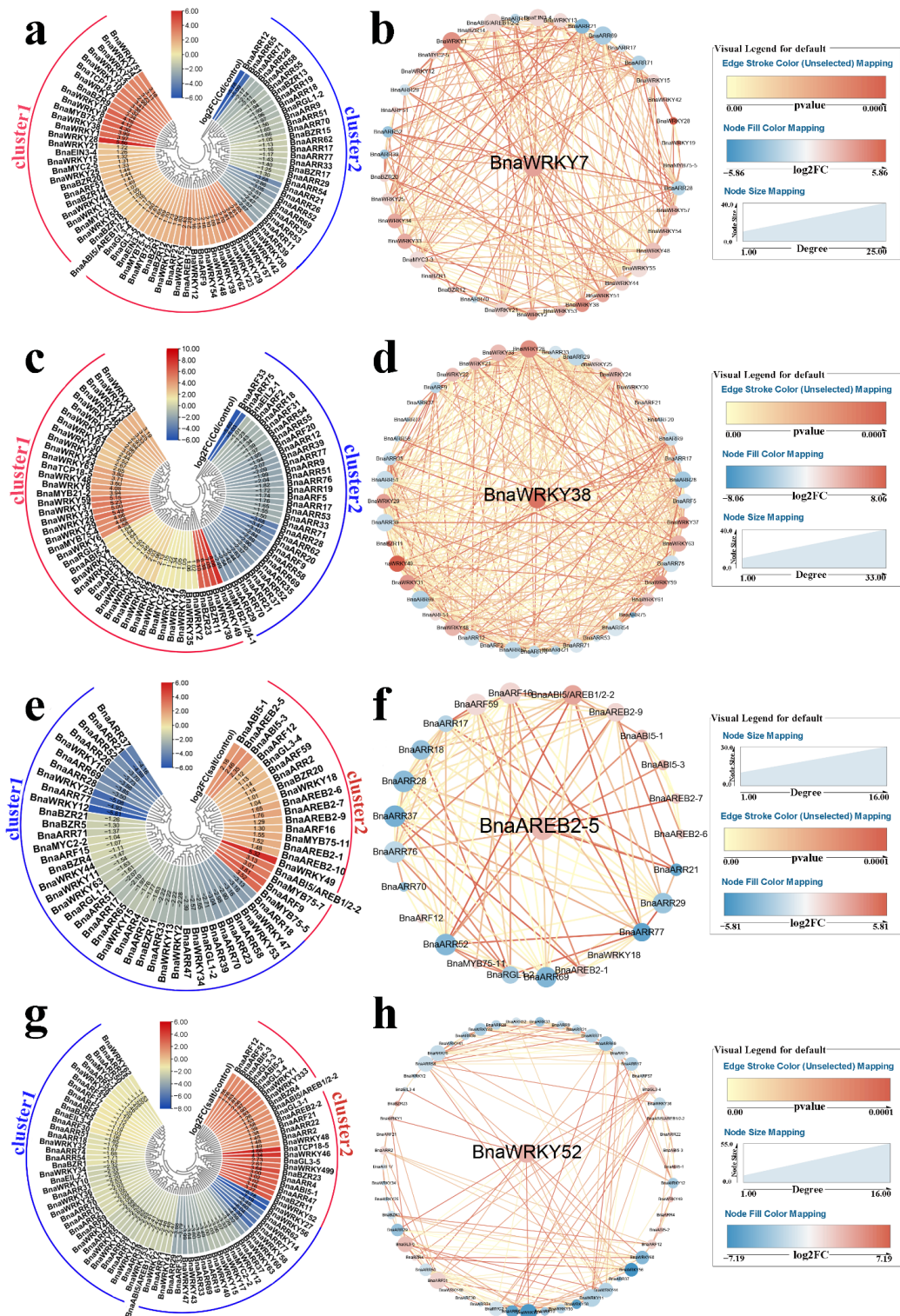


Figure 1. Expression profiles of *B. napus* hormone-related TFs in response to Cd and salt toxicity. Cycle nodes represent genes and the size of the node represents the power of the inter-relationship among nodes by degree value; Color of the node represents log₂FC value; Red indicates up-regulated genes and blue indicates down-regulated genes; Edges between nodes represent *p*-value. (a) Expression analysis of *B. napus* hormone-related TFs in response to Cd toxicity in shoots. (b) Co-expression network

analysis of differentially expressed *B. napus* hormone-related *TFs* in response to Cd toxicity in shoots. (c) Expression analysis of *B. napus* hormone-related *TFs* in response to Cd toxicity in roots. (d) Co-expression network analysis of differentially expressed *B. napus* hormone-related *TFs* in response to Cd toxicity in roots. (e) Expression analysis of *B. napus* hormone-related *TFs* in response to salt toxicity in shoots. (f) Co-expression network analysis of *B. napus* hormone-related *TFs* in response to salt toxicity in shoots. (g) Expression analysis of *B. napus* hormone-related *TFs* in roots in response to salt toxicity. (h) Co-expression network analysis of *B. napus* hormone-related *TFs* in response to salt toxicity in roots.

K is an essential nutrient for plant growth and development [37]. Here, K shortage altered the expression of 121 phytohormone-related *TFs* (Figure 2a,c). In shoots, genes in cluster 1 were induced; in contrast, genes in cluster 2 were inhibited (Figure 2a). Among them, *BnaWRKY21* was identified as a key gene (Figure 2b). Fewer genes were regulated by K stress in the roots (Figure 2c), and *BnaGL3-4* was identified as a likely core gene (Figure 2d). N is another important nutrient required for crop growth [38]. Here, two phytohormone-related *TFs* were under the control of N in shoots (Figure 2e). *BnaWRKY49* expression was markedly down-regulated under low N stress in roots, while seven other genes were induced (Figure 2f). Among the DEGs, *BnaWRKY32* was identified as a hub gene (Figure 2g).

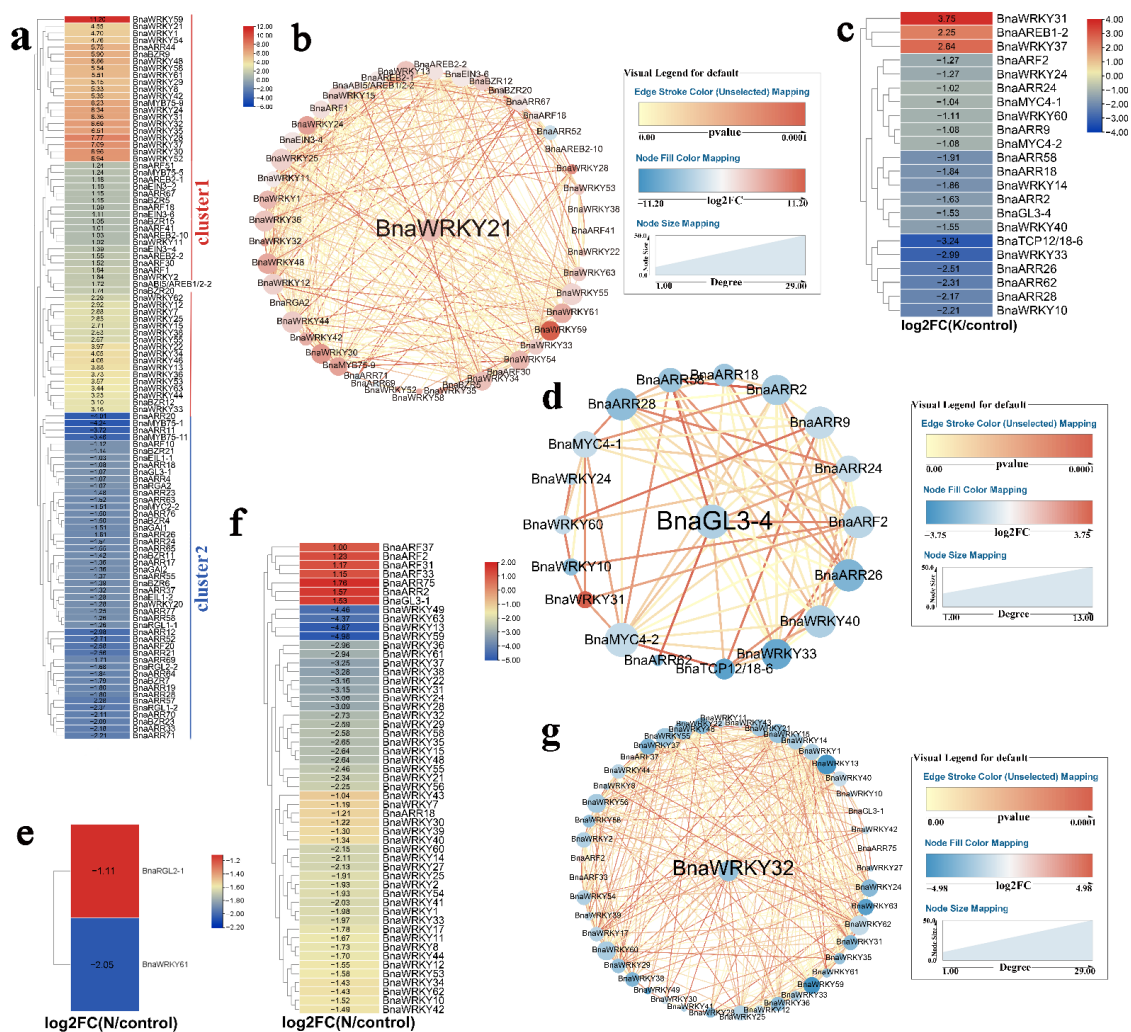


Figure 2. Expression profiles of *B. napus* hormone-related *TFs* in response to K and N starvation. Cycle nodes represent genes and the size of the node represents the power of the inter-relationship among

nodes by degree value; Color of the node represents log₂FC value; Red indicates up-regulated genes and blue indicates down-regulated genes; Edges between nodes represent *p*-value. (a) Expression analysis of *B. napus* hormone-related TFs in response to K starvation in shoots. (b) Co-expression network analysis of differentially expressed *B. napus* hormone-related TFs in response to K starvation in shoots. (c) Expression analysis of *B. napus* hormone-related TFs in response to K starvation in roots. (d) Co-expression network analysis of differentially expressed *B. napus* hormone-related TFs under in response to K starvation in roots. (e) Expression analysis of *B. napus* hormone-related TFs in response to N starvation in shoots. (f) Expression analysis of *B. napus* hormone-related TFs in response to N starvation in roots. (g) Co-expression network analysis of differentially expressed *B. napus* hormone-related TFs in response to N starvation in shoots and roots.

2.4. Expression Profiles of Hormone-Related TFs in Rapeseed with Different PH and Stem Breaking Resistance (SBR)

Both PH and SBR are crucial agronomic traits [39]. Comparative transcriptome analysis between dwarf (df59, ed1, Ldt, and dwf) and wild-type (WT) rapeseeds was performed to define candidate genes. Compared with df59, nine hormone-related TFs were down-regulated in WT rapeseeds, while 18 were up-regulated (Figure 3a). As shown in Figure 3b, *BnaWRKY42* was identified as a hub gene among the above DEGs. In ed1, *BnaMYC2-2*, *BnaARR21*, and *BnaARR71* were down-regulated, while 12 other TFs (especially *BnaWRKY36* and *BnaWRKY59*) were up-regulated (Figure 3c). The key gene *BnaARR21* was appraised according to the gene co-expression network (Figure 3d). In Ldt, five rapeseed hormone-related TFs exhibited low expression (Figure S7a). *BnaWRKY29*, *BnaAREB2-6*, and *BnaWRKY38* were clearly increased in dwf (Figure S7b). Compared with rapeseeds with low SBR during flowering (FL), the expression levels of six phytohormone-related TFs were significantly altered with high SBR during flowering (FH) (Figure S7c). Seven genes were down-regulated in rapeseeds with high SBR during silique development (SH) compared to those with low SBR (SL) (Figure S7d).

2.5. Identification of Weighted Gene Co-Expression Network Analysis (WGCNA) Modules and Hub Genes Associated with Target Traits

WGCNA uses data from all genes to identify gene sets of interest, rather than genes only showing differential expression, and to analyze significant associations with phenotypes. WGCNA has two main advantages: i.e., loss of fewer genes and the ability to collate many genes into gene sets and identify their association with phenotypes without multiple hypothesis testing [40]. Therefore, we used WGCNA to analyze the RNA-sequencing data of Cd, salt, K, and N treatments and six dwarf mutants to identify hub genes and their target genes involved in stress adaptation and PH regulation.

Modules associated with Cd were identified using WGCNA, with the “green” module ($r = -0.89$ and $p < 0.05$) showing a high negative correlation with chlorophyll content (SPAD) and the “purple” module ($r = 0.94$ and $p < 0.05$) showing positive correlation with biomass (Figure 4a, Tables S4-1 and S4-2). A co-expression network was constructed to identify hub genes. *BnaWRKY60*, *BnaWRKY27*, *BnaWRKY56*, and *BnaARR39* were determined in response to Cd (Figure 4b,c). TFs regulate target genes by binding to specific *cis*-elements. Furthermore, WRKYs share the ability to bind to W-box *cis*-regulatory elements [41]. In the above two modules, heavy metal transport/detoxification superfamily proteins (BnaHMTSPs) were identified (Table S5) [42]. In the “green” module, *BnaHMTSPs* (*BnaC07g28710D* and *BnaA07g36500D*) contained W-boxes and may be targets of *BnaWRKY27*, *BnaWRKY56*, and *BnaWRKY60* (Figure 4d). *BnaARR39* likely regulates *BnaHMTSPs* (*BnaA06g39500D*, *BnaA07g36500D*, *BnaC07g10100D*, *BnaC07g28710D*, and *BnaC08g20630D*) through RR binding to *cis*-elements (Figure 4e). WGCNA was also applied to investigate the relationship between modules and salinity. The “salmon” ($r = -0.83$ and $p < 0.05$) and “blue” modules ($r = -0.91$ and $p < 0.05$) were negatively correlated with biomass and leaf area, respectively (Figure 5a). *BnaARR8* and *BnaARR14* were the two most important genes in the

“salmon” module (Figure 5b and Table S4-3), while *BnaBZR22*, *BnaAREB2-6*, *BnaAREB2-7*, and *BnaABI5/AREB1/2-2* were hub genes in the “blue” module (Figure 5c and Table S4-4).

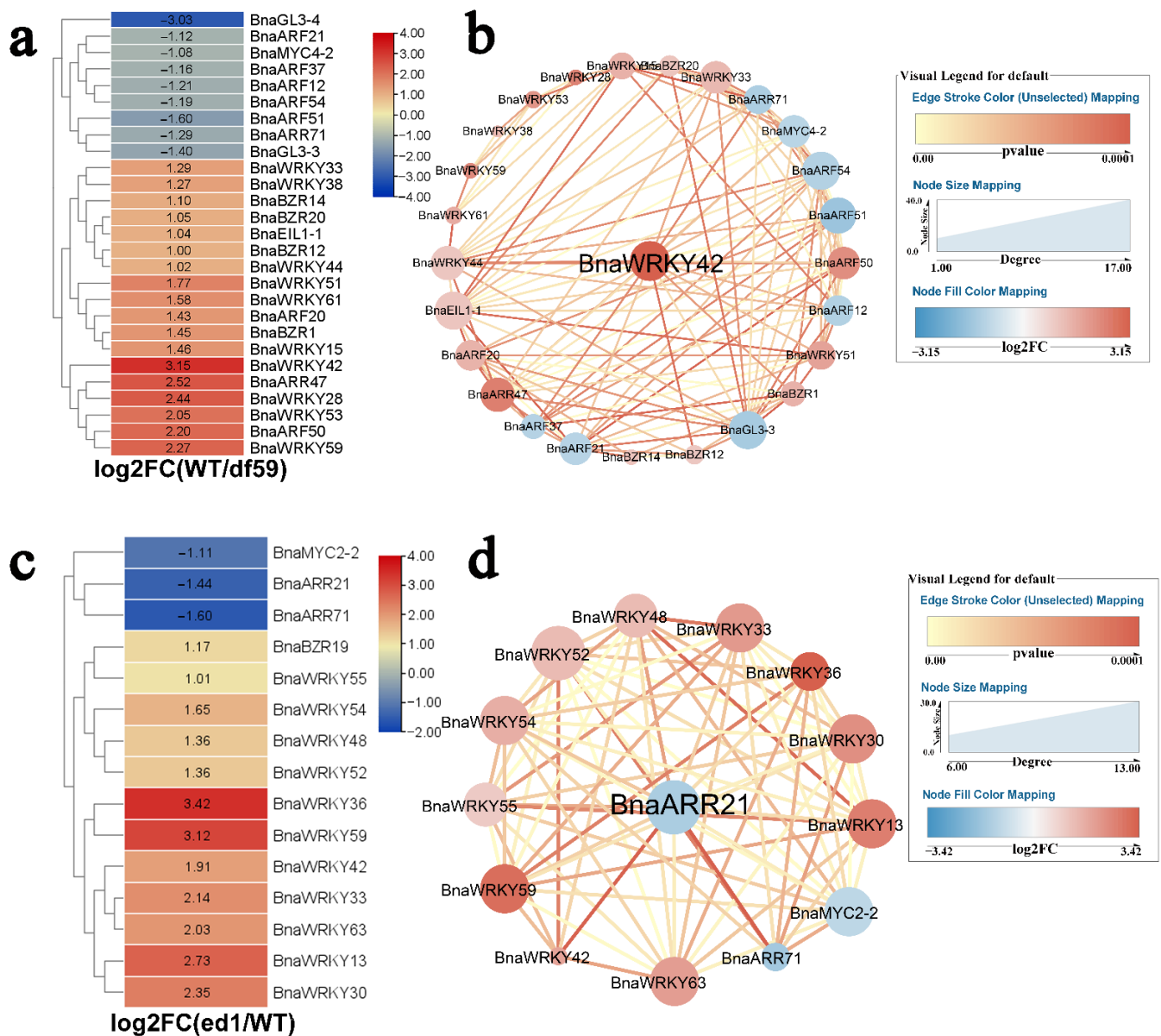


Figure 3. Expression profiles of *B. napus* hormone-related TFs in response to PH. Cycle nodes represent genes and the size of the node represents the power of the inter-relationship among nodes by degree value; Color of the node represents log₂FC value; Red indicates up-regulated genes and blue indicates down-regulated genes; Edges between nodes represent *p*-value. (a) Expression analysis of *B. napus* hormone-related TFs in a conventional rapeseed cultivar (Ningyou 18) and a dwarf mutant (df59). (b) Co-expression network analysis of differentially expressed *B. napus* hormone-related TFs in WT and df59. (c) Expression analysis of hormone-related TFs in an extreme dwarf mutant of rapeseed (ed1) and WT. (d) Co-expression network analysis of differentially expressed *B. napus* hormone-related TFs in ed1 and WT.

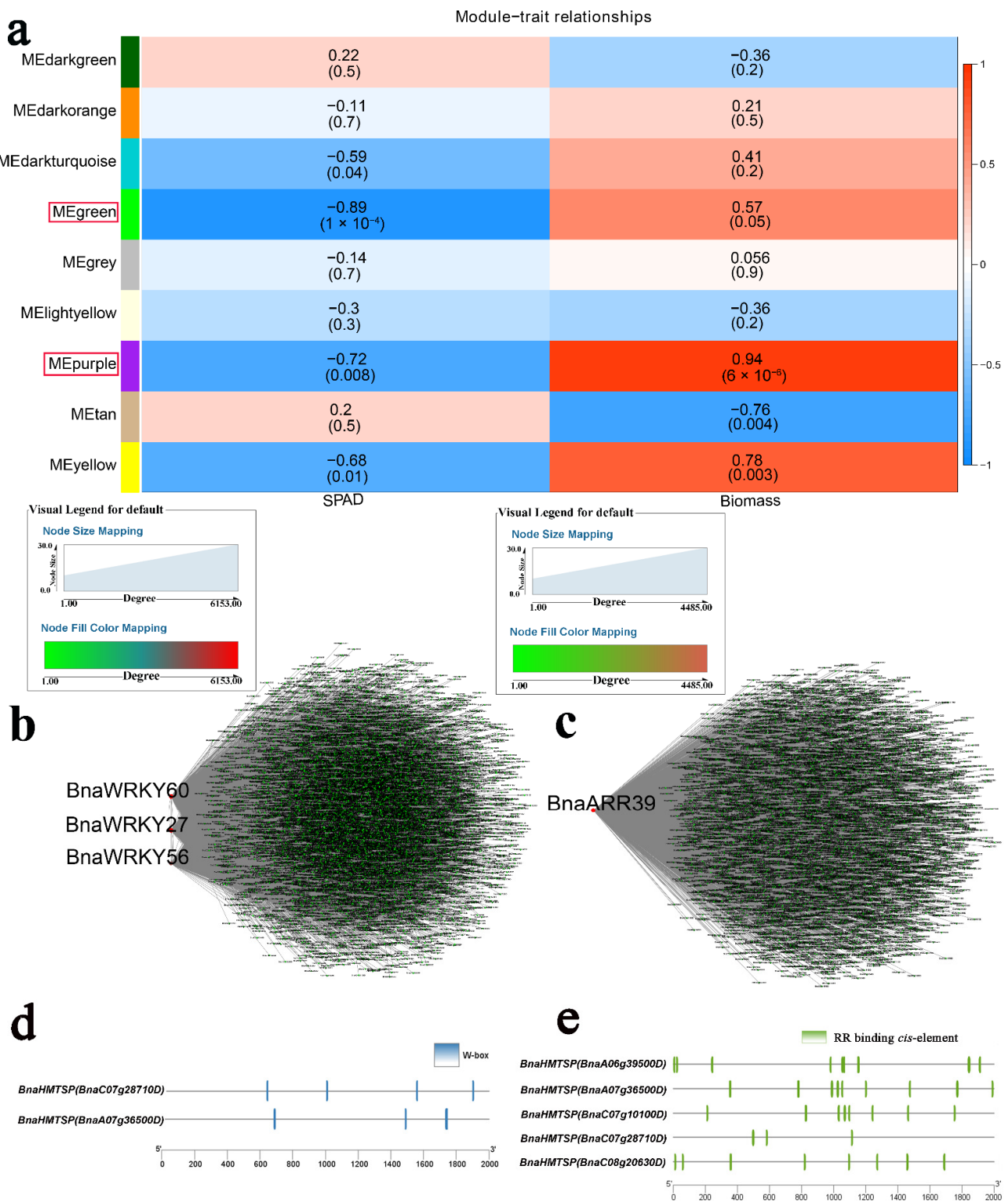


Figure 4. WGCNA of rapeseed genes in response to Cd stress. (a) Module eigengene correlation with traits (SPAD and biomass). Left panel shows modules. (b) Cytoscape representation of the relationship of hormone-related TFs in “green” module. Key genes are represented by large red circles. (c) Cytoscape representation of the relationship of hormone-related TFs in “purple” module. Key genes are represented by large red circles. (d) W-box cis-element in promoter of *BnaHMTSPs*. (e) RR binding cis-element genes in promoter of *BnaHMTSPs*.

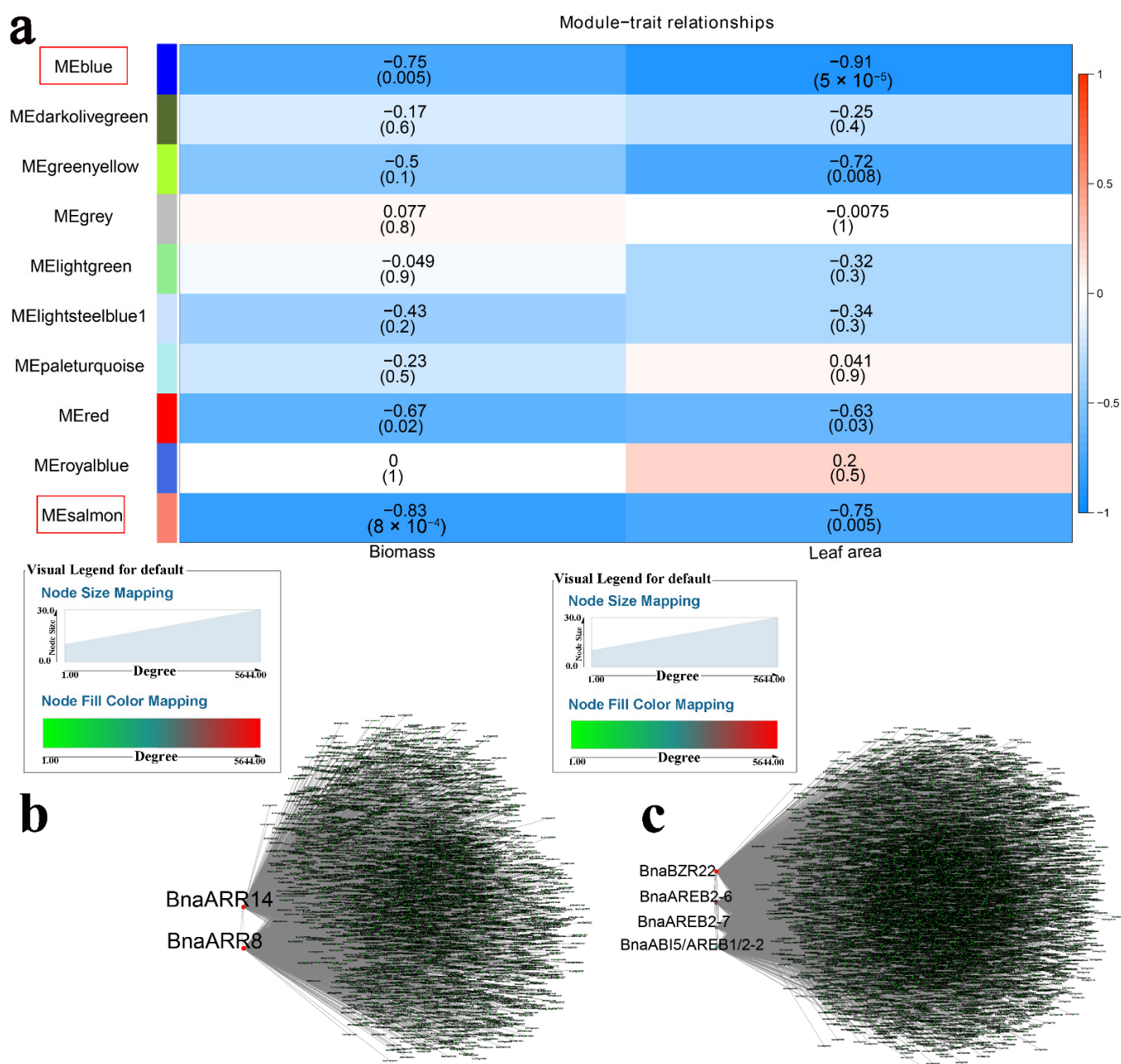


Figure 5. WGCNA of rapeseed genes in response to salt. (a) Module-trait correlation showing the significance of module eigengene correlation with traits (biomass and leaf area). Left panel shows modules. (b) Cytoscape representation of the relationship of hormone-related *TFs* in “salmon” module. Key genes are represented by large red circles. (c) Cytoscape representation of the relationship of hormone-related *TFs* in “blue” module. Key genes are represented by large red circles.

WGCNA was used to identify hub genes in response to K stress. As shown in Figure 6a, the “lightcyan” module ($r = 0.92$ and $p < 0.05$) was positively correlated with biomass, while the “turquoise” module ($r = -0.92$ and $p < 0.05$) was negatively correlated with SPAD. Gene interaction networks were constructed for these modules (Figure 6b,c and Tables S4-5 and S4-6). Three key genes (*BnaEIL1-2*, *BnaWRKY56*, and *BnaARR14*) were selected for their high connectivity (Figure 6b,c). Four K^+ transport genes (*BnaKUP5*, two *BnaKUP6s* and *BnaPCP*) [43,44] were found in the “turquoise” module, and W-boxes and RR binding cis-elements were present in their promoters, suggesting they may be the targets of *BnaWRKY56* and *BnaARR14* (Figure 6d). Under N stress conditions, the “green” module ($r = -0.83$ and

$p < 0.05$) was negatively correlated with SPAD (Figure 7a). *BnaBZR1* and *BnaBZR14* were identified as critical genes in the “green” module (Figure 7b and Table S4-7).

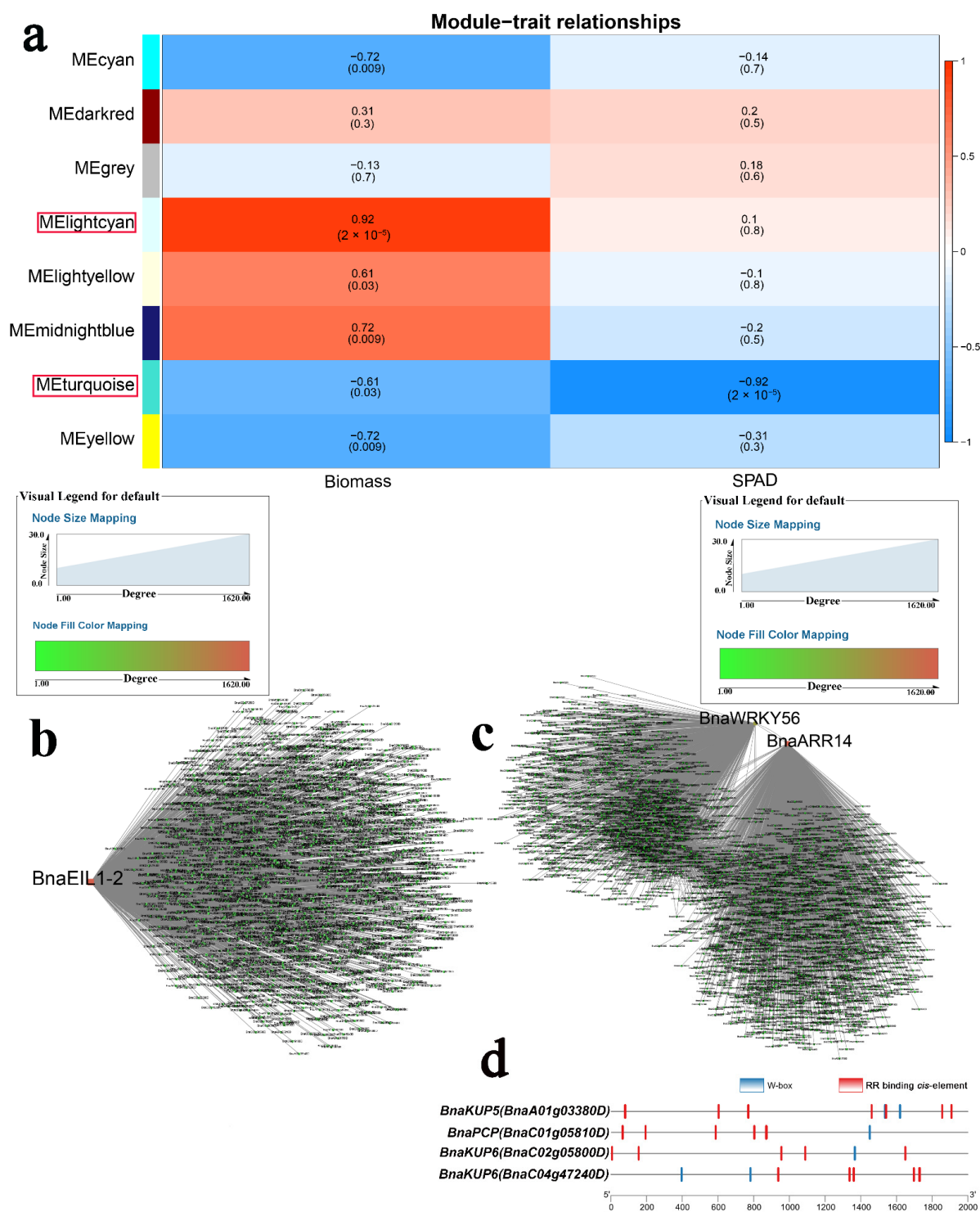
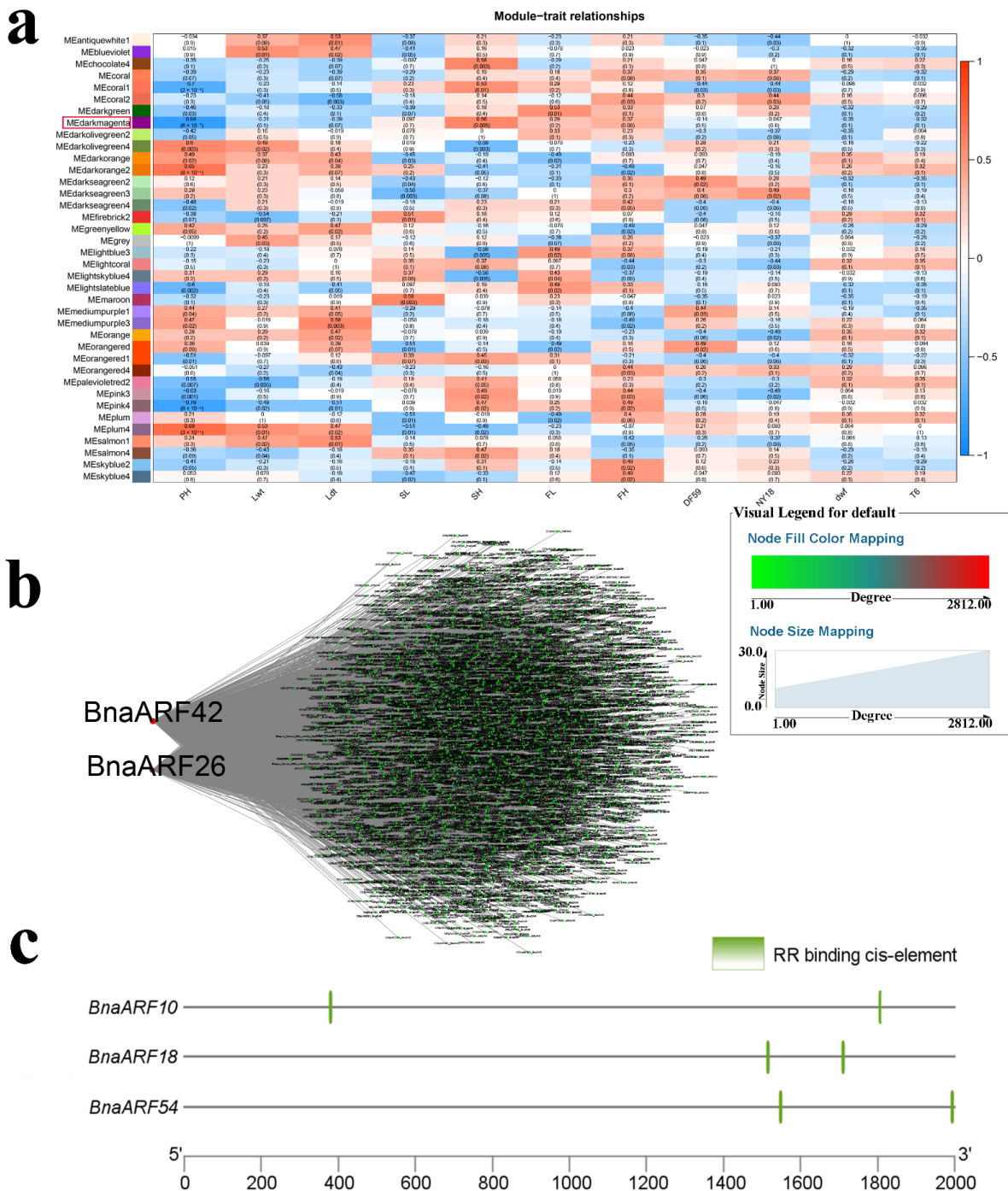


Figure 6. WGCNA of rapeseed genes in response to K starvation. (a) Module–trait correlation showing the significance of module eigengene correlation with traits (biomass and SPAD). Left panel shows modules. (b) Cytoscape representation of the relationship of hormone-related TFs in “lightcyan” module. Key genes are represented by large red circles. (c) Cytoscape representation of the relationship of hormone-related TFs in “turquoise” module. Key genes are represented by large red circles. (d) W-box and RR binding cis-element in promoters of *BnaKUP5*, *BnaPCP*, and *BnaKUP6*.

Here, two ARF-binding *cis*-elements were found in *BnaARF10*, *BnaARF18*, and *BnaARF54*, indicating they were the targets of *BnaARF42* and *BnaARF26* (Figure 8c and Table S5).



to the multiple stresses simultaneously. In total, 21 TFs (i.e., *BnaARF2*, *BnaARR18*, *BnaWRKY1*, *BnaWRKY2*, *BnaWRKY10*, *BnaWRKY12*, *BnaWRKY13*, *BnaWRKY15*, *BnaWRKY25*, *BnaWRKY29*, *BnaWRKY33*, *BnaWRKY34*, *BnaWRKY42*, *BnaWRKY44*, *BnaWRKY48*, *BnaWRKY53*, *BnaWRKY55*, *BnaWRKY56*, *BnaWRKY60*, *BnaWRKY62*, and *BnaWRKY63*) were affected by the four conditions (Table S6-1). *BnaWRKY13* was identified as a DEG in FH/FL, ed1/WT, and SH/SL (Table S6-2). *BnaWRKY38* likely regulated PH in df59 and dwf. *BnaWRKY44*, *BnaARR47*, and *BnaWRKY15* were DEGs in WT/df59 and Ldt/Lwt. *BnaWRKY42*, *BnaWRKY59*, *BnaWRKY33*, and *BnaARR71* may be involved in the dwarfing of df59 and ed1.

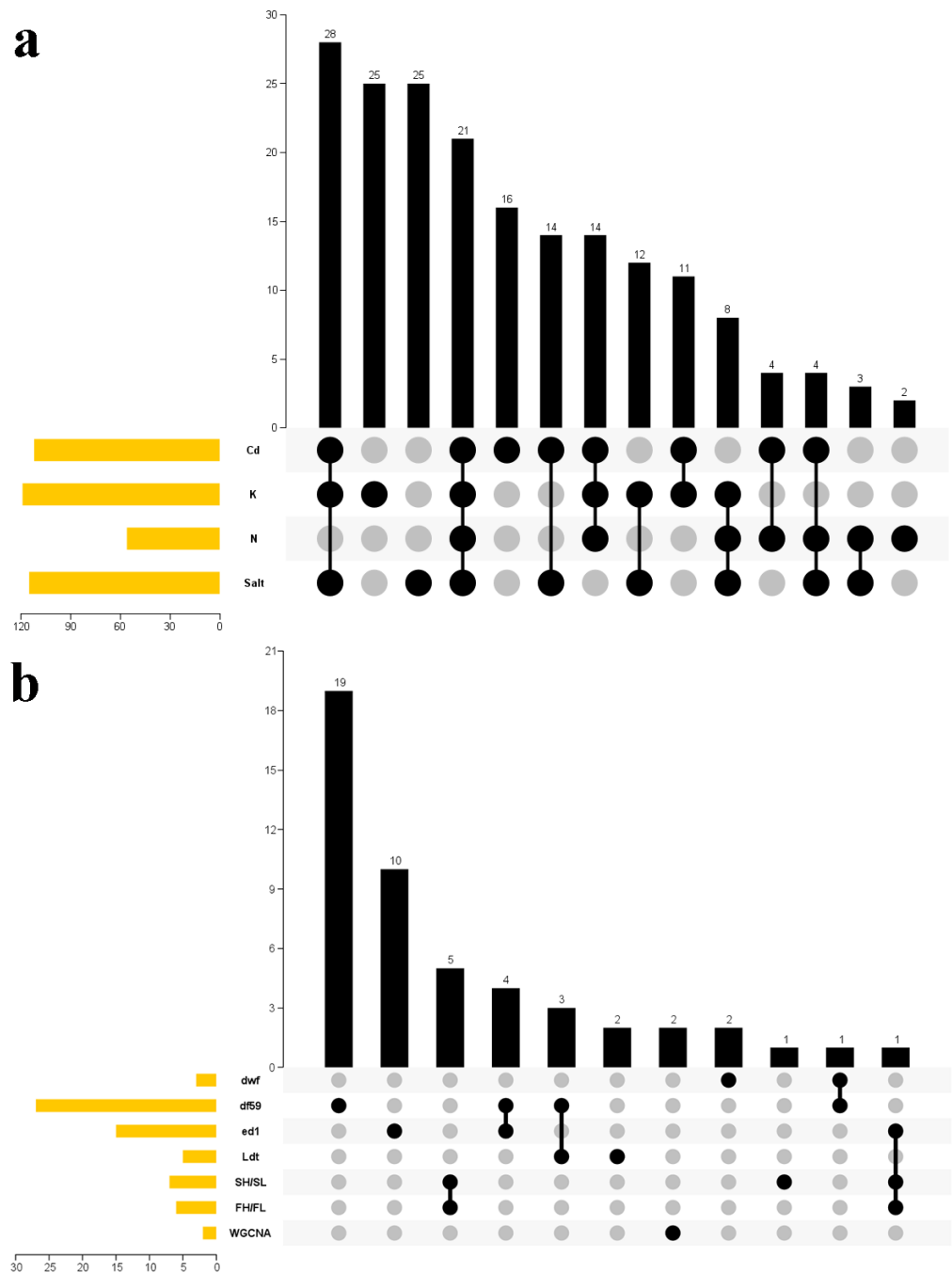


Figure 9. Venn diagram indicating various functions of *B. napus* hormone-related TFs in stress adaptation and PH regulation. (a) Number of rapeseed hormone-related TFs in response to Cd, K, N, and salt stress. (b) Number of rapeseed hormone-related TFs identified in rapeseed dwarf mutants.

3. Discussion

3.1. Comparison of Plant Hormone-Related TFs among Brassicaceae

Genes within a family usually exhibit obvious variations during their evolutionary history, which contribute to gene family division, expansion, and functional divergence [46]. Brassicaceae separated from *Arabidopsis* approximately 43.2 million years ago and underwent genome triplication, with *B. napus* ($2n = 38$), *B. carinata* ($2n = 34$), and *B. juncea* ($2n = 36$) arising from the genome hybridization of *B. nigra* ($2n = 16$), *B. oleracea* ($2n = 18$), and *B. rapa* ($2n = 20$) [47]. New evidence suggests a possible link between polyploidy and enhanced stress tolerance [48]. Notably, amphidiploid species *B. juncea*, *B. napus*, and *B. carinata* are more tolerant to stress than diploids such as *B. oleracea*, *B. nigra*, and *B. rapa* [49]. There are 82 phytohormone-related TFs in *Arabidopsis* [50–57]. In our study, 318, 314, 308, 223, 216, 203, 289, and 162 hormone-related TFs were identified in *B. napus*, *B. carinata*, *B. juncea*, *B. nigra*, *B. oleracea*, *B. rapa*, *C. sativa* and *C. rubella* (non-model Brassicaceae species), respectively (Figure S1a), nearly 3.8, 3.8, 3.7, 2.7, 2.6, 2.4, 3.5, and 1.9 times higher than that in *Arabidopsis*. Furthermore, more segmental hormone-related TFs were found in the non-model Brassicaceae species than in *Arabidopsis* (Figure S1c); *B. napus*, *B. carinata*, *B. juncea*, and *C. sativa* have undergone whole-genome duplication [58–62]. Therefore, the expansion and evolution of TFs in the above species may have been induced by gene and genome-wide duplications. In addition, the number of SA- and SL-related TFs in the above eight species was more than 5.5 times higher than that in *Arabidopsis*, suggesting pivotal roles of SA and SL in the stress tolerance of these species.

Gene functions are always associated with conserved domains [63]. Several distinct domains, including C2, HSF, and RALF, were identified in some Brassicaceae hormone-related TFs (Figure S3). Notably, C2, which is associated with calcium-binding [64], was found in BolGL3-1; HSF, which participates in the regulation of biotic and abiotic stress [65], was found in BjuARF4; and RALF, which is involved in biotic and abiotic stress responses [66], was found in BniBZR1. These results indicate that most Brassicaceae hormone-related TFs shared conserved functions, while several genes may vary among Brassicaceae species.

A total of 1291 gene pairs were identified between *A. thaliana* and non-model Brassicaceae species (Figures S1c and S6), and the functions of Brassicaceae hormone-related TFs were predicted based on homologous *Arabidopsis* TFs. For instance, *AtARF6* and *AtBZR1* regulate hypocotyl and stem elongation in *Arabidopsis* [67] and homologous genes (*BcaARF20*, *CruARF4*, and *BolBZR12*) may share similar roles; *CsaARR63* and *BnaARR49* may control defense response in Brassicaceae based on the homologous gene *AtARR6* [68].

3.2. Putative Functions of *B. napus* Hormone-Related TFs in Regulating Stress Adaptation and PH

We found that most *BnaWRKY* DEGs were up-regulated, while *BnaARRs* were down-regulated in both shoots and roots under Cd and K treatment (Figures 1a,c and 2a,c). We speculated that Cd poisoning and K starvation may induce SA expression and inhibit CTK expression in rapeseed, thereby enhancing plant Cd resistance and K import and translocation. We also found that salt stress and N deficiency led to a significant decrease in the expression of most *BnaWRKYs* in the shoots and roots (Figures 1e,f and 2e,f). Therefore, we concluded that salinity and limited N supply may restrict transcription of SA, thereby enhancing plant salt resistance and N uptake. Taken together, our results showed that most phytohormone TFs were responsive to diverse abiotic stresses, implying essential roles in the resistance or adaptation of rapeseed plants to stress.

Previous studies have shown that phytohormone-related TFs are essential for stress adaptation [69]. Various TFs, such as *AtWRKY13*, *SbWRKY50*, *AtARR22*, *SIBZR1*, and *AtAREB2*, are associated with plant survival under Cd and salt tolerance and K and N starvation [70–72]. However, whether they respond to Cd, salt, K, and N stress and regulate PH in rapeseed remains unknown. Here, candidate phytohormone-related TFs were identified in rapeseed through DEG co-expression analysis and WGCNA. *BnaWRKY7*, 27, 38, 56, 60, and *BnaARR39* were identified as key Cd-related genes (Figures 1b,d and 4). *BnaWRKY52*, *BnaBZR22*, *BnaARR8*, 14, *BnaAREB2-5*, -6, -7, and *BnaABI5/AREB1/2-2* were identified as

salt stress candidate genes (Figures 1f,h and 5). *BnaGL3-4*, *BnaARR14*, *BnaWRKY21*, 56, and *BnaEIL1-2* were identified as K deficiency candidate genes (Figures 2b,d and 6). *BnaWRKY32*, *BnaBZR1*, and *BnaBZR14* were identified as responsive to N stress (Figures 2g and 7). *AtWRKY53* and *AtARR10* are reported to negatively regulate plant responses to drought and salt stress [73,74]. Based on orthologous relationship analysis, we found that the orthologous gene of *AtWRKY53* in *B. napus* was *BnaWRKY7* and the orthologous gene of *AtARR10* was *BnaARR8*.

Various genes and gene families involved in PH regulation have been characterized in plants. Different from our study, however, previous research has primarily focused on specific TFs. In *A. thaliana*, *WRKY46*, *WRKY54*, *WRKY70*, *ARF6*, and *BZR1* regulate cell elongation and PH [75,76]. To further analyze the regulation of phytohormone TFs on PH, we performed DEG co-expression analysis and WGCNA. Results revealed that PH was mediated by *BnaWRKY42*, *BnaARR21*, *BnaARF26*, and *BnaARF42* (Figures 3b,d and 7). Therefore, these genes are likely important for oilseed architecture and stress adaptation.

K- and Cd-related genes (*BnaWRKY56* and *BnaWRKY60*) identified through WGCNA (Figures 4 and 6) responded to all four stress conditions. Furthermore, most key genes revealed through DEG co-expression analysis and WGCNA may play core roles in regulating oilseed resistance to three stresses (Figure 9a). *BnaWRKY38*, *BnaWRKY42*, and *BnaWRKY59*, which may regulate PH, were identified in two dwarf mutants (Figure 9b). In addition, *BnaWRKY38* was identified as a key gene in response to Cd stress, and *BnaWRKY42* was differentially expressed under all four stress conditions. This suggests that the phytohormone TFs can simultaneously regulate PH and stress adaptation. Therefore, future studies should focus on the potential functions of these key genes.

4. Methods and Materials

4.1. Identification and Chromosome Locations of Hormone-Related TFs in Brassicaceae

The protein sequences of *Arabidopsis* hormone-related TFs were used as queries to BLAST the *B. napus*, *B. oleracea*, *B. rapa*, *B. nigra*, *B. juncea*, *B. carinata*, *C. rubella*, and *C. sativa* genomes. We retrieved the phytohormone TF gene sequences using the following databases: Arabidopsis Information Resource (TAIR10, <https://www.arabidopsis.org/>, accessed on 15 May 2022) for *Arabidopsis*, Genoscope (<http://www.genoscope.cns.fr/brassicapapus/>, accessed on 15 May 2022) for *B. napus*, Bol base v1.0 (<http://119.97.203.210/bolbase/index.html>, accessed on 15 May 2022) for *B. oleracea*, Brassica Database (BRAD) v1.1 (<http://brassicadb.org/brad/>, accessed on 15 May 2022) for *B. carinata*, *B. rapa*, *B. juncea*, and *B. nigra*, National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov, accessed on 15 May 2022), EnsemblPlants (<http://plants.ensembl.org/index.html>, accessed on 15 May 2022), and Phytozome v10 (<http://phytozome.jgi.doe.gov/pz/portal.html>, accessed on 15 May 2022) for *C. sativa* and *C. rubella*. The characteristic domains were confirmed using the Batch Web CD-Search Tool (<https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi>, accessed on 15 May 2022) and visualized using the “Gene Structure View (Advanced)” tool in TBtools (v1.09876) to confirm their highly conserved segments. The “Gene Location Visualize from GTF/GFF” tool in TBtools (v1.09876) was used to visualize chromosomal locations, named according to their chromosome order [77].

4.2. Phylogeny, Gene Structure, and Syntenic Analyses of Brassicaceae Hormone-Related TFs

After aligning the full-length protein sequences using ClustalW with default parameters, MEGA X (v10.1.6, University of Pennsylvania, Philadelphia, PA, USA) was used to construct the phylogenetic tree with the maximum-likelihood method. Using generic feature format version 3 files of TFs, gene structure analyses were completed using ‘Visualize Gene Structure (Advanced)’ in TBtools (v1.09876). We performed syntenic analysis, with results visualized using the “One Step MCScanX” and “Advanced Circos” functions in TBtools (v1.09876) [77]. Genes were determined as duplicates in each genome based on the following [78]: (1) Aligned gene sequences were more than 70% identical, and the length of matching sequences was at least 70% of the longer gene; (2) Duplicates on different chro-

mosomes were characterized as segmental duplications. The “Simple Ka/Ks Calculator (NG)” function in TBtools (v1.09876) was used to calculate Ka, Ks, and Ka/Ks values.

4.3. Transcriptome Analysis, GCNA, and WGCNA of Brassicaceae Hormone-Related TFs

The transcriptome data can be found in published papers [79–86], and all the data that are required to reproduce these findings can be shared by contacting the corresponding author. Using fastp software (v0.20.1), we evaluated the overall sequencing quality of raw reads and removed low-quality reads. Hisat2 (v2.1.0) and SAMtools (v1.6) were used to align the high-quality reads to the *B. napus* reference genome sequence (http://cbi.hzau.edu.cn/cgi-bin/rape/download_ext, accessed on 15 May 2022). The expression levels of high-confidence genes in each sample were calculated with Stringtie (v1.3.3b). DEGs were defined using the R package “edgeR”, with $p < 0.05$, false-discovery rate (FDR) < 0.05 , and $|\log_2(\text{fold change})| \geq 1$. GCNA was completed with the cor. test function in R (v4.1) and visualized with Cytoscape (v3.8.2, <https://cytoscape.org/download.html>, accessed on 13 April 2022) based on our previously described protocol [87]. According to our earlier study [87], the R WGCNA package (v1.51) was used to perform WGCNA with high-quality genes. By calculating the module eigengene value, significant module-trait relationships with PH, SPAD, biomass, and leaf area were identified. Cytoscape (v3.8.2, <https://cytoscape.org/download.html>, accessed on 13 April 2022) was used to visualize gene co-expression networks.

5. Conclusions

In this study, 2115 phytohormone-related TFs were systematically identified in nine Brassicaceae species. Their chromosome locations, gene/protein structures, and phylogenetic and syntenic relationships were characterized. Genes responding to Cd, salt, K, and N adaptation in *B. napus* were investigated through differential expression analysis and DEG co-expression network analysis. In addition, WGCNA indicated that 15 and two phytohormone-related TFs and their potential target genes responded to stress and PH regulation, respectively. Taken together, *BnaWRKY56* and *BnaWRKY60* were identified as potential hub genes of rapeseed resistance to stress. *BnaWRKY42* may play an essential role in regulating PH. Our results showed that SA-related *BnaWRKY* TFs may be crucial for regulating PH and stress adaptation in rapeseed. The above-mentioned candidate genes should be validated in future studies.

Supplementary Materials: The supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms23158762/s1>.

Author Contributions: Data curation, S.M., Y.H. and J.H.; Visualization, S.M. and X.L.; Writing—original draft, L.Z., L.H. and J.H.; Writing—review & editing, L.Z., K.Z. and J.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Chinese Postdoctoral Science Foundation (2021M692944); Research Start-Up Project (32212399 and 32213006); Famous Teachers in Central Plains (22610002); and Application of Molecular Design Breeding of Oil Crops and Intelligent Auxiliary Information System in Supercomputing Ecology, Henan Key Project of Science and Technology (202102110006).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used in this study can be found in published papers. The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

TFs: transcription factors; PH: plant height; DEGs: differentially expressed genes; WGCNA: weighted gene co-expression network analysis; GCNA: gene co-expression network analysis; SBR: stem breaking resistance.

References

1. Ali, S.; Baloch, A.M. Overview of Sustainable Plant Growth and Differentiation and the Role of Hormones in Controlling Growth and Development of Plants under Various Stresses. *Recent Pat. Food Nutr. Agric.* **2020**, *11*, 105–114. [[CrossRef](#)] [[PubMed](#)]
2. Blazquez, M.A.; Nelson, D.C.; Weijers, D. Evolution of Plant Hormone Response Pathways. *Annu. Rev. Plant Biol.* **2020**, *71*, 327–353. [[CrossRef](#)]
3. Okushima, Y.; Overvoorde, P.J.; Arima, K.; Alonso, J.M.; Chan, A.; Chang, C.; Ecker, J.R.; Hughes, B.; Lui, A.; Nguyen, D.; et al. Functional genomic analysis of the AUXIN RESPONSE FACTOR gene family members in *Arabidopsis thaliana*: Unique and overlapping functions of ARF7 and ARF19. *Plant Cell* **2005**, *17*, 444–463. [[CrossRef](#)] [[PubMed](#)]
4. Kiba, T.; Yamada, H.; Mizuno, T. Characterization of the ARR15 and ARR16 response regulators with special reference to the cytokinin signaling pathway mediated by the AHK4 histidine kinase in roots of *Arabidopsis thaliana*. *Plant Cell Physiol.* **2002**, *43*, 1059–1066. [[CrossRef](#)]
5. Jakoby, M.; Weisshaar, B.; Droge-Laser, W.; Vicente-Carabajosa, J.; Tiedemann, J.; Kroj, T.; Parcy, F. bZIP transcription factors in *Arabidopsis*. *Trends Plant Sci.* **2002**, *7*, 106–111. [[CrossRef](#)]
6. Silverstone, A.L.; Jung, H.S.; Dill, A.; Kawaide, H.; Kamiya, Y.; Sun, T.P. Repressing a repressor: Gibberellin-induced rapid reduction of the RGA protein in *Arabidopsis*. *Plant Cell* **2001**, *13*, 1555–1566. [[CrossRef](#)]
7. Wang, Z.F.; Ying, T.J. Research progress of ethylene signal transduction in plants. *Zhi Wu Sheng Li Yu Fen Zi Sheng Wu Xue Xue Bao J. Plant Physiol. Mol. Biol.* **2004**, *30*, 601–608.
8. Wang, Z.Y.; Nakano, T.; Gendron, J.; He, J.; Chen, M.; Vafeados, D.; Yang, Y.; Fujioka, S.; Yoshida, S.; Asami, T.; et al. Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. *Dev. Cell* **2002**, *2*, 505–513. [[CrossRef](#)]
9. Boter, M.; Ruiz-Rivero, O.; Abdeen, A.; Prat, S. Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and *Arabidopsis*. *Genes Dev.* **2004**, *18*, 1577–1591. [[CrossRef](#)]
10. Kim, K.C.; Lai, Z.; Fan, B.; Chen, Z. *Arabidopsis* WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. *Plant Cell* **2008**, *20*, 2357–2371. [[CrossRef](#)]
11. Martin-Trillo, M.; Cubas, P. TCP genes: A family snapshot ten years later. *Trends Plant Sci.* **2010**, *15*, 31–39. [[CrossRef](#)] [[PubMed](#)]
12. Meng, L.; Wang, Z.; Yao, S.; Liu, A. Seedlings of large-seeded plants are considered to withstand drought stresses efficiently. *J. Cell Sci.* **2015**, *128*, 3922–3932. [[CrossRef](#)] [[PubMed](#)]
13. Wang, J.; Wang, R.; Mao, X.; Li, L.; Chang, X.; Zhang, X.; Jing, R. TaARF4 genes are linked to root growth and plant height in wheat. *Ann. Bot.* **2019**, *124*, 903–915. [[CrossRef](#)] [[PubMed](#)]
14. Kieber, J.J.; Schaller, G.E. Cytokinin signaling in plant development. *Development* **2018**, *145*, dev149344. [[CrossRef](#)] [[PubMed](#)]
15. Huang, X.; Hou, L.; Meng, J.; You, H.; Li, Z.; Gong, Z.; Yang, S.; Shi, Y. The Antagonistic Action of Abscisic Acid and Cytokinin Signaling Mediates Drought Stress Response in *Arabidopsis*. *Mol. Plant* **2018**, *11*, 970–982. [[CrossRef](#)]
16. Shi, F.; Wang, M.; An, Y. Overexpression of a B-type cytokinin response regulator (OsORR2) reduces plant height in rice. *Plant Signal. Behav.* **2020**, *15*, 1780405. [[CrossRef](#)]
17. Skubacz, A.; Daszkowska-Golec, A.; Szarejko, I. The Role and Regulation of ABI5 (ABA-Insensitive 5) in Plant Development, Abiotic Stress Responses and Phytohormone Crosstalk. *Front. Plant Sci.* **2016**, *7*, 1884. [[CrossRef](#)]
18. De Melo, B.P.; Lourenço-Tessutti, I.T.; Paixão, J.F.R.; Noriega, D.D.; Silva, M.C.M.; de Almeida-Engler, J.; Fontes, E.P.B.; Grossi-de-Sa, M.F. Transcriptional modulation of AREB-1 by CRISPRa improves plant physiological performance under severe water deficit. *Sci. Rep.* **2020**, *10*, 16231. [[CrossRef](#)]
19. Tyler, L.; Thomas, S.G.; Hu, J.; Dill, A.; Alonso, J.M.; Ecker, J.R.; Sun, T.P. DELLA proteins and gibberellin-regulated seed germination and floral development in *Arabidopsis*. *Plant Physiol.* **2004**, *135*, 1008–1019. [[CrossRef](#)]
20. Liao, Z.; Yu, H.; Duan, J.; Yuan, K.; Yu, C.; Meng, X.; Kou, L.; Chen, M.; Jing, Y.; Liu, G.; et al. SLR1 inhibits MOC1 degradation to coordinate tiller number and plant height in rice. *Nat. Commun.* **2019**, *10*, 2738. [[CrossRef](#)]
21. Salih, H.; He, S.; Li, H.; Peng, Z.; Du, X. Investigation of the EIL/EIN3 Transcription Factor Gene Family Members and Their Expression Levels in the Early Stage of Cotton Fiber Development. *Plants* **2020**, *9*, 128. [[CrossRef](#)] [[PubMed](#)]
22. Wang, L.; Ko, E.E.; Tran, J.; Qiao, H. TREE1-EIN3-mediated transcriptional repression inhibits shoot growth in response to ethylene. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 29178–29189. [[CrossRef](#)] [[PubMed](#)]
23. Cui, X.; Gao, Y.; Guo, J.; Yu, T.; Zheng, W.; Liu, Y.; Chen, J.; Xu, Z.; Ma, Y. BES/BZR Transcription Factor TaBZR2 Positively Regulates Drought Responses by Activation of TaGST1. *Plant Physiol.* **2019**, *180*, 605–620. [[CrossRef](#)]
24. Castorina, G.; Consonni, G. The Role of Brassinosteroids in Controlling Plant Height in Poaceae: A Genetic Perspective. *Int. J. Mol. Sci.* **2020**, *21*, 1191. [[CrossRef](#)]

25. Huang, H.; Liu, B.; Liu, L.; Song, S. Jasmonate action in plant growth and development. *J. Exp. Bot.* **2017**, *68*, 1349–1359. [[CrossRef](#)] [[PubMed](#)]
26. Kranz, H.D.; Denekamp, M.; Greco, R.; Jin, H.; Leyva, A.; Meissner, R.C.; Petroni, K.; Urzainqui, A.; Bevan, M.; Martin, C.; et al. Towards functional characterisation of the members of the R2R3-MYB gene family from *Arabidopsis thaliana*. *Plant J.* **1998**, *16*, 263–276. [[CrossRef](#)] [[PubMed](#)]
27. Zhong, Q.; Hu, H.; Fan, B.; Zhu, C.; Chen, Z. Biosynthesis and Roles of Salicylic Acid in Balancing Stress Response and Growth in Plants. *Int. J. Mol. Sci.* **2021**, *22*, 11672. [[CrossRef](#)]
28. Dong, J.; Chen, C.; Chen, Z. Expression profiles of the *Arabidopsis* WRKY gene superfamily during plant defense response. *Plant Mol. Biol.* **2003**, *51*, 21–37. [[CrossRef](#)]
29. Zheng, A.; Sun, F.; Cheng, T.; Wang, Y.; Xie, K.; Zhang, C.; Xi, Y. Genome-wide identification of members of the TCP gene family in switchgrass (*Panicum virgatum* L.) and analysis of their expression. *Gene* **2019**, *702*, 89–98. [[CrossRef](#)]
30. Kamal, K.Y.; Khodaeiaminjan, M.; Yahya, G.; El-Tantawy, A.A.; Abdel, E.D.; El-Esawi, M.A.; Abd-Elaziz, M.; Nassrallah, A.A. Modulation of cell cycle progression and chromatin dynamic as tolerance mechanisms to salinity and drought stress in maize. *Physiol. Plant.* **2021**, *172*, 684–695. [[CrossRef](#)]
31. Hadi, F.; Ayaz, M.; Ali, S.; Shafiq, M.; Ullah, R.; Jan, A.U. Comparative effect of polyethylene glycol and mannitol induced drought on growth (in vitro) of canola (*Brassica napus*), cauliflower (*Brassica oleracea*) and tomato (*Lycopersicon esculentum*) seedlings. *Int. J. Biosci.* **2014**, *4*, 34–41.
32. Kang, N.Y.; Cho, C.; Kim, J. Inducible expression of *Arabidopsis* response regulator 22 (ARR22), a type-C ARR, in transgenic *Arabidopsis* enhances drought and freezing tolerance. *PLoS ONE* **2013**, *8*, e79248. [[CrossRef](#)] [[PubMed](#)]
33. Feng, Y.; Cui, R.; Wang, S.; He, M.; Hua, Y.; Shi, L.; Ye, X.; Xu, F. Transcription factor BnaA9.WRKY47 contributes to the adaptation of *Brassica napus* to low boron stress by up-regulating the boric acid channel gene BnaA3.NIP5;1. *Plant Biotechnol. J.* **2020**, *18*, 1241–1254. [[CrossRef](#)] [[PubMed](#)]
34. Wu, J.; Yan, G.; Duan, Z.; Wang, Z.; Kang, C.; Guo, L.; Liu, K.; Tu, J.; Shen, J.; Yi, B.; et al. Roles of the *Brassica napus* DELLA Protein BnaA6.RGA, in Modulating Drought Tolerance by Interacting with the ABA Signaling Component BnaA10.ABF2. *Front. Plant Sci.* **2020**, *11*, 577. [[CrossRef](#)]
35. Ramirez, D.; Abellán-Victorio, A.; Beretta, V.; Camargo, A.; Moreno, D.A. Functional Ingredients from Brassicaceae Species: Overview and Perspectives. *Int. J. Mol. Sci.* **2020**, *21*, 1998. [[CrossRef](#)]
36. Hanley, M.E.; Hartley, F.C.; Hayes, L.; Franco, M. Simulated seawater flooding reduces oilseed rape growth, yield and progeny performance. *Ann. Bot.* **2020**, *125*, 247–254. [[CrossRef](#)]
37. Ashley, M.K.; Grant, M.; Grabov, A. Plant responses to potassium deficiencies: A role for potassium transport proteins. *J. Exp. Bot.* **2006**, *57*, 425–436. [[CrossRef](#)]
38. Stahl, A.; Vollrath, P.; Samans, B.; Frisch, M.; Wittkop, B.; Snowden, R.J. Effect of breeding on nitrogen use efficiency-associated traits in oilseed rape. *J. Exp. Bot.* **2019**, *70*, 1969–1986. [[CrossRef](#)]
39. Wei, C.; Zhu, L.; Wen, J.; Yi, B.; Ma, C.; Tu, J.; Shen, J.; Fu, T. Morphological, transcriptomics and biochemical characterization of new dwarf mutant of *Brassica napus*. *Plant Sci.* **2018**, *270*, 97–113. [[CrossRef](#)]
40. Zeng, F.; Shi, M.; Xiao, H.; Chi, X. WGCNA-Based Identification of Hub Genes and Key Pathways Involved in Nonalcoholic Fatty Liver Disease. *Biomed. Res. Int.* **2021**, *2021*, 5633211. [[CrossRef](#)]
41. Eulgem, T.; Rushton, P.J.; Robatzek, S.; Somssich, I.E. The WRKY superfamily of plant transcription factors. *Trends Plant Sci.* **2000**, *5*, 199–206. [[CrossRef](#)]
42. Tian, H.; Baxter, I.R.; Lahner, B.; Reinders, A.; Salt, D.E.; Ward, J.M. *Arabidopsis* NPCC6/NaKR1 is a phloem mobile metal binding protein necessary for phloem function and root meristem maintenance. *Plant Cell* **2010**, *22*, 3963–3979. [[CrossRef](#)] [[PubMed](#)]
43. Li, W.; Xu, G.; Alli, A.; Yu, L. Plant HAK/KUP/KT K(+) transporters: Function and regulation. *Semin. Cell Dev. Biol.* **2018**, *74*, 133–141. [[CrossRef](#)] [[PubMed](#)]
44. Kuang, Q.; Purhonen, P.; Hebert, H. Structure of potassium channels. *Cell. Mol. Life Sci.* **2015**, *72*, 3677–3693. [[CrossRef](#)] [[PubMed](#)]
45. Li, S.B.; Xie, Z.Z.; Hu, C.G.; Zhang, J.Z. A Review of Auxin Response Factors (ARFs) in Plants. *Front. Plant Sci.* **2016**, *7*, 47. [[CrossRef](#)] [[PubMed](#)]
46. Yahya, G.; Menges, P.; Ngandiri, D.A.; Schulz, D.; Wallek, A.; Kulak, N.A.; Mann, M.; Cramer, P.; Savage, V.; Raeschle, M.; et al. *Scaling of Cellular Proteome with Ploidy*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 2021.
47. Raza, A.; Razzaq, A.; Mehmood, S.S.; Hussain, M.A.; Wei, S.; He, H.; Zaman, Q.U.; Xuekun, Z.; Hasanuzzaman, M. Omics: The way forward to enhance abiotic stress tolerance in *Brassica napus* L. *GM Crops Food* **2021**, *12*, 251–281. [[CrossRef](#)]
48. Das, L.S.; Dutta, S.; Schaffner, A.R.; Das, M. Gene duplication and stress genomics in Brassicas: Current understanding and future prospects. *J. Plant Physiol.* **2020**, *255*, 153293.
49. Purty, R.S.; Kumar, G.; Singla-Pareek, S.L.; Pareek, A. Towards salinity tolerance in Brassica: An overview. *Physiol. Mol. Biol. Plants* **2008**, *14*, 39–49. [[CrossRef](#)]
50. Muller, B.; Sheen, J. *Arabidopsis* cytokinin signaling pathway. *Sci. STKE* **2007**, *2007*, m5. [[CrossRef](#)]
51. Li, S. The *Arabidopsis thaliana* TCP transcription factors: A broadening horizon beyond development. *Plant Signal. Behav.* **2015**, *10*, e1044192. [[CrossRef](#)]
52. Zhang, Y.; Ji, T.T.; Li, T.T.; Tian, Y.Y.; Wang, L.F.; Liu, W.C. Jasmonic acid promotes leaf senescence through MYC2-mediated repression of CATALASE2 expression in *Arabidopsis*. *Plant Sci.* **2020**, *299*, 110604. [[CrossRef](#)] [[PubMed](#)]

53. Huang, P.Y.; Catinot, J.; Zimmerli, L. Ethylene response factors in *Arabidopsis* immunity. *J. Exp. Bot.* **2016**, *67*, 1231–1241. [[CrossRef](#)] [[PubMed](#)]
54. Lachowiec, J.; Mason, G.A.; Schultz, K.; Queitsch, C. Redundancy, Feedback, and Robustness in the *Arabidopsis thaliana* BZR/BEH Gene Family. *Front. Genet.* **2018**, *9*, 523. [[CrossRef](#)] [[PubMed](#)]
55. Hwang, K.; Susila, H.; Nasim, Z.; Jung, J.Y.; Ahn, J.H. *Arabidopsis* ABF3 and ABF4 Transcription Factors Act with the NF-YC Complex to Regulate SOC1 Expression and Mediate Drought-Accelerated Flowering. *Mol. Plant* **2019**, *12*, 489–505. [[CrossRef](#)]
56. Chen, H.I.; Li, P.F.; Yang, C.H. NAC-Like Gene GIBBERELLIN SUPPRESSING FACTOR Regulates the Gibberellin Metabolic Pathway in Response to Cold and Drought Stresses in *Arabidopsis*. *Sci. Rep.* **2019**, *9*, 19226. [[CrossRef](#)]
57. Wang, L.; Wang, B.; Yu, H.; Guo, H.; Lin, T.; Kou, L.; Wang, A.; Shao, N.; Ma, H.; Xiong, G.; et al. Transcriptional regulation of strigolactone signalling in *Arabidopsis*. *Nature* **2020**, *583*, 277–281. [[CrossRef](#)]
58. Chalhoub, B.; Denoeud, F.; Liu, S.; Parkin, I.A.; Tang, H.; Wang, X.; Chiquet, J.; Belcram, H.; Tong, C.; Samans, B.; et al. Plant genetics. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science* **2014**, *345*, 950–953. [[CrossRef](#)]
59. Song, X.; Wei, Y.; Xiao, D.; Gong, K.; Sun, P.; Ren, Y.; Yuan, J.; Wu, T.; Yang, Q.; Li, X.; et al. *Brassica carinata* genome characterization clarifies U's triangle model of evolution and polyploidy in Brassica. *Plant Physiol.* **2021**, *186*, 388–406. [[CrossRef](#)]
60. Liu, S.; Liu, Y.; Yang, X.; Tong, C.; Edwards, D.; Parkin, I.A.; Zhao, M.; Ma, J.; Yu, J.; Huang, S.; et al. The *Brassica oleracea* genome reveals the asymmetrical evolution of polyploid genomes. *Nat. Commun.* **2014**, *5*, 3930. [[CrossRef](#)]
61. Parkin, I.A.; Koh, C.; Tang, H.; Robinson, S.J.; Kagale, S.; Clarke, W.E.; Town, C.D.; Nixon, J.; Krishnakumar, V.; Bidwell, S.L.; et al. Transcriptome and methylome profiling reveals relics of genome dominance in the mesopolyploid *Brassica oleracea*. *Genome Biol.* **2014**, *15*, R77. [[CrossRef](#)]
62. Yang, J.; Liu, D.; Wang, X.; Ji, C.; Cheng, F.; Liu, B.; Hu, Z.; Chen, S.; Pental, D.; Ju, Y.; et al. The genome sequence of allopolyploid *Brassica juncea* and analysis of differential homoeolog gene expression influencing selection. *Nat. Genet.* **2016**, *48*, 1225–1232. [[CrossRef](#)]
63. Xu, G.; Guo, C.; Shan, H.; Kong, H. Divergence of duplicate genes in exon-intron structure. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 1187–1192. [[CrossRef](#)] [[PubMed](#)]
64. Nalefski, E.A.; Falke, J.J. The C2 domain calcium-binding motif: Structural and functional diversity. *Protein Sci.* **1996**, *5*, 2375–2390. [[CrossRef](#)] [[PubMed](#)]
65. Zhang, Q.; Geng, J.; Du, Y.; Zhao, Q.; Zhang, W.; Fang, Q.; Yin, Z.; Li, J.; Yuan, X.; Fan, Y.; et al. Heat shock transcription factor (Hsf) gene family in common bean (*Phaseolus vulgaris*): Genome-wide identification, phylogeny, evolutionary expansion and expression analyses at the sprout stage under abiotic stress. *BMC Plant Biol.* **2022**, *22*, 33. [[CrossRef](#)]
66. Sharma, A.; Hussain, A.; Mun, B.; Imran, Q.M.; Falak, N.; Lee, S.; Kim, J.Y.; Hong, J.K.; Loake, G.J.; Ali, A.; et al. Comprehensive analysis of plant rapid alkalization factor (RALF) genes. *Plant Physiol. Biochem.* **2016**, *106*, 82–90. [[CrossRef](#)]
67. Liu, K.; Li, Y.; Chen, X.; Li, L.; Liu, K.; Zhao, H.; Wang, Y.; Han, S. ERF72 interacts with ARF6 and BZR1 to regulate hypocotyl elongation in *Arabidopsis*. *J. Exp. Bot.* **2018**, *69*, 3933–3947. [[CrossRef](#)]
68. Bacete, L.; Mérida, H.; López, G.; Dabos, P.; Tremousaygue, D.; Denancé, N.; Miedes, E.; Bulone, V.; Goffner, D.; Molina, A. *Arabidopsis* Response Regulator 6 (ARR6) Modulates Plant Cell-Wall Composition and Disease Resistance. *Mol. Plant-Microbe Interact.* **2020**, *33*, 767–780. [[CrossRef](#)]
69. Chen, L.; Yang, H.; Fang, Y.; Guo, W.; Chen, H.; Zhang, X.; Dai, W.; Chen, S.; Hao, Q.; Yuan, S.; et al. Overexpression of GmMYB14 improves high-density yield and drought tolerance of soybean through regulating plant architecture mediated by the brassinosteroid pathway. *Plant Biotechnol. J.* **2021**, *19*, 702–716. [[CrossRef](#)]
70. Zhang, P.; Wang, R.; Ju, Q.; Li, W.; Tran, L.P.; Xu, J. The R2R3-MYB Transcription Factor MYB49 Regulates Cadmium Accumulation. *Plant Physiol.* **2019**, *180*, 529–542. [[CrossRef](#)]
71. Sheng, Y.; Yan, X.; Huang, Y.; Han, Y.; Zhang, C.; Ren, Y.; Fan, T.; Xiao, F.; Liu, Y.; Cao, S. The WRKY transcription factor, WRKY13, activates PDR8 expression to positively regulate cadmium tolerance in *Arabidopsis*. *Plant Cell Environ.* **2019**, *42*, 891–903. [[CrossRef](#)]
72. Lv, J.; Li, Y.; Liu, Z.; Li, X.; Lei, X.; Gao, C. Response of BpBZR genes to abiotic stress and hormone treatment in *Betula platyphylla*. *Plant Physiol. Biochem.* **2020**, *151*, 157–165. [[CrossRef](#)] [[PubMed](#)]
73. Sun, Y.; Yu, D. Activated expression of AtWRKY53 negatively regulates drought tolerance by mediating stomatal movement. *Plant Cell Rep.* **2015**, *34*, 1295–1306. [[CrossRef](#)]
74. Nguyen, K.H.; Ha, C.V.; Nishiyama, R.; Watanabe, Y.; Leyva-Gonzalez, M.A.; Fujita, Y.; Tran, U.T.; Li, W.; Tanaka, M.; Seki, M.; et al. *Arabidopsis* type B cytokinin response regulators ARR1, ARR10, and ARR12 negatively regulate plant responses to drought. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3090–3095. [[CrossRef](#)] [[PubMed](#)]
75. Lan, J.; Lin, Q.; Zhou, C.; Ren, Y.; Liu, X.; Miao, R.; Jing, R.; Mou, C.; Nguyen, T.; Zhu, X.; et al. Small grain and semi-dwarf 3, a WRKY transcription factor, negatively regulates plant height and grain size by stabilizing SLR1 expression in rice. *Plant Mol. Biol.* **2020**, *104*, 429–450. [[CrossRef](#)] [[PubMed](#)]
76. Oh, E.; Zhu, J.Y.; Bai, M.Y.; Arenhart, R.A.; Sun, Y.; Wang, Z.Y. Cell elongation is regulated through a central circuit of interacting transcription factors in the *Arabidopsis* hypocotyl. *eLife* **2014**, *3*, e03031. [[CrossRef](#)]
77. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant* **2020**, *13*, 1194–1202. [[CrossRef](#)]

78. Zheng, L.; Yang, Y.; Ma, S.; Wang, W.; Zhang, J.; Yue, C.; Wang, Y.; Song, M.; Hao, X.; Huang, J. Genome-Wide Identification of Brassinosteroid Signaling Downstream Genes in Nine Rosaceae Species and Analyses of Their Roles in Stem Growth and Stress Response in Apple. *Front. Genet.* **2021**, *12*, 640271. [[CrossRef](#)]
79. Zhu, K.; Xu, S.; Li, K.; Chen, S.; Zafar, S.; Cao, W.; Wang, Z.; Ding, L.; Yang, Y.; Li, Y.; et al. Transcriptome analysis of the irregular shape of shoot apical meristem in dt (dou tou) mutant of *Brassica napus* L. *Mol. Breed.* **2019**, *39*, 66. [[CrossRef](#)]
80. Zhou, T.; Yue, C.; Huang, J.; Cui, J.; Liu, Y.; Wang, W.; Tian, C.; Hua, Y. Genome-wide identification of the amino acid permease genes and molecular characterization of their transcriptional responses to various nutrient stresses in allotetraploid rapeseed. *BMC Plant Biol.* **2020**, *20*, 151. [[CrossRef](#)]
81. Zheng, M.; Hu, M.; Yang, H.; Tang, M.; Zhang, L.; Liu, H.; Li, X.; Liu, J.; Sun, X.; Fan, S.; et al. Three BnaIAA7 homologs are involved in auxin/brassinosteroid-mediated plant morphogenesis in rapeseed (*Brassica napus* L.). *Plant Cell Rep.* **2019**, *38*, 883–897. [[CrossRef](#)]
82. Zhang, Z.H.; Zhou, T.; Tang, T.J.; Song, H.X.; Guan, C.Y.; Huang, J.Y.; Hua, Y.P. A multiomics approach reveals the pivotal role of subcellular reallocation in determining rapeseed resistance to cadmium toxicity. *J. Exp. Bot.* **2019**, *70*, 5437–5455. [[CrossRef](#)]
83. Zhang, G.B.; Meng, S.; Gong, J.M. The Expected and Unexpected Roles of Nitrate Transporters in Plant Abiotic Stress Resistance and Their Regulation. *Int. J. Mol. Sci.* **2018**, *19*, 3535. [[CrossRef](#)] [[PubMed](#)]
84. Wang, X.; Zheng, M.; Liu, H.; Zhang, L.; Chen, F.; Zhang, W.; Fan, S.; Peng, M.; Hu, M.; Wang, H.; et al. Fine-mapping and transcriptome analysis of a candidate gene controlling plant height in *Brassica napus* L. *Biotechnol. Biofuels* **2020**, *13*, 42. [[CrossRef](#)]
85. Li, H.; Cheng, X.; Zhang, L.; Hu, J.; Zhang, F.; Chen, B.; Xu, K.; Gao, G.; Li, H.; Li, L.; et al. An Integration of Genome-Wide Association Study and Gene Co-expression Network Analysis Identifies Candidate Genes of Stem Lodging-Related Traits in *Brassica napus*. *Front. Plant Sci.* **2018**, *9*, 796. [[CrossRef](#)] [[PubMed](#)]
86. Cui, J.; Hua, Y.; Zhou, T.; Liu, Y.; Huang, J.; Yue, C. Global Landscapes of the Na⁺/H⁺ Antiporter (NHX) Family Members Uncover their Potential Roles in Regulating the Rapeseed Resistance to Salt Stress. *Int. J. Mol. Sci.* **2020**, *21*, 3429. [[CrossRef](#)]
87. Huang, J.; Ma, S.; Zhang, K.; Liu, X.; Hu, L.; Wang, W.; Zheng, L. Genome-Wide Identification of Gramineae Brassinosteroid-Related Genes and Their Roles in Plant Architecture and Salt Stress Adaptation. *Int. J. Mol. Sci.* **2022**, *23*, 5551. [[CrossRef](#)] [[PubMed](#)]