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Comprehensive Genome-Wide Identification and Characterization of the AP2 Subfamily in *Beta vulgaris* L. in Response to Exogenous Abscisic Acid

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Abstract: APETALA2 (AP2) belongs to transcription factor (TF) families, with crucial roles in regulating plant growth, development, and stress responses. In order to explore the characteristics of sugar beet (*Beta vulgaris* L.) AP2s (*BvAP2s*) in response to drought stress hormone abscisic acid (ABA), genome-wide identification, and the phylogeny, gene structure and promoter precursor analysis of the *BvAP2s* were employed to predict their potential functions. It is shown that there are a total of 13 *BvAP2* genes in the *Beta vulgaris*. Based on the primary amino acid sequence, the *BvAP2s* can be further subdivided into euAP2, euANT and basalANT. In addition, cis-acting element analysis showed that *BvAP2s* contained several abiotic stress-related elements, including those associated with ABA and drought stress. Roots are the first to perceive stress signals, and ABA-treated beetroot transcriptome and downstream gene prediction of *BvAP2s* revealed that *BVRB_4g074790*, *BVRB_6g128480* and *BVRB_7g179610* may play an important role involved in ABA signaling pathways during the stress response by regulating downstream GRAM genes, LEAs and U-boxes. Additionally, quantitative real-time polymerase chain reaction (qRT-PCR) further confirmed the downregulation of these three *BvAP2s* in response to ABA induction in sugar beet roots. These findings provide a basis for future utilization of *BvAP2s* in developing drought-tolerant *Beta vulgaris* varieties.

Keywords: *Beta vulgaris*; AP2; TF; exogenous ABA; drought stress

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1. Introduction

Abscisic acid (ABA) is a key plant hormone that plays a crucial role in regulating growth, development, and stress responses. ABA is intricately involved in various stress-related physiological activities, including the regulation of stomatal closure and osmotic adjustment [1]. The accumulation of ABA is directly related to the drought resistance of different plants, and it is often used as one of the indicators for drought resistance identification [2]. Plants can accurately adjust ABA levels in reaction to both internal and external environmental shifts. This regulation enables the control of various downstream transcription factors (TFs) and other ABA-responsive genes through core signal pathways, thereby fulfilling diverse biological functions [3]. The expression of ABA-responsive genes is tightly regulated by specific TFs and cis-acting elements [4]. Studies have demonstrated that ABA is involved in responding to osmotic stress by inducing TFs such as MYB, AP2/ERF and NAC to bind to cis-acting elements on ABA-responsive genes [5–7]. Specifically, *GmERF75*, members of the AP2/ERF family, have been identified that may have positive regulatory functions in *Arabidopsis* ABA signaling transduction under stressful conditions [7].

The AP2/ERF TF family is one of the largest TF families in plants, characterized by a conserved AP2 DNA binding domain consisting of 57−66 amino acids [8]. A comprehensive analysis of exon, intron and motif structure across the AP2/ERF family has led to its classification into three primary subfamilies: AP2, ERF and RAV. The AP2 subfamily proteins feature two repeated AP2 domains, the ERF subfamily proteins contain a single AP2 domain, and the RAV subfamily proteins encompass both the AP2 and B3 domains [9,10]. Moreover, the amino acid sequence of the AP2 domain indicates that the AP2 subfamily can be further subdivided into euAP2, euAINTEGUMENTA (euANT) and basalANT [11].

The AP2 TFs in *Arabidopsis thaliana* are the most extensively studied within the AP2 family, with the first discovered AP2 gene (AP2-1) associated with perianth organs [12]. In addition, the AP2 gene subfamily, which is induced by ABA, played a role in plant defense against both biotic and abiotic stresses. In Arabidopsis thaliana, it has been observed that overexpression of RAP2.6 results in increased sensitivity to exogenous ABA and abiotic stresses during seed germination and early seedling growth [13]. Furthermore, *ORA47*, an AP2 domain transcription factor in Arabidopsis thaliana, is believed to play a role in the production of jasmonic acid (JA) and ABA, as well as in the regulation of various phytohormone genes involved in response to wounding and water stress [14]. Recently, it has been demonstrated that silencing *TaAP2-10* decreases wheat's ability to resist stripe rust fungus. Additionally, the expression of *TaAP2-10* is significantly increased by external factors like ABA [15]. Consequently, AP2 subfamily genes exert a positive or negative influence on plant responses to stress by participating in the synthesis of endogenous ABA or being induced by exogenous ABA. Currently, the AP2 gene subfamily has been investigated and analyzed genome-wide in many plants, including Arabidopsis [16], rice [17], wheat [10], Populus [18], pepper [19] and buckwheat [20], and there is a lack of comprehensive information regarding the AP2 family in sugar beet.

Sugar beet (*Beta vulgaris* L.; 2n = 18) is a biennial herbaceous plant and a primary sugar crop in China [21], serving as an essential raw material for the food, pharmaceutical, and other industries. Notably, the northeast, northwest, and north China regions where sugar beet cultivation is prevalent, are particularly exposed to drought, cold and other environmental stresses [22], which have become significant constraints on sugar beet production in China [23]. Compared with other crops, sugar beet is very sensitive to drought during the seedling stage [24]. It has been demonstrated that drought stress has a significant impact on the physiological and morphological traits of sugar beet roots, as well as on taproot yield [25]. In our previous research involving strand-specific sequencing of the ABA signaling pathway and its regulatory network in sugar beet, we identified a key gene, *BVRB_4g074790* (AP2), involved in the regulation of oxidation reduction and cell wall organization [26]. To further explore the role of AP2 genes in stress tolerance in sugar beet, we conducted a genome-wide identification of the AP2 gene subfamily in sugar beet, and an in-depth analysis of their phylogeny, chromosomal localization, and gene structure and expression patterns in roots under ABA treatment in this study. The findings will contribute to a better understanding of the sugar beet AP2 gene family and lay the foundation for future analysis of the function of desirable genes in sugar beet and, ultimately, for the breeding of improved sugar beet varieties.

2. Materials and Methods

2.1. Identification and Phylogenetic Analysis of BvAP2s in Sugar Beet

The whole genome sequence, protein sequence and gene annotation file (gff3) of *Beta vulgaris* was downloaded from the Ensembl Plants database (https://plants.ensembl.org/ accessed on 15 September 2023). The Hidden Markov Model structural domain (PF00847) was obtained from the Pfam database (http://pfam.xfam.org/ accessed on 15 September 2023) in this study [10]. The Pfam database and NCBI-CDD database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi accessed on 18 September 2023) were consulted to validate the domains of alternative sugar beet AP2 subfamily members. The amino acid sequences of AP2 subfamily members from Arabidopsis and Spinach were sourced

from the plant TF database PlantTFDB (http://planttfdb.gao-lab.org/ accessed on 18 September 2023).

The protein sequences of the selected AP2 subfamily members from sugar beet, Arabidopsis and spinach were subjected to multiple sequence alignment and uploaded to TBtools software (V1.120) to construct a phylogenetic tree using the neighbor joining (NJ) method [27]. The generated phylogenetic evolutionary tree data were uploaded to iTOL (https://iTOL.embl.de/ accessed on 20 September 2023) for editing and visualization.

2.2. Property Analysis and Subcellular Localization Prediction of BvAP2s

The Expasy online tool (http://www.expasy.org/tools/ accessed on 22 September 2023) was employed to analyze amino acid composition, theoretical isoelectric point (PI), protein molecular weight (MW), and instability index [28]. Subcellular localization prediction of the *BvAP2* genes was conducted using the online software Cell-PLoc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/ accessed on 23 September 2023).

2.3. Conserved Motifs and Gene Structure of BvAP2s

The exon and intron structure for members of the *BvAP2* genes was obtained from the GFF3 annotation file of the sugar beet genome. The online website MEME (https://meme-suite.org/meme/tools/meme accessed on 24 September 2023) was used to analyze protein conserved motifs. TBtools software was utilized to visualize the gene structure and conserved motifs, with the maximum number of conserved motifs set to 20 and other parameters set to default values [27].

2.4. Chromosome Distribution and Cis-Acting Elements of BvAP2s

The chromosome distribution and potential duplicate gene pairs of the *BvAP2s* were predicted using TBtools. The upstream sequences (2000 bp) of *BvAP2s* start codons were extracted, and the cis-acting elements were predicted and visualized using TBtools [27].

2.5. Expression Analysis of BvAP2s under ABA Treatment and Transcriptional Target Gene Network Prediction

Transcriptome data of beetroot under ABA treatment [26] were used to analyze the expression patterns of *BvAP2s* using the ChiPlot website (https://www.chiplot.online, accessed on 30 September 2023). Differentially expressed transcription units (TUs) were identified based on the following criteria: (1) a differential expression fold change $|\log2FC| \geq 2$; (2) a Benjamini–Hochberg adjusted p value/false discovery rate (FDR) was less than 0.05. The *BvAP2* TFs binding site of the significantly expressed genes was predicted on the PlantTFDB website (with a cutoff threshold of 10^{-5} for selecting predicted results). The resulting predictions were imported into Gephi 0.9.7 software, and the data were visualized using the "Fruchterman Reingold" layout to highlight key nodes based on their calculated average node weight. Functional prediction of the significantly expressed genes regulated by *BvAP2s* TFs was conducted on the NCBI website.

2.6. Plant Materials and ABA Treatment

Sugar beet accession KWS9147 was germinated in vermiculite, and the seedlings were subsequently transferred into Hoagland solution (pH 5.8) for cultivation in a controlled environment chamber (light intensity 300 μmol/(m^2·s), a 16/8 h photoperiod, temperature of 24 ± 2 °C and relative humidity of 40 ± 5%). After 4 weeks of growth, treatment with 100 mg/L ABA (approximately 0.38 mmol/L) was conducted. Root samples were collected at 1, 6, 12 and 24 h after treatment, with three replicates in both control (normal conditions) and ABA treatment [26]. Subsequently, all samples were promptly frozen in liquid nitrogen and stored at −80 °C for RNA extraction.

2.7. RNA Extraction and qRT-PCR

Total RNA was extracted using the TRIzol reagent (Tiangen Biotech, Beijing, China, DP424) and reverse transcribed to cDNA. The qRT-PCR was performed using the Super-Real PreMix Plus (SYBR Green) (Tiangen Biotech, Beijing, China, FP205) Fluorescence Quantification Kit in three independent biological replicates and technical replicates. The primers used are described in Table 1, and the endogenous control gene were *BvGADPH* (*BVRB_5g110740*) and *Bvactin* (*BVRB_1g005390*). The relative expression was analyzed using the $2^{-\Delta\Delta Ct}$ method [29]. One-way anova was used to identify significant differences at p value ≤ 0.05 using Graphpad Prism 9.5.0 software.

Table 1. The primers and sequences for qRT-PCR.

Gene	Forward Primer (5′→3′)	Reverse Primer (5′→3′)
BVRB_4g074790	CGCATTCCCACAAGCTCAAC	CGACCCAGAACTATGCTCCA
BVRB_6g128480	CGGTGGAGATCAATCAGGTGT	CCAATGACAGTAGTAGAAGAGGG
BVRB_7g179610	ATTGCCCTCGGAACACCATT	TGCAGTAGAGAAGACGGGGA
BVRB_5g110740 (BvGADPH)	GCTTTGAACGACCACTTCGC	ACGCCGAGAGCAACTTGAAC
BVRB_1g005390 (*BvActin*)	TGCTTGACTCTGGTGATGGT	AGCAAGATCCAAACGGAGAATG

3. Results

3.1. Genomic Identification and Phylogenetic Analysis of BvAP2 Genes

The sugar beet *BvAP2s* gene family was identified at the whole-genome level using the protein functional domain PF00847, resulting in the screening of 13 AP2 proteins, which were subsequently validated in the NCBI-CDD database. AP2 subfamily genes are characterized by more than two AP2 protein domains. As shown in Table 2, the lengths of BvAP2 protein sequences vary from 307aa (BVRB_8g198510) to 715aa (BVRB_4g074790) amino acids. The molecular weight ranges from 34.11 to 77.87 kDa, with isoelectric points ranging from 5.61 to 8.51. All BvAP2s are unstable and prone to degradation. Subcellular localization prediction indicated that most BvAP2s are located in the cytoplasm and nucleus (Table 2). Previous studies demonstrated that both AP2/ERF family members MdSHINE2 and NtERF172 are localized to the nucleus [30,31].

Table 2. Genomic information and protein characterization of *BvAP2* subfamily genes.

Gene ID	Number of Amino Acids	Molecular Weight (Da)	Isoelectric Point (pI)	Instability Index	Subcellular Localization
BVRB_1g014630	562	61,898.82	6.58	49.18	Cytoplasm Nucleus
BVRB_2g045840	471	51,016.85	8.51	45.41	Cytoplasm Nucleus
BVRB_4g072290	602	65,389.26	5.98	52.27	Cytoplasm Nucleus
BVRB_4g074790	715	77,866.42	6.08	40.33	Cytoplasm Nucleus
BVRB_4g096070	456	51,690.36	5.86	67.11	Cytoplasm Nucleus
BVRB_6g128480	696	77,721.18	6.54	53.62	Nucleus
BVRB_6g133850	542	59,922.27	6.36	45.56	Cytoplasm Nucleus
BVRB_6g141950	677	76,121.8	7.45	43.9	Nucleus

Table 2. *Cont.*

Gene ID	Number of Amino Acids	Molecular Weight (Da)	Isoelectric Point (pI)	Instability Index	Subcellular Localization
BVRB_7g170060	515	57,210.08	6.03	55.59	Cytoplasm Nucleus
BVRB_7g179610	431	47,779.66	6.18	43.45	Nucleus
BVRB_8g191760	343	39,213.45	6.61	41.28	Cytoplasm Nucleus
BVRB_8g198510	307	34,115.59	5.61	41.97	Nucleus
BVRB_013350	578	64,804.72	6.33	66.04	Nucleus

To elucidate the phylogenetic relationships among *BvAP2s*, we constructed a phylogenetic tree by introducing 30 members of AP2s from *Arabidopsis thaliana* and 13 members from *Spinacia oleracea*. As shown in Figure 1, based on the previous classification of *AP2* genes [32], we observed that *BvAP2s* mainly belong to the euAP2, euANT and basalANT groups. Among them, eight *BvAP2* genes with the presence of an additional ten amino acid residue motif within the first AP structural domain were recognized as ANT group members; the baselANT group included two members, while the euANT group had six members lacking the aforementioned motif. No *BvAP2s* were grouped into the same basal branch, which may suggest their structural and functional diversity. In addition, *BVRB_6g141950* and *Sp 049430* have five and three AP2 structural domains, respectively, and they have not been classified into the above known groups. It is illustrated that *AP2* genes sharing similar structural features were accurately clustered into specific subfamilies, which indicated that the *AP2* gene family has high homology across different species. Meanwhile, different BvAP2s may play distinct roles in the biological processes of sugar beet.

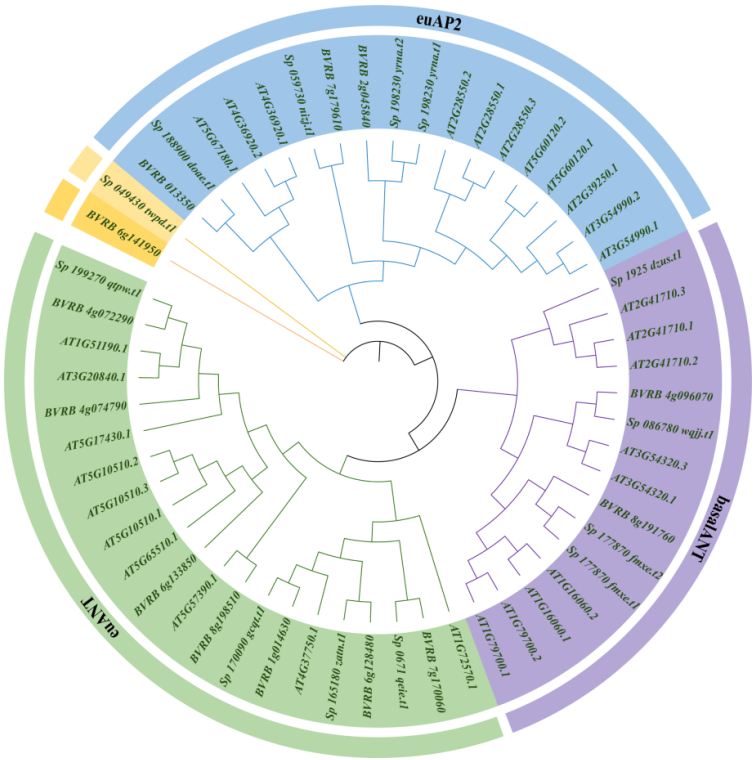

Figure 1. Phylogenetic tree of *AP2* gene subfamily across *Arabidopsis thaliana* (At), *Spinacia oleracea* (Sp) and *Beta vulgaris* (Bv).

3.2. Gene Structures and Conserved Motifs of *BvAP2s*

MEME was used to analyze protein motifs in the 13 AP2 protein sequences in sugar beet (Figure 2A). A total of six conserved motifs were identified and designated motif 1-6, with lengths ranging from 8 to 60 amino acids. Motifs 1, 2, 3 and 5 were found in almost all the members of the *AP2* subfamily, indicating their conservation within the *BvAP2* subfamily. The types and distribution of motifs were different in *BvAP2* genes, but several combined patterns of motifs, such as motif 2-3-1-5, appear frequently. Members within the same subgroup displayed similar motifs, lengths and structures, which suggests functional similarities within each group. The motif arrangements of *BvAP2s* exhibit certain similarities, yet there are also notable differences between groups; for example, motif 6 was unique to the euANT subfamily.

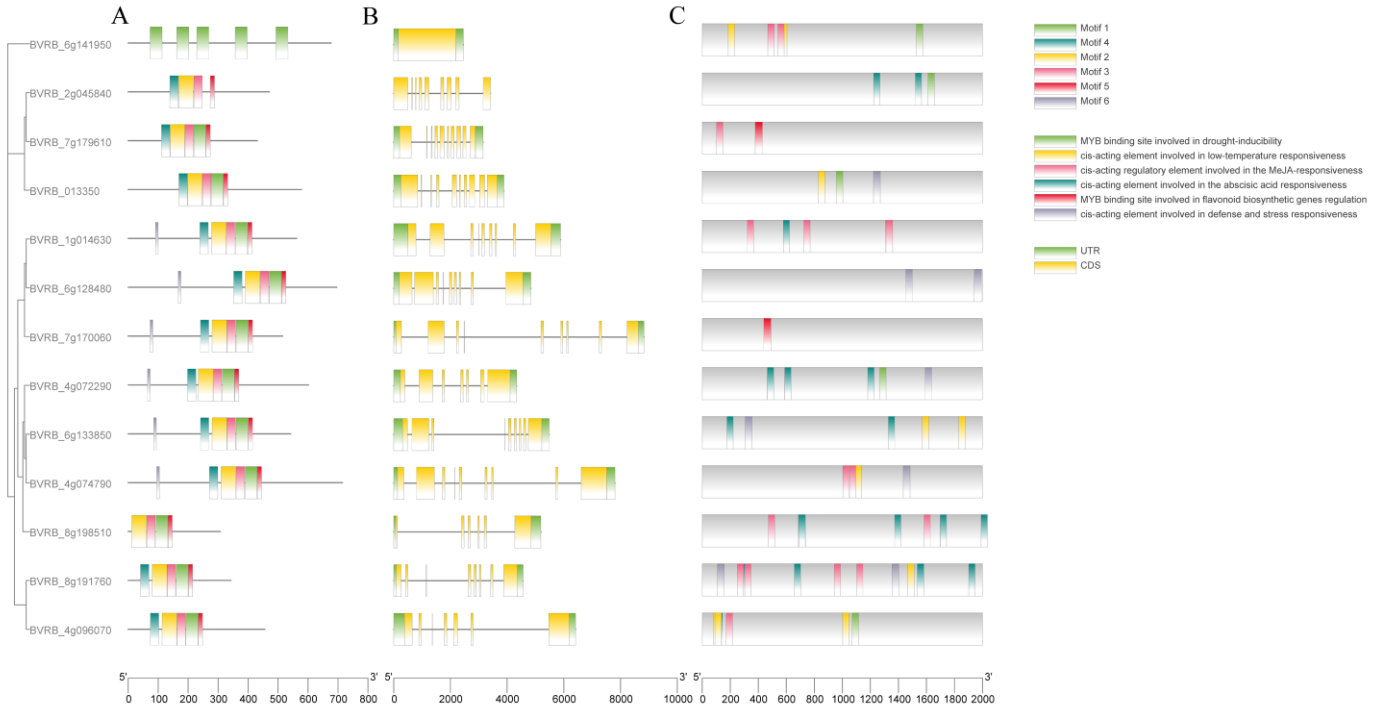

Figure 2. Phylogenetic relationships and conserved motifs (**A**), cis-acting elements (**B**), and exon/intron structures (**C**) of *BvAP2s*. The exons are represented by green rectangles, the introns by yellow rectangles, and the solid lines indicate the positions of the introns. The scale bar indicates the length of the corresponding gene.

In order to gain more insights into gene evolution, the exon–intron organization of *BvAP2* genes was investigated (Figure 2B). Notably, *BVRB_6g141950* exhibited a distinctive profile compared to other *BvAP2* members, as revealed by the gene structure analysis. Among the *BvAP2* genes, all other 12 members except *BVRB_6g141950* contain introns, and the number of exons is 5-9. Admittedly, there are subtle differences in the genetic structure between the subgroups. Most of the euAP2 and euANT genes had eight and nine introns, respectively.

3.3. Cis-Acting Elements in *BvAP2s* Promoters

Cis-acting elements in promoter regions are crucial for the regulation of gene transcription and abiotic stress response. To elucidate the response of *BvAP2s* to environmental signals, we analyzed the cis-regulatory elements in the promoters of the *BvAP2s*. The results showed that there are six known key cis-acting elements in the upstream 2000 bp sequences of *BvAP2s*' promoters (Figure 2C). Predominant elements include the abscisic acid responsive element (ABRE), the MYB binding site involved in drought response (MBS), the low-temperature responsive element (LTR), the MYB binding site for flavonoid biosyn-

thesis regulation, the MeJA-responsive element (CGTCA-element), and the TC-rich repeats involved in defense and stress response. ABREs were found in seven BvAP2 gene promoter sequences, including *BVRB_2g045840*, *BVRB_1g014630* and *BVRB_4g072290*. And we have identified MBS in the promoter sequences of five BvAP2 genes, including *BVRB_2g045840*, *BVRB_6g141950* and *BVRB_013350* (Figure 2C). These findings show the vital function of *BvAP2s* in counteracting environmental stress, especially drought stress.

3.4. Chromosome Localization and Collinearity among BvAP2 Subfamily Genes

The nine *BvAP2* genes are unevenly distributed across five sugar beet chromosomes (Figure 3). The 3 genes on chr 6 constitute the largest cluster, while chr1, chr 4, chr 7 and chr 8 each have 1–2 genes. In addition, *BVRB_013350*, *BVRB_2g045840*, *BVRB_4g096070* and *BVRB_7g179610* are located on the scaffold rather than on chromosomes. It is noteworthy that the majority of *BvAP2s* are distributed in proximity to the chromosome termini.

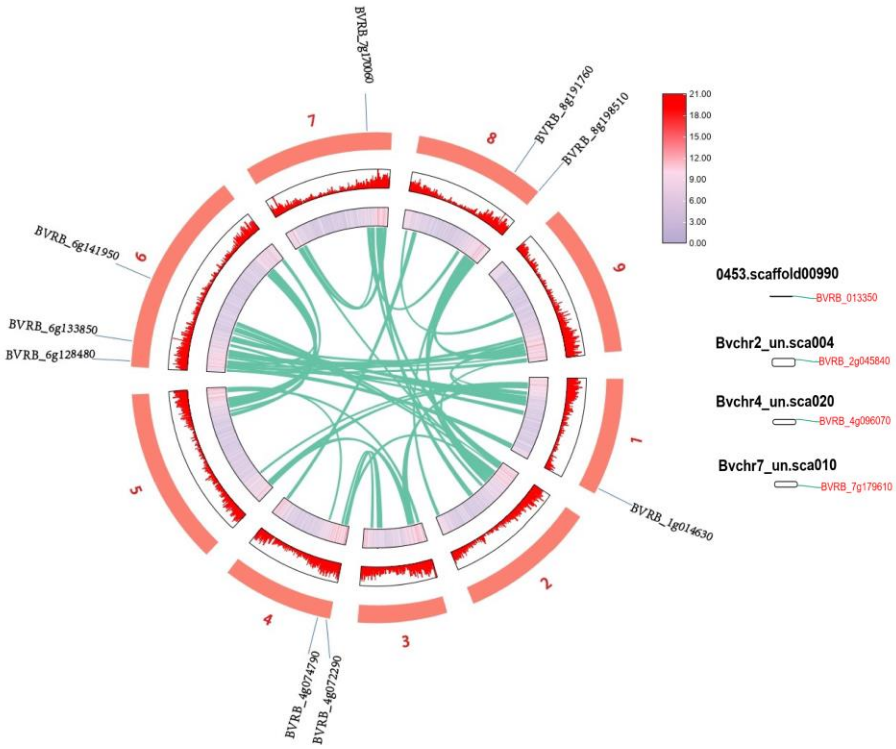

Figure 3. Location and collinearity relationships of *BvAP2s*. The green line represents the gene pairs replicated in tandem.

Gene duplication plays a significant role in gene repetition and evolution. The collinearity analysis of sugar beet genes (Figure 3) revealed a high structural similarity between duplicated pairs of *BvAP2s*, such as *BVRB_1g014630* and *BVRB_6g128480*.

3.5. Responsive Patterns of BvAP2s under Exogenous ABA Treatment

Crop roots are the first to perceive stressors such as drought and osmotic stress, promptly responding to mitigate their effects [33]. To investigate the potential biological roles of AP2s in beetroot, we analyzed their transcript changes in transcriptome data under ABA treatments (Figure 4). The analysis revealed that after exogenous ABA treatment, the expression patterns of *BvAP2s* in sugar beet roots varied. Specifically, seven *BvAP2s* (*BVRB_1g014630*, *BVRB_4g072290*, *BVRB_4g074790*, *BVRB_6g128480*, *BVRB_7g170060* *BVRB_7g179610* and *BVRB_8g198520*) were significantly downregulated, while *BVRB_8g191760* showed significant upregulation. Additionally, a subset of *BvAP2* genes displayed significantly elevated expression at a specific time point. For instance, *BVRB_4g074790* and *BVRB_4g096070* demonstrated significantly elevated expression at 1 h post-treatment. In

conclusion, members of the *BvAP2* gene family are differentially regulated by ABA, suggesting their potential involvement in the ABA signal transduction pathway as TF to modulate the expression of key downstream genes.

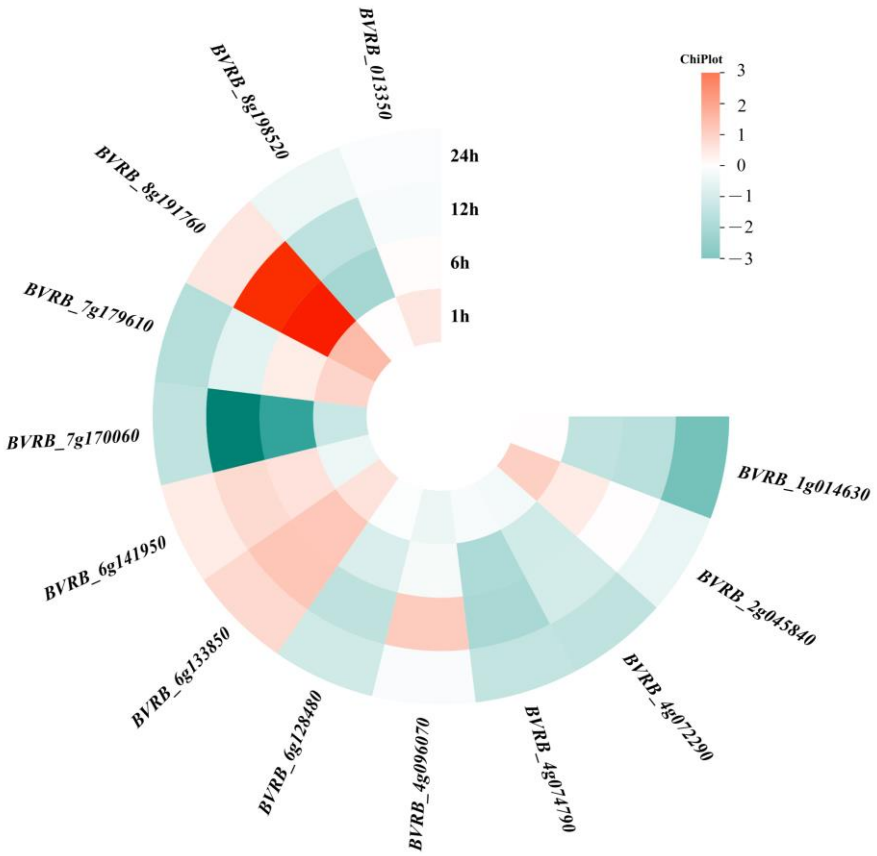

Figure 4. Heat map illustrating the expression pattern of the *BvAP2* gene subfamily in response to ABA treatments.

3.6. Target Genes Regulated by BvAP2 TFs Involved in ABA Response

Due to the limited number of differentially expressed genes in the early stages of exogenous ABA treatment, we screened sugar beet roots for differential gene expression after 12 h and 24 h of treatment. It identified a total of 105 significantly expressed genes (Table S1). The regulatory network of *BvAP2s* was mapped in conjunction with the prediction of transcription factor binding sites in the promoter region of the differential expressed genes (2000 bp upstream) using the PlantTFDB database (Figure 5). Our analysis revealed that four *BvAP2* TFs (*BVRB_4g074790*, *BVRB_6g128480*, *BVRB_6g133850* and *BVRB_7g179610*) regulate 60 of these significantly expressed genes (Table S2). Specifically, *BVRB_4g074790* was found to regulate 56 significantly expressed genes, while *BVRB_6g128480*, *BVRB_6g133850* and *BVRB_7g179610* regulate 6, 6 and 2 significantly expressed genes, respectively. The downstream-regulated target genes were primarily associated with phytohormone signal transduction, glutathione metabolism and peroxide scavenging metabolic pathways. Previous studies have highlighted the important role of exogenous ABA in improving the antioxidant capacity and drought resistance physiology of maize by enhancing antioxidant enzyme activity and stabilizing the AsA and GSH redox state [34]. The above results indicate that these pathways may play a significant role in the stress response, and that sugar beet *BvAP2s* may interact with genes involved in ABA signaling and the regulation of reactive oxygen species metabolism, potentially contributing to drought and other stress response processes.

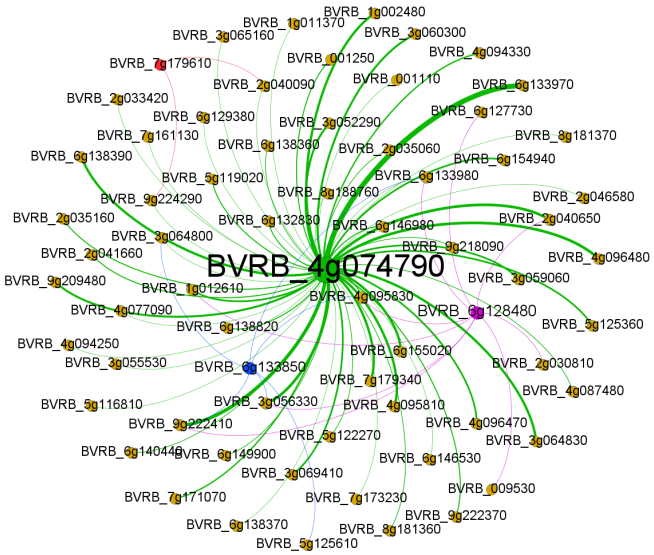

Figure 5. Regulatory network of *BvAP2* TFs and their key target genes induced by exogenous ABA. The brown dots represent genes that are significantly expressed in response to ABA, while the green, blue, red, and purple dots represent *BvAP2* transcription factors (TFs) that regulate the aforementioned genes. The size of the dots represents the number of *BvAP2*-regulated genes, while the thickness of the lines indicates the number of *BvAP2*-regulated sites in the promoter regions of the significantly expressed genes.

3.7. Expression Profiles of BvAP2 Genes under ABA

Combined with the results of these experiments, a total of three *BvAP2s* (*BVRB_4g074790*, *BVRB_6g128480* and *BVRB_7g179610*) were screened for both significant ABA-induced expression and the ability to regulate key downstream genes. To better understand the expression patterns of these three *BvAP2s* under ABA treatment in sugar beet, we performed quantitative real-time PCR (qRT-PCR) analysis. Our results demonstrated that the expression levels of *BVRB_4g074790*, *BVRB_6g128480* and *BVRB_7g179610* were found to be down-regulated by exogenous ABA, with the most pronounced downregulation observed at 24 h. This responsive profile was consistent with the transcriptomic changes, also suggesting that our findings were reliable (Figure 6).

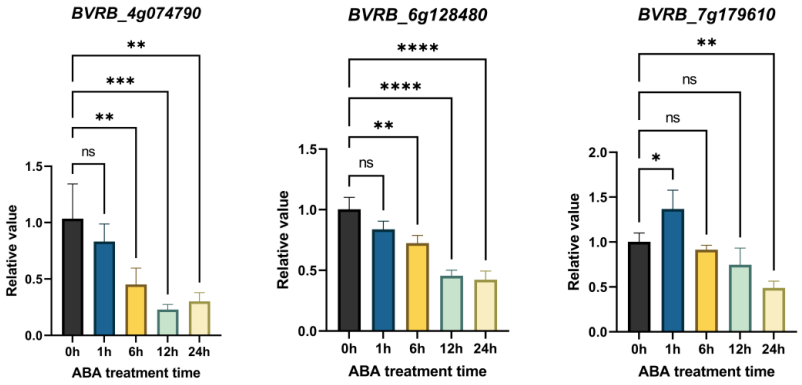

Figure 6. Expression profile of *BVRB_4g074790*, *BVRB_6g128480* and *BVRB_7g179610* under ABA treatment by qRT-PCR. Statistically significant differences were determined using Tukey's test at p-value ≤ 0.05. The greater the number of asterisks, the more significant the change in the relative expression of the gene. The asterisks indicate significant differences compared with control (0 h). The "ns" indicates that the observed difference is not statistically significant. Data were normalized by the *BvNADPH* and *Bvactin* gene using the $2^{-\Delta\Delta Ct}$ method.

4. Discussion

The growth and development of plants under various environmental stresses are regulated by genetic factors. For example, the response of plants to drought stress involves a complex process governed by numerous genes and signaling pathways [2]. Among these genetic factors, the AP2/ERF gene family has garnered considerable attention due to its diverse functions, including roles in abiotic stress resistance, hormone signal transduction, and the regulation of plant metabolites. The significance of the AP2/ERF transcription factor family is widely acknowledged across many plant species [30]. Recent evidence highlights the novel apple MdSHINE2 transcription factor, which activates the expression of wax-related genes to increase cuticular wax load and deposition. This enhances cuticular wax impermeability and alters surface patterns, thereby enhancing plant sensitivity to ABA and drought tolerance [35]. Sugar beet, as one of the most important sugar crops worldwide, is impacted by various abiotic stresses, including drought, which can affect its growth, development, yield and quality. However, the corresponding molecular response mechanisms remain unknown. Among the various regulatory factors, AP2 appears to play a crucial role in sugar beet's response to ABA, which is an essential phytohormone playing a key role in root architecture and plant stress responses [26]. Thus, in our study, sugar beet *AP2s* were genomically identified, and the ABA-induced members were further analyzed using transcriptome data and qRT-PCR.

We identified 13 *BvAP2* genes and conducted a comprehensive study of their phylogeny (Figure 1), sequence structure, chromosomal localization (Table 2) and expression patterns under ABA treatment (Figure 4). TFs possess motifs and domains involved in various activities, including protein interaction, transcriptional activity, and DNA binding [36]. Motif analysis revealed six conserved motifs in BvAP2 proteins (Figure 2A). Among them, motifs 1 and 5 constitute one AP2 domain, while motifs 2 and 4 were components of the other AP2 domain. This pattern of BvAP2 proteins is consistent with observations from studies in other species, such as wheat [10]. Promoter cis-acting element analysis is a valuable method for predicting gene function [37]. In this study, we found that *BvAP2* promoters contain various motifs associated with plant developmental stages, stress responses and hormone regulation (Figure 2B). For instance, the ABREs (abscisic acid-responsive elements) are widely distributed upstream of seven *BvAP2* genes, with five genes containing multiple MBS, including *BVRB_2g045840* and *BVRB_4g072290*. The high abundance and widespread presence of ABRE components suggest an important role in ABA response to abiotic stresses, including cold, drought and salt [38]. ABA is a well-known anti-stress plant hormone that regulates many developmental processes [39]. For instance, the *ZmEREB160* gene has been implicated in the ABA signaling pathway to improve drought resistance [40]. Additionally, five *BvAP2s* have drought-inducible MBS elements, including *BVRB_2g045840*, *BVRB_6g141950* and *BVRB_013350*. This may imply a relationship and interaction of AP2 TFs and the complex ABA regulatory network.

The root system is the main organ that responds, senses and maintains crop yield under drought conditions [41]. Furthermore, the root system can act as a sensor for water deficit conditions and send signals to shoots above ground [42]. Signaling cascades transmit chemical signals to the shoot, triggering molecular responses that result in biochemical and morphological changes. These changes help plants protect against water loss and withstand stressful conditions [43]. AP2/ERF TFs are essential for plant roots in response to abiotic and biotic stresses and in signaling pathways. ABA serves as an important long-distance trafficking messenger responsible for the perceptions of environmental stimuli and the activation of signaling transduction [44]. Moreover, the AP2/ERFs are indispensable for ABA-dependent stress responses as well. *ANT* [45], *RAP2.6L* [46] and *RAP2.6* [13] in *Arabidopsis* are induced in the ABA signaling pathway, increasing its resistance such as drought and salt tolerance. In our study, all tested *BvAP2* genes positively respond to ABA treatment (Figure 4). Exogenous ABA treatment resulted in a significant increase in *BvAP2* genes expression at the middle (6 h and 12 h) and late (24 h) stages of treatment, compared to the early stage of treatment (1 h). This suggests that maintaining water homeostasis under

prolonged drought stress in sugar beet requires more *BvAP2* genes expression [47,48]. Specifically, *BVRB_1g014630*, *BVRB_4g074790*, *BVRB_6g128480*, *BVRB_7g170060* and *BVRB_7g179610* were significantly down-regulated in beetroot, while gene *BVRB_8g191760* was significantly up-regulated (Figure 4). The varying degrees of induction of the *BvAP2* genes suggest their potential involvement in ABA-dependent signaling pathways under drought stress. This finding aligns with the results of previous studies in pearl millet [49].

To further investigate the characteristics of the *BvAP2* genes by ABA application, this study simultaneously screened the other genes significantly expressed in beetroot after ABA treatment (12 and 24 h) and predicted the regulatory relationship of *BvAP2s* with them. The results suggest that *BVRB_4g074790*, *BVRB_6g128480*, *BVRB_6g133850* and *BVRB_7g179610* may play key roles in regulating of these significantly expressed genes (Figure 5). Among the key downstream genes regulated by *BvAP2s*, most are associated with ABA-mediated stress responses. For instance, *BVRB_6g149900* encodes GEM-like protein 5, a member of the GRAM domain family involved in the ABA signaling pathway [50]. *OsABAR1* is a newly discovered protein in rice that contains a unique GRAM structural domain. It enhances the plant's ability to tolerate drought and salt stress by activating the ABA-dependent pathway. Additionally, when *OsABAR1* is overexpressed, rice plants become more responsive to externally applied ABA [51]. Furthermore, *BVRB_3g060300* belongs to the LEA gene family. Overexpression of *BnaCPK5* enhances drought stress tolerance in oilseed rape, partly by regulating the expression of LEA-like *RD29B* through phosphorylation of two core ABA signaling components [52]. Abiotic stress and ABA-inducible LEA Group 4 from *Brassica napus* also plays a key role in salt and drought tolerance [53]. Additionally, *BVRB_5g116810* is part of the U-box gene family. Previous studies have shown that U-box 10 negatively regulates abscisic acid response in *Arabidopsis* [54], while the U-box E3 ubiquitin ligase *PalPUB79* positively regulates ABA-dependent drought tolerance via ubiquitinating *PalWRKY77* in *populus* [55]. The significant expression of *BvAP2s* in beetroot induced by ABA suggested that *BvAP2s* may participate in stress response, including drought, by regulating downstream key genes and through ABA-dependent pathways.

Transcriptome changes and quantitative RT-PCR (qRT-PCR) can intuitively illustrate the characteristics of *BvAP2* genes in sugar beet suffering from ABA treatments. Three *BvAP2* genes (*BVRB_4g074790*, *BVRB_6g128480*, and *BVRB_7g179610*) were selected due to their responsiveness to ABA and their involvement in transcription factor regulatory networks. It was found that the down-regulation of gene expression was positively correlated with the increasing duration of ABA treatment. Prior research has shown that AP2/ERFs interrupt ABA signaling, resulting in reduced sensitivity on root growth inhibition and stomata closure, the inhibition of ABA signaling and drought tolerance by *ERF18/ORA47* in *Arabidopsis thaliana* [14]. It can be deduced that these three *BvAP2s* may be induced by ABA while exerting a negative regulatory effect on abiotic stresses such as drought and may be involved in the regulation of functional genes for signal transduction, compound metabolism, and other processes in response to drought stress in sugar beet.

5. Conclusions

In this study, 13 members of the *AP2* gene subfamily were identified from the whole beet genome of sugar beet. These genes exhibited differential responses to ABA treatment. The majority of *BvAP2* member promoters contain cis-acting elements of ABA response motifs and drought-inducible elements, and other stress-related elements. Notably, *BVRB_4g074790*, *BVRB_6g128480* and *BVRB_7g179610* were strongly induced by ABA, and may constitute a complex regulatory network with their downstream target genes, such as GRAMs, LEAs and U-boxes, involved in ABA-mediated stress regulation in sugar beet. This study provided a solid basis for further research on the function of *BvAP2s* in sugar beet.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14081273/s1, Table S1: Genes showing significant expression changes following ABA treatment; Table S2: The regulatory relationship and promoter binding sites of 4 BvAP2s on 60 significantly expressed genes.

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