Genome-wide identification of *CaARR-Bs* transcription factor gene family in pepper and their expression patterns under salinity stress

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Abstract

In plants, *ARR-Bs* transcription factors play a crucial role in regulating cytokinin signal transduction, abiotic stress resistance, and plant development. A number of adverse environmental conditions have caused severe losses for the pepper (*Capsicum annuum* L.) - a significant and economically important vegetable. Among the transcription factors of type *B-ARRs* family, multiple members have different functions. In pepper, only a few members of the *ARR-Bs* family have been reported and characterized. The current study aimed to characterize *ARR-Bs* transcription factors in *C. annuum* at genomic and proteomic levels and investigate their regulation under salinity stress. A total of ten genes, named *CaARR1* to *CaARR10*, belonging to the largest subfamily of type-B ARRs, were discovered in *C. annum*. To investigate the *CaARR-Bs* family in *C. annuum* on a genome-wide scale, the analysis relied on the known *ARR-Bs* genes in Arabidopsis. An analysis of homologous alignments of candidate genes, including their phylogenetic relationships, gene structures, conserved domains, and qPCR expression profiles, was conducted. In comparison with other plant *ARR-Bs* proteins, *CaARR-Bs* proteins showed gene conservation and potentially specialized functions. Furthermore, tissue-specific expression profiles revealed that CaARR-Bs genes are differentially expressed, suggesting they are functionally distinct. *CaARR-Bs* proteins had a typical conserved domain, including AAR-like (pfam: PF00072) and Myb DNA binding (pfam: PF00249) domains. Ten of the *CaARR-Bs* genes were asymmetrically mapped on seven chromosomes in Pepper. Additionally, the phylogenetic tree of *CaARR-Bs* genes from *C. annuum* and other plant species revealed that *CaARR-Bs* genes were classified into four clusters, which may have evolved conservatively. Furthermore, the utilization of quantitative real-time qRT-PCR for expression analysis indicated that nine *CaARR-Bs* genes displayed differential expression in both examined tissues, namely roots, and leaves. These genes exhibited diverse expression patterns and were found to be highly responsive to salinity stress. In particular, the expression of *CaARR3*, *CaARR5*, *CaARR6*, *CaARR7*, and *CaARR8* showed significant expression levels in roots. These findings enhance our comprehension of the functions performed by *CaARR-Bs* genes in pepper plants and establish a solid groundwork for future investigations into their involvement in growth and development processes under salinity stress conditions.

Keywords: *Capsicum annuum*, *CaARRs*-type B transcription factor, Gene structure, Phylogenetic analysis, Expression analysis, and Salinity stress.

1. Introduction

Peppers (*Capsicum annuum* L.) are important and widely grown vegetables belonging to the family Solanaceae, which includes eggplants, tomatoes, potatoes, etc. In temperate and subtropical regions worldwide, *C. annuum* is commonly cultivated as a seasoning vegetable. Peppers distribution, growth, and development are influenced by abiotic stresses such as drought, high salinity, and temperature extremes. Consequently, it is imperative to understand the mechanisms that contribute to Pepper's tolerance of such conditions (Chen et al., 2015).

Cytokinins are adenine derivatives, which are important plant hormones that affect nearly every aspect of plant development and growth. Among their functions, they promote chloroplast growth, delay senescence and regulate shoot and root development (Mok & Mok, 1994; Haberer & Kieber, 2002; Kakimoto, 2003; Werner et al., 2003). Signals are transduced using two-component phosphorelay systems, and elements of these systems act in response to cytokinin and ethylene hormones, as well as to red light and osmosis (Schaller, 2000; Hutchison & Kieber, 2002; Hwang, Chen & Sheen, 2002; Mason et al., 2005). Initially, the two-component systems were discovered in bacteria, which are controlled by His sensors kinase and response regulators (Mizuno, 1997; Stock, Robinson & Goudreau, 2000). A close correlation has also been found between endogenous cytokinin levels and pleiotropic developmental dysregulation (Ferreira & Kieber, 2005). A two-component signaling pathway is mediated by CKs through histidine kinases (HKs) and response regulators (RRs).

There are two types of Arabidopsis response regulators, type-A and type-B, according to their domain structure and sequence (Imamura et al., 1999). Numerous studies indicate that type-B *ARRs* contain DNA binding and receiver domains (Sakai, Aoyama & Oka, 2000; Lohrmann et al., 2001; Hosoda et al., 2002). Based on their core receiver domain structures and C-terminal domain sequences, Arabidopsis' genome contains 23 authentic response regulators (ARRs), divided into four types: A, B, C, and pseudo (Schaller et al., 2007). DNA-binding and receiver domains distinguish Type-B ARRs as transcription factors (Lohrmann et al., 2001; Hosoda et al., 2002). There is compelling evidence that type-A ARR genes are direct targets of phosphorylated *ARR-B* regulators (Hwang, Chen & Sheen, 2002; Imamura et al., 2003). The *ARR-Bs* have also been shown to positively regulate cytokinin signaling (Mizuno, 2004). In this study, the type-B authentic response regulator is unified as *ARR-Bs*. ARRs are phosphorylated by HKs upon perception of a stress signal, leading to changes in their DNA-binding activity and subsequent regulation of target genes. Several studies have demonstrated the involvement of ARRs in plant responses to salt stress. For instance, in Arabidopsis thaliana, ARR2 and ARR12 were found to be upregulated in response to salt stress, and loss-of-function mutants of ARR2 displayed increased sensitivity to salt stress, indicating the importance of ARR2 in salt stress tolerance (Mizuno, 2004). Similarly, in rice, the overexpression of OsRR22, a member of the ARR family, enhanced salt stress tolerance by upregulating the expression of stress-responsive genes (Zhang, 2009).

Lately, the pepper genome has been sequenced (Kim et al., 2014; Qin et al., 2014; Magdy et al., 2019; Magdy & Ouyang, 2020). A wide range of RNA molecules derived from several tissues, including root, shoot, leaf, flower, and fruit are also available. Using these data sets, pepper improvement and basic research can identify and functionalize a gene family from a global perspective. The purpose of this study was to identify all potential ARR-B genes encoded in the pepper genome. Routine bioinformatic approaches were conducted to examine the chromosomal distribution, phylogenetic relationships, and gene structure of the identified *CaARR-Bs* genes. Additionally, functional predictions were made based on gene expression analysis across various developmental stages and in response to salinity stress. These comprehensive results serve as a crucial basis for future investigations focused on gene family exploration and the functional characterization of *ARR-Bs* in pepper plants.

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2. Materials and Methods

2.1 Identification of members of the *CaARR-Bs* gene family in Pepper

Using the TAIR.org database, we were able to retrieve Arabidopsis thaliana structural domains for *ARR-Bs* genes. The obtained sequences were used as probes for homology searches using BLASTp (Wang et al., 2020a). BLASTP searches selected the sequences of *CaARR-Bs* genes to the online pepper genomics database (http://peppersequence.genomics.cn/), where the genome database of the *C. annuum* cultivar Znula was selected (Qin et al., 2014). The search yielded Pepper *ARR-Bs* candidate genes. The physicochemical properties of the *ARR-Bs* family genes were estimated using Expasy (http://web.expasy.org/) (Gasteiger et al., 2005). Finally, the subcellular localization analysis of the pepper *CaARR-Bs* gene family was performed using Cell-Ploc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/). The *CaARR-Bs* gene family protein sequences were aligned with *A. thaliana ARR-B* genes by MAFFT aligner (Katoh & Standley, 2013) using the embedded algorithms in Geneious Prime. Subsequently, the rooted phylogenetic trees were generated using the maximum likelihood methods (ML). The ML tree was constructed using FastTree V2 (Kumar et al., 2018), embedded in Geneious Prime.

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2.2 Chromosomal location, Gene structure, domain annotation, and secondary structure analysis

The chromosomal location of *CaARR-Bs* genes throughout the pepper genome was investigated using the MG2C V2 online tool (Chao et al., 2021). GSDS was used to identify *CaARR-Bs* gene exon-intron structures by comparing the genomic sequences *vs* cDNA sequences (http://gsds.cbi.pku.edu.cn/). The *CaARR-Bs* and the Myb DNA binding domains (a domain that distinguishes the *ARR-A* from *ARR-B* genes) were detected using CDD (Marchler-Bauer et al., 2015), SMART, pfam and InterProScan databases (Wang et al., 2022). SPOMA was used to predict CaARR-Bs secondary structures (Sapay, Guermeur & Deléage, 2006).

2.3 Potential *cis*-element analysis in promoter regions

A BLASTn search was conducted on the pepper genome using the cultivar Znula as the query sequences to identify *CaARR-Bs* genes. For each gene, a 1500 bp upstream the initiation codon was retrieved and analyzed for *cis*-elements using PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

2.4 Plant materials and salinity stress treatment

Seeds of the pepper cultivar Gedeon F1 (*Capsicum annuum* L.; https://www.syngenta.com.eg/) were sterilized with 1 % sodium hypochlorite for 30 min, washed with sterile water, and then sown in perlite beds at 28º C (Qin et al., 2014). Seedlings were grown under 16 h/light at 25 °C and eight h/night at 18 °C with a relative humidity of 60% until they had six leaves. Plants were irrigated with Hoagland solution at half-strength pH 5.6. Leaves and roots were harvested from the seedlings with three biological replicates as control. For the salt stress experiment, five-week-old plants were irrigated with 200 mM NaCl (Wang et al., 2022). After 12 hours and 24 hours of treatment, each sample (3-4 leaves) was collected in three biological replicates. Liquid nitrogen was used to rapidly freeze samples, followed by -80°C storage until RNA extraction.

2.5 RNA expression and qPCR analysis

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Total RNA was extracted from pepper tissues (leaves and roots) subjected to different salt stress treatments using the EasyPure® Plant RNA Kit (TransGen Biotech, Beijing, China) following the instructions provided by the kit manufacturer. The quality and quantity of the extracted RNA were evaluated using electrophoresis on 2% agarose gels and a Quantus™ Fluorometer (Promega, USA). The cDNA synthesis was performed using SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and adjusted to a concentration of 100 ng µL-1. During the PCR amplification, a range of 108 to 147 base pairs was targeted while avoiding the conserved region (Table 1).

Quantitative real-time RT-PCR (qRT-PCR) was performed using TransStart® Green qPCR SuperMix (TransGen Biotech, Beijing, China). The amplification reactions were performed following: 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 55 °C for 20 s, and 72 °C for 30 s. Melting curve analysis, performed by increasing the temperature from 55 to 95 °C (0.5 °C per 10 s), and a gel electrophoresis of the amplified fragments confirmed that the product contained single amplicons. In each experiment, the relative fold differences were calculated using the $^{\Delta\Delta}$ Ct. Normalization was conducted using GAPDH Ct values, amplified using 5'-ATGATGATGTGAAAGCAGCG-3' and 5'-TTTCAACTGGTGGCTGCTAC-3' as a reference gene (Arce-Rodríguez & Ochoa-Alejo, 2015). In this experiment, three biological replicates per sample were used.

3. Results

3.1 Identification of the *CaARR-Bs* family genes members in Pepper

The identification of the *CaARR-Bs* family genes in the *C. annuum* genome (cultivar Gedeon F1) based on the pepper genome sequences database was performed using BLASTp database search to query *Arabidopsis thaliana ARR-Bs* genes. In total, ten sequences were putative as pepper *ARR-Bs* genes (*CaARRs*). The chromosome location, exon number, and genomic and physiochemical characteristics of each gene are in Table (2). In the current study, the shortest putative open reading frame (ORF) was 1374, while the longest was 2025, with the amino acid length of *CaARRs* proteins ranging from 389 to 684, a molecular weight (Mw) of 43.09 to 75.16 kDa, and a theoretical isoelectric point (PI) varying from 5.39 to 9.47. In addition, the presence of the ARR-like domain was verified using the Pfam database, and its position was unfixed among all copies, while all copies were sublocalized in the nucleus.

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3.2 Alignment and phylogenetic analysis of *CaARR-Bs*

Phylogenetics were conducted to confirm the identified *CaARRs* copies and determine the evolutionary affinity of the *CaARR-Bs* genes and *ARR-Bs* genes of Arabidopsis. With full-length amino acid sequences, the alignment of the *ARR-BS* domain was conducted and used to perform an unrooted phylogenetic tree with a bootstrap test. The analyzed *ARR-Bs* proteins were grouped into five distinct clusters. The *CaARR-Bs* proteins in Pepper were clustered into four of the five subclusters with strong bootstrap support (Fig. 1). Functional divergence could have resulted from the presence or absence of species-specific *CaARR-Bs*.

3.3 Chromosomal location and duplication event of *CaARR-Bs* genes

As indicated by the starting and ending positions of *CaARR-Bs* genes on the chromosomes in Table (1), the genomic DNA sequences of each *CaARR-Bs* gene were mapped to the chromosomal location (Fig. 2). With few exceptions, *CaARR-Bs* genes were mainly found at the extremities of their respective chromosomes. The *CaARR-Bs* genes were asymmetrically distributed on chromosomes 1, 5, 6, 7, 9, 11, and 12, and none were found on chromosomes 2, 3, 4, 8, and 10. The *CaARR* 1 and 2 genes were

located on chromosome 1, *CaARR* 3 and 4 were located on chromosome 5 with a separation of 10 Mb, and *CaARR5* was on chromosome 6. In addition, *CaARR* 6 and 7 located on chromosome 7 with a separation of <1 Mb, while chromosome 9 had *CaARR8*, chromosome 11 had *CaARR9*, and *CaARR10* was in chromosome 12 (Fig. 2).

3.4 Predicted secondary structures of *CaARR-Bs* proteins

The prediction of the secondary structure of *CaARR-Bs* proteins was conducted. The primary constituent forms of the secondary structure of the pepper *ARR-Bs* protein, with α-helices, β-turns, and extended chains, were investigated and measured. According to structural predictions, pepper *ARR-Bs* gene family members contain α-helices, irregularly coiled, extended chains, and β-turns. The most average proportion for alpha-helices was around 34.1 ± 8%; the highest percentage was from *CaARR6* (84.7%) and the lowest was from *CaARR2* (26.3%). Most genes have averaged around 21.6 ± 4% β-turns; the highest percentage was from *CaARR3* (26.3%) and the lowest was from *CaARR5* (13.8%). The results show that the irregular coiling average record was 23.3 ± 6%, as the highest proportion was for *CaARR7* (30.3%), while the lowest was for *CaARR6* (13.7%; Table 3).

3.5 Gene structure *CaARR-Bs* proteins

For each gene in the CaARR-Bs gene family, exon-intron distribution and conserved motifs were analyzed. For each gene, several isoforms were detected with the exception for *CaARR2* and *CaARRs* 5 – 10 (unique isoform). The *CaARR1* recorded two isoforms where one was complete and a shorter isoform (x_2: 1431 bp and 477 aa). The *CaARR3* recorded three isoforms: one complete and two shorter isoforms (x_2: 1992 bp and 664 aa; x_3: 1956 bp and 652 aa). Finally, the *CaARR4* recorded five isoforms where one was complete, and five shorter isoforms (x_2: 1785 bp and 595 aa; x_3: 1896 bp and 632 aa; x_4: 1899 bp and 633 aa; x_5: 1908 bp and 636 aa).

There were differences in exonic and intronic regions between the 10 *CaARR-Bs*. Exons ranged from 5 to 11 while introns ranged from 4 to 10. The CaARR-Bs genes were clustered according to their sequence homology into three groups, showing similar gene structures within each group. Regardless of their chromosomal location, one cluster included *CaARR6 and CaARR8*, another included *CaARR7*, *CaARR1*, and *CaARR5*, and the other included *CaARR2*, *CaARR4*, and *CaARR9* (Fig. 3).

3.6 Potential *cis*-element analysis in promoter regions of *CaARR-Bs* genes

To further characterize the potential regulatory mechanisms of *CaARR-Bs* , 1500 bp upstream sequences from the translation start sites were analyzed. Based on their biological significance, *cis*-elements in *CaARR-Bs* were identified and categorized. The promoter sequences of 10 CaARR-Bs genes contained 146 possible cis-elements. Three main groups of cis-acting elements were identified: hormones, environmental stress, and photoresponses. The *CaARR-Bs* promoters included transcription factors binding sites related to abscisic acid responsiveness, auxin responsiveness, defense and stress responsiveness, endosperm expression, enhancer-like involved in anoxic specific inducibility, light responsiveness, low temperature responsiveness, meristem expression, zein metabolism regulation, MeJA responsiveness, Gibberellin responsiveness, ATBP 1 transcription factor, MYB binding site involved in drought inducibility, MYB binding site involved in flavonoid biosynthetic genes regulation, MYB binding site involved in light responsiveness, MYBHv1 binding site, and Salicylic acid responsiveness (Fig. 4). According to the *cis*-component, *CaARR-Bs* genes can respond to different abiotic stresses.

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3.7 *CaARR-Bs* gene expression profiles in response to salinity stress

The expression level of the *CaARR-Bs* was measured with qRT-PCR for seedlings exposed to salt stress. The expression patterns of *CaARR-Bs* genes varied among stress levels and showed considerable variation in expression patterns of the *CaARR-Bs* genes over time in the roots of plants more than in leaves. A heat map was generated to show the expression profiles while the expression differences were color-indicated (Fig. 5). In response to salinity stress, the expression of patterns of *CaARR2* and *CaARR9* in the roots were up-regulated after 12 and 24 h; additionally, *CaARR5* and *CaARR6* were up-regulated in roots after 24 and 12 h, respectively. Meanwhile, the *CaARR3*, *CaARR4*, *CaARR7*, and *CaARR8* were highly expressed in control samples and were down-regulated after 12 and 24 h of treatments. In the leaves, the expression level of *CaARR1* was the only expressed *CaARR* gene, and only in control samples, followed by *CaARR5*, which showed slight expression after 24 h of salinity treatment. The expression of *CaARR2*, *CaARR6*, and *CaARR9* increased slightly after 24 h of salinity treatment. The expression of *CaARR3*, *CaARR4*, *CaARR7*, and *CaARR8* was down-regulated in 24h. The expression level on the leaves of *CaARR6*, *CaARR5*, and *CaARR67* was decreased, while *CaARR3* was induced after 12 h and 24 h of stress. The *CaARR10* was the only copy that showed no amplification curves during the qPCR.

4. Discussion

The His-Asp phosphorelay signaling pathway in prokaryotic cells is controlled by ARRs (Suzuki et al., 1998). On the basis of their phylogeny, the ARR-B gene family evolved from nonvascular plants (bryophytes) such as phylostratum, while several orthologous genes exist in the plants (Cuming et al., 2007). The ARR-B genes have exhibited evolutionary conservation across all the selected species. This conservation is evident in their expansion within higher plants. ARRs have been classified as types A and B based on their conserved domains (Imamura et al., 1999). An N-terminal DNA-binding domain and a receiver domain indicate that ARR-B functions as a transcription factor (Ishida et al., 2008). A key component of cytokinin signaling pathways, type *B-ARRs* genes have been linked to plant response to various environmental stresses; in response to different stress conditions, superoxide anion and hydrogen peroxide contents were measured as antioxidant enzyme activity (Nakamichi et al., 2009). There are *ARR-Bs* transcription factors identified in Arabidopsis genomes (Mason et al., 2004; Ramírez-Carvajal, Morse & Davis, 2008), rice (Schaller et al., 2007), peach (Zeng et al., 2017), pear (Ni et al., 2017), soybean (He et al., 2022), and fragrant rice (Rehman et al., 2022). Despite this, little is known about *ARR-Bs* in Pepper. Studies have shown that gene organization plays an important role in how multiple gene families evolve (Xu et al., 2012; Ullah et al., 2019; Wang et al., 2020; Arce-Rodríguez, Martínez & Ochoa-Alejo, 2021; Ahiakpa et al., 2022). In the current study, ten *CaARR-Bs* genes in pepper have been identified and characterized by their structure, *cis*-elements in the promoter regions, chromosomal location, gene duplication, and phylogeny. In addition, salinity stress affects the expression profile of different tissues. Thus, in this study, the CaARR-Bs genes were comprehensively analyzed to determine their biological function.

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From the public genomic data, we derived the genomic sequences, protein sequences, and chromosomal locations of the identified *CaARR-Bs* genes (Chao et al., 2021). Following the standard protocol, the secondary structures of *CaARR-Bs* proteins were putatively predicted (Sapay, Guermeur & Deléage, 2006) and functionally characterized with proper tools (Wang et al., 2022). Resulting in a total of ten predicted *CaARR-Bs* genes were found on seven chromosomes, suggesting that the gene family show a segmental repetition. During plant genome evolution, duplication or large-scale segmental duplication is thought to produce gene families (Cannon et al., 2004). Many transcription factor families have been reported in gene duplication events, including *HD-ZIP*, *C2H2-ZF*, *SlARR-B*, *MYB*, and *NAC* (Liu et al., 2015; Chen et al., 2015; Arce-Rodríguez, Martínez & Ochoa-Alejo, 2021).

All the *CaARR-Bs* homologous gene pairs identified using the pepper genome *versus* the Arabidopsis genome showed tight phylogenetic clustering; their topologies are more closely related, suggesting they are more closely related. In addition, intron numbers were related to *CaARR-Bs* gene classifications. The duplication of genes is crucial to genomic expansion and realignment (Kumar, Tyagi & Sharma, 2011). Based on the phylogenetic tree and synteny analysis, the results were consistent. Genetic evolution has been attributed to gene or genome duplication events as the primary source of variation in the CaARR-Bs family gene. The result showed that segmental duplication events promote the evolution *of CaARR-Bs* genes (Yang, Tuskan & Cheng, 2006; Yang et al., 2008; Gillis et al., 2009).

Further clarifying the roles of *CaARR-Bs* promoter regions in response to abiotic stresses, we also identified several conserved *cis*-regulatory elements. An analysis of *cis*-acting elements in pepper type-*B ARRs* genes showed a close relationship between these genes and growth, hormonal signal transduction, and abiotic stress resistance. Several *cis*-elements involved in drought resistance are found in the promoter region of *ARR-B*, and a triple mutant lacking all three of these genes was reported in Arabidopsis (Nguyen et al., 2016). Further, *ARR-B* belongs to the helix-loop-helix family and are nuclear-localized transcription factors, as evidenced by their helix-loop-helix structure in the *CaARR-Bs* domain (Hosoda et al., 2002). The *ARR-Bs* regulators target type-A *ARRs* genes directly, which are activated by phosphorylated *ARR-Bs* (Hwang & Sheen, 2001; Imamura et al., 2003).

In Capsicum, the involvement of two-component response regulators (ARRs) has been identified as key components in these signaling pathways. Salt-responsive genes are regulated by ARRs, which mediate the plant's adaptive response. The involvement of ARRs in salt stress signaling pathways can be attributed to their ability to regulate downstream stress-responsive genes. ARRs function by interacting with other transcription factors and *cis*-acting elements in the promoter regions of target genes, thereby modulating their expression. This regulatory mechanism enables ARRs to orchestrate the activation of stress-responsive genes involved in ion homeostasis, osmotic regulation, antioxidative defense, and other adaptive processes that contribute to salt stress tolerance in Capsicum (Urao et al., 2000).

There is a close relationship between the expression profiles of genes and their biological functions. Despite this, *ARR-B* expression patterns in different tissues have rarely been studied in Pepper. Studies have shown that ARR-B transcription factors are involved in cytokinin signal transduction; in addition, they may play a role in root development and drought- and salinity-tolerance (Garay-Arroyo et al., 2012; Kiryushkin et al., 2019; Seo et al., 2020). Based on the qPCR analysis, we found that these genes had broad expression profiles in Pepper, *CaARR2*, *CaARR5*, *CaARR6*, and *CaARR9* were highly expressed in the roots. while *CaARR2*, *CaARR4*, *CaARR6*, and *CaARR9* were down-regulated in the shoots. Interestingly, the Arabidopsis ortholog of *SlARR-B1* regulates sodium accumulation in tomato shoots (Mason et al., 2010). Several gene families have been identified, and their expression profiles have been characterized according to the stress responses and phytohormone responses of different plant species (Ye et al., 2009; Zhu et al., 2013; Zhao et al., 2016; Xia et al., 2017; Wang et al., 2017; Liu et al., 2020; Zhang et al., 2020; He et al., 2020).

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Conclusion

To conduct functional studies, it is essential first to characterize and classify gene families. During the present study, 10 *CaARR-Bs* genes were identified and classified. Seven of the twelve chromosomes of *C. annuum* contained uneven distributions of genes. According to the phylogenetic analysis, most *CaARR-Bs* presented possible orthologs in Arabidopsis, indicating a common evolutionary origin.

Moreover, different salinity stress levels induced different expression levels of *CaARR-Bs* genes in leaves and roots, supporting the theory that *CaARR-Bs* have functionally divergent functions. By integrating our results, we identified *CaARR-Bs* candidates that might contribute to regulating salt stress resistance and shed new light on *CaARR-Bs* transcription factors' role in secondary metabolism. To better understand how *CaARR-Bs* function and how they are regulated in *Capsicum* spp., more interspecific functional characterization of *CaARR-Bs* genes is required.

Conflict of Interest

The author declares no Conflict of Interest.

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Figure Legend

Figure 1. (**A**) Alignment of the *ARR* domain sequences from 10 putative *ARRs-B* genes in pepper and 21 *ARRs-B* genes from *Arabidopsis thaliana*. *ARRs-B* and Myb DNA binding motifs on amino acid sites are marked at the top, and sequence identities are shown below. (**B**) An unrooted phylogenetic tree displays *CaARRs-B* genes' relationships in *C. annuum* and *A. thaliana*. Different colors indicate the five different groups. Numbers at nodes represent bootstrap values based on 1000 replicates.

Figure 2. Chromosomal distribution of *CaARRs-B* gene genes in Pepper. The scale is in million bases (Mb). Chromosomes without *CaARRs* genes are not shown.

Figure 3. A schematic diagram of the 10 *CaARRs*-B gene structures showing exons and introns structures.

Figure 4. The number of various *cis*-elements on the promoters of each *CaARRs-B* gene. Promoter sequences (-1500 bp) of 10 *CaARRs-B* genes were analyzed.

Figure 5. Expression profiles of 9 pepper *ARRs-B* genes in different tissues, a case-oriented PCA based on the complete qPCR profile (left), and a gene-oriented heatmap (right) generated using the heat

mapper tool were shown. Blue, white, and red colors correspond to low, moderate, and high fold change levels.