

Article

Genome-Wide Identification of the Maize ARR-B Gene Family and Expression Analysis Response to Low-Phosphorus in Maize (*Zea mays* L.)

Shuang Li ^{1,2,3,†} , Litao Yi ^{1,2,†} , Shaoxian Liu ^{1,2} , Han Lv ^{1,2,3}, Aiping Yin ^{1,2,3}, Qingtao Zeng ^{1,2,3}, Xiaonian Jing ^{1,2,3}, Nanqing Ma ^{1,2,3}  and Chaofeng Li ^{1,2,3,*} 

¹ Maize Research Institute, Southwest University, Chongqing 400715, China; ls18855077417@126.com (S.L.); 13068332389@163.com (L.Y.); sxliu123@163.com (S.L.); lv03162046@163.com (H.L.); yaaap8910@163.com (A.Y.); 15893521629@163.com (Q.Z.); 18584308909@163.com (X.J.); 19556577205@163.com (N.M.)

² College of Agronomy and Biotechnology, Southwest University, Chongqing 400715, China

³ Engineering Research Center of South Upland Agriculture, Ministry of Education, Chongqing 400715, China

* Correspondence: cfli1988cas@swu.edu.cn

† These authors contributed equally for this manuscript.

Abstract

Cytokinin signaling is essential for plant growth, development, and responses to environmental stresses. As key transcription factors in this pathway, type-B response regulators (ARR-Bs) activate the expression of primary cytokinin-responsive genes. However, the systematic identification of the ARR-B family in maize and its functional roles in responses to phosphorus (P) nutritional stress remain largely unclear. In this study, the maize ARR-B (ZmARR-B) transcription factor family was systematically identified and comprehensively characterized at the genome-wide level. A total of 12 ZmARR-B genes were identified and comprehensively characterized in terms of chromosomal localization, physicochemical properties, protein structure, phylogenetic relationships, gene organization, conserved motifs, promoter cis-regulatory elements, and collinearity. Based on systematic measurements of leaf inorganic phosphate (Pi) concentrations in 70 maize inbred lines from southwestern China, the lines 082, B73, and Ye107 were selected as representative materials. By integrating long-term (14 d) and short-term (0.5–12 h) low-P treatments with transcriptome sequencing and qRT-PCR validation, we systematically characterized the expression dynamics of ZmARR-B family members under P deficiency. The results showed that ZmARR-B genes exhibited markedly differentiated low-P-responsive expression patterns among distinct genetic backgrounds. *ZmARR-B03* was transiently induced during the early phase of low-P treatment (0–6 h), followed by a rapid decline in transcript abundance. This characteristic pulse-like expression pattern suggests that *ZmARR-B03* may function as a rapid-response regulator during early P-starvation signal transduction. In contrast, *ZmARR-B06* exhibited sustained transcriptional upregulation throughout the low-P treatment period, suggesting a potential role in long-term adaptive regulation under P-deficient conditions. Notably, the number of low-P-responsive ZmARR-B members was significantly greater in the P-efficient inbred line 082 than in the P-sensitive line Ye107, indicating that ZmARR-B-mediated P-starvation responses are strongly genotype dependent. This study provides an important foundation for elucidating the molecular mechanisms by which cytokinin signaling, through type-B ARRs, regulates phosphate nutritional adaptation in maize. It also identifies potential candidate genes for the genetic improvement of P-efficient maize germplasm.



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1. Introduction

Maize (*Zea mays* L.) is among the world's most extensively cultivated and productive cereal crops. Its applications span food, animal feed, industrial processing, and bioenergy production, making it vital for global food security and sustainable agricultural development [1]. Phosphorus (P) is a vital macronutrient that supports plant growth, development, and reproductive processes. It is involved in the biosynthesis and metabolism of nucleic acids, phospholipids, and diverse phosphorylated metabolites, and is indispensable for photosynthesis, energy transduction, and signal transduction [2]. As a typical C4 crop with high photosynthetic efficiency and biomass accumulation capacity, maize has a substantial demand for P. However, compared with other mineral nutrients, soil P generally exhibits low availability, resulting in limited P acquisition efficiency by plants [3]. Approximately 70% of arable soils worldwide are estimated to contain insufficient available P to support normal crop growth and development [4]. Consequently, P deficiency is a major nutritional constraint on plant growth and yield formation. Elucidating the molecular mechanisms underlying maize responses to P deficiency and identifying functional genes associated with low-P tolerance are therefore of considerable theoretical and practical importance for breeding high-yielding, P-efficient maize cultivars.

Cytokinins (CKs) are key phytohormones that regulate a broad range of plant processes, such as cell division and differentiation, shoot apical meristem maintenance, leaf senescence, and nutrient transport and distribution [5]. Cytokinin signaling is mediated through a multistep two-component phosphorelay system analogous to bacterial two-component signaling systems [6]. In *Arabidopsis thaliana*, this pathway comprises three major classes of core components: histidine kinase receptors (*Arabidopsis* histidine kinases, AHKs), histidine phosphotransfer proteins (*Arabidopsis* histidine phosphotransfer proteins, AHPs), and response regulators (*Arabidopsis* response regulators, ARRs) [7]. Upon cytokinin perception, AHK receptors undergo autophosphorylation at a conserved histidine residue. The phosphate group is subsequently transferred through the receiver domain of the receptor to a histidine residue on an AHP protein. Phosphorylated AHPs then translocate to the nucleus and transfer the phosphate group to a conserved aspartate residue in the N-terminal receiver domain of ARR proteins, thereby modulating ARR activity and downstream transcriptional responses.

Based on their domain architecture and biological functions, ARR proteins are divided into type-A and type-B subfamilies. Type-A ARRs typically consist of a receiver domain followed by a short C-terminal region. Their expression is rapidly induced by cytokinins, and they primarily act as negative-feedback modulators of cytokinin signaling. By contrast, type-B ARRs (ARR-Bs) serve as key positive transcriptional regulators in this pathway [8]. They possess a conserved N-terminal receiver domain that accepts the phosphate group from AHPs and undergoes conformational changes, as well as a C-terminal GARP DNA-binding domain and a glutamine-rich transcriptional activation domain [9]. Following phosphorylation-mediated activation, ARR-B proteins specifically recognize and bind cis-regulatory elements in the promoters of downstream target genes through their GARP domains, thereby activating target gene transcription. Thus, ARR-Bs function at the downstream output stage of cytokinin signaling [10], converting upstream hormonal inputs into specific transcriptional responses and exerting central regulatory functions in plant growth, organogenesis, and stress adaptation.

Since the completion of the *Arabidopsis* genome sequence, the ARR-B gene family has been systematically investigated in this model species. Eleven type-B ARR members have been identified in *Arabidopsis*, including *ARR1*, *ARR2*, *ARR10-ARR14*, and *ARR18-ARR21* [11]. These proteins exhibit both functional redundancy and pronounced specialization. For example, *ARR1*, *ARR10*, and *ARR12* act as important transcriptional activators in cytokinin-mediated regulation of root meristem development and vascular tissue differentiation [12]. *ARR2* participates in the regulation of leaf senescence and pathogen defense responses [13], whereas *ARR14* and *ARR18* have distinct roles in gametophyte development and embryogenesis. In addition, ARR-B transcription factors participate in nitrogen nutritional responses. *ARR1* can directly bind to the promoter of the nitrate transporter gene *AtNRT2.1* and activate its expression [10], thereby affecting nitrogen uptake and assimilation. In rice (*Oryza sativa*), genome-wide identification and expression profiling have shown that ARR-B genes exhibit differential expression patterns in roots, stems, leaves, and panicles, with several members responding to drought, salinity, and cytokinin treatments [14,15]. Chen et al. (2025) [15] provided important insights into the evolutionary characteristics of the ARR-B gene family in the Poaceae. Their study systematically characterized the ARR-B family across six Poaceae species, providing an important genomic framework for elucidating the evolutionary history of ARR-B genes in grasses. However, the evolutionary conservation and diversification of ARR-B genes across broader plant lineages, particularly among monocots, dicots, and woody plants, remain poorly characterized. Moreover, the potential roles of ARR-B genes in plant responses to phosphorus (P) deficiency and phosphate (Pi) starvation remain largely unexplored. In soybean, *GmRR1* has been demonstrated to participate in low-P responses; disruption of *GmRR1* significantly improves P acquisition efficiency and biomass accumulation by modifying root system architecture [16].

Cytokinin also plays important roles in plant responses to P deficiency. P starvation can suppress cytokinin biosynthesis and reduce local cytokinin concentrations in root tips, thereby alleviating cytokinin-mediated inhibition of root hair formation and lateral root development and promoting root system architectural remodeling [17]. *IPS1* is an important gene involved in the regulation of plant P homeostasis. Exogenous cytokinin application markedly represses the expression of *AtIPS1* and other P-deficiency-induced genes, thereby negatively regulating long-distance inorganic phosphate transport [18]. Furthermore, cytokinin participates in cross-kingdom signaling between plants and arbuscular mycorrhizal fungi. These fungi expand the P-absorbing zone of the root system, whereas cytokinin can regulate fungal colonization and arbuscule development, thereby indirectly influencing P acquisition through the symbiotic pathway [19].

Therefore, elucidating the expression patterns and regulatory mechanisms of ARR-B transcription factors under low-P stress will be important for improving phosphorus use efficiency, and developing nutrient-efficient maize germplasm.

2. Materials and Methods

2.1. Identification of Maize ARR-B Family Members

The maize B73 reference genome assembly (Zm-B73-REFERENCE-NAM-5.0.55), including genome, protein, and gene annotation sequences, was obtained from Phytozome v13 (<https://phytozome-next.jgi.doe.gov/>; accessed on 10 May 2026). *Arabidopsis* ARR-B protein sequences were downloaded from PlantTFDB (<https://planttfdb.gao-lab.org/>; accessed on 15 May 2026) [20] and employed as queries in an initial BLASTP search against the maize proteome available in Phytozome v13.

To further identify candidate proteins, the hidden Markov model (HMM) for the CheY-like receiver domain (REC domain; PF00072) was constructed using HMMER 3.0 [21]

implemented in TBtools v2.485 [22] and used to scan the maize proteome. The intersection of the BLASTP and HMMER results was retained as the initial candidate set. A maize-specific ARR-B HMM profile was subsequently generated from the initial candidates and used to search the maize proteome again with HMMsearch.

Conserved domains in candidate proteins were verified using the SMART database (<https://smart.embl.de/>, accessed on 18 May 2026) and the NCBI Conserved Domain Database (<https://www.ncbi.nlm.nih.gov/cdd>, accessed on 19 May 2026). Candidate ZmARR-B proteins were initially identified based on sequence similarity and the presence of characteristic ARR-B domains. The conserved domain architecture of the candidate proteins was subsequently analyzed to further confirm their molecular identity and structural characteristics. For loci with multiple transcripts, the isoform encoding the longest complete open reading frame was selected as the representative protein. The remaining proteins were defined as core members of the maize ARR-B family.

2.2. Chromosomal Distribution and Synteny Analysis of Maize ARR-B Genes

Chromosomal localization, visualization, and synteny analyses of ZmARR-B genes were performed using TBtools v2.485. The chromosomal coordinates of the 12 ZmARR-B genes were obtained from the GFF3 annotation file, and their distribution across the 10 maize chromosomes was visualized using the “Gene Location Visualize” tool. According to their chromosomal positions and order, the genes were named ZmARR-B01–ZmARR-B12.

Intraspecific collinearity analysis was conducted using the “One Step MCScanX” function in TBtools v2.485 [22] based on the maize GFF3 annotation and protein sequence files. Interspecific synteny analyses were further performed between maize and *A. thaliana*, rice, sorghum, and *Populus trichocarpa*. Circular synteny maps were generated using the “Advanced Circos” function to visualize duplication events and the evolutionary conservation of ZmARR-B genes across species.

2.3. Multiple Sequence Alignment and Phylogenetic Analysis of Maize ARR-B Genes

To investigate the evolutionary relationships of the maize ARR-B family, four representative species were selected: *A. thaliana* v10, *Oryza sativa* v7.0, *Sorghum bicolor* v5.1, and *P. trichocarpa* v4.1. Maize ARR-B protein sequences were used as queries to retrieve homologous ARR-B proteins from these species through BLASTP searches in Phytozome.

All ARR-B protein sequences were aligned with the MUSCLE algorithm implemented in MEGA 11 [23]. A maximum-likelihood phylogenetic tree was then generated under the Poisson substitution model, using partial deletion of missing data, a 90% site-coverage cutoff, and 1000 bootstrap replicates. The phylogenetic tree was visualized and annotated using the iTOL web server (<https://itol.embl.de/>; accessed on 21 May 2026). In addition, the 12 maize ARR-B proteins were aligned using DNAMAN software v10 with default settings to examine conserved regions and domain organization.

2.4. Physicochemical Characterization, Subcellular Localization, and Protein Structure Prediction

ExPASy ProtParam [24] (<https://web.expasy.org/protparam/>; accessed on 23 May 2026) was used to determine the amino acid length, theoretical molecular weight and isoelectric point, aliphatic index, instability index, and grand average of hydropathicity (GRAVY) of the 12 ZmARR-B proteins. Proteins with an instability index ≤ 40 were considered stable, whereas those with an index >40 were considered unstable. Positive and negative GRAVY values indicate overall hydrophobicity and hydrophilicity, respectively.

CELLO v2.5 [25] (<http://cello.life.nctu.edu.tw/>; accessed on 24 May 2026) was employed to predict the subcellular localization of the proteins. SOPMA [26] (<https://npsa.lyon.inserm.fr/>; accessed on 25 May 2026) was used to estimate the proportions of α -helices, extended strands, β -turns, and random coils in their secondary structures.

Homology-based three-dimensional models were generated using SWISS-MODEL [27] (<https://swissmodel.expasy.org/>; accessed on 26 May 2026).

2.5. Conserved Motif, Domain, and Gene Structure Analyses

Gene structure data, including exon, intron, and untranslated region (UTR) annotations, were obtained from the maize GFF3 file and visualized with TBtools v2.485 [22]. Conserved protein motifs were detected using the MEME Suite web server [28] (<https://meme-suite.org/>; accessed on 28 May 2026), with the motif number limited to 10 and motif lengths constrained to 6–50 amino acids; other settings remained at their default values. The phylogenetic tree, motif profiles, and exon–intron structures were then combined and displayed using TBtools' "Gene Structure View (Advanced)" module [22] to evaluate the structural conservation and divergence of ZmARR-B family members.

2.6. Promoter Cis-Regulatory Element Analysis

For each *ZmARR-B* gene, a 2000-bp sequence upstream of the translation start site (ATG) was extracted as the putative promoter region. Cis-regulatory elements were identified using PlantCARE [29] (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>; accessed on 29 May 2026), focusing on motifs related to phytohormone and light responses, stress tolerance, and tissue-specific expression. The predicted elements were visualized with TBtools v2.485 [22].

2.7. Plant Materials and Growth Conditions

Maize inbred lines B73, 082 (characterized by relatively high foliar Pi accumulation), Ye107 (characterized by relatively low foliar Pi accumulation), and M73 were maintained in our laboratory. Plants were grown under controlled conditions with a light intensity of 15,000–20,000 lux, a 14 h light/10 h dark photoperiod, day/night temperatures of 28/24 °C, and a relative humidity of 65%.

To investigate the responses of *ZmARR-B* genes to low-phosphorus (LP) stress, transcriptome sequencing was performed using four maize inbred lines subjected to LP treatment. For the long-term LP treatment, three maize inbred lines (082, Ye107, and B73) were hydroponically cultured in Hoagland nutrient solution (Coolaber Technology Co., Ltd., Beijing, China) supplemented with 5 µM KH₂PO₄ from the V3 stage. The complete Hoagland medium contains 250 µM KH₂PO₄, 2 mM Ca(NO₃)₂·4H₂O, 1.25 mM NH₄NO₃, 0.1 mM KCl, 0.65 mM K₂SO₄, 0.65 mM MgSO₄, 10.0 mM H₃BO₃, 0.5 mM (NH₄)₆Mo₇O₂₄, 1.0 mM MnSO₄, 0.1 mM CuSO₄·5H₂O, 1.0 mM ZnSO₄·7H₂O, and 0.1 mM Fe-EDTA [30]. Following 14 days of LP treatment, whole seedlings were harvested for total RNA extraction. For the short-term LP treatment, the maize inbred line M73 was cultured under the same hydroponic conditions, and whole seedlings were collected at 0, 0.5, 1, 3, 6, and 12 h after the initiation of LP treatment for total RNA extraction. Plants maintained in a complete nutrient solution under otherwise identical growth conditions served as the corresponding control group. This experimental design enabled us to characterize both sustained and early transcriptional responses of *ZmARR-B* genes to P deficiency across genetically diverse maize inbred lines and to identify candidate genes exhibiting rapid or persistent responses to Pi deprivation. The RNA-seq libraries were constructed from qualified total RNA and sequenced on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) using paired-end 150-bp (PE150) sequencing according to the manufacturer's protocols. Briefly, RNA quality assessment, mRNA enrichment, fragmentation, cDNA synthesis, end repair, adapter ligation, and PCR amplification were performed to generate sequencing-ready libraries. The raw reads were processed using the BMK-Cloud bioinformatics platform (Biocloud, www.biocloud.net) to remove adapter sequences and low-quality reads and obtain high-quality clean reads. The clean reads were aligned to the

maize reference genome, followed by transcript abundance quantification and differential expression analysis between control and LP-treated samples. These analyses enabled the identification of ZmARR-B genes exhibiting transcriptional responses to P deficiency and provided candidate genes for further functional characterization.

Plants used for seedling-stage physiological and biochemical analyses were grown in soil. Plants in the control group (NP) were cultivated in nutrient soil, whereas plants in the low-P group (LP) were cultivated in low-P soil obtained from the Maize Research Institute, Southwest University (Xiema, Beibei, Chongqing, China). The field soil contained 8.9, 3.3, and 117 mg·kg⁻¹ available N, P, and K, respectively, with an organic matter content of 14.6 g·kg⁻¹. According to the nutrient classification criteria of the Second Chinese Soil Survey, the field soil was classified as low-P soil because its available P concentration was below 5 mg·kg⁻¹, whereas its available N and K contents were within the appropriate ranges. The nutrient soil contained 10, 27.5, and 110 mg·kg⁻¹ available N, P, and K, respectively. The normal-P group (NP) treatment was prepared by thoroughly mixing the nutrient soil with the low-P soil at a 1:1 (v/v) ratio, whereas the LP treatment consisted of the same amount of low-P soil.

2.8. Acid Phosphatase Activity Assay

Acid phosphatase (ACP) activity was determined using an ACP activity assay kit based on a disodium phenyl phosphate microplate method [31]. Fresh maize tissue (0.2 g) was homogenized in 1 mL of physiological saline and kept on ice for 15–30 min. The homogenate was then filtered through gauze and centrifuged at 10,000 rpm for 20 min. After centrifugation, the supernatant was collected, the total extract volume was recorded, and aliquots were stored at –20 °C until further analysis.

Samples were diluted as recommended by the manufacturer. In a 1.5-mL micro-centrifuge tube, 30 µL of the diluted sample, 180 µL of distilled water, 60 µL of acidic substrate working solution, and 30 µL of ACP assay buffer were added in sequence. The reaction mixture was incubated at 37 °C for 15 min, followed by a 10-min equilibration at room temperature. Absorbance was measured at 510 nm using a microplate reader after blank correction. A standard curve was constructed using phenol standards, and phenol production in the samples was calculated from the regression equation. ACP activity was expressed as the amount of phenol produced per unit extraction volume per minute and was further normalized to fresh weight (nmol·min⁻¹·g⁻¹ FW), according to the manufacturer's instructions.

2.9. Determination of Inorganic Phosphate Content

Inorganic phosphate (Pi) levels were measured using a Tissue Inorganic Phosphorus Content Assay Kit [32] (AKIC002M; Boxbio, Beijing, China). Fresh maize tissue (0.1 g) was homogenized in 1 mL of extraction buffer on ice and centrifuged at 10,000 × g for 10 min at 4 °C. The resulting supernatant was collected and maintained on ice until analysis.

Sample, standard, and blank wells were set up in a 96-well plate, and the reagents and chromogenic solution were added following the manufacturer's protocol. After thorough mixing, the plate was incubated at 40 °C for 10 min, cooled to room temperature, and left to stand for an additional 10 min. Absorbance was recorded at 660 nm within 30 min after distilled-water blanking. Pi concentrations were derived from the standard curve, normalized to tissue fresh weight, and reported as µg·g⁻¹ FW.

2.10. Total RNA Extraction and qRT-PCR Analysis

Total RNA was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The RNA pellets were resuspended in 80 µL of RNase-free ddH₂O, and RNA concentration and purity were assessed using a nucleic acid quantification instrument. RNA integrity

was examined by electrophoresis on a 1% agarose gel. High-quality RNA samples were stored at -80°C until use.

First-strand cDNA was generated using the NovoScript[®] Plus All-in-one 1st Strand cDNA Synthesis SuperMix with gDNA Purge kit (E047-01B; Novoprotein, Shanghai, China). qRT-PCR was conducted with SYBR Green chemistry, using ZmACTIN3 as the reference gene. Three biological replicates, together with technical replicates, were analyzed for each treatment. Each 20- μL reaction comprised 10 μL of $2\times$ NovoScript[®] SYBR qPCR SuperMix Plus (E096; Novoprotein, Shanghai, China), 1 μL each of forward and reverse primers (1 μM), 1 μL of cDNA, and 7 μL of RNase-free ddH₂O.

The thermal protocol included an initial denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 30 s, 60°C for 20 s, and 72°C for 30 s. A melting-curve analysis was subsequently performed from 60 to 95°C , with fluorescence recorded at 0.5°C intervals. Amplification was carried out on a Bio-Rad CFX96 real-time PCR system (CFX96 Touch; Bio-Rad, Hercules, CA, USA). Relative transcript abundance was determined using the $2^{-\Delta\Delta C_t}$ method [33]. Primer sequences are provided in Supplementary Table S1.

2.11. Statistical Analysis

All experiments were conducted with at least three independent biological replicates. Results are expressed as means $\pm$ standard deviations (SD). Statistical analyses were performed using SPSS Statistics 27.0. Two-group comparisons were conducted using a two-tailed Student's *t*-test, with asterisks indicating significant differences. For comparisons involving multiple groups, one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used. Different lowercase letters represent significant differences among treatments at $p < 0.05$.

3. Results

3.1. Identification and Physicochemical Characterization of Maize ARR-B Family Members

Using the amino acid sequences of *A. thaliana* type-B response regulators (ARR-Bs) as queries, we identified 12 candidate maize ARR-B family members by integrating BLASTP homology searches in Phytozome v13, hidden Markov model (HMM)-based searches using HMMER 3.0, and conserved-domain validation. Based on their physical positions on maize chromosomes, these genes were designated ZmARR-B01 to ZmARR-B12.

The physicochemical properties of the ZmARR-B proteins are summarized in Table 1. Protein length varied substantially, ranging from 73 amino acids (aa) for ZmARR-B07 to 788 aa for ZmARR-B05. Except for ZmARR-B08, which was predicted to be a small protein of 8.41 kDa, the remaining members had predicted molecular weights ranging from 33.65 to 86.48 kDa. Theoretical isoelectric point analysis showed that most ZmARR-B proteins had pI values below 7.0 and were therefore predicted to be acidic proteins. ZmARR-B09 was the only predicted basic protein, with a pI of 9.17.

Based on the instability index, using 40 as the threshold, ZmARR-B06 and ZmARR-B08 were predicted to be stable proteins, whereas the other members were predicted to be unstable. This result suggests potential variation in protein stability among family members. Aliphatic index analysis showed that, with the exception of the relatively low value of ZmARR-B02 (67.52) and the high value of ZmARR-B08 (101.25), the indices of the other proteins ranged from 70 to 100, indicating variation in predicted thermal stability. All ZmARR-B proteins showed negative GRAVY values, indicating that they are predominantly hydrophilic.

Subcellular localization prediction indicated that most ZmARR-B proteins are likely localized to the nucleus, consistent with their proposed roles as transcriptional regulators. In contrast, ZmARR-B08 and ZmARR-B11 were predicted to localize to the cytoplasm and

chloroplast, respectively, suggesting possible differences in subcellular localization and functional diversification.

Table 1. Basic physicochemical characteristics of maize ARR-B family members.

Gene	Gene ID	Chr	CDS (bp)	Protein (aa)	MW/Da	PI	Instability Index	Aliphatic Index	GRAVY	Localization
ZmARR-B01	Zm00001eb009140	Chr01	2055	685	73,429.60	6.38	49.31	77.82	−0.69	nucl
ZmARR-B02	Zm00001eb046540	Chr01	753	251	33,649.00	6.79	47.49	67.52	−0.62	nucl
ZmARR-B03	Zm00001eb086410	Chr02	1179	393	44,074.60	6.52	40.84	72.14	−0.47	nucl
ZmARR-B04	Zm00001eb144290	Chr03	1755	585	65,635.30	6.21	44.61	79.9	−0.43	nucl
ZmARR-B05	Zm00001eb227390	Chr05	2367	789	86,477.50	5.76	44.57	76.79	−0.68	nucl
ZmARR-B06	Zm00001eb234170	Chr05	1896	633	85,104.00	5.98	37.13	80.02	−0.59	nucl
ZmARR-B07	Zm00001eb257860	Chr05	2031	677	73,063.20	5.63	48.89	81.2	−0.65	nucl
ZmARR-B08	Zm00001eb275790	Chr06	216	73	8412.00	4.92	26.14	101.25	−0.51	cyto
ZmARR-B09	Zm00001eb365420	Chr08	1758	586	66,358.20	9.17	44.55	82.27	−0.24	nucl
ZmARR-B10	Zm00001eb374760	Chr09	2010	670	72,502.00	5.87	45.11	81.08	−0.58	nucl
ZmARR-B11	Zm00001eb388280	Chr09	2016	672	73,467.80	5.41	44.91	79.57	−0.73	chlo
ZmARR-B12	Zm00001eb399830	Chr09	2061	687	74,002.00	6.45	45.93	76.99	−0.71	nucl

nucl, cyto, and chlo are abbreviations for nucleus, cytoplasm, and chloroplast, respectively.

3.2. Chromosomal Distribution and Protein Structural Analysis of Maize ARR-B Genes

Chromosomal mapping revealed that the 12 ZmARR-B genes were irregularly distributed across the 10 maize chromosomes (Figure 1). Chromosomes 5 and 9 each harbored three genes, chromosome 1 contained two, and chromosomes 2, 3, 6, and 8 each carried one gene. No ZmARR-B genes were identified on chromosomes 4, 7, or 10.

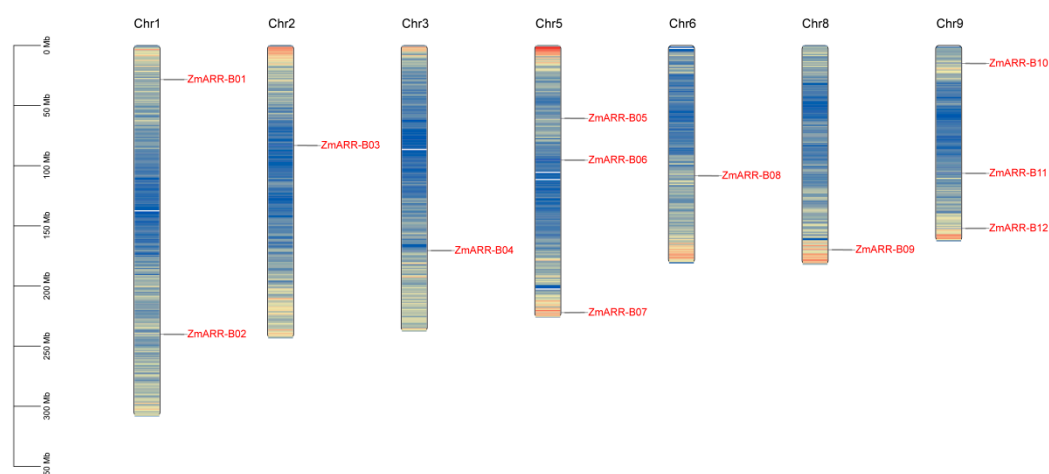


Figure 1. Chromosomal distribution of ARR-B family members in maize. Red labels denote ARR-B gene loci, while lines indicate their corresponding chromosomal positions. The number above each chromosome represents its chromosome number.

Secondary structure prediction results are shown in Supplementary Table S2. ZmARR-B proteins were mainly composed of α -helices, extended strands, and random coils, whereas β -turns were not predicted. Random coils represented the largest proportion, followed by α -helices and extended strands, suggesting that these proteins may possess relatively high conformational flexibility.

Three-dimensional homology modeling showed that, despite differences in protein length and peripheral structural regions, most ZmARR-B proteins contained a relatively conserved REC domain (Figure 2). Members within the same phylogenetic subgroup displayed relatively similar predicted three-dimensional conformations, indicating comparable structural and potentially functional characteristics. Notably, because of its short sequence length, ZmARR-B08 exhibited a simplified predicted structure and lacked portions of the typical peripheral structural regions.

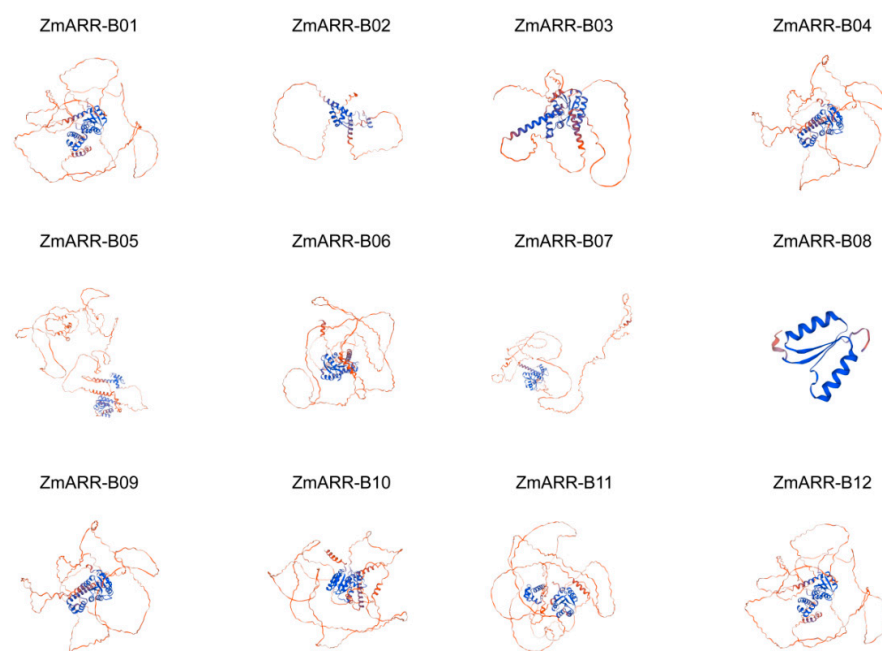


Figure 2. Predicted three-dimensional structures of maize ARR-B proteins. The N- and C-termini are shown in blue and red, respectively. α -Helical regions are highlighted in blue.

3.3. Phylogenetic and Multiple Sequence Alignment Analyses of the Maize ARR-B Family

To investigate evolutionary relationships within the ARR-B family, a total of 81 homologous ARR-B proteins from maize, *A. thaliana*, rice, sorghum, and *Populus trichocarpa* were collected and used to construct a maximum-likelihood phylogenetic tree (Figure 3). Based on tree topology and branch support, these proteins were classified into five groups (Groups I–V). Group V was the largest group, comprising 47 members, whereas Group II was the smallest group, containing only three members.

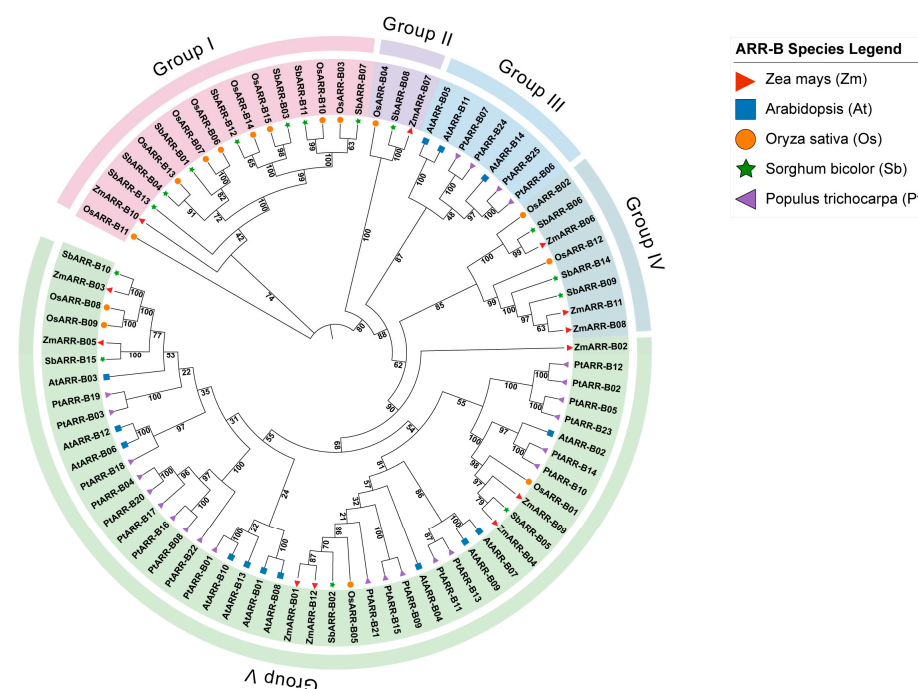


Figure 3. Phylogenetic relationships among ARR-B proteins from five plant species. The outer, middle, and inner circles indicate ARR-B subfamilies, gene names, and corresponding phylogenetic clades, respectively. Symbols on the right identify the species of origin: *Zea mays* (Zm), *Arabidopsis thaliana* (At), *Oryza sativa* (Os), *Sorghum bicolor* (Sb), and *Populus trichocarpa* (Pt).

Phylogenetic analysis showed that maize ARR-B proteins generally clustered more closely with homologs from rice and sorghum than with those from *A. thaliana* and *P. trichocarpa*. This pattern is consistent with the evolutionary relationship between monocotyledonous and dicotyledonous plants. In particular, ARR-B proteins from different species clustered closely within Group V, suggesting that some members of this subgroup may retain relatively conserved functions across plant species.

Candidate ZmARR-B proteins were initially identified based on sequence similarity and the presence of characteristic ARR-B domains. Multiple sequence alignment revealed that most ZmARR-B proteins possess a conserved N-terminal REC domain and a C-terminal MYB-like DNA-binding domain (Supplementary Figure S1). Notably, ZmARR-B08 was substantially shorter than the other family members and lacked a complete MYB-like DNA-binding domain as well as part of the REC domain, although several conserved residues within the REC domain were retained. These structural features suggest that ZmARR-B08 may represent an atypical ARR-B member with pronounced structural divergence, and its molecular function requires further experimental validation.

3.4. Gene Structure and Conserved Motif Analyses of the Maize ARR-B Family

Gene structure analysis revealed diversity in exon–intron organization among ZmARR-B family members, whereas genes within the same phylogenetic clade generally displayed similar structures (Figure 4). Most ZmARR-B genes contained both 5' and 3' untranslated regions (UTRs), and coding sequence length was generally positively associated with protein length. Structural variation among members was mainly attributable to differences in exon and intron number and UTR length. For example, *ZmARR-B08* encoded a short protein and exhibited a relatively simplified gene structure, whereas *ZmARR-B05* encoded a 788-aa protein and possessed a relatively complex multi-exon, multi-intron organization.

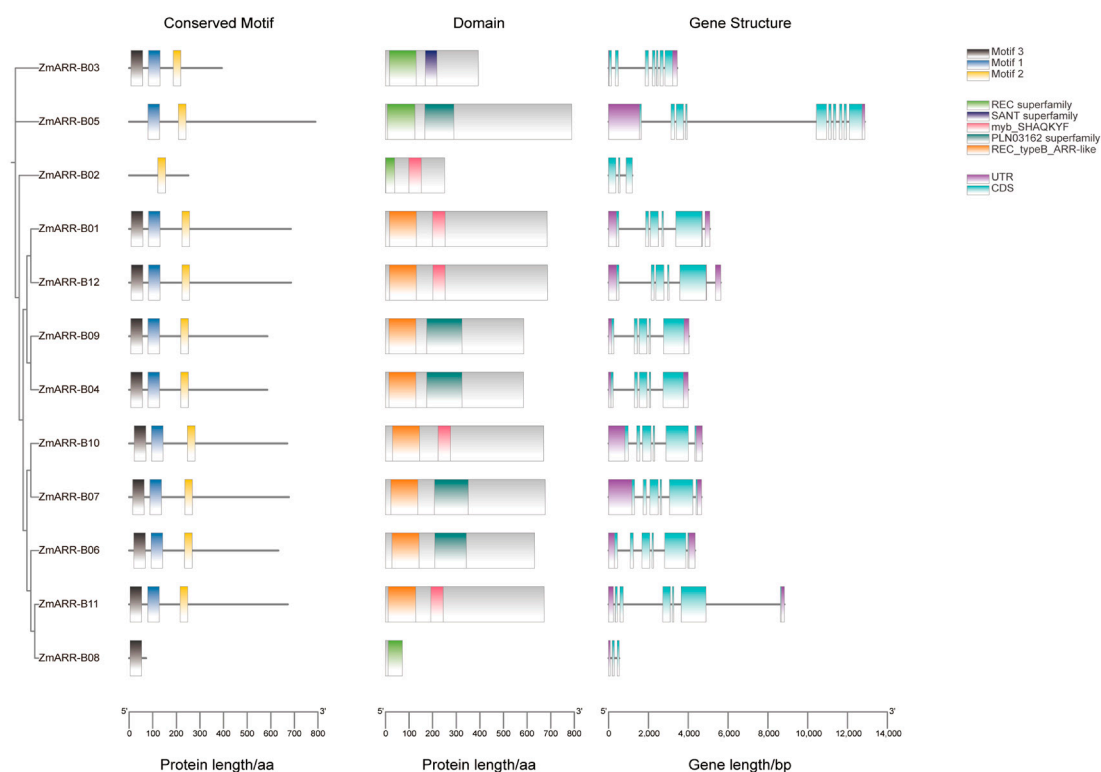


Figure 4. Gene structures, conserved domains, and motifs of the ZmARR-B family. The left, middle, and right panels show the conserved amino acid motifs, ARR-B domains, and gene structures, respectively. Gene structures include coding sequences (CDSs), introns, and untranslated regions (UTRs).

Conserved motif analysis further revealed characteristic domain compositions of ZmARR-B proteins. Motif 1 corresponded closely to the REC_typeB_ARR-like domain (PLN03162 superfamily) and was present in most ZmARR-B members, representing a major conserved motif of this family. This region corresponds to the N-terminal response regulator receiver domain of type-B ARR proteins and is involved in receiving phosphate groups transferred by upstream histidine phosphotransfer proteins. Motif 2 corresponded to the myb_SHAQKYF-type DNA-binding domain and was associated with GARP DNA-binding activity. With the exception of *ZmARR-B08*, Motif 2 was widely distributed among family members and constituted a key C-terminal DNA-binding module that may mediate recognition and binding of downstream target-gene promoters. Motif 3 partially corresponded to regions associated with the REC and SANT superfamilies and may contribute to structural or functional divergence among specific family members.

Notably, *ZmARR-B08* encoded only 72 aa and showed a markedly different motif composition from the other members, including the absence of a complete Motif 2 and MYB-like DNA-binding domain. Together with its predicted cytoplasmic localization, these features suggest that *ZmARR-B08* may not retain the full functions of a canonical type-B ARR transcription factor, or may represent a truncated, functionally divergent, or annotation-pending gene model.

3.5. Cis-Regulatory Element Analysis of Maize ARR-B Gene Promoters

To investigate potential transcriptional regulatory mechanisms of ZmARR-B genes, cis-regulatory elements within the 2000-bp upstream regions of their translation start sites were predicted (Figure 5). Light-responsive, phytohormone-responsive, abiotic stress-responsive, defense- and stress-responsive, and tissue-specific regulatory elements were widely detected in ZmARR-B promoters. However, the types, numbers, and combinations of these elements varied substantially among family members.

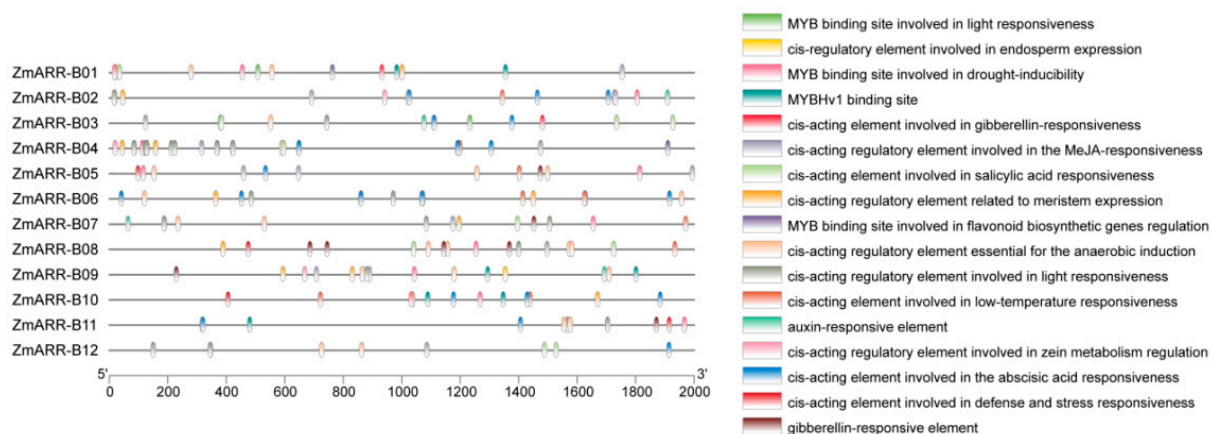


Figure 5. Cis-regulatory elements in the promoter regions of maize ARR-B genes. The left panel illustrates the positional distribution of these elements, whereas the right panel presents their corresponding types.

MYB-binding sites were broadly distributed in the promoters of most ZmARR-B genes, suggesting that their transcription may be influenced by MYB-related regulatory networks. *ZmARR-B01* contained relatively few predicted cis-elements, mainly light-responsive elements. In contrast, the promoters of *ZmARR-B05*, *ZmARR-B06*, and *ZmARR-B07* were enriched in phytohormone- and stress-responsive elements, including elements associated with gibberellin, jasmonic acid, and abscisic acid responses. These results suggest that these genes may participate in multiple hormone signaling and stress-response processes. Although *ZmARR-B08* displayed an unusual protein structure and predicted

subcellular localization, its promoter still contained several hormone- and stress-responsive elements, indicating that its transcription may be regulated by environmental cues.

Collectively, the diversity of promoter cis-regulatory elements provides clues to transcriptional regulatory divergence among ZmARR-B family members.

3.6. Synteny Analysis of the Maize ARR-B Family

To investigate the expansion pattern and evolutionary relationships of the ZmARR-B family, intraspecific and interspecific synteny analyses were performed. Three pairs of paralogous genes were identified within maize: *ZmARR-B09/ZmARR-B04*, *ZmARR-B08/ZmARR-B11*, and *ZmARR-B07/ZmARR-B10* (Figure 6). Each pair was located in a closely related phylogenetic clade and exhibited relatively similar gene structures, suggesting that they may have originated from genome duplication events. Their precise duplication types and functional relationships require further analysis in conjunction with duplication patterns and expression profiles.

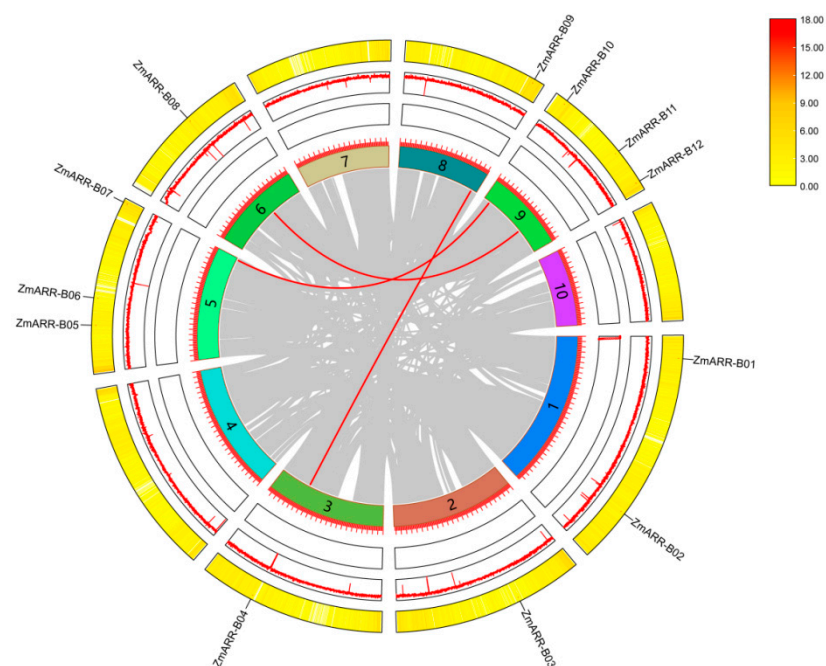


Figure 6. Synteny analysis of the maize ARR-B gene family. Black labels on the outer circle denote the corresponding ARR-B loci. Gray lines represent syntenic gene pairs, whereas bold red lines indicate duplicated pairs within the maize ARR-B family. Numbers in the inner circle identify the chromosomes.

Interspecific synteny analysis identified 3, 8, 10, and 7 homologous ARR-B gene pairs between maize and *A. thaliana*, rice, sorghum, and *P. trichocarpa*, respectively (Figure 7). More syntenic gene pairs were retained between maize and the monocots rice and sorghum than between maize and the dicots *A. thaliana* and *P. trichocarpa*. Maize and sorghum shared the largest number of homologous gene pairs. These findings indicate relatively high conservation of ARR-B loci in monocots and are consistent with the close evolutionary relationship between maize and sorghum.

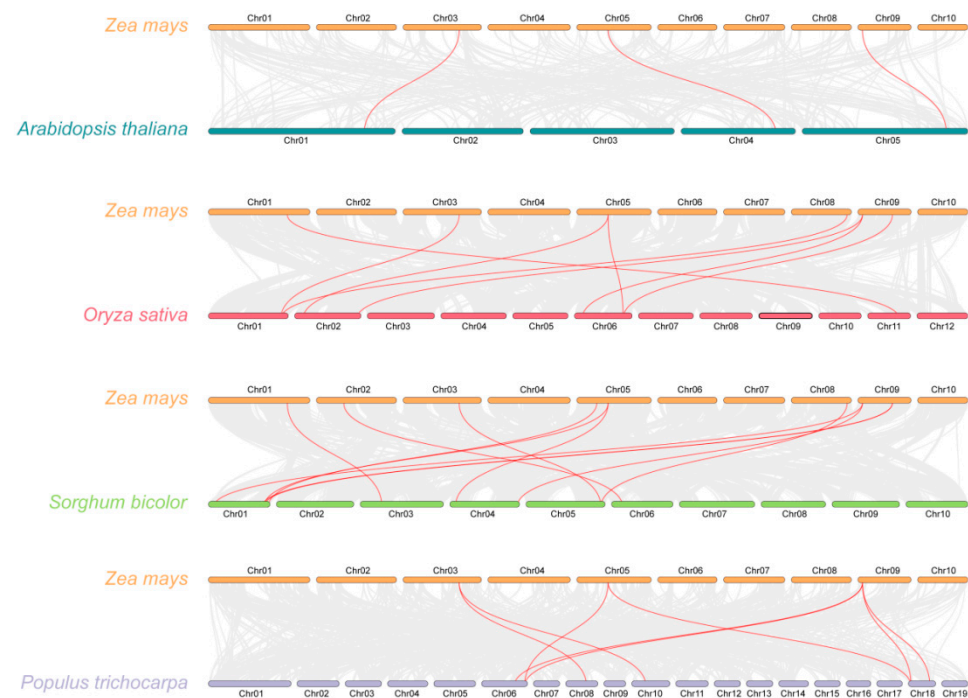


Figure 7. Synteny relationships between maize ARR-B genes and those of other plant species. Gray lines denote syntenic gene pairs between maize and *Arabidopsis thaliana*, *Oryza sativa*, *Sorghum bicolor*, or *Populus trichocarpa*. Bold red lines indicate co-syntenic ARR-B gene pairs shared between maize and the corresponding species.

3.7. Leaf Inorganic Phosphate Content, Classification, and Low-Phosphate Tolerance of Maize Inbred Lines from Southwestern China

To preliminarily screen maize inbred lines with contrasting inorganic phosphate (Pi) accumulation, 70 core inbred lines collected from southwestern China were grown, and leaf Pi concentrations were measured at the three-leaf stage (Figure 8). Based on the distribution of Pi concentrations, the lines were classified into high-Pi ($>0.015 \text{ mmol} \cdot \text{g}^{-1}$), intermediate-Pi ($0.012\text{--}0.015 \text{ mmol} \cdot \text{g}^{-1}$), and low-Pi ($<0.012 \text{ mmol} \cdot \text{g}^{-1}$) groups. The maize inbred lines 082, B73, and Ye107, which exhibit relatively high, intermediate, and low foliar Pi accumulation, respectively, were selected for subsequent analyses.

The low-P tolerance of these three lines was assessed under normal-phosphate (NP) and low-phosphate (LP) soil conditions. Plant growth status and associated growth and physiological traits were evaluated at 7, 14, and 21 d after seedling emergence (Figure 9). Under LP conditions, the growth of 082 was only mildly inhibited. In contrast, B73 and Ye107 displayed pronounced P-deficiency symptoms, including leaf chlorosis and anthocyanin-associated purple coloration, at 14 and 21 d, with Ye107 exhibiting the most severe phenotype.

Quantitative analysis further showed that LP treatment significantly reduced plant height and stem diameter in all three lines. The reduction was greatest in Ye107, intermediate in B73, and lowest in 082. Changes in leaf Pi content and acid phosphatase (ACP) activity were consistent with phenotypic and growth-related differences, indicating that 082 had stronger low-P adaptive capacity, whereas Ye107 was more sensitive to P deficiency.

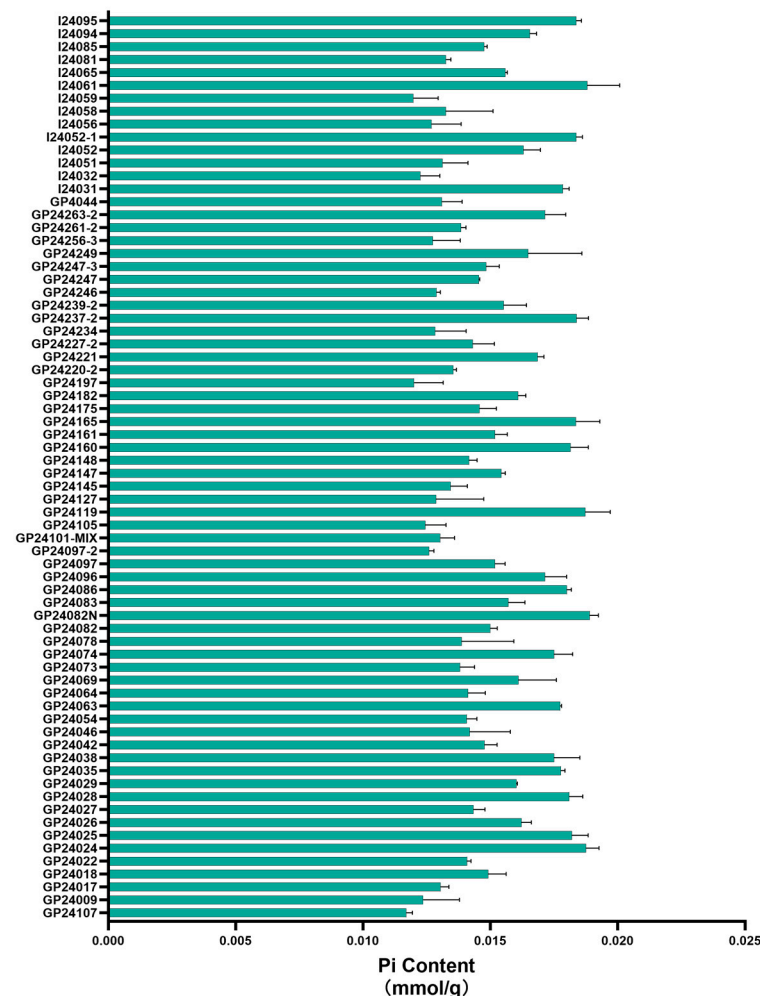


Figure 8. Determination of inorganic phosphorus content in the leaves of three-leaf-stage seedlings of 70 major maize inbred lines in the south-western China region.

3.8. Expression Analysis of Maize ARR-B Family Members in Response to Low-Phosphate Stress

To analyze the responses of the ZmARR-B family to LP stress, maize inbred lines 082, B73, and Ye107 were subjected to normal-phosphate (NP) and LP hydroponic treatments for 14 d. Expression profiles of ZmARR-B family members were then analyzed based on RNA-seq data (Figure 10). The results showed that different ZmARR-B genes exhibited distinct transcriptional responses to LP stress. The expression of ZmARR-B04 was upregulated under LP conditions, suggesting that it may participate in the regulation of low-P responses. In contrast, ZmARR-B03, ZmARR-B06, ZmARR-B09, and ZmARR-B12 were downregulated under LP treatment, indicating that their transcription may be repressed by P deficiency. Notably, transcriptional upregulation or downregulation alone does not directly establish a positive or negative regulatory role; the specific functions of these genes require further validation through genetic and molecular biological experiments.

ZmARR-B01 and ZmARR-B07 maintained relatively high transcript abundance across the different materials, although their expression patterns differed among inbred lines, suggesting that they may be regulated in a genetic background-dependent manner. ZmARR-B02, ZmARR-B05, ZmARR-B08, ZmARR-B10, and ZmARR-B11 exhibited low transcript abundance under both NP and LP conditions, indicating that these genes may be weakly expressed in the tested tissues and developmental stage or may be relatively insensitive to LP stress. Consistent with the phenotypic results, the line 082 exhibited the greatest number of LP-induced or LP-repressed ZmARR-B genes, followed by Ye107, whereas B73

showed the fewest responsive members. These results suggest that the transcriptional responses of the ZmARR-B family to P starvation are strongly genotype dependent and may be associated with differences in P-use efficiency among maize germplasms.

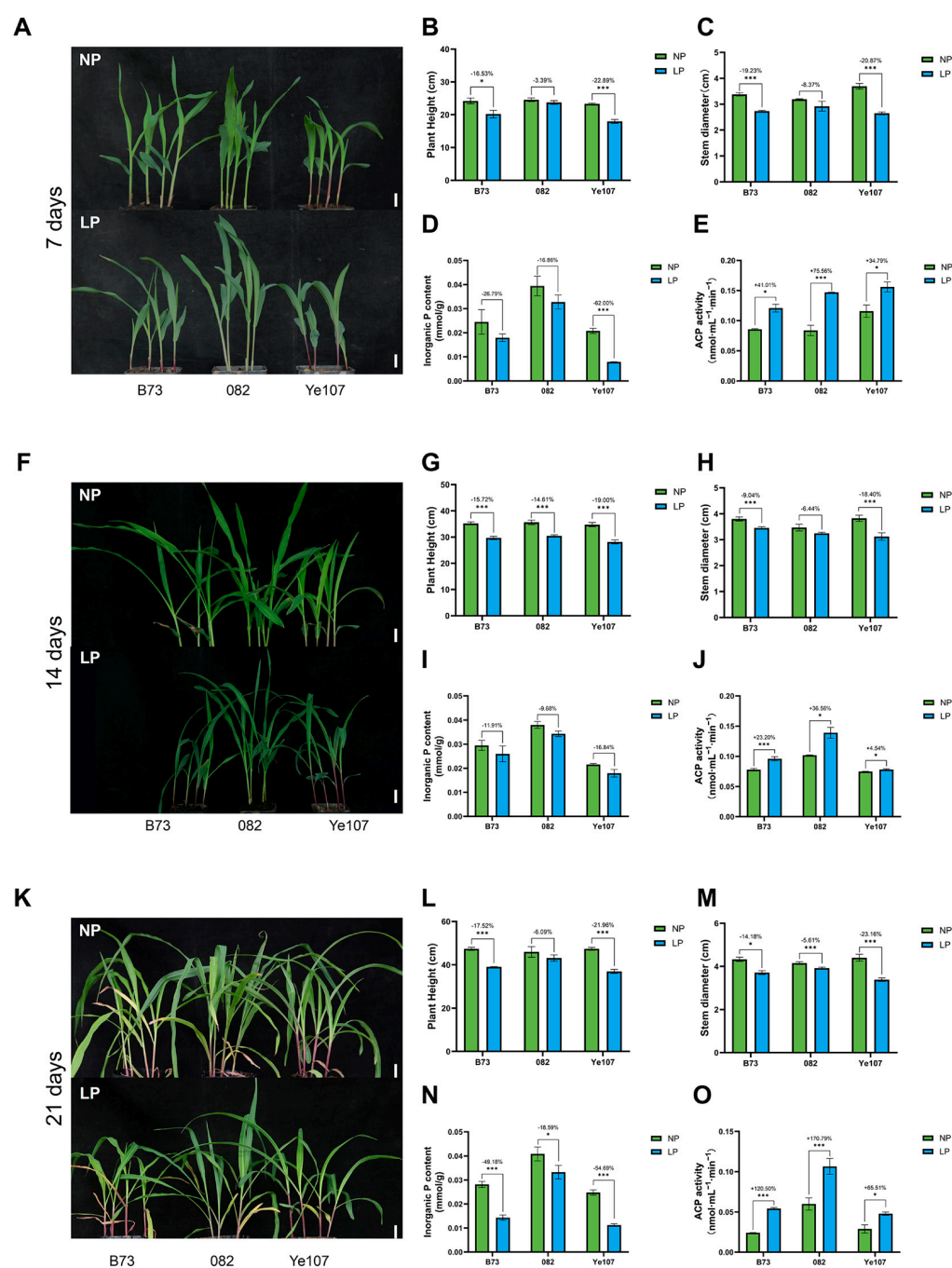


Figure 9. Analysis of low phosphorus tolerance in maize inbred lines 082, B73, and Ye107. Phenotypic observation of the above-ground parts of maize inbred lines after 7 (A), 14 (F), and 21 (K) days of normal- and low-phosphorus treatments. Plant height of maize inbred lines after 7 (B), 14 (G), and 21 (L) days of normal- and low-phosphorus treatments. Stem diameter of maize inbred lines after 7 (C), 14 (H), and 21 (M) days of normal- and low-phosphorus treatments. Inorganic P content of maize inbred lines after 7 (D), 14 (I), and 21 (N) days of normal- and low-phosphorus treatments. ACP activity of maize inbred lines after 7 (E), 14 (J), and 21 (O) days of normal- and low-phosphorus treatments. Asterisks above bars indicate a statistically significant difference between the control and treatment groups (Student's *t* test): * $p < 0.05$; *** $p < 0.001$.

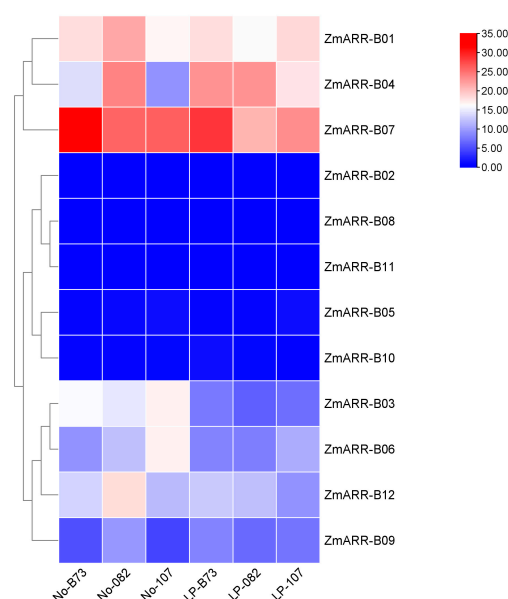


Figure 10. Expression profiles of the maize ARR-B gene family in three inbred lines under varying phosphorus treatments. Red and blue represent relatively high and low transcript levels, respectively. Each treatment included three biological replicates. NP denotes maize seedlings grown under normal-phosphorus conditions, whereas LP refers to seedlings cultivated under low-phosphorus treatment.

To validate the RNA-seq results, *ZmARR-B01*, *ZmARR-B04*, *ZmARR-B06*, and *ZmARR-B07* were selected for qRT-PCR analysis. The qRT-PCR results were generally consistent with the overall expression trends observed in the transcriptome data (Figure 11). Under LP conditions, *ZmARR-B01*, *ZmARR-B06*, and *ZmARR-B07* showed an overall downregulation trend, whereas *ZmARR-B04* was generally upregulated. The induction of *ZmARR-B04* was more pronounced in Ye107 but was reduced in 082. These results further indicate that ZmARR-B family members display differentiated low-P-responsive expression patterns across distinct genetic backgrounds.

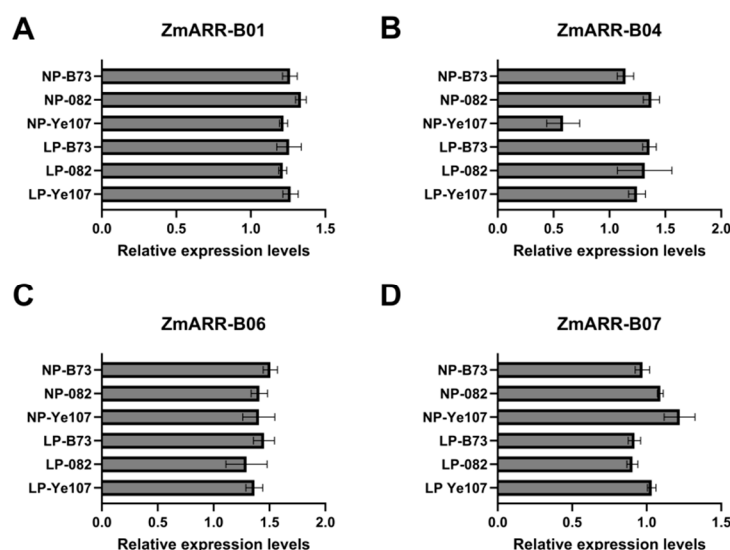


Figure 11. Relative expression of maize ARR-B genes determined by qRT-PCR. Relative expression levels of ZmARR-B01 (A), ZmARR-B04 (B), ZmARR-B06 (C), and ZmARR-B07 (D) under NP and LP conditions. NP denotes maize seedlings grown under normal-phosphorus conditions, whereas LP refers to seedlings cultivated under low-phosphorus treatment.

Our preliminary observations indicated that M73 exhibits a more pronounced transcriptional response to short-term phosphorus deficiency than B73, suggesting that this inbred line may provide a sensitive genetic background for investigating early transcriptional responses to P limitation. Meanwhile, the M73 genome is highly similar to the B73 reference genome, and the B73 genome annotation was considered suitable for transcriptome profiling and functional annotation of M73. Therefore, to further identify *ZmARR-B* genes potentially involved in the early response to P deficiency and to characterize their dynamic transcriptional responses under P-limited conditions, an additional short-term low-phosphorus (LP) treatment was performed using the maize inbred line M73. Whole seedlings were collected at 0, 0.5, 1, 3, 6, and 12 h after treatment for RNA-seq analysis (Figure 12). The results revealed distinct temporal expression patterns among *ZmARR-B* family members under short-term LP stress. *ZmARR-B01*, *ZmARR-B03*, and *ZmARR-B12* exhibited transient induction followed by a decline in expression, whereas *ZmARR-B06* and *ZmARR-B07* displayed sustained upregulation. The remaining members showed low expression levels at the examined time points.

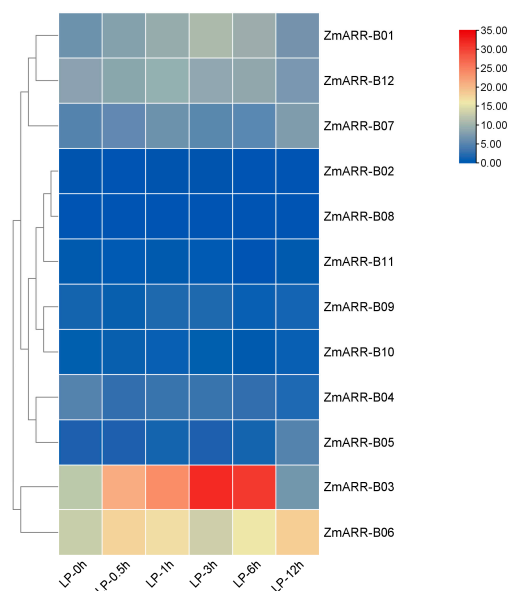


Figure 12. Expression heatmap of the *ZmARR-B* gene family following short-term low-phosphorus treatment (0, 0.5, 1, 3, 6, and 12 h). Expression values were calculated as $\log_2(\text{FPKM} + 1)$. Red and blue indicate relatively high and low expression levels, respectively. Each time point included three biological replicates.

Among these genes, *ZmARR-B03* exhibited the most pronounced early response to LP stress. Its transcript abundance increased significantly during the 0–6 h treatment period but declined rapidly at 12 h, showing a characteristic pulse-like expression pattern. This result suggests that *ZmARR-B03* may participate in early P-starvation signaling responses. In contrast, *ZmARR-B06* maintained relatively high expression throughout the treatment period, indicating that it may contribute to sustained adaptive regulation under LP stress. The precise biological functions of these candidate genes remain to be elucidated through further studies, including genetic transformation, mutant analysis, and target-gene identification.

4. Discussion

Type-B response regulators (ARR-Bs) belong to the plant-specific GARP transcription factor superfamily and constitute key transcriptional output components of the cytokinin signaling pathway. Through their N-terminal receiver (REC) domains, ARR-B

proteins receive phosphorelay signals from upstream histidine phosphotransfer proteins, whereas their C-terminal GARP DNA-binding domains regulate the expression of primary cytokinin-responsive genes and other downstream targets. Consequently, ARR-Bs play important roles in nutrient sensing, root and shoot development, floral transition, chloroplast development, and circadian-clock regulation [34–36]. In *A. thaliana*, *ARR1*, *ARR10*, and *ARR12* regulate root meristem activity and vascular differentiation [8], whereas rice ARR-B members have been implicated in cytokinin responses and abiotic stress adaptation [37]. However, the systematic identification of the maize ARR-B family and its potential roles in phosphate-starvation responses have remained largely unexplored. Here, 12 candidate maize ARR-B (ZmARR-B) members were identified at the genome-wide level. Their phylogenetic relationships, collinearity, protein features, promoter cis-regulatory elements, and expression profiles under P deficiency were comprehensively analyzed, providing initial insights into their evolution and potential roles in maize P nutritional adaptation.

Phylogenetic analysis classified 81 ARR-B homologous proteins from maize, *A. thaliana*, rice, sorghum, and *P. trichocarpa* into five groups (Groups I–V). ARR-B members from monocotyledonous and dicotyledonous species generally formed relatively distinct clusters, reflecting sequence divergence during species evolution. Interspecies synteny analysis further showed that maize retained the largest number of collinear ARR-B gene pairs with sorghum, followed by rice, whereas fewer collinear pairs were identified between maize and the dicot species *A. thaliana* and *P. trichocarpa*. This pattern is consistent with known phylogenetic relationships and suggests that some ARR-B loci have been relatively well conserved among monocots. Nevertheless, syntenic relationships and differences in gene number alone cannot establish lineage-specific functional expansion of the maize ARR-B family. Further analyses incorporating duplication mode, duplication age, selective pressure, and cross-species functional comparisons will be required to clarify the evolutionary history of this family.

Physicochemical and structural analyses revealed pronounced variation among ZmARR-B members in amino acid length, theoretical isoelectric point, instability index, and aliphatic index, suggesting potential divergence in protein stability, conformation, and regulatory properties. Most members were predicted to be acidic proteins with instability indices above 40. Although these predictions imply that some proteins may have relatively high turnover potential, sequence-based instability indices do not directly reflect in vivo protein half-life or degradation rates. Experimental validation, including protein stability and ubiquitin-mediated degradation assays, is therefore needed. Similar to observations in the tomato ARR-B family [38], the substantial structural heterogeneity of maize ARR-B proteins may support differentiated regulatory functions across developmental stages, tissues, and environmental conditions.

Secondary-structure prediction indicated that ZmARR-B proteins are mainly composed of α -helices, extended strands, and random coils, with random-coil regions accounting for the largest proportion. Intrinsically flexible or disordered regions often facilitate conformational plasticity, transcriptional activation, and protein–protein interactions. This feature may therefore be compatible with the role of ARR-B proteins as transcriptional regulators integrating multiple signaling inputs. Homology-based three-dimensional modeling indicated that most ZmARR-B proteins retained a typical REC domain, and members within the same phylogenetic subgroup displayed similar overall conformations, suggesting partial conservation of molecular function. Conserved-motif analysis further showed that Motif 1 corresponded to the REC_typeB_ARR-like domain, whereas Motif 2 was associated with the myb_SHAQKYF/GARP DNA-binding domain. Together, the REC and GARP domains provide the structural basis for ARR-B proteins to receive phosphorelay signals and regulate target-gene transcription.

Notably, ZmARR-B08 encodes a short protein lacking a complete myb_SHAQKYF domain and is predicted to localize predominantly to the cytoplasm. This member may represent a structurally simplified, non-canonical ARR-B protein; alternatively, it may reflect a truncated transcript, an inaccurate gene model, or a functionally degenerated paralog. Because it lacks the complete DNA-binding module characteristic of canonical ARR-B transcription factors, its classification as a conventionally active ARR-B transcription factor cannot be inferred from sequence prediction alone. Its true structure and function should be further examined through full-length cDNA cloning, domain validation, experimental subcellular localization, and protein-interaction assays. Domain loss or rearrangement can promote functional divergence in plant protein families, but whether ZmARR-B08 has acquired a non-canonical signaling function requires direct experimental evidence.

Promoter cis-regulatory element analysis revealed that the upstream regions of ZmARR-B genes contain diverse light-responsive elements, MYB-binding sites, abscisic acid (ABA)-, jasmonic acid (JA)-, and gibberellin (GA)-responsive elements, as well as elements associated with stress and defense responses. These results suggest that ZmARR-B transcription may be jointly influenced by endogenous hormonal and environmental cues. In particular, the promoters of *ZmARR-B05*, *ZmARR-B06*, and *ZmARR-B07* were enriched in stress- and hormone-responsive elements, suggesting that these genes may function at regulatory nodes integrating multiple signals. By contrast, *ZmARR-B01* contained fewer predicted element types, potentially indicating a relatively simplified or condition-specific transcriptional regulatory pattern. Nevertheless, cis-element prediction only indicates potential regulatory capacity and does not demonstrate transcription factor–promoter binding. Yeast one-hybrid assays, dual-luciferase reporter assays, electrophoretic mobility shift assays, and ChIP-qPCR should be used to validate the functions of key cis-elements.

Rather than duplicating the comparative genomic scope of Chen et al. (2025) [15], our phylogenetic analysis extends the taxonomic framework beyond the Poaceae by incorporating representative monocot, dicot, and woody plant species, including *Zea mays*, *O. sativa*, *S. bicolor*, *A. thaliana*, and *P. trichocarpa*. This broader phylogenetic framework provides an opportunity to evaluate the evolutionary conservation and lineage-specific diversification of ARR-B proteins across more distantly related plant lineages and to distinguish conserved features from lineage-specific characteristics.

Phosphorus deficiency is a major nutrient stress limiting maize growth and yield formation [39]. Plants commonly adapt to P deficiency through root system architectural remodeling, enhanced Pi uptake and transport, increased acid phosphatase activity, and improved intracellular P remobilization [40–42]. Cytokinin is an important hormone coordinating growth with nutrient status and may participate in root developmental regulation and P homeostasis under P-deficient conditions [17–19]. In the present study, the line 082 (characterized by relatively high foliar Pi accumulation) exhibited less severe growth inhibition under low-P soil conditions, whereas B73 and Ye107 developed more evident P-deficiency symptoms, including leaf chlorosis and anthocyanin-associated purple pigmentation. Ye107 was the most severely affected genotype. Changes in plant height, stem diameter, shoot fresh weight, Pi content, and acid phosphatase activity further supported substantial differences in low-P adaptation among the three inbred lines. These results suggest that 082 may possess stronger P acquisition, transport, reutilization, or growth-maintenance capacity. However, its physiological basis requires further investigation through analyses of root architecture, rhizosphere P mobilization, expression of Pi transport-related genes, and P flux.

RNA-seq analysis under long-term LP treatment revealed differentiated expression profiles of ZmARR-B genes among the maize inbred lines. *ZmARR-B04* was transcriptionally induced under LP conditions, whereas *ZmARR-B03*, *ZmARR-B06*, *ZmARR-B09*,

and *ZmARR-B12* were downregulated in some genotypes. Importantly, LP-induced or LP-repressed expression alone cannot establish whether a gene acts as a positive or negative regulator of P-deficiency responses. Such changes may reflect participation in stress perception, signal transduction, feedback regulation, or growth–defense trade-offs. The overall consistency between qRT-PCR and RNA-seq results supports the reliability of the obtained expression profiles. In particular, *ZmARR-B04* was more strongly induced in Ye107 but downregulated in 082, indicating that the same gene can be subjected to distinct transcriptional regulation in different genetic backgrounds. This genotype-dependent response may arise from promoter sequence variation, differences in upstream regulators, altered hormonal homeostasis, or genetic-background effects on P-signaling pathways.

Notably, the line 082 contained the largest number of LP-responsive *ZmARR-B* members, followed by the line Ye107, whereas B73 had the fewest responsive members. This result suggests that the P-efficient genotype may possess more active or finely tuned cytokinin-associated transcriptional reprogramming. Together with its stronger low-P tolerance phenotype, the data imply that *ZmARR-B*-mediated signaling may be associated with P-use efficiency in maize. However, a larger number of responsive genes does not necessarily confer superior adaptation. The causal relationship between *ZmARR-B* responsiveness and P efficiency should be assessed using expanded germplasm panels, association analysis, superior allelic variation mining, and functional validation.

Short-term LP treatment further revealed temporal expression divergence within the *ZmARR-B* family. *ZmARR-B01*, *ZmARR-B03*, and *ZmARR-B12* were transiently induced and subsequently downregulated. Among them, *ZmARR-B03* increased rapidly during the 0–6 h treatment period and declined markedly at 12 h, displaying a pulse-like expression pattern. Such transient transcriptional activation may enable plants to rapidly initiate early P-starvation signaling while avoiding the metabolic costs of sustained high expression. Therefore, *ZmARR-B03* represents a promising candidate linking early P-starvation perception with cytokinin signaling. In contrast, *ZmARR-B06* was continuously induced during short-term LP treatment but appeared to be downregulated under long-term LP conditions, suggesting time-dependent regulation. It may participate in early adaptive responses and subsequently be attenuated by feedback regulation or transition into a different regulatory state during prolonged stress. This finding highlights the importance of dynamic expression analysis across distinct durations of nutrient stress.

In summary, this study systematically identified and preliminarily characterized the structure, evolution, and low-P-responsive expression patterns of the maize *ZmARR-B* family. It identifies *ZmARR-B03* and *ZmARR-B06* as candidate genes potentially involved in distinct phases of low-P adaptation. Future research should prioritize the generation of overexpression, knockout, or genome-edited lines for *ZmARR-B03*, *ZmARR-B04*, and *ZmARR-B06*; evaluate root architecture, P uptake and transport efficiency, P reutilization efficiency, and yield-related traits under P deficiency; identify direct target genes using ChIP-seq, DAP-seq, yeast one-hybrid, and dual-luciferase assays; and quantify cytokinin levels together with changes in related signaling components. These efforts will help elucidate the molecular mechanism by which the cytokinin–ARR-B module contributes to P nutritional adaptation in maize and may provide valuable candidate genes for molecular breeding of P-efficient maize.

5. Conclusions

This study identified 12 candidate *ZmARR-B* genes at the genome-wide level and characterized their evolutionary relationships, structural features, promoter cis-regulatory elements, and expression patterns under phosphate (P) deficiency. Most members displayed the conserved REC and Myb-type/GARP DNA-binding domains characteristic

of type-B response regulators, whereas ZmARR-B08 showed an atypical truncated structure requiring further validation. Phylogenetic, synteny, and promoter analyses indicated evolutionary and regulatory diversification within the ZmARR-B family. Expression profiling under low-phosphate conditions revealed strong genotype- and time-dependent responses. *ZmARR-B03* exhibited transient early induction, suggesting a potential role in initial P-starvation signaling, whereas *ZmARR-B06* showed sustained upregulation during short-term P deficiency, indicating possible involvement in adaptive regulation. The maize inbred line 082, which exhibits relatively high foliar Pi accumulation, contained more low-P-responsive ZmARR-B members than Ye107, which exhibits relatively low foliar Pi accumulation. These findings provide candidate genes, particularly *ZmARR-B03* and *ZmARR-B06*, for functional validation and future breeding of P-efficient maize.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16181756/s1>, Figure S1: Multiple sequence alignment of maize ARR-B proteins; Table S1: Primers sequences used for RT-qPCR analysis; Table S2: Prediction of the secondary structure of ZmARR-B proteins.

Author Contributions: Conceptualization, S.L. (Shuang Li) and C.L.; methodology, S.L. (Shuang Li), L.Y. and C.L.; software, S.L. (Shaoxian Liu), S.L. (Shuang Li) and H.L.; validation, S.L. (Shuang Li), A.Y. and Q.Z.; formal analysis, L.Y., S.L. (Shaoxian Liu) and H.L.; resources, X.J., N.M. and C.L.; data curation, S.L. (Shuang Li), S.L. (Shaoxian Liu) and C.L.; writing—original draft preparation, S.L. (Shuang Li), S.L. (Shaoxian Liu) and C.L.; writing—review and editing, L.Y. and C.L.; supervision, H.L., A.Y., Q.Z. and C.L. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Transcriptomic data from the low-phosphorus treatment have been deposited in the Genome Sequence Archive of the BIG Submission Portal at the National Genomics Data Center (NGDC; <https://ngdc.cnpc.ac.cn/>) under BioProject accession PRJCA044786.

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

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