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Genome-Wide Identification, Characterization and Expression Profile of F-Box Protein Family Genes Shed Light on Lateral Branch Development in Cultivated Peanut (*Arachis hypogaea* L.)

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Abstract: F-box proteins are a large gene family in plants, and play crucial roles in plant growth, development, and stress response. To date, a comprehensive investigation of F-box family genes in peanuts, and their expression pattern in lateral branch development has not been performed. In this study, a total of 95 F-box protein family members on 18 chromosomes, named *AhFBX1-AhFBX95*, were identified in cultivated peanut (*Arachis hypogaea* L.), which were classified into four groups (Group I–IV). The gene structures and protein motifs of these peanut FBX genes were highly conserved among most FBXs. We found that significant segmental duplication events occurred between wild diploid species and the allotetraploid of peanut FBXs, and observed that AhFBXs underwent strong purifying selection throughout evolution. *Cis*-acting elements related to development, hormones, and stresses were identified in the promoters of *AhFBX* genes. In silico analysis of *AhFBX* genes revealed expression patterns across 22 different tissues. A total of 32 genes were predominantly expressed in leaves, pistils, and the aerial gynophore tip. Additionally, 37 genes displayed tissue-specific expression specifically at the apex of both vegetative and reproductive shoots. During our analysis of transcriptome data for lateral branch development in spreading and erect varieties, namely M130 and JH5, we identified nine deferentially expressed genes (DEGs). Quantitative real-time PCR (qRT-PCR) results further confirmed the expression patterns of these DEGs. These DEGs exhibited significant differences in their expression levels at different stages between M130 and JH5, suggesting their potential involvement in the regulation of lateral branch development. This systematic research offers valuable insights into the functional dissection of AhFBX genes in regulating plant growth habit in peanut.

Keywords: cultivated peanut; F–box protein; bioinformatics; expression analysis

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1. Introduction

The ubiquitin-protease system plays a crucial role in protein degradation in eukaryotes. Within the cell, ubiquitin E3 ligase participates in various physiological processes by regulating the ubiquitination of regulatory proteins [1,2]. The Skp-Cullin-F box (SCF) complex, a subset of ligase E3, is characterized by the involvement of F-box in substrate recognition. SKP1 further specifically binds with F-box motif of N-terminal, ultimately assisting in protein degradation [3,4]. The F-box protein family is one of the largest protein families in plants and its encoded genes play critical roles in plant growth and development.

They are also involved in the transduction of plant hormone and light signals [5]. F-box proteins usually contain only one F-box domain (ILSRLPTKHLARTSCVSKRWR) and are generally consisted of 40–50 amino acids. Notably, this family is distinguished by the presence of at least one F-box structural domain at the N-terminal end and an interacting secondary structure at the C-terminal end of the protein [6]. These features allow for the categorization of the protein into different subfamilies, each with divergent functions. To date, a large number of F-box protein family members have been identified in various plant species, such as *Arabidopsis thaliana* (694), rice (687), maize (359), alfalfa (972), soybean (725), tomato (139), eggplant (389), and poplar (337), among others [7–14].

The expression pattern and gene function of some F–box protein genes in plant growth and development have been clarified. For instance, in *Arabidopsis thaliana*, *MAX2* has been identified as a positive regulator of photomorphogenesis. By constructing a *pleiotropic photosignaling* (*pps*) mutant, the expression of *MAX2* was reduced, leading to repression of shoot lateral branching [15]. Song et al. demonstrated that the development of plant leaf morphology is influenced by the expression of *At1g27340* (*LCR*), using a constructed *Arabidopsis* miR394 mutant [16]. Interestingly, abnormal leaf development was observed when this gene was expressed either excessively low or high. Similarly, Marrocco et al. confirmed the crucial role of *EID1* in shaping plant photomorphogenesis by manipulating photopigments in *Arabidopsis thaliana* [17]. Additionally, Qin et al. substantiated that the F-box protein family gene *QDtbn1* acts as a negative regulatory factor, determining the number of tassel branches in maize [18]. Currently, the availability of the peanut genomes allows for the exploration of various gene families in the entire genome [19–22], including the SNARE [23], SAUR [24], PIF [25], and aquaporin [26] families. However, a comprehensive study on the of F-box protein family in peanuts has yet to be conducted.

The peanut is a vital oilseed crop that provides high-quality vegetable oil and proteins, making a significant contribution to economic value. The variations in plant varieties depend on the nutritional and reproductive growth patterns, as well as the differences in the angle of lateral branches and main stem [27]. Therefore, peanut plants can be classified into four branching habits: erect, bunch, spreading and prostrate [28,29]. The plant ideotype plays a crucial role in optimizing the utilization of light energy, which greatly affects yield, cultivation methods, and suitability for machine harvesting [30]. Therefore, studying plant branching is essential for cultivating optimal crop varieties. Previous research on staple cereal crops had successfully developed high-yield versions by studying genes related to branch development [31,32]. Similarly, the growth pattern of lateral branches, particularly the first lateral branch, is an important agronomic trait that determines the plant architecture and production in peanuts. However, the study of branch habit genes in peanuts is still limited. Fortunately, our previous research has identified three candidate genes of the F-box family associated with lateral branch angle (LAB) using genome-wide association study (GWAS) and bulk segregant analysis (BSA). Of these genes, *Araip.E64SW* could be involved in lateral branch development, potentially leading to a spreading or prostrate appearance [33]. Therefore, the aim of this study is to investigate the expression patterns of F-box protein genes in various tissues of cultivated peanuts, with a focus on their patterns during lateral branch development.

To comprehensively study F-box protein genes and their potential involvement in lateral branch development, we have identified F-box protein genes using bioinformatics approaches, and integrated the expression data from various tissues of "Tifrunner" cultivar and previous transcriptomic data from different stages of branching development. This process highlights the potential *AhFBX* genes related to lateral branch development in peanut. The main objectives of this research are: (1) assessing the quantity, physicochemical properties, conserved domain features, gene structure, and evolutionary origins of AhFBXs; (2) explicating the computationally predicted expression patterns of *AhFBXs* across different peanut tissues; (3) pinpointing the potential *AhFBX* candidates related to lateral branch development; and (4) elucidating the expression patterns of these candidate genes in branching development across diverse plant types. With this investigation,

our goal is to provide gene resources that enhance our understanding of the molecular mechanisms in which F-box associated genes contribute to peanut branching development and impact on plant architecture. This study holds significant value in the formulation of plant architecture.

2. Materials and Methods

2.1. Genome–Wide Identification and Chromosome Localization

The complete genomic data for cv. Tifrunner was procured from Peanutbase (http://peanutbase.org/, accessed on 12 April 2023). Using procedure outlined by Zhao et al. [34], we used the conserved domain (accession: PF00646) of the F-box protein family to execute F-box protein sequence detection through HMMER v3.3.2 (http://hmmer.org/, accessed on 12 April 2023) with a query threshold of E-value $\leq 1 \times 10^{-10}$. Once redundant sequences were excluded, potential sequences were submitted to the SMART (http://smart.embl-heidelberg.de/, accessed on 12 April 2023), Pfam database (http://pfam-legacy.xfam.org/, accessed on 12 April 2023), and CDD (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi, accessed on 12 April 2023) databases, in order to validate whether the retrieved protein sequences incorporated the appropriate conserved domains. Family members were labeled according to their sequential appearance on the respective genomic chromosomes, ranging from AhFBX1 to AhFBXn. The chromosomal location diagram was constructed using MapChart 2.32 [35], based on the physical positioning of *AhFBX* genes in the reference genome.

2.2. Physicochemical Properties, Phylogenetic Tree and Gene Structure Analysis

The physicochemical attributes of the entire gene family were compued using Expasy (http://www.expasy.org/tools/protparam, accessed on 13 April 2023) [36], predicting such properties as the amino acid count, molecular weight, isoelectric point (pI), instability index, aliphatic index, lipophilicity and hydrophilicity. The subcellular localization was predicted using Cello (http://cello.life.nctu.edu.tw/, accessed on 13 April 2023). From the *Arabidopsis* Information Resource website (TAIR, http://www.arabidopsis.org/index.jsp, accessed on 26 July 2022), a total of 90 AtFBXs exhibiting complete conserved domains (with E-value $< 1 \times 10^{-7}$) were retrieved. Subsequently, a phylogenetic analysis of AhFBXs and AtFBXs was performed using MEGA X (Bootstrap = 1000 replicates) [37]. The conserved motifs, along with gene structure, were predicted using MEME (http://meme-suite.org/tools/meme, accessed on 13 April 2023) [38] and the online platform Gene Structure Display Server (GSDS) 2.0 (http://gsds.gao-lab.org/, accessed on 13 April 2023) [39].

2.3. Prediction of Cis–Acting Elements in the Promoter Region

An analysis was conducted on the upstream promoter region of AhFBXs, which spans a 2000 bp sequence, to predict its *cis*-acting elements within the promoter region. This was accomplished using the PlantCARE resource (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 14 April 2023). Subsequently, a comprehensive map of the *cis*-acting elements was constructed with the aid of the GSDS 2.0 [39].

2.4. Collinearity and Estimation of Ka/Ks Ratios Analysis

Gene collinearity and Ka/Ks values were examined using the one-step MCScanX module (E-value $< 1 \times 10^{-5}$) from the TBtools software (version 1.132). The basic non-synonymous/synonymous mutation ratio (Ka/Ks) calculator were employed to analyze gene collinearity and Ka/Ks values, respectively [40–42]. These tools provided enhanced clarity in interpreting evolutionary patterns. The Ks value can be utilized to estimate the divergence time of duplication events, where the divergence time (T) equals Ks divided by twice the neutral substitution rate (λ). The λ is estimated at 8.12×10^{-9} for peanut [19].

2.5. In Silico Expression Analysis of AhFBX Genes in Different Tissues

Tissue-specific expression patterns of *AhFBXs* were examined using data expressed as fragments per kilobase of exon per million aligned fragments (FPKM) data. This data acquired from https://dev.peanutbase.org/expression/expr_tissue_Hyp.html, accessed on 17 May 2023 [43], was transformed via the application of $\log_2$(FPKM+1). Subsequent normalization was performed using the z-score method, denoted by the equation $(\sum(x^2) - (\sum x)^2/n)/n$. Within this equation, 'x' represents the raw value, while 'n' denotes the number of data points. To visualize gene expression, a heatmap was generated using R software (version 4.1.0) (https://www.r-project.org/, accessed on 17 May 2023).

2.6. Plant Materials

The erect variety, Jihua 5 (JH5), and the prostrate germplasm, M130, were selected as materials described previously [33]. They were grown under rigorously controlled environmental conditions within a climatically-controlled chamber, maintained at a stable temperature of 25 °C. Furthermore, they were subjected to a circadian rhythm consisting of 16 h of light succeeded by 8 h of darkness. Samples were systematically collected at five-day intervals up to 30 days after planting (DAP), specifically at 5, 10, 15, 20, 25 and 30 DAP. During these stages, the lateral branch development phase for 'JH5' was denoted as J05, J10, J15, J20, J25 and J30, respectively. Similarly, timescales for the M130 variety were named as M05, M10, M15, M20, M25 and M30. The collected materials were quickly frozen in liquid nitrogen at −80 °C until RNA extraction.

2.7. Transcription Expression Analysis in Lateral Branch Development

Transcriptomic profiles of the lateral branch development for two distinct varieties (JH5 and M130) were analyzed at various stages (J05–J30 and M05–M30) in the growth of the first lateral branches (BioProject: PRJNA675413). The FPKM values were utilized to analyze the expression patterns of *AhFBXs*. After logarithm of two was taken, TBtools software was used to draw the heatmap. Differential expression genes (DEGs) were analyzed using the criteria $|\log2FC| \geq 1$ and $p < 0.05$.

2.8. RNA Extracted and qRT–PCR Analysis

Total RNA was extracted and purified using FastPure Universal Plant Total RNA Isolation Kit (Vazyme, Nanjing, China). RNA purity and quantity were determined using Gel electrophoresis and NanoDrop™ One (ThermoFisher, Waltham, MA, USA). First-strand cDNA synthetization was performed using the Hiscript II QRT SuperMix for qPCR (Vazyme, Nanjing, China). According to the gene CDS sequence, the primers were designed after comparing the specificity and uniqueness in NCBI, qRT-PCR primers were designed using Primer Premier 5 software (Table S1). PCR amplification system and procedure were conducted following the protocol described previously [44]. Three biological replicates were set up, and three first lateral branches were sampled in each stage of the replicates. The relative expression of candidate genes was calculated utilizing $2^{-\Delta\Delta Ct}$ method [45]. GraphPad Prism 8.0 software was used for Student's *t*-test ($p < 0.01$) and correlation analysis ($p < 0.01$).

3. Results

3.1. Identification and Chromosome Location of AhFBXs

To identify *FBX* genes in cultivated peanuts, the HMM profile of FBX (accession: PF00646) was used to search the peanut local protein database. A total of 95 *AhFBX* genes, named *AhFBX1-AhFBX95*, were identified in cultivated peanut (Table S2). All member contained a typical F-box domain (ILSRLPTKHLARTSCVSKRWR) and were used for further analysis. Of these, 94 *AhFBXs* were assigned to 18 chromosomes, excluding Arahy.07 and Arahy.18 (Table S2 and Figure 1). Of these, Arahy.04 and Arahy.14 had 18 and 17 members, respectively. Both Arahy.09 and Arahy.19 displayed 16 members each. In contrast, Arahy.06 only had four members, while Arahy.03, Arahy.08, and Arahy.13 each contained three

members. Arahy.10 had two members. It is worth noting that *AhFBX95* was not found on the chromosome, but on the scaffold_50:16019-23949. As for chromosome distribution, there seemed to be a trend with most *AhFBX* genes being found at either terminal of each chromosome, and a fewer number of genes located centrally or near the middle of the chromosome.

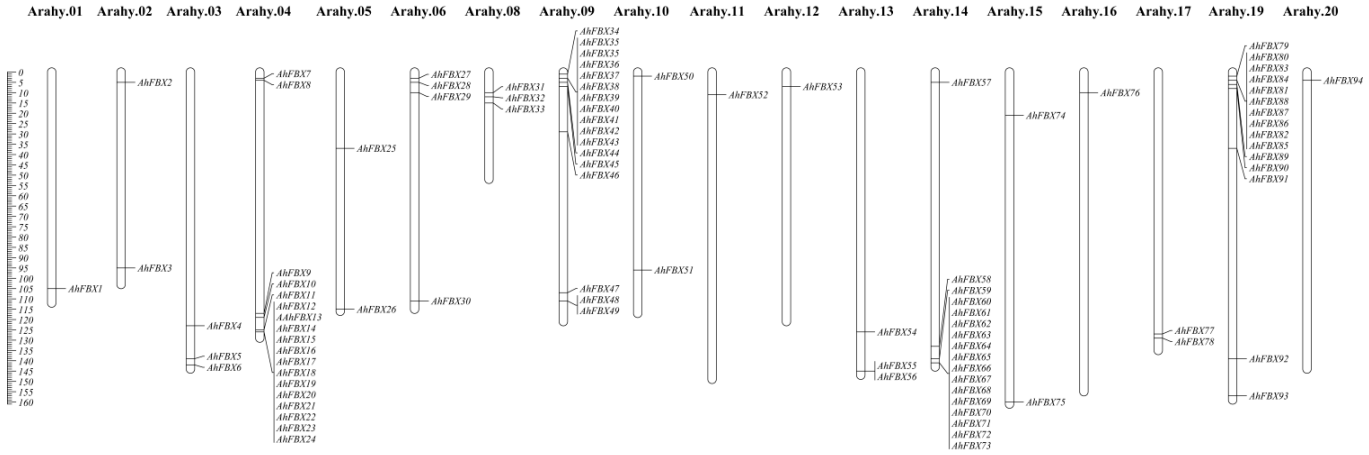

Figure 1. Chromosomes locations of *AhFBX* genes in cultivated peanut. Left ruler indicated chromosome length with Mb unit.

3.2. Physicochemical Properties and Subcellular Localization Prediction of AhFBXs

All of the AhFBX proteins consisted of 212–1372 amino acids (aa), yielding an average of approximately 449 aa per protein. The relative molecular weights, isoelectric point (pI), instability index and aliphatic index ranged from 24.05–157.44 kDa, 4.88–9.78, 7.41–66.74 and 75.23–115.82, with all of these proteins being hydrophilic (Table S2). Subcellular localization prediction (Table S2) indicated that AhFBX proteins were located in various cellular structures (Table S2). Specifically, 51 members (or 53.7%) were found in the cytoplasmic membrane, 24 members (or 25.2%) in the cell nucleus, eight members (or 8%) in chloroplasts, seven members (or 7%) in the extracellular matrix, three members (or 3%) in the cytoplasm, and two members (or 2%) in the mitochondria.

3.3. Classification and Phylogenetic Tree of AhFBXs

A systematic evolutionary analysis with AtFBX proteins underscores that AhFBX proteins were categorized into four subgroups, including Group I (consisting of 7 out of 95 members, 7/95), Group II (28/95), Group III (40/95), and Group IV (20/95) (Figure 2A). Subsequently, the four subgroups were subdivided into eight subfamilies based on the variations in C–terminal structural domains, demonstrated in Figure 2B. These subfamilies were defined as: FBX (with unknown structural domains of F-box protein at the C-terminal), FBA (with F-box associated domains at the C-terminal), FBD (with DNA damage repair and cell cycle checkpoint proteins), FBP (with a protein phosphatase 2, PP2), FBK (with several Kelch repeats), FBT (with a tubby domain, Tub), FBL (with leucine-rich repeats, LRR and FBO (with only one F-box protein, FBO_C).

In Group I, the FBP subfamily, FBA, FBX, and FBD were represented with 3, 2, 1, and 1 members, respectively. Group II consisted of 20 FBA members and 8 FBX members. Group III was comprised of 20 FBD members, 12 FBX members, 4 FBK members, 3 FBL members, and 1 FBA member. Lastly, Group IV contained 8 FBX members, 4 FBA members, 3 FBD members, 3 FBO members, and 2 FBT members. The number of family members for each of the eight subfamilies, listed in descending order, was as follows: FBX (29) > FBA (27) > FBD (24) > FBK (4) > FBP (3)/FBL (3)/FBO (3) > FBT (2). In any case, these structural domains in AhFBX proteins would contribute to the functional analysis of AhFBX proteins.

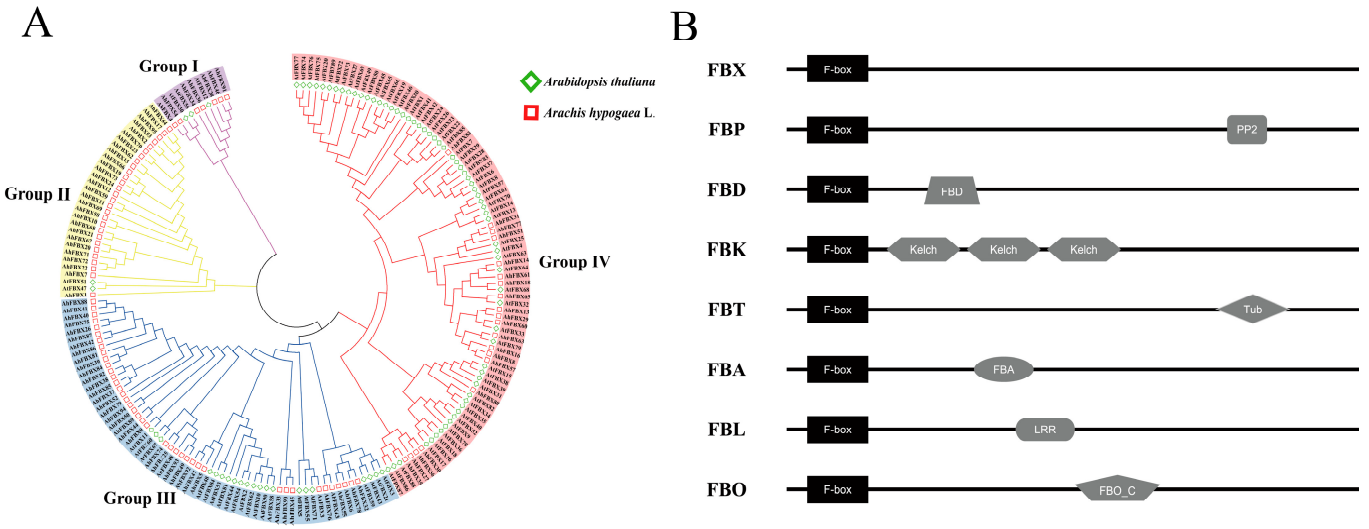

Figure 2. Phylogenetic tree and subfamilies of AhFBX proteins. (**A**) Phylogenetic tree of *Arabidopsis thaliana* and *Arachis hypogaea*. (**B**) Eight subfamilies of F-box protein in peanut.

3.4. Conserved Motifs and Gene Structural of AhFBXs

The AhFBX proteins were identified 10 different motifs, named as motif 1-motif10. As expected, all peanut F-box proteins contained highly representative motif, SKP1 binding (motif 1). The other motifs were also highly conserved and had unknown binding sites (Table S3 and Figure 3A). Of these, 51 (53.7%) AhFBX proteins only contained one motif, 6 (6.3%) proteins had two motifs, 38 (40%) proteins had more than three motifs. The disparity in conservative motifs could be served as a crucial foundation for identification and categorization of peanut F-box proteins. Additionally, AhFBX genes contained 1–15 exons and 1–13 introns. Of these, 53 (55.8%) members had 2–3 exons and 84 (88.4%) members had 1–4 introns. The family members with the highest number of exons and introns are AhFBX39 and AhFBX84. AhFBX39 had 15 exons and 13 introns, while AhFBX84 had 13 each of exons and introns. The members that possessed the same number of exons were grouped together, suggesting a potential correlation between the gene structure and its clustering group.

3.5. Cis–Acting Elements in the Promoter Region of AhFBXs

To predict the potential functions of AhFBXs, we analyzed the cis–acting elements in different gene promoter regions using the sequences located 2000 bp upstream of the CDS. All members mainly divided into three categories (Table S4 and Figure 3B). The first category comprised elements related to plant hormone responses, including the TCA–element, TGACG–motif, MBS, as–1, and others. The second category encompassed elements pertained to growth and development, including but not limited to the ARE, Box–4, GT1–motif, ABRE, TGACG-motif, and more. The third category contained elements that were associated with resistance to various stress, such as MYB, MYC, ERE, and ABRE etc. This distribution of cis–elements suggested that AhFBXs were closely related to plant growth and development and responded to various stresses.

3.6. Collinearity and Estimation of Ka/Ks Ratios of AhFBXs

To gain a better understanding of the duplication events and evolutionary relationship of *AhFBXs*, we conducted collinearity and *Ka*/*Ks* analysis. A total of 133 pairs of collinear genes were identified in inter-species (Table S5 and Figure 4A). A substantial number of segmental duplication events were observed in Aradu.04/Arahy.04, Aradu.09/Arahy.09, Araip.04/Arahy.14 and Araip.09/Arahy.19, suggested that the segmental duplication events played an important role in the evolution of peanuts. Similarly, intra-species collinearity analysis of cultivated peanut revealed that paired genes predominantly existed

in chromosomes Arahy.04/Arahy.14 and Arahy.09/Arahy.19 (Table S6 and Figure 4B). In addition, 19 pairs of duplicated genes had valid *Ka*, *Ks* and *Ka*/*Ks*. Among these, the *Ks* value of *AhFBX* gene pairs ranged from 0.0072 to 0.2182, indicating that large-scale *AhFBX* gene duplication events occurred as far back as 13.4 million years ago (MYA), and as recently as 0.44 MYA. The *Ka*/*Ks* values for all gene pairs were less than 1, with the exception of *AhFBX36*/*AhFBX83*, indicated that these genes likely underwent substantial purifying selection throughout evolution (Table S7 and Figure 4C).

Figure 3. Conserved motifs, gene structure and *cis*–elements of promotor region in *AhFBXs*. (**A**) Conserved motifs and gene structure of *AhFBXs*. (**B**) Different numerical value represents the number of components involved in hormonal response elements, development–related elements and stress–related elements.

3.7. In Silico Expression Patterns of AhFBXs in Different Tissues

To explore the expression patterns of *AhFBX* genes in different tissues, we analyzed the expression profiles of 22 tissues (Table S8 and Figure 5). Of these genes, 24 genes showed relative higher expression levels in the different tissues. For example, in Group I, *AhFBX46* showed highest expression level in nodule. In Group II, *AhFBX1*, *AhFBX2* and *AhFBX7* displayed highest expression level in pistil, seed pattee 6 and pericarp pattee 6, respectively. In Group III, *AhFBX26*, *AhFBX32*, *AhFBX40*, *AhFBX55*, *AhFBX75* and *AhFBX94* had highest expression level in seed pattee 10, seed pattee 5, vegetative shoot tip, lateral_leaf, stamen and seed pattee 8, respectively. In Group IV, *AhFBX31* had highest expression level in perianth. Additionally, a part of homoeologous genes from same group exhibited a similar expression pattern. For instance, *AhFBX46* and *AhFBX91* in Group I, *AhFBX7*, *AhFBX64*

and *AhFBX95* in Group II, *AhFBX45* and *AhFBX90*, *AhFBX38* and *AhFBX90* in Group III, *AhFBX26* and *AhFBX75*. The variation in expression patterns of homoeologous genes could be a result of polyploidization and duplication events that occurred during their evolution.

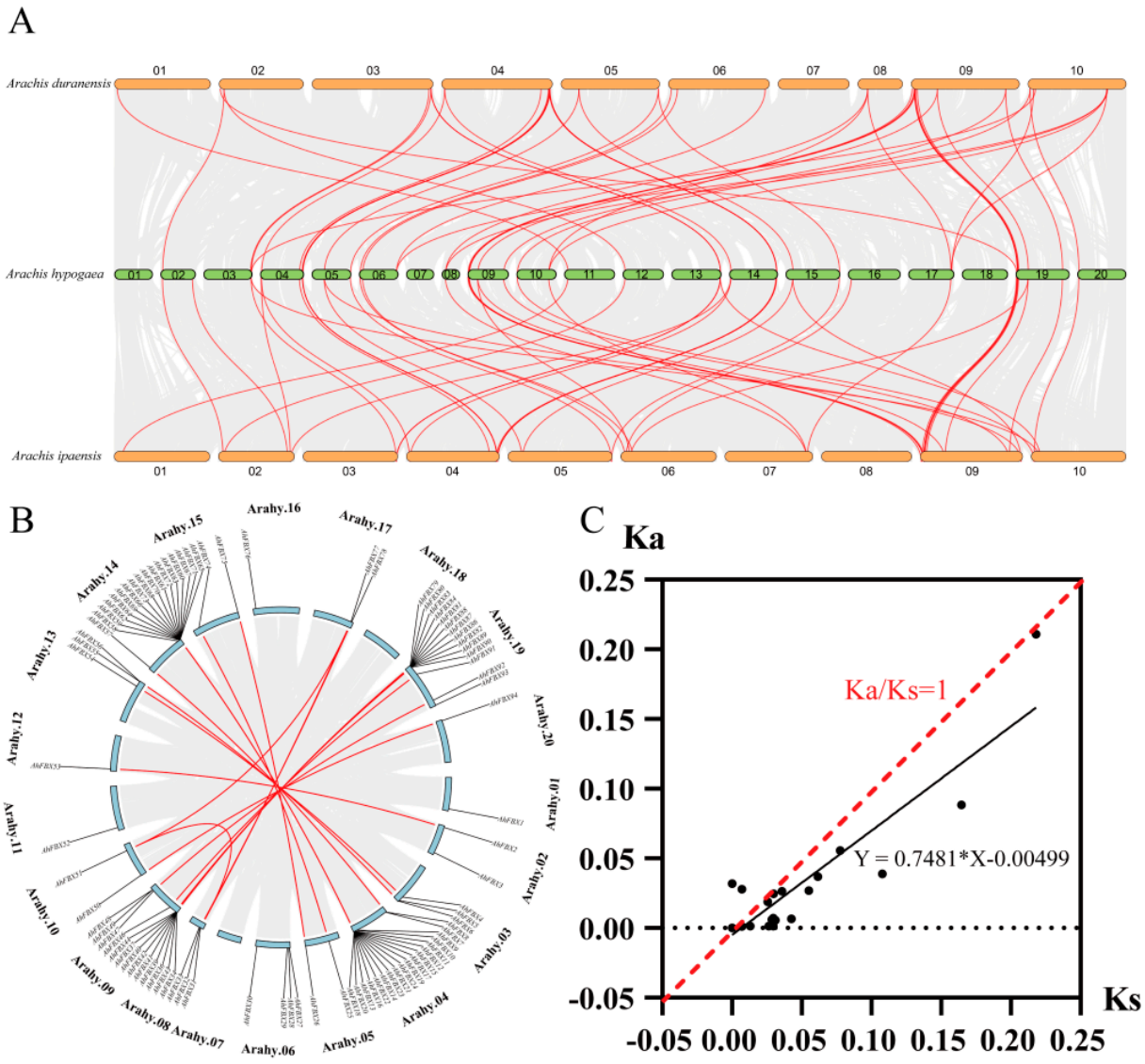

Figure 4. Collinearity plots in inter–species and intra–species, and *Ka*/*Ks* ratio of *AhFBXs* in peanut. (**A**) Red line represented the *AhFBX* genes in colinear blocks between *Arachis hypogaea* and its diploid progenitors. (**B**) Red line represents the *AhFBX* genes in colinear blocks in *Arachis hypogaea*. (**C**) *Ka*/*Ks* ratio of duplicated *AhFBX* gene pairs in peanut. The black dots represent the value of *Ka*/*Ks*. The red line represent *Ka*/*Ks* = 1.

3.8. Expression of AhFBX Genes during the Lateral Branch Development

According to the transcriptomic data of lateral branch development in JH5 and M130, we analyzed the expression patterns of 95 *AhFBX* genes and identified nine differentially expressed genes (DEGs) at different stages of lateral branch development (Table S9 and Figure 6A). To validate the transcriptomic data of DEGs, we performed qRT-PCR to determine their expression patterns. Each DEG displayed highest expression at a specific stage in JH5 and M130 (Table S10 and Figure 6B). For instance, *AhFBX31* showed highest expression at 20DAP in JH5, while *AhFBX45* had highest expression at 5DAP in M130. Comparing the two varieties, *AhFBX31* in M130 exhibited significantly higher expression than in JH5 at each period ($p < 0.01$). Additionally, the expression level of *AhFBX31* in M130 was highest at 15DAP and 25DAP, whereas it was relatively lower at 5DAP, 10DAP, and 30DAP. *AhFBX40*,

AhFBX45, *AhFBX46*, *AhFBX90* and *AhFBX91* displayed significantly higher expression in M130 compared to JH5 at 5DAP ($p < 0.01$). Similarly, *AhFBX46*, *AhFBX90*, and *AhFBX91* showed significantly higher expression in M130 compared to JH5 at 15DAP ($p < 0.01$). On the other hand, *AhFBX8* and *AhFBX57* had significantly higher expression in JH5 compared to M130 at 20DAP ($p < 0.01$). Correlation analysis further confirmed a strong positive correlation between qRT-PCR and FPKM values (r = 0.8801, $p < 0.0001$). Based on these findings, it is hypothesized that the differential expression of these *AhFBX* genes are associated with the developmental changes observed in lateral branch development.

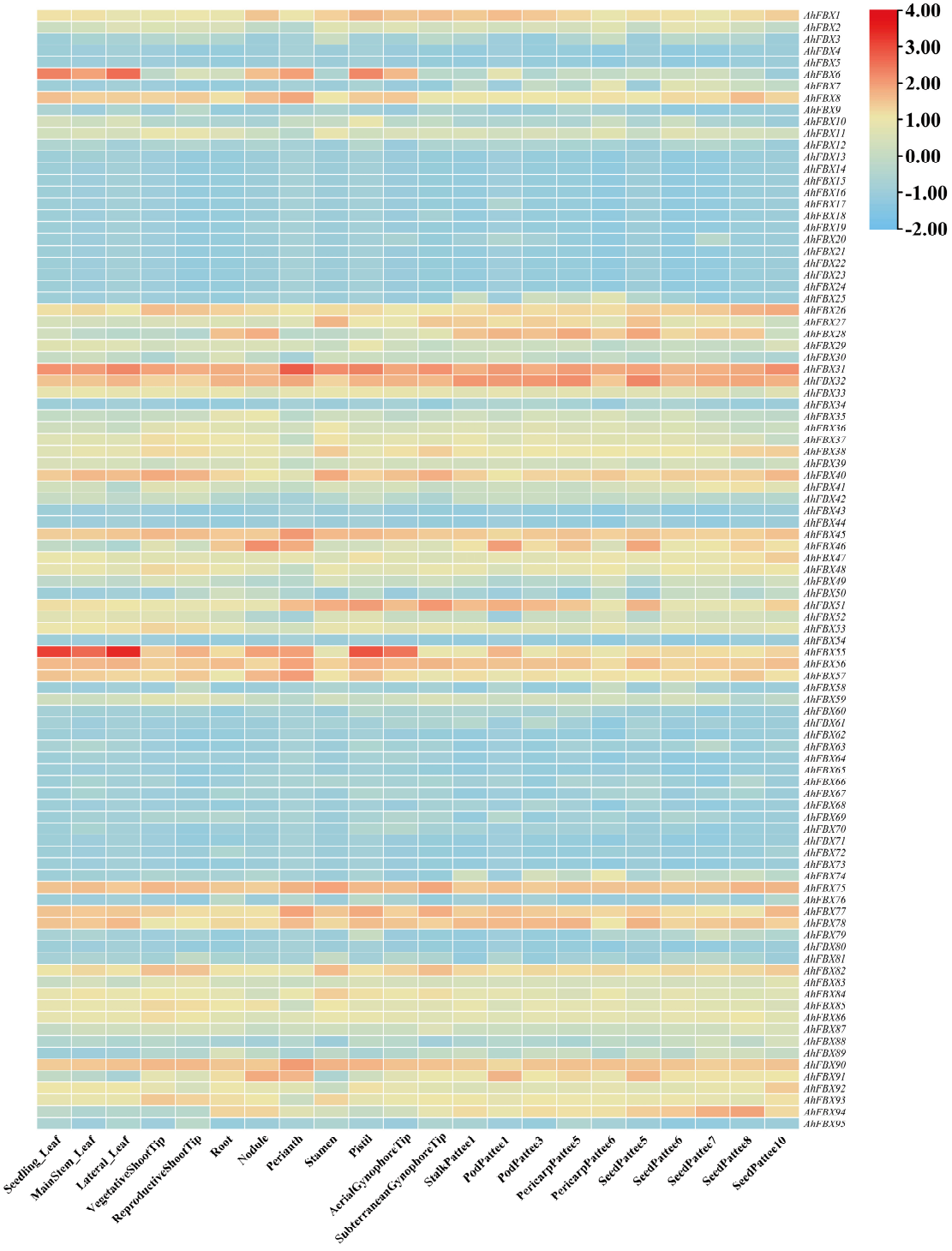

Figure 5. Expression patterns of *F-box* genes in 22 different tissues of cv. Tifrunner. The red and blue colors indicated the higher expression and lower expression values, respectively.

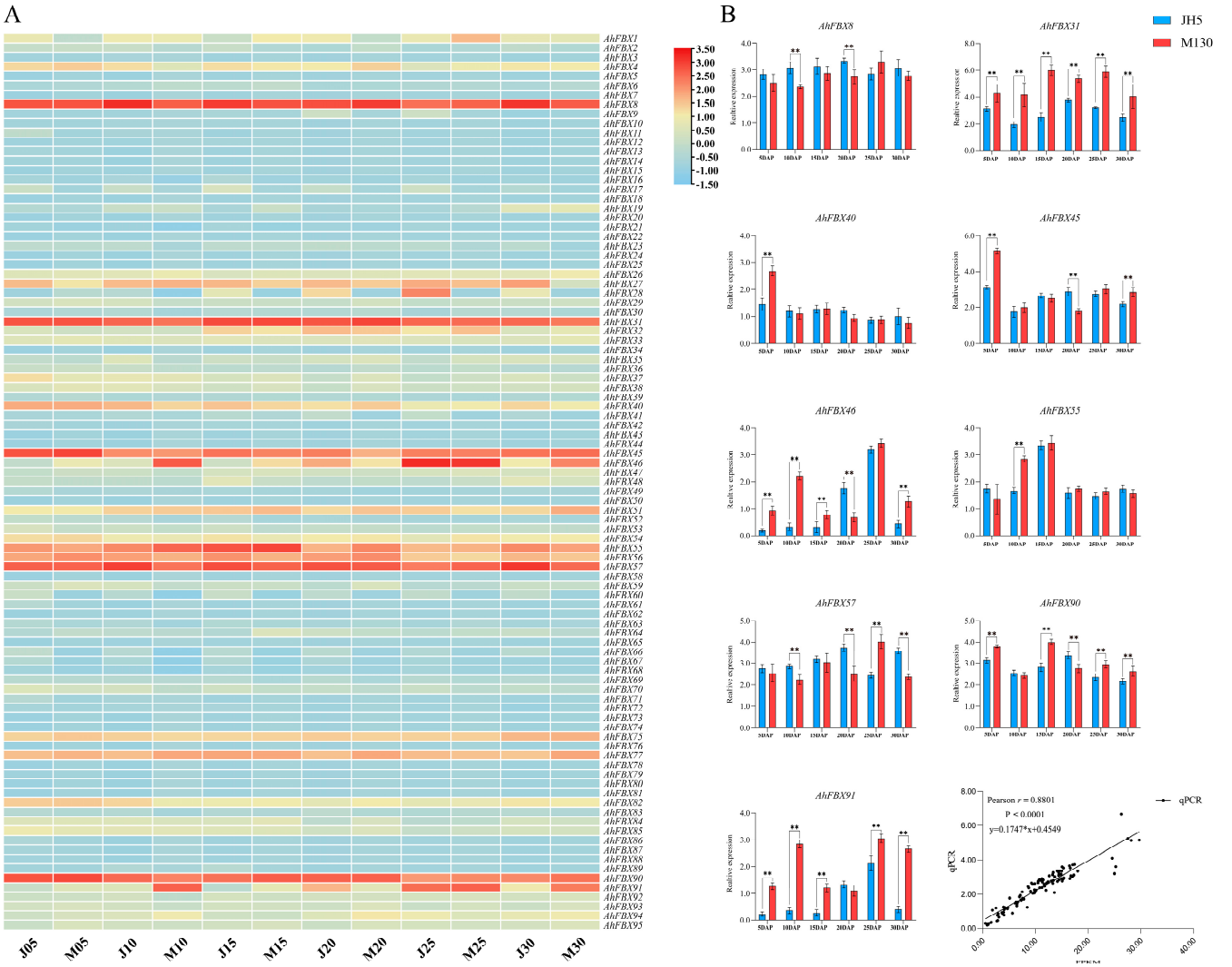

Figure 6. (**A**) Expression patterns of F-box genes in different stages first lateral branches leaves of two distinct varieties. (**B**) Expression patterns and correlation analysis of *AhFBXs* by qRT-PCR and FPKM. The red bar diagram represented expression pattern during the lateral branches developments of JH5. The blue bar diagram showed expression pattern during the lateral branches developments of M130. The line chart exhibited the FPKM values of *AhFBX* DEGs. The X axes of the bar diagram indicated days after planting (DAPs). The Y axes of the bar diagram indicated the relative expression levels of DEGs, respectively. The X and Y axes of the linear regression plot showed the relative expression levels of qRT-PCR and the FPKM values of transcriptomic data, respectively. ** *p* value less than 0.01.

4. Discussion

The F-box protein superfamily, primarily found in plants, plays a crucial role in a wide range of physiological and biochemical processes. The heterogeneity of F-box protein structures is predominantly due to variations in their C-terminal domains which are primarily responsible for substrate recognition and binding via interaction with SCF subunits. These proteins partake in different signal transduction pathways, thereby helping regulate vital cellular processes, rendering the F-box proteins one of the largest families of regulatory proteins. The identification of F-box proteins through whole-genome sequencing, however, can vary substantially among species, largely due to differences in chromosome number and evolutionary lineage. Moreover, the subfamily composition within the F-box protein family also exhibit diversity across species. Previous researches reported the discovery of 694 F-box proteins in *Arabidopsis*, 687 in rice, 359 in maize, 927 in alfalfa,

and 725 in soybean [5–11,46]. In our study, we identified 95 genes in peanut, shorter than other crops, since some incomplete, variant, and putative F-box domain proteins were excluded. A previous investigation on maize revealed the highest number of F-box protein subfamilies with a total of 12 subfamilies, which include FBX, FBT (Tub), FBD, FBW (WD40), FBL (LRR), FBK (Kelch-type), FBP (PP2), FBDUF (DUF295), TLH, FBA, JmjC, and ALH [9]. Alfalfa, on the other hand, boasts the highest number of F-box protein subfamilies among legumes, with 15 subfamilies. These comprised of F-box, FBA, LRR, FBD, Kelch, DUF, PP2, TUB, WD40, PAS, Actin, GSH-synth ATP, ARM, JmjC, and LysM [10]. In our study, we observed the presence of merely eight subfamilies within the F-box protein family in peanut, a substantially smaller quantity than in maize and alfalfa. Thus, we speculated that the restricted count of F-box protein family members and subfamilies in peanut may be accredited to the significant homology between the A and B subgenomes of cultivated peanut, the abundance of repetitive sequences, and the diminished genome heterozygosity [19].

Distinct subfamilies demonstrating individual domains illustrate diverse functions integral to plant growth and development. Prior research predominantly delved into the role of the F-box protein family members in stress response processes, while investigations related to growth and development have not been extensively conducted. Consequently, it is essential to examine the equilibrium between target genes involved in stress response and those related to growth and development. For instance, a study on the FBK subfamily revealed that *OsFBK12*, an F-box protein encompassing the Kelch repeats domain in rice, collaborates with OSK1 to form the SCF complex. This results in the degradation of *S*-ADENOSYL-*L*-METHIONINE SYNTHETASE1 (SAM1) impacting the Ethylene (ETH) content and influencing the leaf senescence process in rice [47]. Similarly, in *Arabidopsis*, the Kelch repeats F-box (KFB) proteins KFB01, KFB20, and KFB50 interact with phenylalanine ammonia lyase (PAL) isoenzymes. The regulation, either through an increase or decrease of KFB expression, impacted the activity of PAL, which subsequently affected the rate-limiting process in the phenylpropanoid pathway. This eventually determined the lignin content in cells [48]. The Tub domain, noted as a distinct feature of the FBT subfamily, is recognized in numerous plant proteins. Overexpression of *AtTLP9* in *Arabidopsis* had been shown to enhance sensitivity to abscisic acid (ABA), suggesting a possible role of the Tub domain in the ABA signaling pathway [49]. The PP2 (PHLOEM PROTEIN 2) domain is a characteristic recognition domain of the FBP subfamily. PP2 proteins are involved in vascular formation, transport of plant nutrients and macromolecules, and signal transduction processes, and are closely related to nutrient transport. PP2 proteins are involved in wound healing, resistance to biotic stress, and nutrient transport in plants. It is speculated that the FBP subfamily could be involved in regulating the formation of phloem in plants and participating in nutrient transport pathways [50,51]. The LRR (leucine-rich repeats) domain has been confirmed to be involved in plant root development and immunity in *Arabidopsis thaliana* [52]. In the present study, we had also identified these associated structural domains of peanut F-box protein, speculated that *AhFBXs* genes could be involved in stress response and plant development processes.

Whole-genome duplication (WGD) events can provide an explanation for the large number of members within the F-box protein family and their diverse range of functions. Additionally, collinearity and the estimation of *Ka*/*Ks* ratios can assist researchers in indirectly confirming the allotetraploid origin of the peanut genome. By analyzing the *Ka*/*Ks* ratios of various F-box genes, it can be inferred that the cultivated peanut had undergone two significant WGD events throughout its evolutionary history. The first event occurred around 60 MYA when legume crops diversified into different species [53]. The second event took place approximately 2.16 MYA, resulting in the formation of the current allotetraploid peanut [19]. In this study, 19 gene pairs were identified through intraspecific collinearity analysis. Through *Ka*/*Ks* analysis of these gene pairs, it was determined that the duplication timeframe of family members ranged from 13.4 MYA to 0.34 MYA. Among the selected gene pairs, 11 pairs demonstrated differentiation occurring after 2.16 MYA,

while 8 pairs indicated differentiation occurring before 2.16 MYA. These findings suggested that the cultivated peanut differentiation also experienced the WGD event. Thus, the F-box proteins have undergone both the differentiation of the legume crop genome and the duplication event within the peanut gene during the process of evolution.

The F-box protein family displays a wide range of structural and functional diversity, which is evident in the diversity of their expression patterns. In our study, *AhFBX* genes revealed abundant variation of *cis*-elements, main including light response, plant hormone and stress responses, such as ARE, G-box, AuxRE, MYB, BOX, and TCA-element, etc. These findings suggested that *AhFBX* genes could be played roles in various physiological and biochemical processes. Of particular interest was the BOX element, a light-responsive element composed of TAATTA. The promoter region of the *rbcS-3A* gene in pea contains several BOX elements, which contribute to the regulation of the light-controlled molecular switch [54]. Another important *cis*-element was the G-box element, which was widely involved in light responses and had a core sequence of CCACGTGG. The G-box binding protein (GBF) in the *rbcS-1A* gene of *Arabidopsis thaliana* regulated photomorphogenesis [55]. Additionally, the G-box element participated in the regulation of circadian rhythm by binding to the pseudo response regulator (PRR) in *Arabidopsis thaliana* [56]. In our study, we identified 41 BOX elements and 20 G-box elements in the differentially expressed genes of lateral branch development, speculated that the lateral branch development of peanut could be regulated by light, ultimately influencing the growth habit of the lateral branch.

The lateral branching habit not only influences the penetration of peanut pegs to produce pods but also affects planting density per unit area [57]. However, to date, F-box protein genes related to lateral branch development were still unknown in peanuts. The systematic research of peanut F-box protein genes would help to promote the further study of lateral branch development and peanut growth habit. In the present study, we obtained the expression patterns of 9 DEGs related to the lateral branch development using our previously released RNA-seq data on NCBI. Based on phylogenetic analysis results, these DEGs were divided into three Groups (I, III and IV). In tissue-specific expression analysis, *AhFBX46*, *AhFBX91* and *AtFBX12* (At2g24250) derived from Group I. Among these, *AtFBX12* exhibited specific expression during seed development and was associated with the mitochondrial protein pathway [58]. Moreover, *AhFBX46* and *AhFBX91* also had higher specifically expressed in seeds of peanut, suggested that their involve in seed growth and development. Group III consisted of *AhFBX40*, *AhFBX45*, *AhFBX55*, *AhFBX90*, *AtFBX58* (At3g50080), and *AtFBX5* (At1g23390). Previous studies showed that *AtFBX58* (At3g50080) in *Arabidopsis* regulates lateral root formation [59], and *AtFBX5* (At1g23390) was a negative regulator involved in the synthesis of brassinosteroids and flavonoids, as well as responsive to leaf, stem, pod development, and environmental stresses [60]. In the present study, *AhFBX40* was specific expressed in vegetative shoot tip. *AhFBX45* and *AhFBX90* were specific expression in perianth. *AhFBX55* was specific expression in lateral leaves. Thus, we suggested that these genes may have similar functions to *AtFBX5.* Group IV consisted of *AhFBX8*, *AhFBX31*, *AhFBX57*, and *AtFBX79* (At4g35930). In previous studies, the FBS mutant *fbs4-1* of *Arabidopsis* (At4g35930) was linked to stomata development [61]. However, in our study, three genes were specific expression in perianth. For lateral branch development, nine DEGs displayed different expression pattern in developmental changes of lateral branches. Obviously, *AhFBX31* had significant change trend at 5DAP-30DAP between JH5 and M130. Other DEGs had not showed continuous significant change trend at 15DAP-25DAP. Our previous research found that the lateral branch length of M130 was more than JH5 [62]. Thus, we speculated that *AhFBX31* as a candidate gene of lateral branch development, could be involved in the formation of spreading growth habit. The gene function of *AhFBX31* needs to be further verified. These findings suggested that peanut F-box protein genes may play an important role in the growth processes of peanut development, especially in the process of lateral branch development.

5. Conclusions

In this study, a comprehensive analysis of the F-box protein family in *Arachis hypogaea* was conducted, resulting in the identification of a total of 95 *AhFBX* genes, which were categorized into four subgroups and eight subfamilies according to the phylogenetic relationship and conserved domain. Collinearity analysis indicated that segmental duplication events played a pivotal role in the evolution of the AhFBX family. Furthermore, *Ka*/*Ks* analysis demonstrated that strong purifying selection influenced the evolution of AhFBXs. Differential expression patterns of peanut F–box protein family genes in different tissues, and they were involved in the regulation of peanut growth and development. Among them, *AhFBX8*, *AhFBX31*, *AhFBX45*, *AhFBX46*, *AhFBX55*, *AhFBX57*, *AhFBX90* and *AhFBX91* showed specific expression in lateral branch development. These genes hold promise for future functional studies of F-box protein genes and their significance in shaping peanut's lateral branch.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10030255/s1, Table S1: Sequences of the primers used in this study; Table S2: Statistics of physical location of *AhFBXs* on peanut chromosomes; Table S3: Motif sequence of *AhFBXs*; Table S4: *Cis*-element analysis of *AhFBXs* gene promoters; Table S5: Syntenic relationships between *Arachis hypogaea* and *Arachis duranensis*, *Arachis ipaensis*; Table S6: Synteny analysis of the cultivated peanut; Table S7: *Ka*/*Ks* analysis and years of genetic evolution; Table S8: Published transcriptome data of the various tissues in cultivated peanuts; Table S9: Transcriptome expression data of *AhFBXs* in the lateral branch development; Table S10: The relative expression data of nine *AhFBX* genes.

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