BMC Plant Biology

RESEARCH

Open Access

WRKY transcription factors modulate flowering time in four *Arachis* species: a bioinformatics analysis

Xiao Fang[1], Lubin Liu[2], Meiran Li[2], Hui Song[2*] and Yihui Zhou[1*]

Abstract

Background WRKY proteins are important transcription factors (TFs) in plants, involved in growth and development and responses to environmental changes. Although WRKY TFs have been studied at the genome level in *Arachis* genus, including oil crop and turfgrass, their regulatory networks in controlling flowering time remain unclear. The aim of this study was to predict the molecular mechanisms of WRKY TFs regulation flowering time in *Arachis* genus at the genome level using bioinformatics approaches.

Results The flowering-time genes of *Arachis* genus were retrieved from the flowering-time gene database. The regulatory networks between WRKY TFs and downstream genes in *Arachis* genus were predicted using bioinformatics tools. The results showed that WRKY TFs were involved in aging, autonomous, circadian clock, hormone, photoperiod, sugar, temperature, and vernalization pathways to modulate flowering time in *Arachis duranensis*, *Arachis ipaensis*, *Arachis monticola*, and *Arachis hypogaea* cv. Tifrunner. The WRKY TF binding sites in homologous flowering-time genes exhibited asymmetric evolutionary pattern, indicating that the WRKY TFs interact with other transcription factors to modulate flowering time in the four *Arachis* species. Protein interaction network analysis showed that WRKY TFs interacted with FRUITFULL and APETALA2 to modulate flowering time in the four *Arachis* species. WRKY TFs implicated in regulating flowering time had low expression levels, whereas their interaction proteins had varying expression patterns in 22 tissues of *A. hypogaea* cv. Tifrunner. These results indicate that WRKY TFs exhibit antagonistic or synergistic interactions with the associated proteins.

Conclusions This study reveals complex regulatory networks through which WRKY TFs modulate flowering time in the four *Arachis* species using bioinformatics approaches.

Keywords *Arachis* genus, Flowering time, Protein interaction, WRKY transcription factor

Background

WRKY transcription factors (TFs) modulate plant growth and development and response to abiotic and biotic stress by regulating downstream genes and forming protein complexes [1–5]. WRKY TFs modulate the plant flowering process through various pathways, including photoperiod, autonomous, vernalization, gibberellin, and aging pathways [4, 6]. AtWRKY71 from *Arabidopsis thaliana* activates *FLOWERING LOCUS T* (*FT*) and *LEAFY* (*LFY*) to promote flowering [7]. AtWRKY75 interacts with DELLA to activate *FT*, accelerating flowering [8].

*Correspondence:
Hui Song
biosonghui@outlook.com
Yihui Zhou
zhouyihui_2006@163.com
[1] School of Animation and Media, Qingdao Agricultural University, 700# Changcheng Road, Qingdao, Shandong 266019, China
[2] College of Grassland Science, Qingdao Agricultural University, 700# Changcheng Road, Qingdao, Shandong 266019, China

Moreover, WRKY184 from *Brassica napus* upregulates *FRUITFULL* (*FUL*) expression to promote flowering [9]. WRKY TFs are also implicated in phytohormone pathways, such as abscisic acid (ABA) and auxin, to modulate flowering time. OsWRKY72 from *Oryza sativa* indirectly activates genes associated with the auxin pathway, such as *AUXIN1*, *AUXIN RESISTANT1*, and *BUD1*, and genes related to the ABA pathway, such as *ABA2* and *ABA INSENSITIVE 4* (*ABI4*), to promote flowering [10]. WRKY TFs regulate salt and cadmium stress to modulate flowering time. For instance, RtWRKY23 from *Reaumuria trigyna* upregulates *HISTONE ACETYLTRANSFERASE 1*, *ANT*, and *MADS AFFECTING FLOWERING 5* expression to alleviate salt stress, resulting in earlier flowering [11]. AtWRKY12 and AtWRKY13 potentially co-regulate flowering time and cadmium stress due to their similar regulatory patterns in modulating flowering time and cadmium stress [4].

Environmental changes, such as nutrient availability, drought stress, and temperature stress, can alter flowering time [4]. However, the role of WRKY TFs in regulating these pathways ultimately modulating flowering time is unclear. This indicates that WRKY TFs may regulate flowering time through additional flowering pathways. Therefore, it is imperative to conduct research to identify flowering-time genes at the genome level and verify whether WRKY TFs regulate these genes. A plant flowering-time gene database, PFGD, was established in the recent past [12]. The database provides a platform to identify WRKY TFs that regulate downstream genes and the proteins associated with these TFs.

Plants in the *Arachis* genus serve as oil, forage and turfgrass crops [13]. Cultivated peanut (*Arachis hypogaea*) and *Arachis monticola* are allotetraploids obtained by crossing by two wild diploids, *Arachis duranensis* and *Arachis ipaensis* [14, 15]. Comparative genomic analyses between diploid and tetraploid peanuts reveal asymmetric evolution in the subgenomes of cultivated peanuts [16–18]. Asymmetric evolution of subgenomes leads to functional bias of genes in a specific subgenome [16, 17, 19]. A homoeologous WRKY pair, AhTWRKY24 from subgenome B and AhtWRKY106 from subgenome A, were identified in *Arachis hypogaea* cv. Tifrunner [20]. DNA affinity purification sequencing data revealed that AhTWRKY24 and AhtWRKY106 regulate approximately an equal number of downstream genes in *A. hypogaea* cv. Tifrunner genome, but they also exhibit specific regulation of distinct downstream genes [20]. These results indicate that asymmetric evolution influences genes regulated by WRKY in *A. hypogaea* cv. Tifrunner.

Although WRKY TFs have been identified at the genome level in members of *Arachis* genus [5, 21], their role in regulating flowering time is yet to be fully elucidated. In this study, flowering-time genes for *A. duranensis*, *A. ipaensis*, *A. monticola*, and *A. hypogaea* cv. Tifrunner were retrieved from PFGD database. This study revealed the regulatory networks of WRKY TFs in the four *Arachis* species through bioinformatics approaches.

Methods

Sequence retrieval

The PFGD database (http://pfgd.bio2db.com/index.html) is a valuable repository for genes that regulate flowering time in plants [12]. The flowering-time genes of the *Arachis* species were retrieved from the PFGD database. The species of the *Arachis* genus included *A. duranensis*, *A. ipaensis*, *A. monticola*, and *A. hypogaea* cv. Tifrunner.

WRKY TFs have been identified in various *Arachis* species [5, 21]. *A. duranensis*, *A. ipaensis*, *A. monticola*, and *A. hypogaea* cv. Tifrunner have 16 WRKY TFs, which are AtWRKY12 and AtWRKY75 homologs and are involved in regulating flowering time [5, 21, 22]. The 16 WRKY TFs in the four *Arachis* species were retrieved from PeanutBase database based on previous studies [5, 21, 22].

The orthologs of the genes in *A. duranensis*, *A. ipaensis*, *A. monticola*, and *A. hypogaea* cv. Tifrunner were identified using the MCScan X program with an e-value of 1E-10 [23]. Similarly, paralogs and homoeologs were identified using the MCScan X program. The homoeologs were identified in *A. monticola* and *A. hypogaea* cv. Tifrunner.

Identification of WRKY TFs regulation flowering-time genes in *Arachis* genus

The 2 kb *cis*-acting regions of flowering-time genes were isolated from the four *Arachis* species and uploaded to the Nesite database to predict the WRKY TF binding sites (TFBSs) [24]. The search parameters were an expected mean number of 0.01, a statistical significance level of 0.95, an 80% homology between known TFBS and motif, and a 20% variation in the distance between TFBS blocks. The protein interaction relationships between the 16 WRKY TFs and flowering-time proteins in the four *Arachis* species were predicted using the STRING database. *A. thaliana* was used as a reference and the protein–protein interaction network analysis was conducted with the default parameters in the STRING database.

Tissue expression profile of *WRKY* and flowering-time genes in *A. hypogaea* cv. Tifrunner

The RNA-seq datasets of 22 tissues of *A. hypogaea* cv. Tifrunner were retrieved from PeanutBase [25, 26]. The raw read counts were aligned to the *A. hypogaea* cv. Tifrunner genome using Bowtie 2 in the TBtools program [23], and expression levels were quantified as fragments per kilobase of transcript per million mapped reads

(FPKM) using RSEM [27]. The expression levels were standardized by $\log_2$ transformation (FPKM + 1). The expression patterns were visualized using TBtools program [23].

Phylogenetic analysis

MAFFT was used to conduct multiple sequence alignments with default parameters [28]. ProtTest was used to estimate the best-fit model for the construction of a phylogenetic tree based on the maximum likelihood method [29]. The IQ-tree program was used to construct the maximum likelihood trees using the best-fit model from the ProtTest program, 10,000 ultrafast bootstraps, and 1000 the SH-like approximate likelihood ratio test. The phylogenetic trees were visualized using the Figtree tool [30].

Results

Multiple flowering-time pathways are identified in *Arachis* genus

The PFGD database comprises 552, 622, 514, and 576 flowering-time genes from *A. duranensis*, *A. ipaensis*, *A. monticola*, and *A. hypogaea* cv. Tifrunner (Accessed on January 23, 2024). Notably, analysis of the *cis*-acting elements showed that 41, 53, 81, and 91 flowering-time genes are potentially regulated by WRKY TFs in *A. duranensis*, *A. ipaensis*, *A. monticola*, and *A. hypogaea* cv. Tifrunner (Fig. 1 and Additional File 1). These four species of *Arachis* genus had similar flowering pathways regulated by WRKY TFs. The flowering pathways included aging, autonomous, circadian clock, hormone, photoperiod, sugar, temperature, and vernalization. However, no previous evidence confirms the involvement of WRKY TFs in sugar and temperature pathways to modulate flowering time.

Homologous flowering-time genes have asymmetric WRKY transcription factor binding sites

The four species in *Arachis* genus have different numbers of flowering-time paralogs. In *A. duranensis*, four pairs of paralogous flowering-time genes contained WRKYTFBSs, whereas 15 flowering-time genes from 15 paralogous pairs contained WRKY TFBSs (Fig. 2 and Additional File 2). Similarly, *A. ipaensis* and *A. hypogaea* cv. Tifrunner had two and six pairs of paralogous flowering-time genes containing WRKY TFBSs, respectively (Fig. 2 and Additional File 2). Moreover, one copy of 12, 13, and 19 paralogous flowering-time gene pairs in *A. ipaensis*, *A. monticola*, and *A. hypogaea* cv. Tifrunner exhibited WRKY TFBSs (Fig. 2 and Additional File 2). These results indicate that evolutionary patterns of WRKY TFBSs in flowering-time genes vary across species.

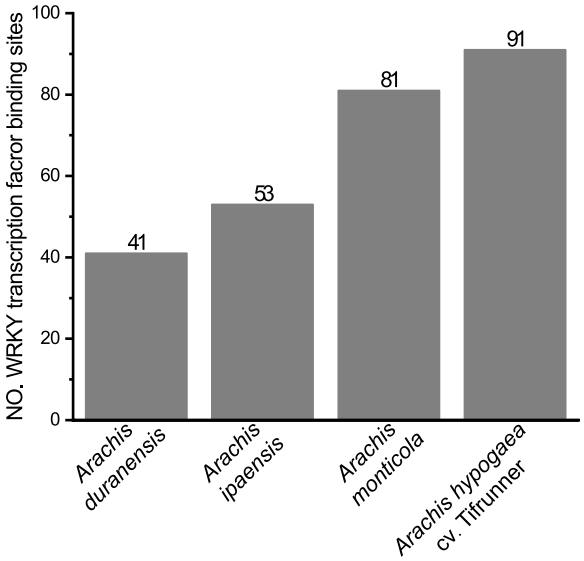

Fig. 1 The flowering-time genes regulated by WRKY transcription factors in the four *Arachis* species

The findings showed that *A. monticola* and *A. hypogaea* cv. Tifrunner have varying number of homoeologs associated with flowering time. *A. monticola* and *A. hypogaea* cv. Tifrunner exhibited two and 14 homoeologous flowering-time gene pairs containing WRKY TFBSs, respectively. Conversely, *A. monticola* and *A. hypogaea* cv. Tifrunner had one copy of 15 and 74 homoeologous flowering-time gene pairs exhibiting WRKY TFBSs (Fig. 2 and Additional File 2). These results indicate the WRKY TFBSs in flowering-time genes exhibit an asymmetric evolutionary pattern between the two species.

Previous studies demonstrated that 16 *Arachis* WRKY TFs are orthologs with AtWRKY12 and AtWRKY75, which regulate flowering time by binding W-box elements of *FUL* and *FT* genes [8, 31]. Conserved orthologs of flowering time were identified across four *Arachis* species through synteny analyses. These genes are mainly implicated in aging, autonomous, and sugar pathways (Table 1). These results indicate that WRKY TFs modulate specific regulatory networks of flowering time in the four *Arachis* species.

WRKY TFs interact with FUL and AP2 to modulate flowering time in *Arachis* genus

The WRKY TF interaction relationships among the four *Arachis* species were predicted using the STRING database. The WRKY TFs, which were orthologs to AtWRKY12, interacted with FUL in the four *Arachis* species (Fig. 3). Similarly, the WRKY TFs, which were orthologs to AtWRKY75, interacted with APETALA2 (AP2) in the four *Arachis* species (Fig. 3). Phylogenetic

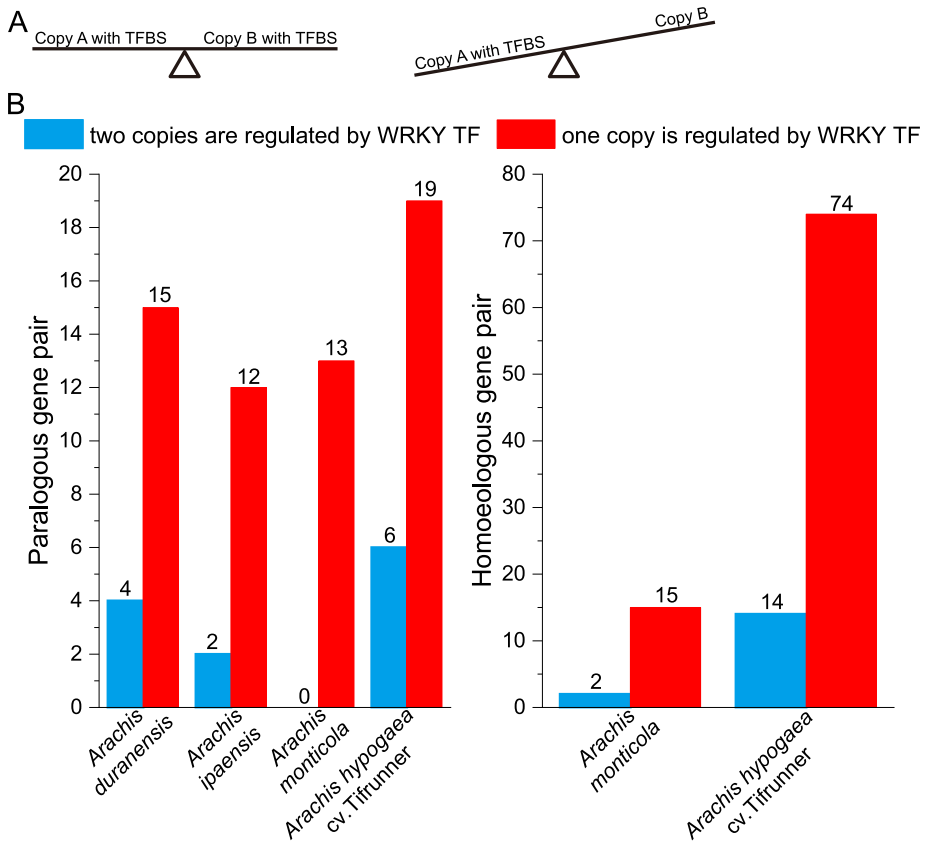

Fig. 2 Homologs potentially regulated by WRKY transcription factors. **a** A schematic representation of symmetric and asymmetric WRKY transcription factor binding site (TFBS) in a homologous pair. **b** The homologs of flowering-time genes exhibiting WRKY TFBSs in the four *Arachis* species

Table 1 WRKY transcription factors potentially regulate conserved orthologs in the four *Arachis* species

duranensis	ipaensis	monticola	Tifrunner	Genome	Arabidopsis	Annotion	Pathway
Aradu.EI58K	No component	EVM0038983	T5C9EA	A	AT5G44160	INDETERMINATE DOMAIN 8, NUTC RACKER	Sugar
No component	Araip.6UE4Z	Not determined	V8NH83	B			
Aradu.F7PQZ	No component	Not determined	Not determined	A	AT1G78580	TREHALOSE-6-PHOSPHATE SYNTHASE 1	Aging
No component	Araip.FER8B	EVM0016112	PK6QLT	B			
Aradu.FY71B	No component	Not determined	8JQ024	A	AT3G11910	UBIQUITIN-SPECIFIC PROTEASE 13	Autonomous
No component	Araip.QTU2G	EVM0050491	Not determined	B			
Aradu.NJJ5I	No component	Not determined	Q6VMSA	A	AT3G63010	GA INSENSITIVE DWARF 1B	Aging
No component	Araip.6D3E7	EVM0030129	Not determined	B			
Aradu.SE6Q0	No component	EVM0035381	00XS3D	A	AT5G67180	TARGET OF EARLY ACTIVATION TAGGED 3	Aging
No component	Araip.MAL00	Not determined	Not determined	B			

analyses showed that WRKY TFs and the associated proteins exhibited different evolutionary relationships in the four *Arachis* species. FUL showed a high likelihood of loss in *A. hypogaea* cv. Tifrunner genome compared to the WRKY12 TFs (Fig. 3). Conversely, AP2 was prone to expansion relative to WRKY75 in *A. monticola* and *A. hypogaea* cv. Tifrunner genomes (Fig. 3). Notably, there was no evidence confirming that AtWRKY12 and AtWRKY75 proteins interact with FUL and AP2 to modulate flowering time. The results indicate that WRKY TFs exhibit specific protein interaction relationships to modulate flowering time in the four *Arachis* species.

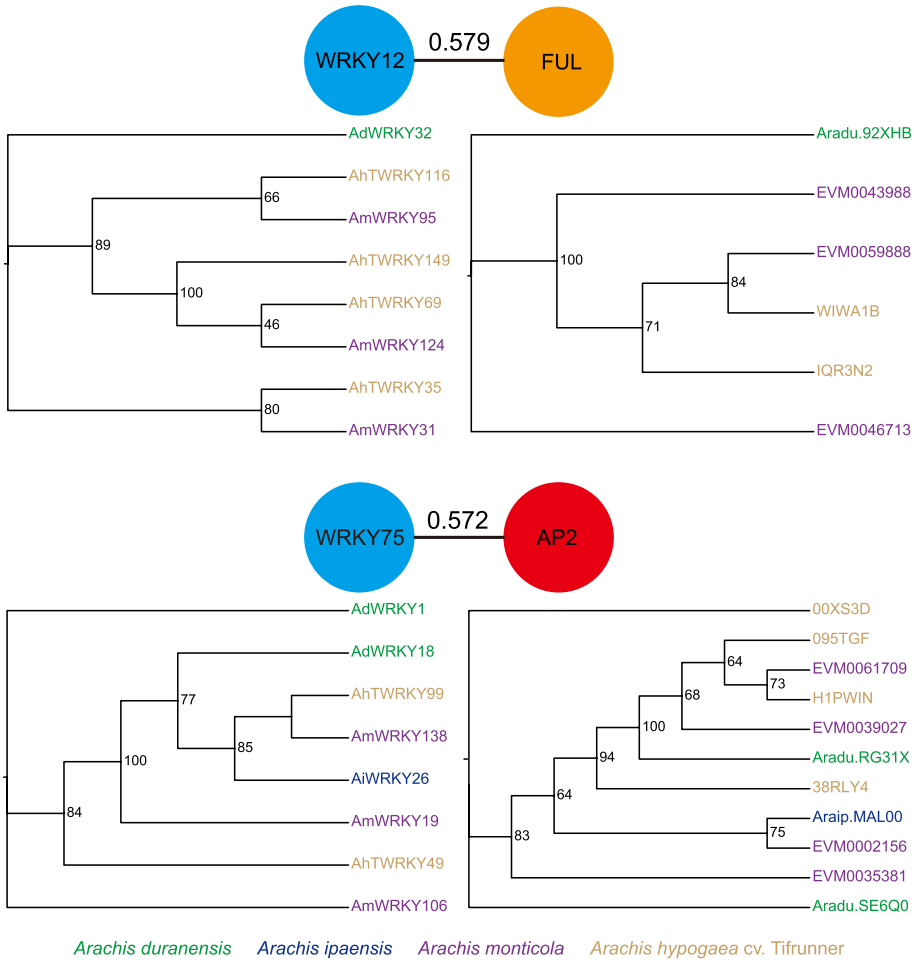

Fig. 3 WRKY transcription factors interact with FUL and AP2 to modulate flowering time in the four *Arachis* species

Varying expression patterns of flowering-time genes in 22 tissues of *A. hypogaea* cv. Tifrunner

WRKY TFs regulating flowering time clustered in group I based on their expression levels in 22 tissues of *A. hypogaea* cv. Tifrunner (Fig. 4). The genes in group I exhibited low expression levels compared to other groups (Fig. 4). Group I had two homoeologous gene pairs, AhTWRKY35 and AhTWRKY116, and AhTWRKY69 and AhTWRKY149. Each homoeologous gene pair exhibited similar expression pattern in 22 tissues (Fig. 4). These results indicate that WRKY homoeologous gene pair share similar regulatory networks. In addition, 18 flowering-time genes were grouped in a subclade based two the WRKY homoeologous gene pairs (Fig. 4), indicating that these genes are potentially regulated by these WRKY homoeologs.

In addition, AhTWRKY49 and AhTWRKY99 (ortholog with WRKY75) indicated potential synergistic interactions with 38RLY4 (AP2), and AhTWRKY35 and AhTWRKY116, and AhTWRKY69 and AhTWRKY149 (ortholog with WRKY12) exhibited potential synergistic interactions with WIWA1B and IQR3N2 (FUL), because they had similar expression patterns (Figs. 3 and 4). Conversely, AhTWRKY49 and AhTWRKY99 exhibited potential antagonistic interactions with 00XS3D, 095TGF, and H1PWIN (AP2) due to the differences in expression patterns (Figs. 3 and 4).

Discussion

Arachis plants are essential sources of oil, proteins, and forage [13]. The genomes of cultivated peanut and its progenitors have been sequenced [15, 17, 32–34]. WRKY TFs have been identified at the genome level in members of *Arachis* genus [5, 21]. However, the functions of WRKY TFs in flowering time have not been elucidated. In this study, WRKY TFs that regulate flowering-time genes and their interaction proteins in *A. duranensis*, *A. ipaensis*, *A. monticola*, and *A. hypogaea* cv. Tifrunner were identified using bioinformatics approaches. The main findings are summarized as follows: Firstly, WRKY

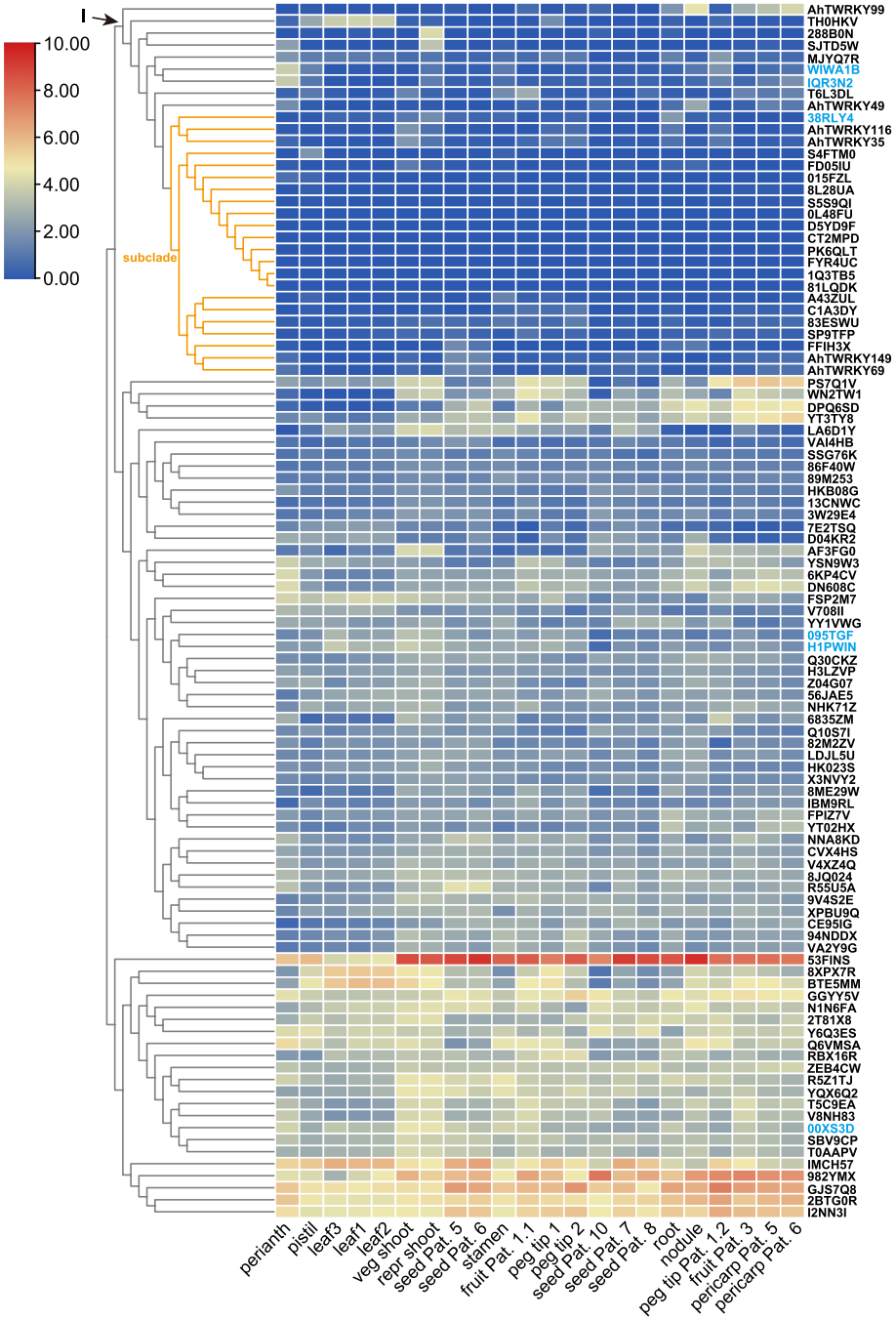

Fig. 4 The expression patterns of WRKY and flowering-time genes in 22 tissues of *Arachis hypogaea* cv. Tifrunner. Blue font indicates interacting proteins with WRKY transcription factors

TFs are involved in aging, autonomous, circadian clock, hormone, photoperiod, sugar, temperature, and vernalization pathways to modulate flowering time in the four *Arachis* species. Secondly, asymmetric WRKY TFBSs were identified in homologs of the flowering-time genes across the four *Arachis* species. Thirdly, two conserved protein complexes involving WRKY TFs interaction with FUL and AP2 modulated flowering time in the four *Arachis* species.

WRKY TFs are primarily involved in flowering pathways and phytohormone pathways to modulate flowering time [4, 35]. The flowering pathways include

photoperiod, autonomous, vernalization, gibberellin, and aging pathways [4, 6]. WRKY TFs regulate flowering time through ABA, auxin, and ET pathways [4, 10, 36]. However, the role and the underlying mechanisms of WRKY TFs in regulating flowering time through sugar and temperature pathways have not been fully elucidated. Previous studies demonstrated that low sugar and high temperature promote flowering, whereas high sugar and low temperature conditions delay flowering in plants [4, 37–39]. These findings imply that WRKY TFs potentially modulate flowering time through sugar and temperature pathways in the four *Arachis* species.

Specific interactions between WRKY TFs and other proteins modulated flowering time in the four *Arachis* species. *Arachis* WRKY TFs interacted with FUL and AP2 proteins to modulate flowering time. FUL, a member of the MADS-box gene family, modulates flowering time and floral meristem development [40, 41]. AP2, a member of the AP2/ERF gene family, regulates the interaction between floral meristem and APETALA1 (AP1), LEAFY (LFY), and CAULIFLOWER (CAL) to modulate flower and floral meristem development [42, 43]. Notably, a regulatory network exists between FUL and AP2. FUL directly and negatively regulates AP2 to downregulate wuschel-related homeobox expression in shoot apical meristem, reducing flowering in monocarpic plants [44]. Furthermore, AP2 downregulates genes implicated in axillary bud dormancy and cytokinin signaling, resulting in global proliferative arrest to affect flowering in monocarpic plants [45]. These findings indicate that WRKY TFs are implicated in the global proliferative arrest pathway in *Arachis* genus.

Arachis WRKY TFs, which regulate flowering time, are orthologs with AtWRKY12 and AtWRKY75 [5]. AtWRKY12 and AtWRKY75 interact with DELLA proteins to modulate flowering time [8, 31]. Moreover, AtWRKY12 activates *FUL*, and AtWRKY75 activates *FT* to promote flowering [8, 31]. Based on these results, we hypothesized that *Arachis* WRKY TFs, orthologous to AtWRKY12, interact with FUL protein and regulate *FUL* expression.

However, our study had some limitations. Firstly, it is challenging to establish one-to-one regulatory relationships between WRKY TF and downstream genes using bioinformatics approaches. This is because WRKY TFs regulate a conserved W-box element shared across several downstream genes. Additionally, our results showed that the number of downstream genes was higher than the number of WRKY TFs in the four *Arachis* species. These findings indicate that one WRKY TF may regulate several downstream genes to modulate flowering time. AtWRKY63 activates *COOLAIR* and *COLDAIR*, leading to the downregulation of *FLOWERING LOCUS C* (*FLC*) expression and consequently accelerating flowering [46]. AtWRKY71 activates *FT* and *LFY* to promote flowering [7]. Our results demonstrated that WRKY TFBSs of homologous downstream genes exhibit asymmetric evolution, suggesting that WRKY TFs interact with other transcription factors to modulate the flowering process in the four *Arachis* species.

Conclusion

In summary, bioinformatics approaches were used in this study to predict the WRKY TFs regulating downstream genes and interaction proteins in the four *Arachis* species. WRKY TFs are involved in multiple pathways to modulate flowering time in the four *Arachis* species. WRKY TFs can interact with FUL and AP2 modulated flowering time in the four *Arachis* species. Although several novel regulatory networks were elucidated in this study, further experimental testing is required to verify these relationships.

Abbreviations

ABA	Abscisic acid
AP1	APETALA1
AP2	APETALA2
CAL	Cauliflower
FLC	Flowering locus C
FPKM	Fragments per kilobase of transcript per million mapped reads
FT	Flowering locus T
FUL	Fruitfull
LFY	Leafy
PFGD	Flowering-time gene database
TF	Transcription factor
TFBS	Transcription factor binding site

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05343-7.

Supplementary Material 1.

Supplementary Material 2.

Authors' contributions

XF, HS and YZ conceived and designed this research. XF, HS and YZ analyzed data and wrote the manuscript. LL and ML executed the data analyses. HS and YZ evaluated the manuscript. All authors gave read and approved the final version.

Funding

This study was funded by Foundation of Shandong Youth & Children of Academy Educational Science (No. 23SCT005), and Qingdao Agricultural University Innovation Program for Master's degree Candidates (QNYCX23001).

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the public databases as followings.
Flowering-time genes from PFGD database: http://pfgd.bio2db.com/index.html.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 9 May 2024 Accepted: 26 June 2024
Published online: 28 June 2024

References

1. Song H, Cao Y, Zhao L, Zhang J, Li S. Review: WRKY transcription factor: Understanding the functional divergence. Plant Sci. 2023;334: 111770.
2. Rushton PJ, Somssich IE, Ringler P, Shen QJ. WRKY transcription factors. Trends Plant Sci. 2010;15(5):247–58.
3. Eulgem T, Rushton PJ, Robatzek S, Somssich IE. The WRKY superfamily of plant transcription factors. Trends Plant Sci. 2000;5(5):199–206.
4. Song H, Duan Z, Zhang J. WRKY transcription factors modulate flowering time and resposne to environmental changes. Plant Physiol Bioch. 2024;210: 108630.
5. Song H, Guo Z, Duan Z, Li M, Zhang J. WRKY transcription factors in *Arachis hypogaea* and its donors: from identification to function prediction. Plant Physiol Bioch. 2023;204: 108131.
6. Strikanth A, Schmid M. Regulation of flowering time: all roads lead to Rome. Cell Mol Life Sci. 2011;68(12):2013–37.
7. Yu Y, Liu Z, Wang L, Kim SG, Seo PJ, Qiao M, et al. WRKY71 accelerates flowering via the direct actication of *FLOWERING LOCUS T* and *LEAFY* in *Arabidopsis thaliana*. Plant J. 2016;85(1):96–106.
8. Zhang L, Chen L, Yu D. Transcription factor WRKY75 interacts with DELLA proteins to affect flowering. Plant Physiol. 2018;176(1):790–803.
9. Yang J, Chen H, Yang C, Ding Q, Zhao T, Wang D. A WRKY transcription factor WRKY184 from *Brassica napus* L. is involved in flowering and secondary wall development in transgenic *Arabidopsis thaliana*. Plant Growth Regul. 2020;92:427–40.
10. Song Y, Chen L, Zhang L, Yu D. Overexpression of *OsWRKY72* gene interferes in the abscisic acid signal and auxin transport pathway of *Arabidopsis*. J Biosci. 2010;35(3):459–71.
11. Du C, Ma B, Wu Z, Li N, Zheng L, Wang Y. *Reaumuria trigyna* transcription factor RtWRKY23 enhances salt stress tolerance and delays flowering in plants. J Plant Physiol. 2019;239:38–51.
12. Wu T, Liu Z, Yu T, Zhou R, Yang Q, Cao R, et al. Flowering genes identification, network analysis, and database construction for 837 plants. Hortic Res. 2024;11(4):uhae013.
13. Song H, Huang Y, Ding L, Duan Z, Zhang J. *Arachis* species: high-quality forage crops-nutritional properties and breeding strategies to expand their utilization and feeding value. Grassland Res. 2023;2(3):212–9.
14. Bertioli DJ, Abernathy B, Seijo G, Clevenger J, Cannon SB. Evaluating two different models of peanut's origin. Nat Genet. 2020;52(6):557–9.
15. Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD, Cannon EKS, et al. The genome sequences of *Arachis duranensis* and *Arachis ipaensis*, the diploid ancestors of cultivated peanut. Nat Genet. 2016;48(4):438–46.
16. Yin D, Ji C, Song Q, Zhang W, Zhang X, Zhao K, et al. Comparison of *Arachis monticola* with diploid and cultivated tetraploid genomes reveals asymmetric subgenome evolution and improvement of peanut. Advanced Science (Weinh). 2019;7(4):1901672.
17. Bertioli DJ, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, et al. The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. Nat Genet. 2019;51(5):877–84.
18. Lu Q, Huang L, Liu H, Garg V, Gangurde SS, Li H, et al. A genomic variation map provides insights into peanut diversity in China and associations with 28 agronomic traits. Nat Genet. 2024;56(3):530–40.
19. Song H, Guo Z, Zhang X, Sui J. *De novo* genes in *Arachis hypogaea* cv. Tifrunner: systematic identification, molecular evolution, and potential contribution to cultivated peanut. Plant J. 2022;111(4):1081–95.
20. Li M, Chen M, Zhang Y, Zhao L, Zhang J, Song H. Identification of the target genes of AhTWRKY24 and AhTWRKY106 transcription factors reveals their regulatory network in *Arachis hypogaea* cv. Tifrunner using DAP-seq. Oil Crop Science. 2023;8(2):89–96.
21. Chen M, Li M, Zhao L, Song H. iphering evolutionary dynamics of WRKY genes in *Arachis* species. BMC Genomics. 2023;24(1):48.
22. Song H, Sun W, Yang G, Sun J. WRKY transcription factors in legumes. BMC Plant Biol. 2018;18(1):243.
23. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, et al. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13(8):1194–202.
24. Shahmuradov IA, Solovyev VV. Nsite, NsiteH and NsiteM computer tools for studying transcription regulatory elements. Bioinformatics. 2015;31(21):3544–5.
25. Clevenger J, Chu Y, Scheffler B, Ozias-Akins P. A developmental transcriptome map for allotetraploid *Arachis hypogaea*. Front Plant Sci. 2016;7:1446.
26. Dash S, Cannon EKS, Kalberer SR, Farmer AD, Cannon SB. PeanutBase and other bioinformatic resources for peanut. In: Stalker, H.T., Wilson, R.F.(Eds.), Peanuts: genetics, processing, and utilization. Academic Press and AOCS Press; 2016. p. 241–252. ISBN: 9781630670382 Copyright © 2016 AOCS Press. Published by Elsevier Inc.
27. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Biotechnol. 2011;12:323.
28. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30(4):772–80.
29. Darriba D, Taboada GL, Doallo R, Posada D. ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics. 2011;27(8):1164–5.
30. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-tree: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32(1):268–74.
31. Li W, Wang H, Yu D. *Arabidopsis* WRKY transcription factors WRKY12 and WRKY13 oppositely regulate flowering under short-day conditions. Mol Plant. 2016;9(11):1492–503.
32. Yin D, Ji C, Ma X, Li H, Zhang W, Li S, et al. Genome of an allotetraploid wild peanut *Arachis monticola*: a de novo assembly. Gigascience. 2018;7(6):66.
33. Chen X, Lu Q, Liu H, Zhang J, Hong Y, Lan H, et al. Sequencing of cultivated peanut, *Arachis hypogaea*, yields insights into genome evolution and oil improvement. Mol Plant. 2019;12(7):920–34.
34. Zhuang W, Chen H, Yang M, Wang J, Pandey MK, Zhang C, et al. The genome of cultivated peanut provides insight into legume karyotypes, polyploid evolution and crop domestication. Nat Genet. 2019;51(5):865–76.
35. Wang H, Chen W, Xu Z, Chen M, Yu D. Functions of WRKYs in plant growth and development. Trends Plant Sci. 2023;28(6):630–45.
36. Yu Y, Qi Y, Xu J, Dai X, Chen J, Dong CH, et al. Arabidopsis WRKY71 regulates ethylene-mediated leaf senescence by directly activating *EIN2*, *ORE1* and *ACS2* genes. Plant J. 2021;107(6):1819–36.
37. Ohto M, Onai K, Furukawa Y, Aoki E, Araki T, Nakamura K. Effects of sugar on vegetative development and floral transition in Arabidopsis. Plant Physiol. 2001;127(1):252–61.
38. Stratonovitch P, Semenov MA. Heat tolerance around flowering in wheat identified as a key trait for increased yield potential in Europe under climate change. J Exp Bot. 2015;66(12):3599–609.
39. Li Y, Cheng RY, Spokas KA, Palmer AA, Borevitz JO. Genetic variation for life history sensitivity to seasonal warming in *Arabidopsis thaliana*. Genetics. 2014;196(2):569–77.
40. Melzer S, Lens F, Gennen J, Vanneste S, Rohde A, Beeckman T. Flowering-time genes modulate meristem determinacy and growth form in *Arabidopsis thaliana*. Nat Genet. 2008;40(12):1489–92.
41. Ferrándiz C, Gu Q, Martienssen R, Yanofsky MF. Redundant regulation of meristem identify and plant architecture by FRUITFULL, APETALA1 and CAULIFLOWER. Development. 2000;127(4):725–34.
42. Feng K, Hou XL, Xing GM, Liu JX, Duan AQ, Xu ZS, et al. Advances in AP2/ERF super-family transcription factors in plant. Crit Rev Biotechnol. 2020;40(6):750–76.

43. Jofuku KD, den Boer BG, Van Montagu M, Okamuro JK. Control of Arabidopsis flower and seed development by the homeotic gene APETALA2. Plant Cell. 1994;6(9):1211–25.
44. Balanzà V, Martínez-Fernández I, Sato S, Yanofsky MF, Kaufmann K, Angenent GC, et al. Genetic control of meristem arrest and life span in Arabidopsis by a FRUITFULL-APETALA2 pathway. Nat Commun. 2018;9(1):565.
45. Martínez-Fernández I, Menezes de Moura S, Alves-Ferreira M, Ferrándiz C, Balanzà V. Identification of players controlling meristem arrest downstream of the FRUITFULL-APETALA2 pathway. Plant Physiol. 2020;184(2):945–59.
46. Hung FY, Shih YH, Lin PY, Feng YR, Li C, Wu K. WRKY63 transcriptional activation of *COOLAIR* and *COLDAIR* regulates vernalization-induced flowering. Plant Physiol. 2022;190(1):532–47.

Publisher's Note