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GENOME-WIDE IDENTIFICATION AND CHARACTERIZATION OF THE *WRKY* GENE FAMILY IN WHITE FONIO (*DIGITARIA EXILIS* STAPF)

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Abstract

White fonio (*Digitaria exilis* Stapf), a traditional West African cereal, exhibits valuable agronomic traits including a short growth cycle and pronounced drought tolerance. However, researchers have poorly characterized its molecular biology, especially regarding key regulatory gene families such as WRKY transcription factors. This study systematically identified and analyzed the *WRKY* gene family in *D. exilis* to clarify its potential role in stress adaptation. We screened the complete *D. exilis* genome using BLASTP and HMMER, identifying 112 non-redundant *DeWRKY* genes. Our comprehensive bioinformatic analyses encompassed phylogenetic classification, chromosomal localization, examination of conserved motifs and gene structures, prediction of cis-acting elements, and collinearity assessment. Unevenly distributed across 18 chromosomes, the 112 *DeWRKY* genes fall into phylogenetic Groups I, II, and III, with Group II constituting the largest clade (44%). This phylogenetic profile aligned with the structure observed in model plants such as *Arabidopsis thaliana*. Conserved motif analysis detected Motif 1 (the WRKY domain) in all members, with Motif 2 and Motif 4 also appearing frequently. Promoter analysis uncovered abundant cis-elements for stress and hormone responses, especially those involved in drought and abscisic acid signaling. Intra-genomic collinearity analysis highlighted the major contribution of segmental duplications to the expansion of the *DeWRKY* family. In summary, this work presents the first genome-wide characterization of the *WRKY* family in *D. exilis*, addressing a notable knowledge gap. Our findings provide important insights into the genomic architecture and potential regulatory functions of *DeWRKY* genes in environmental stress responses, thereby establishing a theoretical basis for future functional studies and molecular breeding programs targeting enhanced stress tolerance in crops.

Key words: *Digitaria exilis*; *WRKY* gene family; Bioinformatics

Introduction

WRKY transcription factors constitute one of the largest transcription factor families in plants, playing pivotal roles in plant growth, development, and responses to various stresses (Li *et al.*, 2025; Wei *et al.*, 2025; Yang *et al.*, 2025). As a core regulatory hub in plants, WRKY proteins coordinate the dynamic balance between growth and stress defense through multi-dimensional molecular mechanisms (Ma & Hu, 2024; Zeng *et al.*, 2024). Members of this family are characterized by a highly conserved N terminal WRKYGQK motif, in which the amino acids W, R, and K are invariant (Yamasaki *et al.*, 2012). At the C terminus of the WRKY domain, two types of zinc finger structures are present: C_2HC(C-X_7-C-X_{23}-HX-C) and C_2H_2(C-$X_{4\text{-}5}$-C-$X_{22\text{-}23}$H-X-H) (Eulgem *et al.*, 2000). Based on the number of WRKY domains and the features of the zinc finger motif, the *WRKY* gene family is classified into three major groups, namely Group I to III (Rushton *et al.*, 2010). The first *WRKY* gene was identified in sweet potato in 1994 (Ishiguro & Nakamura, 1994). Since then, *WRKY* genes have been discovered in many other plant species (Xie *et al.*, 2005; Huang *et al.*, 2012). Accumulating evidence indicates that WRKY transcription factors are crucial regulators in plant abiotic stress responses (Wu *et al.*, 2009; Jiang *et al.*, 2017; Javed & Gao, 2023). For instance, *AtWRKY53* was found to negatively regulate drought tolerance in *A. thaliana* by mediating stomatal movement (Sun & Yu, 2015). Overexpression of *TaWRKY2* and *TaWRKY19* enhanced salt and drought tolerance in transgenic *A. thaliana* (NIU *et al.*, 2012). Overexpression of *SlWRKY6* confers enhanced drought tolerance in tomato (*Solanum lycopersicum*) via boosted antioxidant defenses and ABA-induced stomatal closure (Chen *et al.*, 2024).

White fonio (*D. exilis* Stapf), an indigenous traditional crop in West Africa belong to the family Ranunculaceae, holds significant agricultural and nutritional value (Wang *et al.*, 2021). It has a short growth cycle, maturing as early as 8 weeks after sowing, and exhibits strong drought tolerance along with the ability to grow in poor soils (Gigou *et al.*, 2009; Ballogou *et al.*, 2013). Although the grains are small, they are nutrient dense, rich in protein and dietary fiber. Despite its relatively low yield, this West African crop is harvested in early summer, filling an important dietary gap before the maturation of staples such as sorghum or pearl millet in the region, and thus serves as a vital food source in West Africa (Temple & Bassa, 1991; Fanou *et al.*, 2009). However, compared with traditional cereal crops such as rice and wheat, molecular studies on white fonio remain limited, particularly concerning gene family identification and functional characterization. Whether *WRKY* genes contribute to its drought resistance remains unexplored.

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Therefore, this study aims to identify the *WRKY* gene family in *D. exilis*, which will fill a knowledge gap regarding *WRKY* genes in this species. The findings are expected to provide new insights into the molecular basis of its advantageous traits, such as drought tolerance, and to offer a theoretical foundation for crop improvement and stress resistance breeding (Yuan *et al.*, 2025).

Materials and Methods

Identification of *WRKY* gene family in *D. exilis*: The whole-genome sequence and annotation files of *D. exilis* were downloaded from the Ensembl Plants database (https://plants.ensembl.org/Digitaria_exilis/Info/Index). To identify all potential WRKY family members, a two-step strategy was employed. First, a local BLASTP search was performed using the known WRKY protein sequences from *A. thaliana* downloaded from TAIR website (http://www.arabidopsis.org/) as queries against the *D. exilis* proteome, with an E-value cutoff of $1e^{-5}$. Second, all candidate sequences obtained from the BLAST search were further subjected to domain validation using the HMMER (v3.3.2) software. The hidden Markov model (HMM) profile of the WRKY domain (PF03106) was retrieved from the Pfam database (http://pfam.xfam.org/). Protein sequences that lacked a complete WRKY domain or exhibited abnormal domain architecture were excluded. The remaining non-redundant sequences containing an intact WRKY domain were defined as the *WRKY* gene family in *D. exilis*.

Analysis of physicochemical properties: The physicochemical parameters of the identified WRKY proteins, including molecular weight (MW), theoretical isoelectric point (pI), grand average of hydropathicity (GRAVY), and instability index, were predicted using the ExPASy platform. Subcellular localization was predicted using Plant-mPLoc.

Conserved motif, domain, and gene structure analysis: The conserved motifs within the WRKY proteins were identified using the MEME suite (v5.5.2) with the following parameters: maximum number of motifs set to 15, and optimum motif width set between 6 and 50 amino acids. The conserved WRKY domain was visualized by aligning protein sequences using Clustal W and displayed with GeneDoc. The gene structure (exon-intron organization) was determined by comparing the coding sequences (CDS) with their corresponding genomic DNA sequences using TBtools (v2.376) software.

Phylogenetic analysis: Full-length protein sequences of WRKY members from *D. exilis* and *A. thaliana* were aligned using Clustal W with default parameters. An unrooted phylogenetic tree was constructed using the MEGA (v12.1.2) software with the neighbor-joining (NJ) method. Bootstrap analysis was performed with 1000 replicates to assess the reliability of the tree branches. The tree was subsequently classified into different groups and subgroups based on established classification criteria for *WRKY* families.

Chromosomal localization and synteny analysis: The physical positions of *DeWRKY* genes were extracted from the genome annotation file and mapped onto the *D. exilis* chromosomes using TBtools (v2.376) software. For synteny analysis, MCScanX toolkit in TBtools (v2.376) software was employed to identify syntenic blocks within the *D. exilis* genome (intra-species synteny) and between *D. exilis* and *A. thaliana* genomes (inter-species synteny). The synteny relationships were visualized using Advanced Circos in TBtools (v2.376) software.

Cis-acting regulatory element analysis: The 2000 bp promoter sequences upstream of the transcription start site (TSS) for each *DeWRKY* gene were extracted using TBtools (v2.376) software, and employ the PlantCare website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) to predict promoter cis-acting elements.

Results

Identification of members of the *DeWRKY* gene family and analysis of their protein physicochemical properties: This study identified a total of 112 sequences containing conserved WRKY domains and renamed them as *DeWRKY001* to *DeWRKY112* based on their chromosomal positions (Table 1). As shown in Table 1, physicochemical property analysis revealed that the number of amino acids in the encoded proteins ranged from 65 (*DeWRKY018*) to 826 (*DeWRKY006*), with an average of 345. The molecular weight ranged from 7,708.35 Da (*DeWRKY018*) to 93,244.68 Da (*DeWRKY006*), with an average of 37,025.35 Da. The isoelectric point (pI) ranged from 4.39 to 11.71, with 66 proteins being acidic and 46 proteins being basic. The instability index ranged from 34.15 to 88.20, with three proteins (*DeWRKY018*, *DeWRKY071*, and *DeWRKY091*) having an instability index below 40, indicating that they were stable proteins. The remaining 109 proteins had an instability index above 40, indicating that most of them were unstable proteins. The GRAVY values were all negative, indicating that *D. exilis* WRKY proteins were hydrophilic. The subcellular localization prediction analysis of the *D. exilis* WRKY protein revealed that 93 family members were located in the nucleus, 9 family members in the chloroplasts, 4 family members in the cytoplasm, 2 family members in the extracellular space, 2 family members in the mitochondria, and one each in peroxisomes and endoplasmic reticulum.

Chromosomal localization analysis of the *DeWRKY* gene family: Based on the reference genome of *D. exilis*, we analyzed the chromosomal localization of the *WRKY* gene family (Fig. 1). The 111 *DeWRKY* genes were distributed across 18 chromosomes, with only 1 gene (*DeWRKY*112) failing to be mapped to a specific location. Chromosome 3B was the most densely populated with 17 *WRKY* genes , while chromosome 6A was the lowest density populated with only one *WRKY* gene. From the results of chromosome localization analysis, it could be observed that the *DeWRKY* gene density was highest on chromosomes 3A and 3B, followed by chromosomes 5A and 5B, with a clear bias in their distribution.

Table 1. Identification of *DeWRKY* gene family members and analysis of their protein physicochemical properties.

Gene name	Gene ID	No. of amino acids	Molecular weight	Isoelectric point	Coefficient of instability	Grand average of hydropathicity	Subcellular localization
DeWRKY001	*Dexi1A01G0006590*	387	41988.25	8.98	58.26	-0.658	nucl
DeWRKY002	*Dexi1A01G0024150*	383	39329.03	5.23	47.35	-0.348	nucl
DeWRKY003	*Dexi1B01G0023190*	387	39644.38	5.14	47.05	-0.353	nucl
DeWRKY004	*Dexi1B01G0027480*	567	60097.20	5.07	50.65	-0.712	nucl
DeWRKY005	*Dexi2A01G0011550*	308	32296.29	5.13	60.10	-0.180	chlo
DeWRKY006	*Dexi2A01G0014920*	826	93244.68	8.57	44.90	-0.413	nucl
DeWRKY007	*Dexi2A01G0015390*	283	29816.44	4.92	53.29	-0.202	nucl
DeWRKY008	*Dexi2A01G0015400*	268	28045.38	5.61	59.12	-0.230	nucl
DeWRKY009	*Dexi2A01G0017640*	239	26562.13	8.31	46.00	-0.533	nucl
DeWRKY010	*Dexi2A01G0031450*	306	32642.95	5.42	60.62	-0.799	nucl
DeWRKY011	*Dexi2A01G0036950*	355	36419.31	7.00	63.12	-0.366	nucl
DeWRKY012	*Dexi2B01G0010950*	332	35058.35	5.27	57.88	-0.273	chlo
DeWRKY013	*Dexi2B01G0013940*	306	34612.57	8.99	38.36	-0.547	nucl
DeWRKY014	*Dexi2B01G0014370*	281	29519.01	5.00	60.05	-0.297	nucl
DeWRKY015	*Dexi2B01G0014380*	328	34921.93	6.06	57.75	-0.375	nucl
DeWRKY016	*Dexi2B01G0030340*	362	38575.39	5.32	59.69	-0.797	nucl
DeWRKY017	*Dexi3A01G0000900*	206	22486.60	8.53	56.05	-0.321	nucl
DeWRKY018	*Dexi3A01G0000910*	65	7708.350	9.05	34.15	-1.409	nucl
DeWRKY019	*Dexi3A01G0000930*	281	31147.84	6.39	53.22	-0.678	nucl
DeWRKY020	*Dexi3A01G0000950*	272	29846.78	5.19	78.17	-0.806	nucl
DeWRKY021	*Dexi3A01G0010530*	533	55242.10	5.99	59.40	-0.524	nucl
DeWRKY022	*Dexi3A01G0015280*	322	34776.87	5.51	57.45	-0.594	nucl
DeWRKY023	*Dexi3A01G0016000*	297	32250.05	5.48	46.60	-0.489	mito
DeWRKY024	*Dexi3A01G0016220*	587	61460.34	6.19	49.65	-0.576	nucl
DeWRKY025	*Dexi3A01G0020940*	545	59625.32	5.33	63.10	-0.662	nucl
DeWRKY026	*Dexi3A01G0020950*	276	30757.35	6.76	72.75	-0.792	nucl
DeWRKY027	*Dexi3A01G0021130*	575	60605.38	6.24	54.44	-0.577	nucl
DeWRKY028	*Dexi3A01G0027120*	321	34354.75	7.19	63.85	-0.760	nucl
DeWRKY029	*Dexi3A01G0027470*	319	33979.46	5.60	46.59	-0.516	nucl
DeWRKY030	*Dexi3A01G0027480*	399	42614.62	6.30	54.85	-0.443	extra
DeWRKY031	*Dexi3A01G0030730*	407	43074.28	6.67	53.43	-0.777	nucl
DeWRKY032	*Dexi3A01G0033470*	353	37623.91	10.38	55.01	-0.438	nucl
DeWRKY033	*Dexi3B01G0001020*	227	24362.92	8.15	64.54	-0.678	nucl
DeWRKY034	*Dexi3B01G0001030*	302	34055.89	7.63	43.23	-0.346	nucl
DeWRKY035	*Dexi3B01G0001040*	286	32243.48	6.34	60.20	-0.993	nucl
DeWRKY036	*Dexi3B01G0001050*	285	31420.05	6.00	51.73	-0.692	nucl
DeWRKY037	*Dexi3B01G0001070*	100	11230.59	10.15	88.20	-1.120	nucl
DeWRKY038	*Dexi3B01G0010710*	528	54683.75	6.39	56.30	-0.459	nucl
DeWRKY039	*Dexi3B01G0016290*	292	31677.37	5.59	47.70	-0.477	mito
DeWRKY040	*Dexi3B01G0016560*	568	59169.85	6.68	49.09	-0.538	nucl
DeWRKY041	*Dexi3B01G0018660*	455	48801.05	9.39	45.06	-0.170	nucl
DeWRKY042	*Dexi3B01G0021440*	241	26332.45	8.38	69.65	-0.747	nucl
DeWRKY043	*Dexi3B01G0021450*	266	28867.76	5.09	72.17	-0.678	nucl
DeWRKY044	*Dexi3B01G0021460*	274	30444.97	6.32	73.13	-0.766	nucl
DeWRKY045	*Dexi3B01G0027940*	324	34691.98	7.13	60.32	-0.814	nucl
DeWRKY046	*Dexi3B01G0028450*	319	34012.46	5.42	47.10	-0.521	nucl
DeWRKY047	*Dexi3B01G0028460*	399	42638.57	6.09	55.55	-0.462	nucl
DeWRKY048	*Dexi3B01G0032210*	414	43799.26	6.87	53.62	-0.739	nucl
DeWRKY049	*Dexi3B01G0034930*	351	37408.65	10.36	55.48	-0.429	nucl
DeWRKY050	*Dexi4A01G0003160*	584	60109.54	8.09	50.71	-0.425	nucl
DeWRKY051	*Dexi4A01G0004180*	373	39652.55	5.91	54.41	-0.566	nucl
DeWRKY052	*Dexi4A01G0014060*	226	24319.55	10.10	59.52	-0.705	nucl
DeWRKY053	*Dexi4A01G0014070*	373	39768.82	5.79	65.56	-0.853	nucl
DeWRKY054	*Dexi4A01G0019050*	338	36492.28	9.30	52.02	-0.499	cyto
DeWRKY055	*Dexi4B01G0006730*	374	39885.76	6.16	51.04	-0.652	nucl
DeWRKY056	*Dexi4B01G0014070*	396	43269.87	5.44	58.49	-0.963	nucl
DeWRKY057	*Dexi4B01G0014080*	403	42348.67	5.26	60.83	-0.703	nucl

Table 1. (Cont'd.).

Gene name	Gene ID	No. of amino acids	Molecular weight	Isoelectric point	Coefficient of instability	Grand average of hydropathicity	Subcellular localization
DeWRKY058	*Dexi4B01G0018580*	340	36732.50	9.38	57.87	-0.553	nucl
DeWRKY059	*Dexi5A01G0003670*	523	53308.43	6.92	45.65	-0.284	nucl
DeWRKY060	*Dexi5A01G0013730*	578	60857.53	6.75	46.70	-0.574	cyto
DeWRKY061	*Dexi5A01G0020810*	343	36745.25	6.36	51.15	-0.567	nucl
DeWRKY062	*Dexi5A01G0022190*	308	32350.28	7.64	49.97	-0.431	nucl
DeWRKY063	*Dexi5A01G0025920*	223	22448.59	4.39	54.91	-0.256	cyto
DeWRKY064	*Dexi5A01G0026990*	247	26850.63	4.52	87.83	-0.702	nucl
DeWRKY065	*Dexi5A01G0030790*	258	28989.74	5.43	68.24	-0.938	nucl
DeWRKY066	*Dexi5A01G0030820*	304	32284.87	5.54	56.36	-0.411	nucl
DeWRKY067	*Dexi5A01G0031090*	356	37390.31	5.08	70.01	-0.821	nucl
DeWRKY068	*Dexi5A01G0032270*	194	20808.85	5.21	66.50	-0.761	nucl
DeWRKY069	*Dexi5B01G0003710*	523	53414.62	7.24	47.49	-0.303	nucl
DeWRKY070	*Dexi5B01G0013860*	570	59996.64	6.60	46.87	-0.569	E.R.
DeWRKY071	*Dexi5B01G0018770*	344	35764.37	9.51	36.97	-0.372	nucl
DeWRKY072	*Dexi5B01G0020910*	348	37156.72	6.24	48.28	-0.530	nucl
DeWRKY073	*Dexi5B01G0022310*	296	31078.84	6.19	47.44	-0.338	chlo
DeWRKY074	*Dexi5B01G0026210*	235	23446.69	4.55	57.42	-0.257	cyto
DeWRKY075	*Dexi5B01G0027230*	298	32566.92	4.99	86.98	-0.869	nucl
DeWRKY076	*Dexi5B01G0031070*	258	29102.85	5.44	65.58	-0.974	nucl
DeWRKY077	*Dexi5B01G0031100*	311	32789.44	5.53	54.90	-0.415	nucl
DeWRKY078	*Dexi5B01G0031350*	464	50335.69	6.61	66.69	-1.017	nucl
DeWRKY079	*Dexi5B01G0039900*	415	45453.88	9.22	72.95	-0.629	nucl
DeWRKY080	*Dexi6A01G0019010*	188	20922.51	8.19	48.79	-0.703	nucl
DeWRKY081	*Dexi6B01G0007390*	317	32627.76	10.09	56.31	-0.492	nucl
DeWRKY082	*Dexi6B01G0015930*	482	53251.08	5.44	57.86	-0.691	nucl
DeWRKY083	*Dexi6B01G0017930*	188	20821.35	7.07	49.25	-0.662	nucl
DeWRKY084	*Dexi7A01G0002110*	326	34929.36	9.68	54.42	-0.654	nucl
DeWRKY085	*Dexi7A01G0017530*	430	44567.84	4.77	54.76	-0.365	nucl
DeWRKY086	*Dexi7A01G0018080*	371	38922.34	9.87	53.71	-0.274	pero
DeWRKY087	*Dexi7B01G0003040*	324	34758.18	9.68	60.38	-0.693	nucl
DeWRKY088	*Dexi7B01G0009380*	311	36097.88	9.72	56.50	-0.856	nucl
DeWRKY089	*Dexi7B01G0018210*	423	44138.49	4.80	55.06	-0.391	nucl
DeWRKY090	*Dexi7B01G0018730*	274	28784.82	10.53	61.44	-0.393	nucl
DeWRKY091	*Dexi8A01G0000940*	178	20151.79	8.32	37.55	-0.448	chlo
DeWRKY092	*Dexi8A01G0000970*	284	31186.76	6.39	49.03	-0.690	nucl
DeWRKY093	*Dexi8A01G0000990*	271	29809.84	4.98	76.43	-0.758	nucl
DeWRKY094	*Dexi8A01G0008880*	297	32625.89	4.62	51.81	-0.244	extra
DeWRKY095	*Dexi8B01G0000850*	328	36016.42	5.99	65.81	-0.478	nucl
DeWRKY096	*Dexi8B01G0000870*	149	17236.37	9.68	43.18	-1.023	nucl
DeWRKY097	*Dexi8B01G0000900*	289	31956.76	7.05	53.90	-0.720	nucl
DeWRKY098	*Dexi8B01G0007200*	118	12882.55	9.07	51.41	-0.636	nucl
DeWRKY099	*Dexi9A01G0005090*	348	37093.49	9.40	61.33	-0.272	nucl
DeWRKY100	*Dexi9A01G0008010*	495	52462.60	8.75	58.26	-0.581	chlo
DeWRKY101	*Dexi9A01G0008130*	494	52927.73	10.07	67.63	-0.382	nucl
DeWRKY102	*Dexi9A01G0009340*	353	37773.95	9.98	52.42	-0.474	nucl
DeWRKY103	*Dexi9A01G0015930*	307	32838.18	10.06	53.24	-0.516	nucl
DeWRKY104	*Dexi9A01G0027680*	419	45198.13	5.80	51.96	-0.606	nucl
DeWRKY105	*Dexi9A01G0037290*	219	23886.39	8.88	68.40	-0.743	nucl
DeWRKY106	*Dexi9B01G0007580*	590	63210.51	8.35	48.52	-0.594	chlo
DeWRKY107	*Dexi9B01G0008960*	358	38390.87	10.07	56.77	-0.477	nucl
DeWRKY108	*Dexi9B01G0014070*	259	27847.07	9.39	59.52	-0.115	chlo
DeWRKY109	*Dexi9B01G0026600*	420	45501.54	5.78	48.97	-0.592	nucl
DeWRKY110	*Dexi9B01G0030790*	261	27248.91	11.71	73.50	-0.261	chlo
DeWRKY111	*Dexi9B01G0036070*	219	23824.28	8.71	67.13	-0.723	nucl
DeWRKY112	*DexiUA01G0006450*	332	35058.35	5.27	57.88	-0.273	chlo

Note: cyto: cytoplasm; chlo: chloroplast; mito: mitochondria; plas: plasmodesmata; nucl: nucleus; pero: peroxisome; vacu: vacuoles; extra: extracellular; E.R.: Endoplasmic reticulum

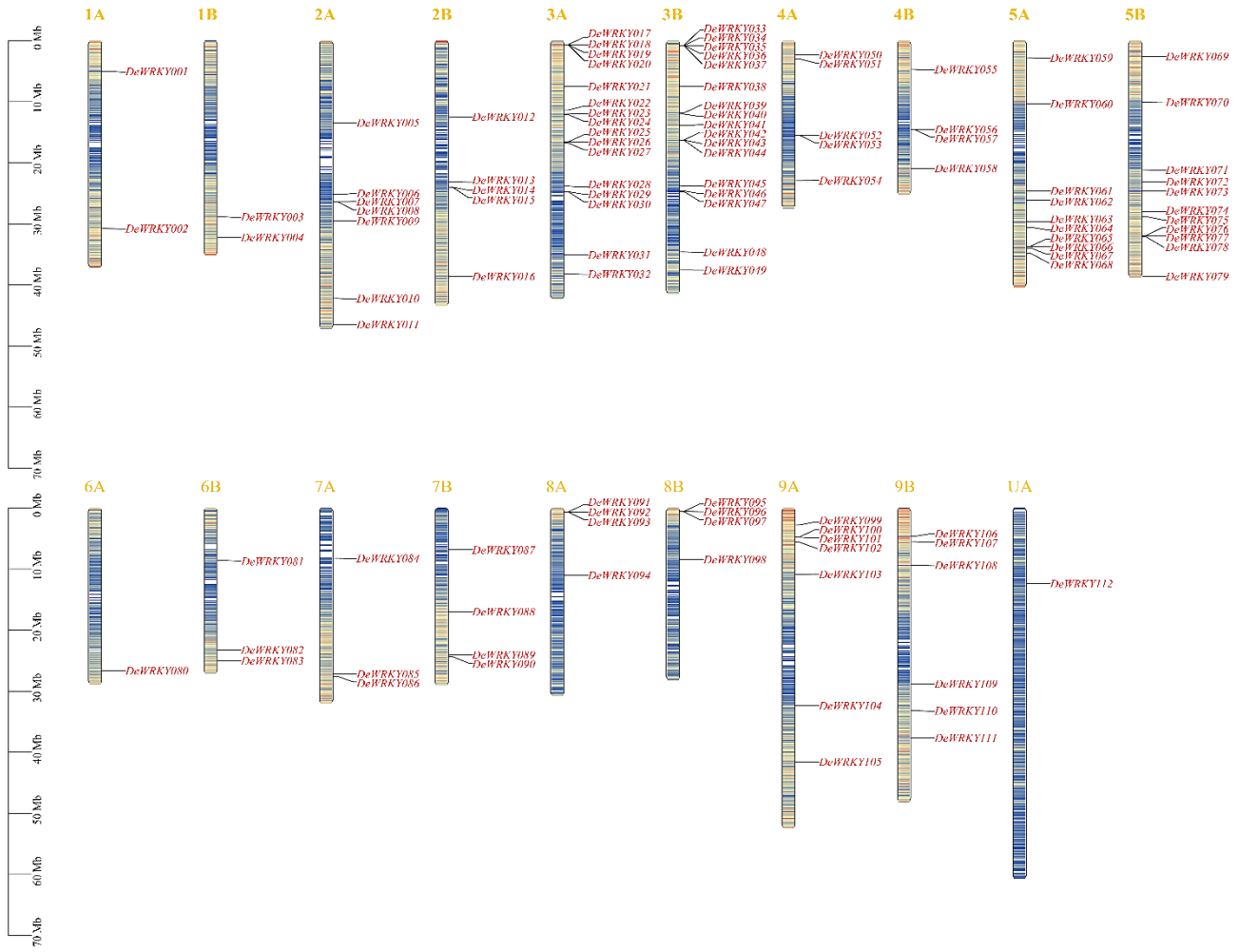

Fig. 1. Chromosomal distribution of *DeWRKY* gene family members.

Conserved motif and gene structure analysis of *DeWRKY* gene family members: Conserved motif analysis of the *DeWRKY* family proteins (Fig. 2) identified a total of 10 motifs. Members within the same phylogenetic clade exhibited highly consistent motif arrangements, reflecting their functional conservation. All 112 family members contained Motif 1 (the canonical WRKY domain), which was therefore identified as the core motif. Motif 2 was identified in 87 family members, and Motif 4 was detected in 84. It is speculated that Motif 1, Motif 2, and Motif 4 together constituted the most critical structural elements in the evolution of the *DeWRKY* family.

Gene structure analysis (Fig. 2) revealed that the number of exons in *DeWRKY* family members ranged from 1 to 11, and introns from 2 to 12. Among them, *DeWRKY081* and *DeWRKY098* contained only 2 exons and 1 intron; *DeWRKY108* possessed the most complex gene structure, with 12 exons and 11 intron.

Phylogenetic analysis of the *DeWRKY* gene family: Using MEGA (v12.1.2) software, a phylogenetic tree was constructed based on the protein sequences of 112 *D. exilis WRKY* genes (*DeWRKY*) and 72 *A. thaliana WRKY* genes (*AtWRKY*) (Fig. 4). Based on the type of N-terminus WRKY domain and the zinc finger motif at the C-terminus, as well as the classification criteria in *A. thaliana*, all 112 *DeWRKY* genes can also be divided into three major categories (Fig. 3): Group I contains 16 members; Group II contains the most members, totaling 50, which can be further divided into five subgroups: IIa (4 members), IIb (11 members), IIc (3 members), IId (15 members), and IIe (17 members); Group III contains 46 members.

Cis-Acting Element Analysis of the *DeWRKY* Gene Family: The promoter region sequence of 2000 bp upstream of the *DeWRKY* gene was obtained, and its cis-acting elements were analyzed (Fig.3). In terms of hormone response, abscisic acid (103 genes), auxin (66 genes), gibberellin (76 genes), and salicylic acid (57 genes), MeJA (105genes) response elements were widely distributed among the family members. Moreover, all genes contained light responsive element, 88 genes contained anaerobic induced sites, 63 genes contained drought-induced sites. The analysis results of cis-acting elements revealed that hormone responsive cis-acting elements were widely distributed, especially abscisic acid and MeJA-responsive elements, with MeJA-responsive elements showing the strongest preference. Defense and stress responsiveness elements in stress responsive elements were almost undistributed. Growth and development elements, except for light responsive element and anaerobic induction elements, were also almost undistributed. It is particularly noteworthy that among various cis-acting elements, MeJA-responsiveness and light responsive elements exhibited the widest distribution and the strongest preference.

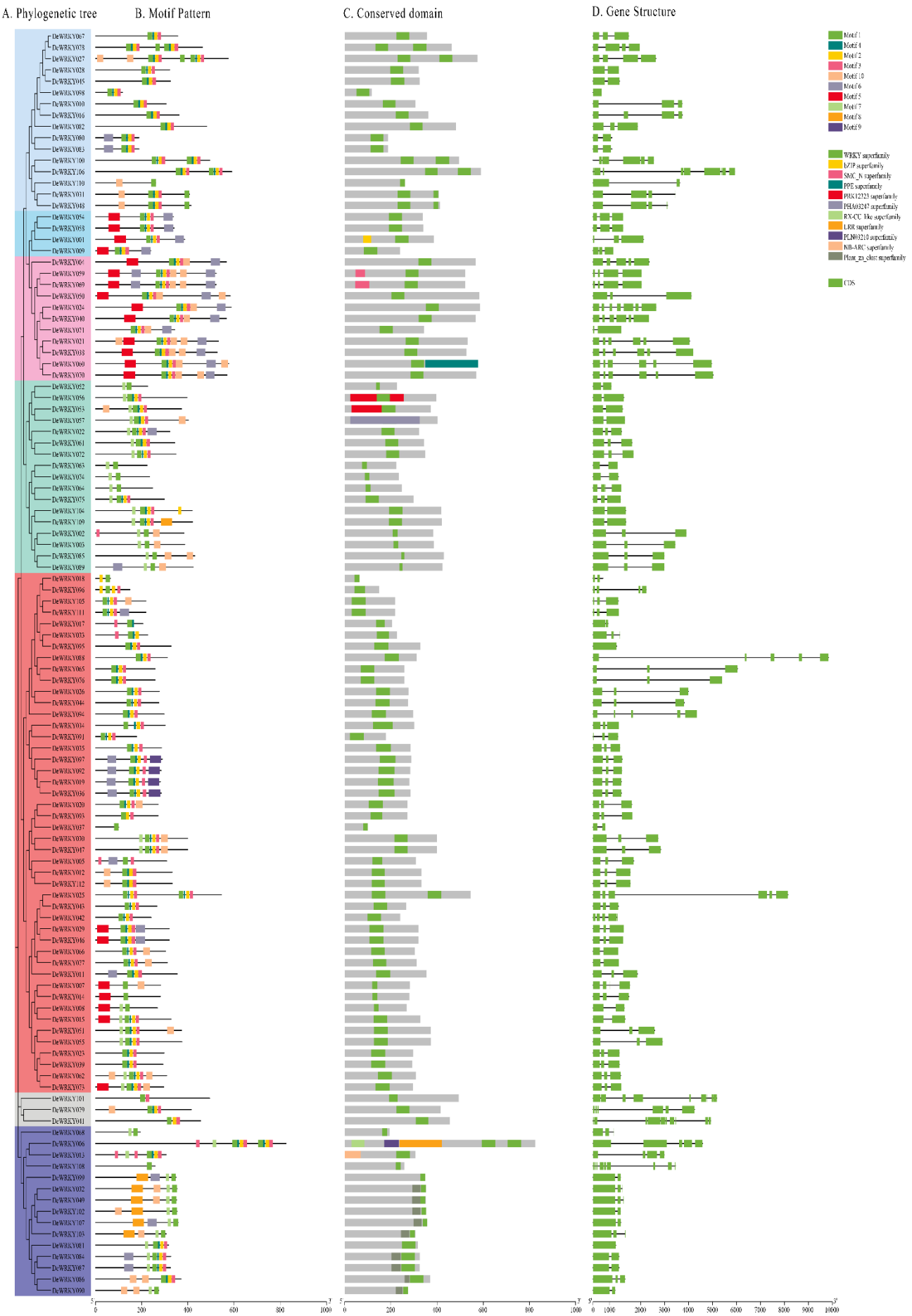

Fig. 2. Motifs pattern and conserved domain and gene structure analysis of *DeWRKY* gene family members.

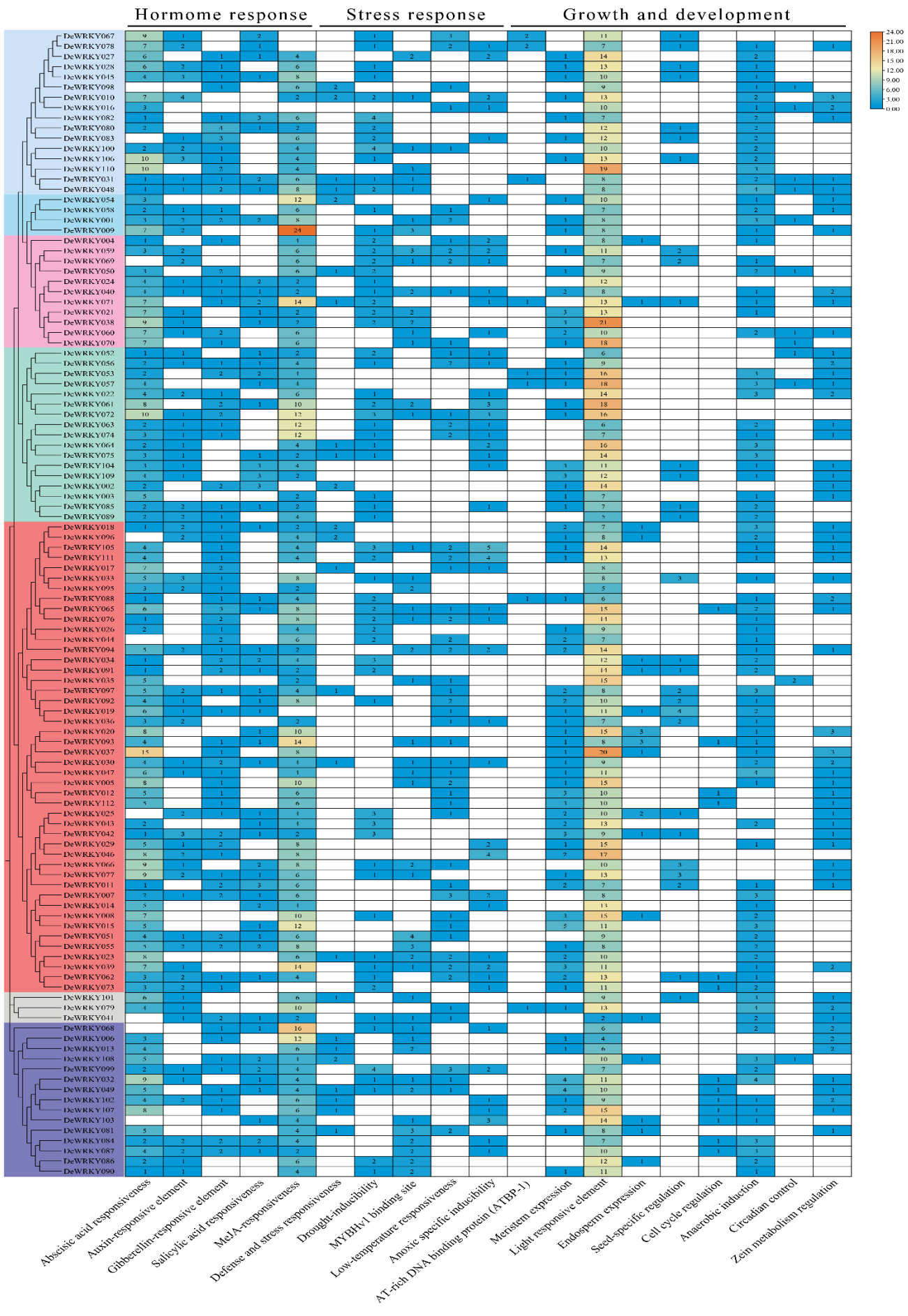

Fig. 3. Analysis of cis-acting elements in the promoter regions of *DeWRKY* genes.

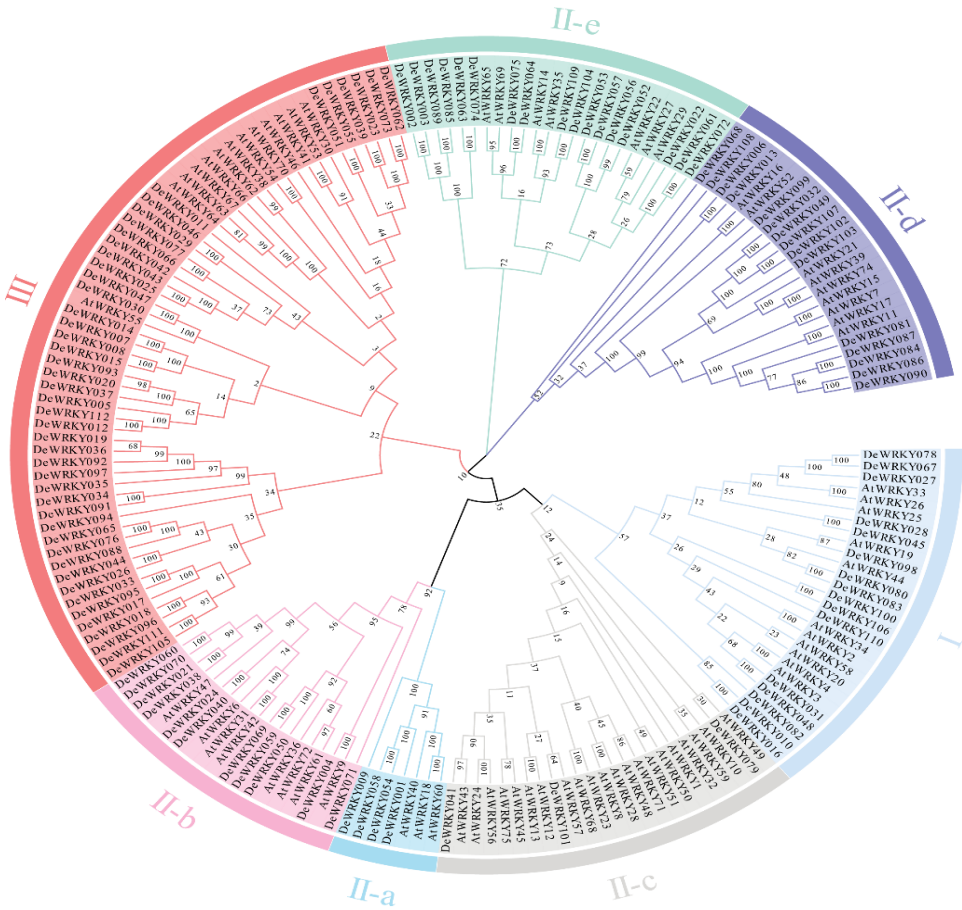

Fig. 4. Phylogenetic analysis of *Digitaria exilis* and *Arabidopsis thaliana* WRKYs proteins.

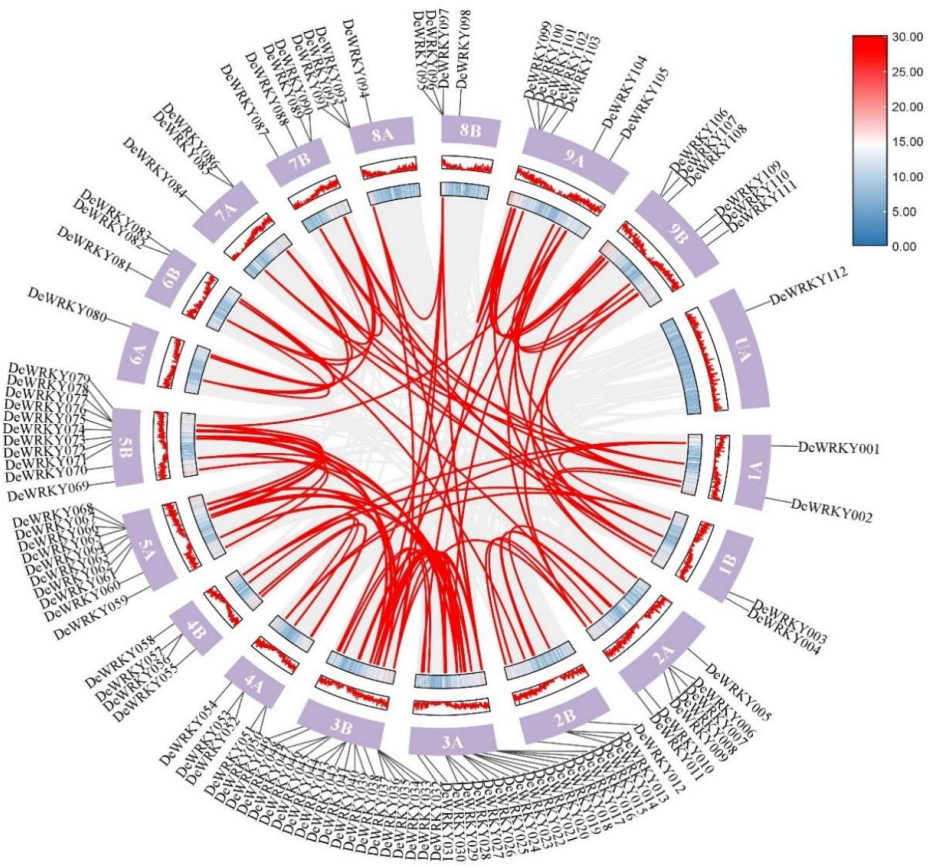

Fig. 5. Collinearity analysis among of *DeWRKY* gene family members.

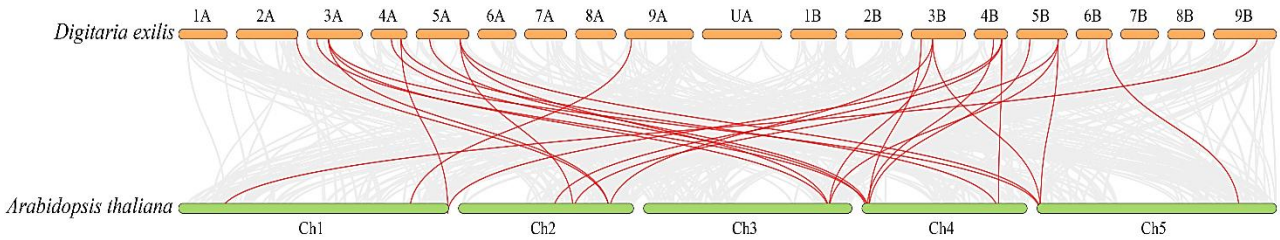

Fig. 6. Collinearity analysis of *Digitaria exilis* and *Arabidopsis thaliana WRKY* genes.

Collinearity analysis among *DeWRKY* gene family members: The collinearity analysis (Fig. 5) revealed 118 pairs of segmental duplication events among 112 *DeWRKY* genes, indicating that this gene family had undergone extensive segmental duplication in the *D. exilis* genome. Cross-species collinearity comparison between *D. exilis* and *A. thaliana* (Fig. 6) identified 27 pairs of orthologous *WRKY* gene pairs. Meanwhile, the results of intraspecific collinearity analysis indicated that most of the homologous gene pairs within the *DeWRKY* gene family were distributed between subgenomes of chromosomes, potentially related to the polyploidization event of *D. exilis*.

Discussion

In this study, we systematically identified the WRKY transcription factor family in white fonio (*D. exilis*), revealing a total of 112 *DeWRKY* genes, which exhibited a notably uneven distribution across 18 chromosomes. Phylogenetic analysis classified the *DeWRKY* genes into groups I, II, and III, with group II being the most abundant (approximately 44%). This classification pattern aligns closely with the *WRKY* family structures in model plants such as *A. thaliana*, supporting the conservation of the *WRKY* gene family during plant evolution (Wu *et al.*, 2005). Chromosomal localization and intra species synteny analysis highlighted the predominant role of gene duplication events in the expansion of the *DeWRKY* family. Multiple gene clusters, such as high density regions on chromosome 3A and chromosome 3B, suggested that whole genome or segmental duplications served as key mechanisms for family amplification. Conserved motif analysis and cis regulatory element prediction further indicated potential functions of *DeWRKY* genes in environmental adaptation, particularly drought tolerance, which is highly consistent with the ecological characteristics of white fonio, including its short growth cycle and drought resistanc (Gigou *et al.*, 2009).

However, this study has several limitations. First, functional experimental validation was lacking; all analyses were based on bioinformatic predictions, and the expression patterns or functions of *DeWRKY* genes were not verified through methods such as qRT-PCR, transgenic approaches, or CRISPR Cas9. Therefore, their roles in stress responses cannot be directly demonstrated. Second, expression profile data are missing; transcriptomic data from tissue specific contexts (e.g., roots, leaves, flowers) or under stress conditions (e.g., drought, salinity) were not integrated, leaving inferences about the functional differentiation of *DeWRKY* genes without empirical support.

Based on the above shortcomings, future research should focus on the following directions. First, functional validation and expression analysis: time course expression analysis under key stress conditions (e.g., PEG simulated drought), combined with tissue specific sampling (roots, leaves, seeds), should be conducted to clarify the expression regulatory networks of *DeWRKY* genes. Second, cross species functional exploration: extending research to crops such as sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*), constructing a comparative phylogenetic tree of *WRKY* genes across multiple species, and screening conserved stress responsive genes for molecular breeding applications.

Conclusion

This study conducted an analysis of the *WRKY* gene family in *D. exilis*, filling a gap in the molecular biology research of traditional West African crops. A total of 112 *DeWRKY* genes were identified, which are unevenly distributed across 18 chromosomes. The physicochemical properties of the encoded proteins, phylogenetic evolution, chromosomal distribution, conserved motifs, and cis regulatory elements were examined, leading to a relatively systematic identification of the *DeWRKY* gene family in *D. exilis*. By integrating phylogenetic, structural, and functional predictive analyses, this work provides new insights into the drought tolerant adaptation of this species and lays a theoretical foundation for stress resistant crop breeding. Future experimental validation would significantly strengthen the potential agricultural application value of this gene family.

Conflict of Interest: The author declare no conflicts of interest.

Author Contribution: Data analysis, article writing and subsequent work, Baolin Han. The Author have read and agreed to the published version of the manuscript.

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