

Genome-wide identification and analysis of WRKY gene family in jute (*Corchorus capsularis*)

Lilan Zhang

Fujian Agriculture and Forestry University

Xuebei Wan

Fujian Agriculture and Forestry University

Yi Xu

Fujian Agriculture and Forestry University

Sylvain Niyitanga

Fujian Agriculture and Forestry University

Jianmin Qi

Fujian Agriculture and Forestry University

Liwu Zhang (✉ zhang_liwu@hotmail.com)

Fujian Agriculture and Forestry University <https://orcid.org/0000-0001-8465-2476>

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Abstract

Background WRKY transcription factor is a kind of transcription factor which plays an important role in plant response to biotic, abiotic stress, plant growth and development. However, little information was available about the WRKY genes in jute (*Corchorus capsularis*).

Results In the present study, 43 jute WRKY (CcWRKY) genes were identified by using Pfam database domain search and BLAST homology alignment based on the transcriptome data of jute. And the gene structure, phylogeny, conserved domain and three-dimensional structure of protein were also analyzed by GSDS2.0, MEGA7.0, DNAMAN5.0, WebLogo 3 and SWISS-MODEL bioinformatics tools. According to the WRKY conserved domain features and the evolution analysis with *Arabidopsis thaliana* , 43 members were divided into three classes: I, II and III containing 9, 28, 6 members, respectively. According to the evolutionary relationship, class II further divided into five subclasses: II-a (2), II-b (7), II-c (7), II-d (6) and II-e (6). Genetic structure analysis showed that exon and intron number of CcWRKY genes had high variability (3-11 exons), even within the same subgroup. Most of the CcWRKY genes were expressed in different tissues, but they were mainly expressed in stem bark and stem stick. After GA 3 stress, the expression of most WRKY genes in GA 3 -sensitive variety "Aidianyehuangma" was significantly different from that of normal variety "Huangma 179". These results indicated that CcWRKY genes play an important role in gibberellin biosynthesis pathway and fiber development.

Conclusions CcWRKY proteins are highly conserved, the length of the gene sequence and the number of introns varied widely, all WRKY genes showed a variety of expression patterns in different tissues, most of the WRKY genes responded to GA 3 stress, which play an important role in gibberellin biosynthesis pathway and bast fiber development.

Background

The WRKY gene family is a transcription factor that exists only in plants. It is mainly involved in transcriptional regulation and signal transduction processes in plants[1]. In the transcriptional regulatory network, WRKY transcription factors bind specific DNA sequences to activate or repress transcription of multiple target genes[2, 3]. The conserved WRKY domain contains approximately 60 amino acid residues. In the WRKY domain, a conserved WRKYGQK hexapeptide sequence is usually followed by a C₂H₂- or C₂HC-type zinc finger structure. According to the number of WRKY domains and the type of zinc finger structure, the WRKY family can be divided into 3 groups: Group I, Group II and Group III[4]. And Group II could be further divided into five subgroups: II-a, II-b, II-c, II-d and II-e. Group I contains two WRKY domains and C₂H₂ zinc finger structure, Group II contains a WRKY domain and a C₂H₂ zinc finger structure, and Group III contains a WRKY domain and a C₂-H-C zinc finger structure[5].

Since the first WRKY gene (*SPF1*) was cloned from sweet potato[6], WRKY genes have been found in many plants, such as *Arabidopsis*[7], rice[8], barley[9], rapeseed[10], soybean[11], corn[12], potato[13], tobacco[14], poplar[15], cotton[16], and cucumber[17]. At present, WRKY transcription factors have been

found to play an important role in plant defense response and growth development. The transcription factor WRKY70 is a common component in salicylic acid (SA)- and jasmonic acid (JA)-mediated signal pathways[18]. Expression of WRKY70 is activated by SA and repressed by JA[18]. In *Arabidopsis*, some WRKY transcription factors are positive regulators of ABA (Abscisic acid)-mediated stomatal closure and hence drought responses[19]. AtWRKY75 in *Arabidopsis thaliana* and OsWRKY31 in rice are both related to the growth of lateral root[20, 21]. The synergistic interaction of ABA-inducible WRKY genes in regulating GAMYB (GA-inducible transcriptional activator)-mediated GA (Gibberellin) signaling in aleurone cells, thereby establishing a novel mechanism for ABA and GA signaling cross-talk[22].

Jute (*Corchorus capsularis* L) is a natural fiber plant, which belongs to the Malvaceae family ($2n = 14$). It is mainly cultivated in Bangladesh, India, China and so on. Although many studies have showed that WRKY gene plays an important role in plant growth development, stress tolerance and fiber development[23], little information was available about the WRKY genes in jute. Therefore, this is the first time to identify and analyze the WRKY transcription factors in jute. The aims were: (1) Identification, phylogenetic and structure analysis of the WRKY gene family in jute; (2) Expression analysis in different tissues and GA₃ stress of WRKY gene family in jute.

Methods

Plant materials

The tested varieties “Huangma 179” and “Aidianyehuangma” were planted in Yangzhong Science and Education Base of Fujian Agriculture and Forestry University, Youxi County, Sanming City, Fujian Province on May 1st, 2017. 10 days after sowing, hypocotyl samples were obtained from three individuals separately. On July 1st, 2017 (60 days after sowing), the stem bark and stem stick were obtained from three individuals separately. On September 1st, 2017(120 days after sowing), the stem barks were obtained from three individuals separately. Then, the samples were immediately frozen in liquid nitrogen and stored in a refrigerator at -80°C for subsequent analysis. Three samples come from each tissue were stored separately as three biological replicates.

To identified the WRKY gene of jute in response to GA₃ stress, six 60-day-after-sowing jute plants were treated with GA₃ stress. The GA₃ stress treatment was carried out for 4 hours and 3 days, respectively. After the GA₃ stress treatment, the stem barks of each jute were obtained. In addition, three jute plants of the same age were sampled separately as control. All samples were immediately frozen in liquid nitrogen and stored in a refrigerator at -80°C for follow-up analysis.

Identification and Analysis of Conservative Domain of CcWRKY

The assembled sequence data have been deposited at the NCBI Sequence Read Archive (SRA, <http://www.ncbi.nlm.nih.gov/Traces/sra>) vide SRA SRP215917. Assembly of CcWRKY genes and ORF analysis were just based on the references of Islam et al.[24] and Zhang, L. et al.[25]. To obtain the WRKY family genes in the jute genome, a local BLASTP search was performed to identify complete WRKY members, using *Arabidopsis* WRKY protein sequences as query sequences. In addition, we also use the conservative domain prediction software Pfam to ensure that all candidate genes contain WRKY conserved domains[26].

The WRKY domain proteins of CcWRKY genes were identified and analyzed by DNAMAN5.0 software. Then, conservative structure prediction is performed in Weblogo (<http://weblogo.berkeley.edu/logo.cgi>). According to SWISS-MODEL[27], the three-dimensional homology of 43 members of WRKY transcription factor in jute were modeled and then the tertiary structure of WRKY protein were identified.

Phylogenetic analysis of CcWRKY protein

According to the software GSDS2.0 (<http://gsds.cbi.pku.edu.cn/>), The schematic diagram of intron and exon structure of CcWRKY were made. Phylogenetic and molecular evolutionary analysis was conducted using MEGA7 (<http://www.megasoftware.net>) [28] with pairwise distance and the neighbor-joining algorithm. The p-distance method was used to compute the evolutionary distances, which were used to estimate the number of amino acid substitutions per site. Conducting 1,000 bootstrap sampling steps[12] established the reliability of each tree. AtWRKY proteins were added to phylogenetic analysis to facilitate the subgroup classification of CcWRKY. WRKY family sequences from *Arabidopsis* were downloaded from TAIR (<http://www.arabidopsis.org/>).

Expression analysis of CcWRKY genes at different growth stages and GA₃ stress

According to the FRKM value of different genes in different tissues (hypocotyls–10d, leaf–60d, root–60d, stem bark–60d, stem stick–60d, stem bark–120d) and response with GA₃ stress on stem bark–60d, heatmap and histogram of WRKY genes were drawn by Excel, SPSS and R language.

RNA extraction and quantitative real-time PCR (qRT-PCR)

The RNA was extracted from the leaves, roots, stem bark and stem stick at the vegetable stage(60-day-after-sowing) of jute with the EZNA Plant RNA Kit (from OMEGA), and cDNA was synthesized by a reverse transcription kit (from Takara). This experiment used the Actin [29] as the reference gene by qRT-PCR. qRT-PCR reaction system: 10 µl of GoTaq® qPCR Master Mix, 0.4 µl of left primer(10µM), 0.4 µl of right primer(10µM), 2µl of cDNA, and 7.2µl of Nuclease-Free Water. Amplifications were performed with an initial 10min step of 95°C followed by 40 denaturation cycles at 95°C for 15s and primer annealing at

60°C for 1min. The melting curve used the default program of the instrument (ABI7500). The qRT-PCR reaction of each gene was repeated 3 times, and a similar result was obtained. In addition, the data obtained by qRT-PCR were calculated and analyzed by the formula $2^{-\Delta\Delta CT}$. The primers were designed to avoid the WRKY conserved domain.

Results

Identification of full length CcWRKY genes

To obtain the WRKY family genes in the jute genome, a local BLASTP search was performed to identify complete WRKY members, using *Arabidopsis* WRKY protein sequences as query sequences. In addition, we also used Pfam conserved domain prediction software to obtain 43 candidate genes containing WRKY domain (named as CcWRKY), as shown in Table 1.

Analysis of conservative domain of CcWRKY genes

The sequences of WRKY domain protein of jute WRKY genes were identified and analyzed by DNAMAN5.0 software. Then, conservative structure prediction was performed in Weblogo. The results showed that the conserved domains of WRKY gene family in jute could be divided into three groups: I, II and III. Group I had nine members. It could be further divided into I-C and I-N subgroups. Group I contain two WRKY domains and zinc finger structures, and the zinc finger structure are $CX_4C_{22-23}HXH$. Group II could be further divided into subgroups II-a, II-b, II-c, II-d and II-e, with 2, 7, 7, 6 and 6 members, respectively. In II-a, II-b, II-d and II-e, the heptapeptide domain and zinc finger structure of WRKY at C-terminal were WRKYGQK and $CX_5C_{23}HXH$, while in II-c, the heptapeptide domain and zinc finger structure of WRKY at C-terminal were WRKYGQK and $CX_4C_{23}HXH$. There were six members in group III. The heptapeptide domain and zinc finger structure of WRKY at C-terminal were WRKYGQK and $CX_7C_{23}HXC$ (Fig 1).

In addition, it was found that there are still mutations in its protein sequence in jute although WRKY transcription factor has a very conserved WRKY domain. Among the 43 members of WRKY transcription factor in jute, the conserved domain of one gene (WRKYGQK) and the zinc finger structure of four genes were all mutated (Table 2). This variation indicated that despite the structurally highly conserved WRKY gene family, some variations still occur in its WRKY domain, which also illustrated that the plant WRKY gene family had diversity in the evolutionary process.

Phylogenetic analysis of CcWRKY protein in diverse species

Comparing the known WRKY region of *Arabidopsis thaliana* WRKY protein with CcWRKY, the WRKY domain sequence of CcWRKY protein was clustered and analyzed using MEGA7 (Fig 2). These CcWRKY proteins can be divided into three groups: I, II and III. And Group II can be divided into II-a, II-b, II-c, II-d and

II-e subgroups. The classifications of phylogenetic tree analysis were consistent with the results of Figure 1 (Table 1).

Structure analysis of intron and exon of WRKY in jute

The numbers of exons and introns of jute WRKY were shown in Figure 3. The numbers of exons varied from 3 to 11. 21 WRKYs (48.84%) contained 3 exons, 5 WRKYs (11.63%) contained 4 exons, 8 WRKYs (18.60%) contained 5 exons, 6 WRKYs (13.95%) contained 6 exons. From the groups, Group II c+d+e and group III were relatively conservative, while Group I and Group II a+b+c's structures were significantly different and changed greatly. Most CcWRKYs in Group II c+d+e and group III contain 3 exons except Ccv40151700 (4 exons) and Ccv40018590(4 exons).

Analysis of tertiary structure of protein

The tertiary structure of protein is further coiled and folded on basis of the secondary structure. The tertiary structure of CcWRKY protein were conducted by SWISS-MODEL. The majority of the 43 amino acid sequences have the similar three-dimensional structure. One representative homology modeling from CcWRKY gene family was shown in Figure 4, consisting of several beta folding. Their tertiary structure were quite similar with *Arabidopsis thaliana*[30]. It had also proved that the CcWRKY gene family is highly conserved in structure.

Expression analysis of CcWRKY genes in different tissues

Tissue specific expression of genes is often considered as markers of specific gene functions in this tissue. Since WRKY genes are related to the bast fiber development of plants[31, 32], we mainly focus on the expression of CcWRKY genes at different stages of stem growth. Based on the RNA-seq data, we used R language to draw the heatmap of the expression patterns of CcWRKY genes in different stem growth stages (Fig. 5). The difference of gene expression is generally represented by colors, red represents high expression and blue represents low expression. The results showed that all the CcWRKY genes were expressed in the stem of jute, and the expression of WRKY genes differ at different stem growth stages. Meanwhile, it proved that there were no pseudogenes in 43 genes. From Figure 5, we could see that 43 genes were divided into two categories. The expressions of 13 genes were lower in the different tissues of jute, and the others were higher. Totally, 10 WRKYs were highly expressed in leaf (60d), 3 WRKYs were highly expressed in hypocotyls (10d), 2 WRKYs were highly expressed in stem stick (60d), 2 WRKYs were highly expressed in stem bark (60d), 14 WRKYs were highly expressed in root (60d), and 12 WRKYs were highly expressed in stem bark (120d). It could be seen that the WRKY genes were mainly expressed in the stem bark of jute. With the continuous growth of jute, the bast fiber of jute will gradually accumulate in the stem bark. Therefore, it is believed reasonably that the WRKY genes are involved in bast fiber development in jute. For example, Ccv40032460 was highly expressed in hypocotyls (10d), lowly

expressed in stem bark (60d), and no expression in stem bark (120d). It suggests that this gene may play a negative regulatory role in jute fiber accumulation.

GA₃ stress analysis of CcWRKY genes involved in cell wall formation

The stem barks were treated with GA₃ stress for “Huangma 179” and “Aidianyehuangma” when the plants were at vigorous growth stages (60 days after sowing). Then, the samples were taken after 4 hours and 3 days, respectively. The samples without GA₃ treatment could be used as control. We analyzed the RNA-seq results of these materials, and then drew the corresponding histogram (Fig. 6), the up column indicated that the gene expressions were up-regulated, and the down column showed the gene expressions were down-regulated. According to our previous research[33], “Aidianyehuangma” is a dwarf variety that sensitive to GA₃. After spraying GA₃, it was found that the plant height of “Aidianyehuangma” could increase[33]. The expression of WRKY genes mostly changed significantly under GA₃ stress, especially for the down regulated genes, as shown in Figure 6. By comparison of expression of CcWRKY genes in different treatment time (4h and 3d) after spraying GA₃, the expression of most of CcWRKY genes (31 genes) changed in the same trend in “Huangma 179”, similar results were found in “Aidianyehuangma”. From these, we found that the expressions of 18 CcWRKY genes of “Aidianyehuangma” were sensitive to the GA₃ stress, which showed significantly different from controls. It indicated that these CcWRKY genes play an important role in the gibberellin biosynthesis pathway and fiber development.

Comparing the expression of WRKY gene in “Huangma 179” and “Aidiantehuangma” in the same treatment, the two showed a similar trend in general, but the trend of the former was more obvious whether it was up or down. Since “Aidianyehuangma” is a dwarf variety that sensitive to GA₃ while “Huangma 179” is a normal variety. This suggested that these co-regulated CcWRKY genes possibly participated in cross-talk between signaling pathways regulating GA₃ stress. Since gibberellin has a close relationship with fiber development [34], the down regulated CcWRKY genes (like Ccv40018580, Ccv40001440, Ccv40052670, Ccv40120290, Ccv40136560, Ccv40154680, and Ccv40170160 etc.) might positively regulated the synthesis of GA₃. This also indicated that CcWRKY genes play an important role in gibberellin biosynthesis pathway and fiber development.

To verified the accuracy of the gene expression, 9 CcWRKY genes were randomly selected for qRT-PCR analysis (Fig. 7). The results of qRT-PCR corresponded to the results of FPKM.

Discussion

CcWRKY transcription factors in jute

WRKY transcription factors are one of the largest families of transcriptional regulators in plants. They play important roles in plant growth and development, as well as defensive in biotic and abiotic stresses [1, 35–37]. In this study, there were at least 43 WRKY members in jute genome, and the numbers were similar to those of sugar beet(40)[38], canola(43)[39] and castor(47)[40]but different from those of other plants, such as *Arabidopsis thaliana*(74), cotton(116)[16], soybean(188)[41], poplar(104)[15], tomato(81)[42], cabbage(145)[43], whose members were more than those of jute. By comparing species, the numbers of WRKY genes in different species is not proportional to their genome size. Nowadays, researchers have suggested that gene duplication, segmental duplication and whole genome duplication play important roles in the mass production of gene families[41]. Unfortunately, due to the lack of data on jute research, genome data also only published a draft, so many problems failed to get a satisfactory answer. We suspected that jute genome WRKY genes were less than other species, perhaps because they did not experience whole genome replication as other species do. However, with the improvement of genome sequencing and assembly, upgrade and update of the search and analysis software, discovery of variable splicing in genomes and the continuous advance of jute related research, we believed that the new WRKY members also existed in the genome of jute.

In general, the locations of introns and exons in the genome may provide important evidences for their evolutionary relationships. In this study, we systematically and comprehensively analyzed the distributions and lengths of exons and introns of the members of the WRKY gene family. By analyzing the gene structures of jute WRKY, it was found that its members consisted of 3 to 11 exons, and nearly half of them were 3 exons. These results provided valuable information for the study of jute and the evolutions of the WRKY gene family in other species. In addition, the members of the WRKY family have similar three-dimensional structures, which were formed by several beta folds. It is similar to the 3D structure of the domains of *Arabidopsis* WRKY protein in the database[30].

CcWRKY genes involved in cell wall formation

Both Jute and cotton are important fibre crops in the world. So, we usually would compare the results of jute with the cotton. According the expressions analysis of CcWRKY genes in different tissues, we could find that the mostly group III CcWRKY genes (Ccv40018580, Ccv40120290, Ccv40170160 and Ccv40154680) were highly expressed during the fiber development except two genes (Ccv40018590 and Ccv40064890). This result in jute was consistent to that in cotton[44]. In our study, most CcWRKY genes expressed differently in the stem bark and hypocotyl. This interesting phenomenon led us to believe that there was a certain connection between WRKY transcription factors and bast fiber development. In recent years, the study[45] has shown that *Arabidopsis* WRKY transcription factor was involved in the formation of secondary cell wall of myeloid parenchyma cells, which could significantly increase plant biomass. Among them, AtWRKY12 could negatively regulated the deposition of lignin, xylan and cellulose in myelocytes. In this study, the expression of Ccv40232460 (CcWRKY12) in the stem decreased with the growth of jute plants. We speculated that this gene has a negative regulatory effect on the accumulation of secondary wall fibers in jute. This was consistent with previous studies in WRKY12[45].

It has long been known that cellulose synthesis in plants was regulated by various phytohormones. GA₃ is an important hormone for plant growth and development throughout the whole life cycle. Therefore, in this study, the gene expression patterns of 43 CcWRKY transcription factor genes under GA₃ stress were systematically analyzed. Because of the great influences of GA₃ on plant heights, the dwarf variety “Aidianyehuangma” was selected as the target of GA₃ stress treatment. The results showed that the expressions of most genes were significantly increased or decreased, indicated that these CcWRKY transcription factor genes were likely to be involved in the defense response under GA₃ stress. At present, it has been shown that gibberellin-mediated signaling cascade regulates cellulose synthesis [34]. Therefore, it will be very interesting to further studied about the relationships between CcWRKY genes, GA₃ stress and fiber development.

In addition, due to the importance of jute economy, it will be very important to study the transcriptional regulation of WRKY proteins in jute. These CcWRKY genes have positive effects on fiber development and response to GA₃ stress, and could promote fiber yield and quality. At present, the researches of transcription factors in the model plants was only a part of them, and it couldn't fully reveal the functions of each transcription factor. And the same transcription factors have different functions in different species because of the different evolutionary directions. Overall, this study provided a theoretical basis for the subsequent study of CcWRKY family and its effects on fiber development by identification and bioinformatic analysis of CcWRKY gene family.

Conclusion

In this study, we have identified the CcWRKY gene family by analyzed the gene structures, subgroup classifications, phylogenetic relationships and three-dimensional structures of protein. The CcWRKY proteins were highly conserved, the lengths of the gene sequences and the numbers of introns and exons varied widely, all WRKY genes showed a variety of expression patterns at different stages of stem development and responded to GA₃ stress, which play important roles in GA₃ physiological regulation and fiber development.

Declarations

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Availability of data and materials

The datasets supporting the results of this publication are included within the article and its additional files.

Authors' contributions

LZ designed and directed the research; XW and YX analyzed the data; LZ wrote the manuscript with input from all authors; SN, JQ and LZ contributed new germplasm and techniques. All authors edited and agreed on the final manuscript.

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, tables are available as a download in the supplemental files section

Abbreviations

GA₃: Gibberellin.

Figures

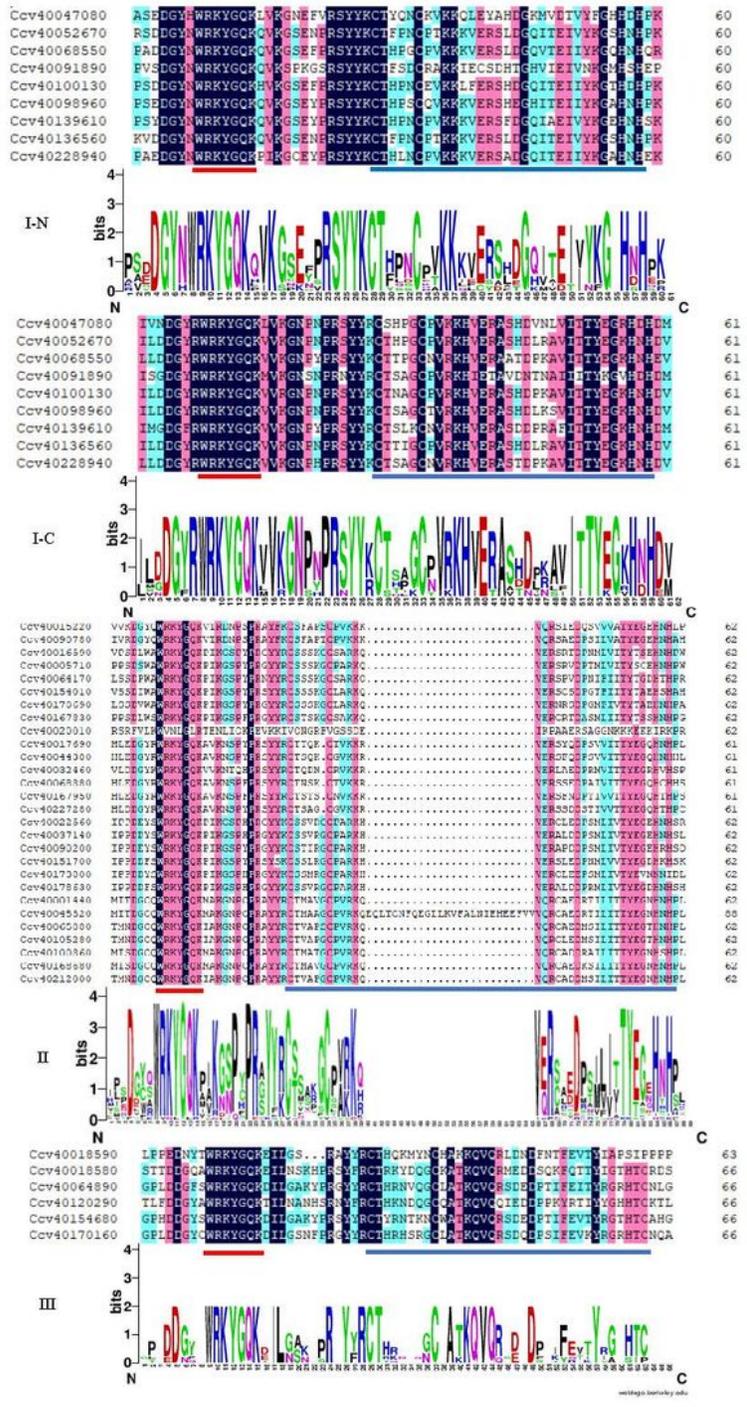


Figure 1

Sequence analysis of WRKY conserved domain of WRKY protein in jute

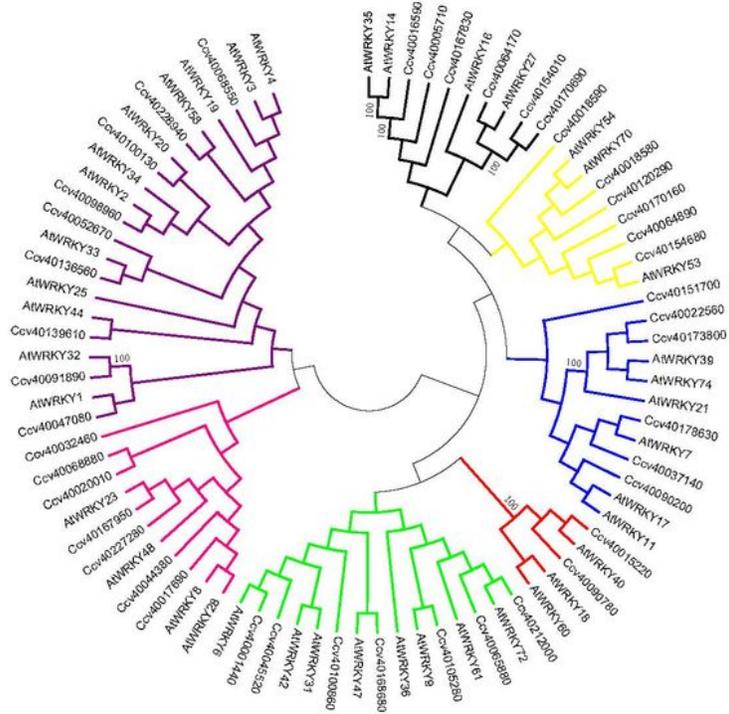


Figure 2

Phylogenetic tree of jute and Arabidopsis WRKY protein

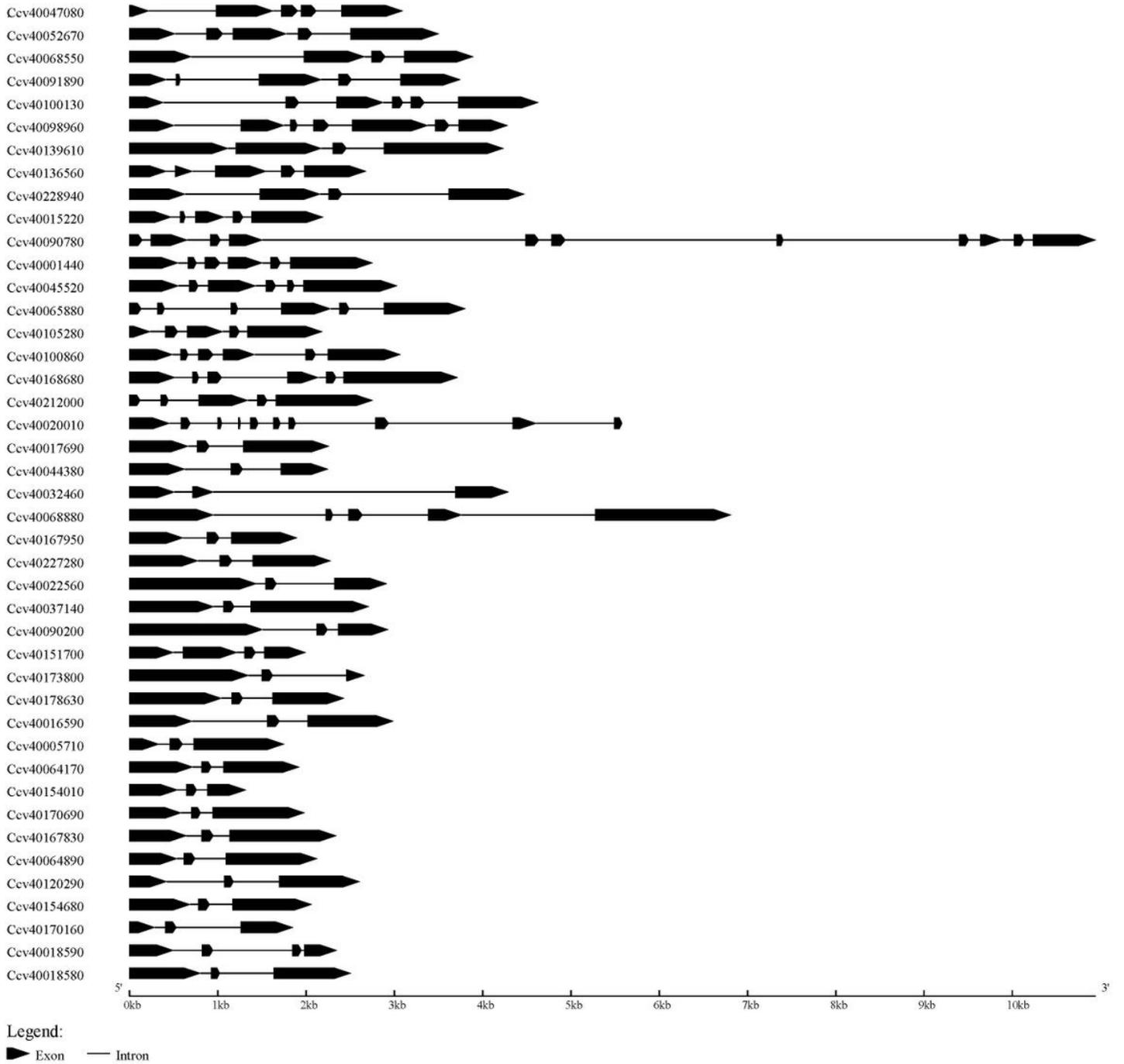


Figure 3

Intron and exon structure of CcWRKY genes in jute

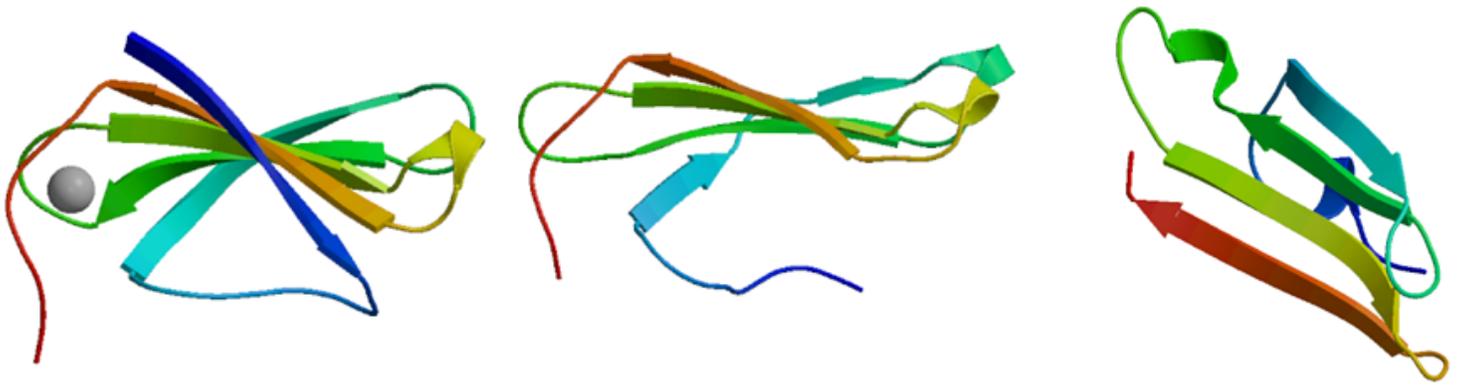


Figure 4

Protein 3D structure prediction of WRKY family in jute

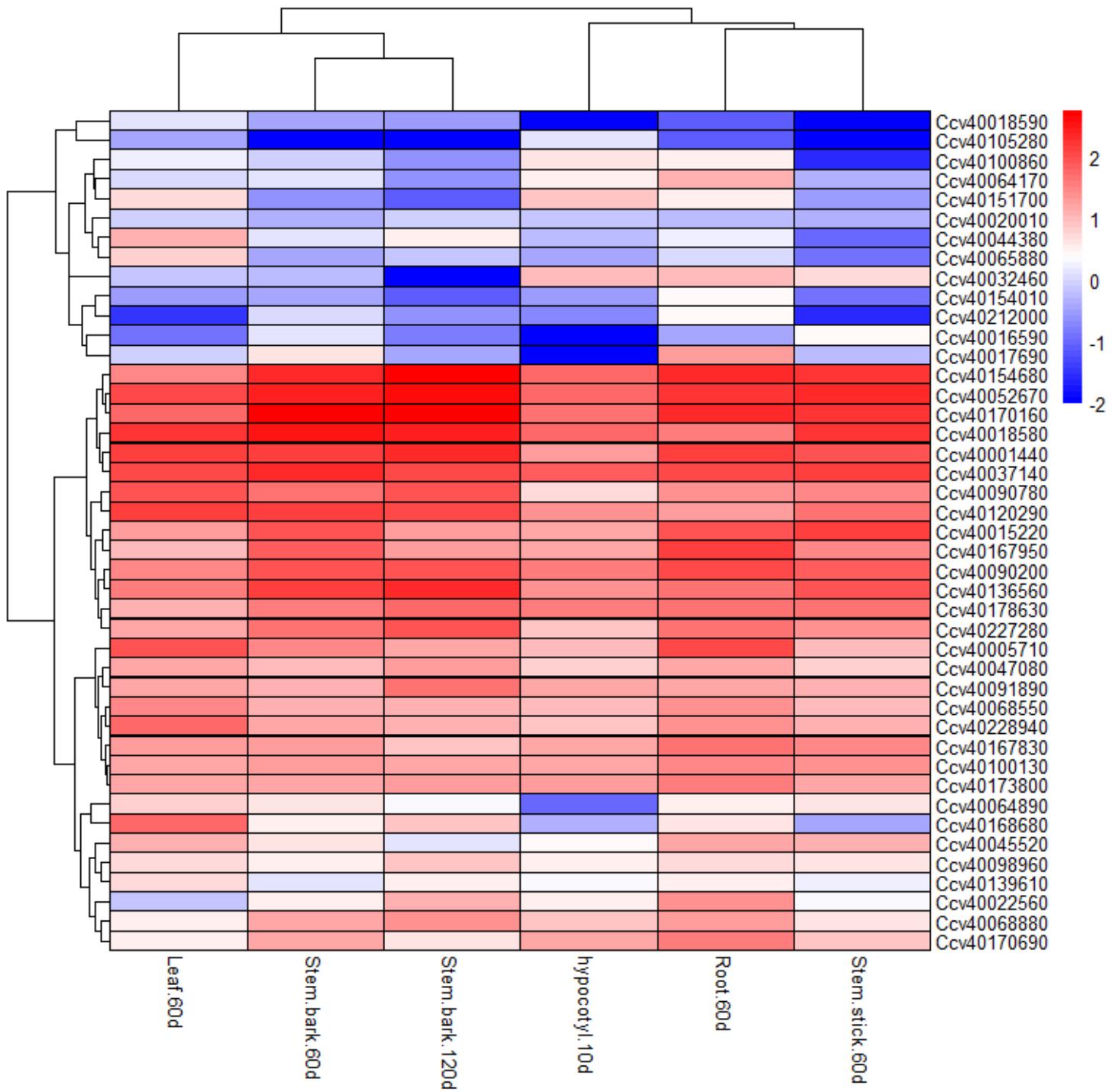


Figure 5

Expression profiles of CcWRKY genes in different tissues

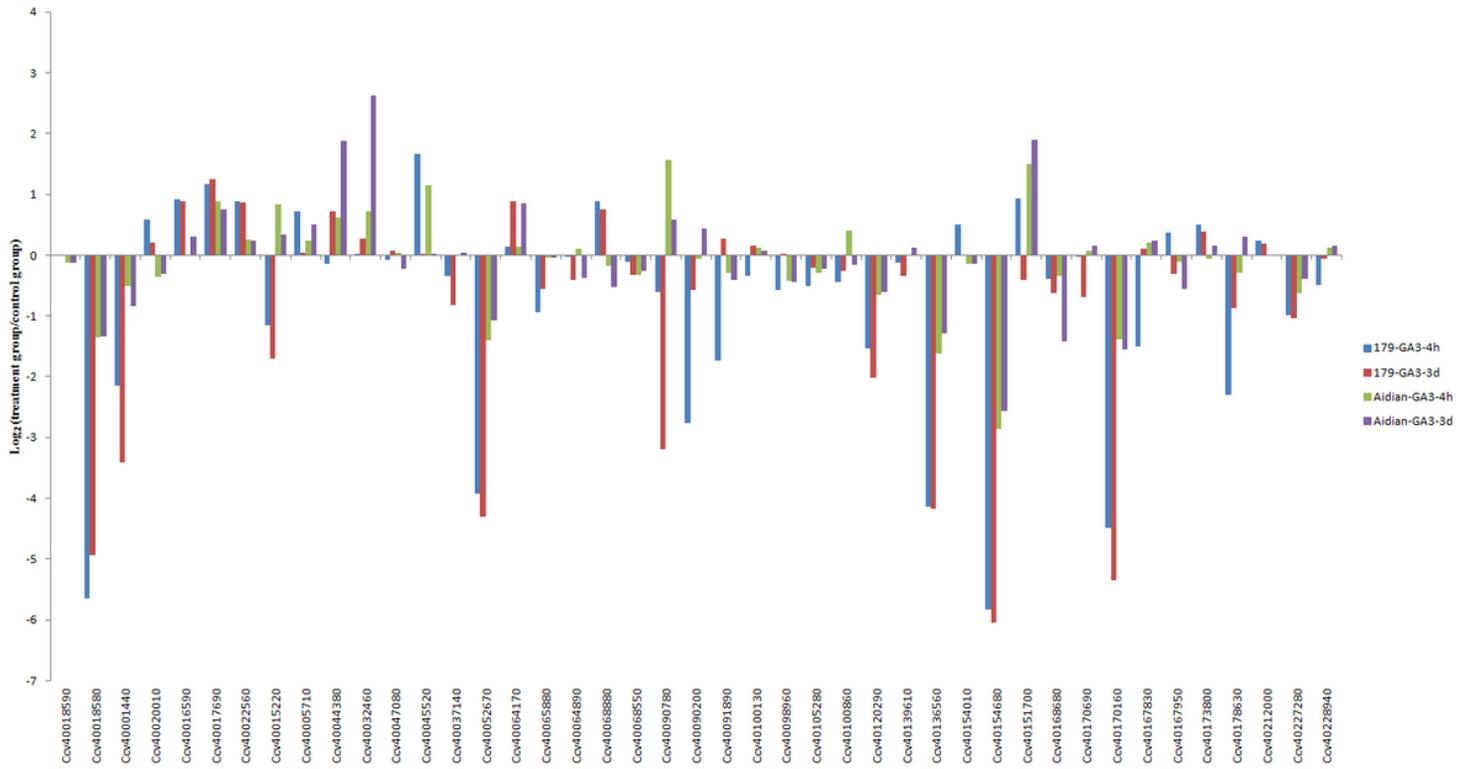


Figure 6

Expression profiles of CcWRKY genes under GA3 stress

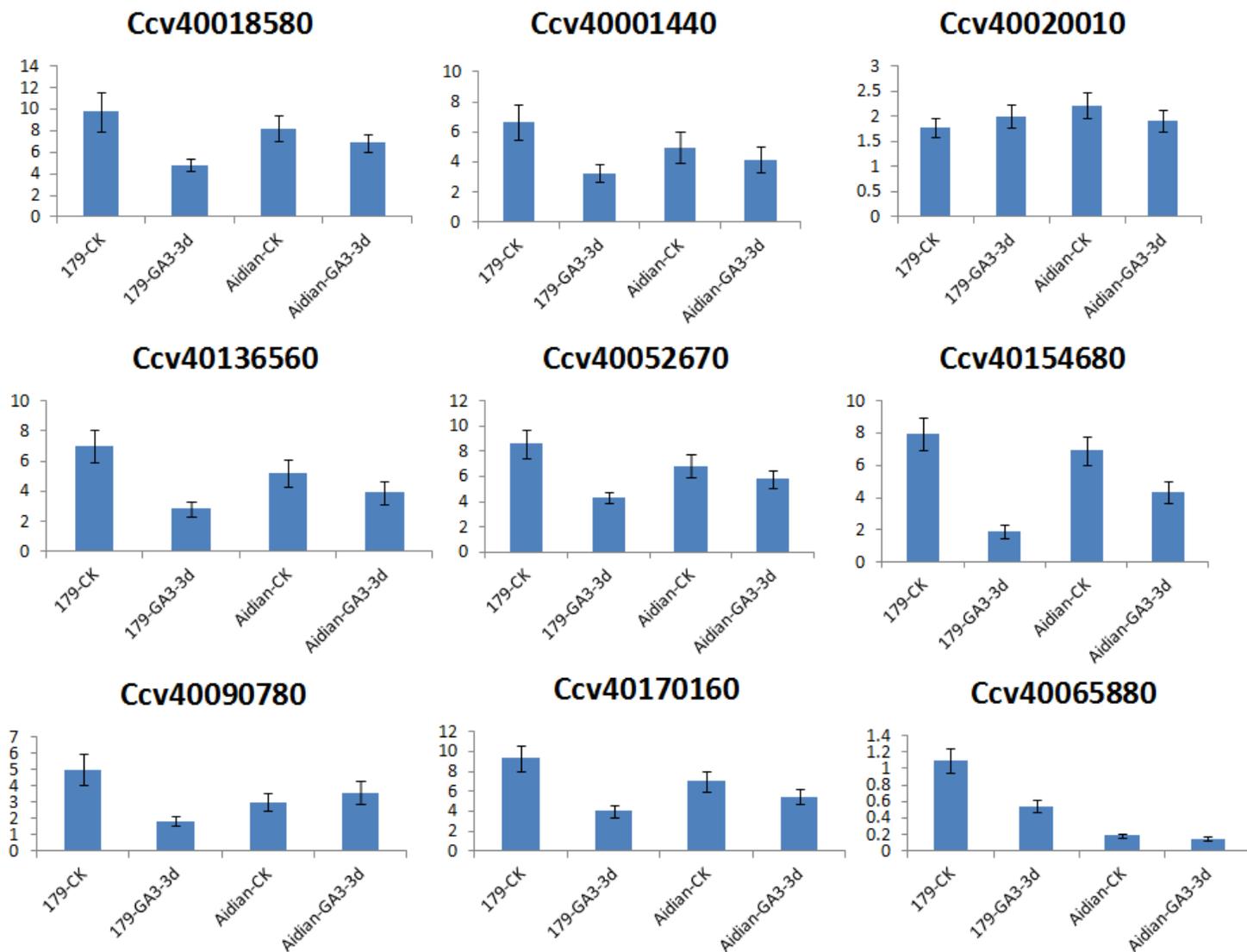


Figure 7

qRT-PCR verification of randomly selected 9 CcWRKY genes (Test varieties: "Huangma 179" and "Aidianyehuangma"; samples: jute stem bark of 60 days after sowing; CK: control, water treatment; 3d: 3d after GA3 stress).

Supplementary Files

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- [Table2.docx](#)
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