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Characterization and Expression Analysis of WRKY Transcription Factors in Groundnut (*Arachis hypogaea L.*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author BS conceived and designed the experiments. Author JG extended real time facility. Authors VRD and RN contributed the reagents and wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Groundnut *(Arachis hypogaea L.), is* an important subsistence oil yielding crop of the semi-arid tropics and often exposed to several environmental cues (high temperature, drought & heavy metal). The WRKY transcription factor (TF) is one of the master regulator, and play vital role in stress responses. However, far less information is available on functional characterization and tolerance mechanism of stress responsive WRKY genes in groundnut till date. In this study, a comprehensive phylogenetic, protein features, gene structure and motif analysis of WRKY TF gene family was carried out. We conducted expression profiling of 10 WRKY genes under high temperature, drought and heavy metal ($CdCl_2$) in various tissues. Majority of the AhWRKY (*Arachis hypogaea WRKY*) proteins were clustered and share close relationship with *Arabidopsis* and *Glycine max*. RT- qPCR analysis of *AhWRKY* genes revealed differential expression either in their

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transcript abundance or in their expression patterns in response to at least one abiotic stress. In particular, *AhWRKY41* expression level was found to be maximum in all the stress conditions. On the other hand, *AhWRKY20* and *AhWRKY22* were down regulated. The obtained data demonstrate that *AhWRKY41* may act as a positive regulator in drought/ high temperature/ heavy metal and would exhibit stress tolerance mechanism by activation of stress-associated gene expression.

Keywords: WRKY; abiotic stress; RT- qPCR; groundnut.

1. INTRODUCTION

Groundnut (*Arachis hypogaea L.*) is an oil yielding crop cultivated worldwide and one of the major grain legumes in tropical and subtropical regions, often exposed to maximum temperature of >40 °C for a short periods during growing season and low or erratic rainfall results in reducing its yield by 40%. However, its productivity is majorly affected by high temperature, drought, and heavy metal, associated with increased population, inherit food demand and global climate change. WRKY superfamily of transcription factors (TFs) are composed of several proteins involved in transcriptional regulation of developmental processes and stress responses [1]. The WRKY TFs are characterized by unique WRKYGQK motif followed by a zinc-finger-like motif C_2H_2 or C_2HC and evolutionarily highly conserved [2]. A single WRKY transcription factor might mediate transcriptional reprogramming associated with several signaling pathways. However, recent advances revealed the enormous significance in eliciting responses induced by abiotic stress conditions. For instance, over-expression of WRKY45 *and* WRKY11 from rice in *Arabidopsis* results in enhanced tolerance to salt and drought [3,4]. Niu and Wang et al. [5,6] identified 53 TaWRKY (*Triticum aestivum WRKY*) genes through wheat ESTs and demonstrated the over-expression of TaWRKY2, TaWRKY10 and TaWRKY19 exhibiting increased stress tolerance. Recent study demonstrated that, WRKY proteins are also involved in regulating developmental processes via auxins, cytokinins, and steroids in the downstream of hormone signaling in the antagonistic functions of salicylic acid (SA) and jasmonic acid (JA)/ethylene (ET) [7]. In *Arabidopsis* expression of *TaWRKY79* from wheat produced longer primary roots than the wild type in the presence of either NaCl or ABA. *OsWRKY31 (Oriza sativa* WRKY*)* and *AtWRKY70 (Arabidopsis thaliana* WRKY*)* were reported to be involved in regulation of disease resistance, root growth, auxin and immune responses, senescence, defense signaling pathways respectively, suggesting their involvement in synchronization of multiple biological processes [8,9]. Transgenic Arabidopsis over- expressing GmWRKY21 gene from *Glycine max* were more tolerant to cold, and induced GmWRKY54 displayed more salt and drought tolerance than those of wild type, whereas over-expression of GmWRKY13 results in increased sensitivity to salt and mannitol stress [10].

More recently, over-expression of GsWRKY20 (*Glycine soja WRKY*), a member of WRKY subgroup III, in alfalfa enhanced both drought and salt tolerance of the transgenic plants [2]. In *Populus simonii,* 20 WRKY genes showed differential response to various biotic and abiotic stress conditions suggesting they could play vital role in imparting stress tolerance [11]. Extensive research on WRKY TFs has been carried out in the recent past in several crop plants such as rice [12], cucumber [13], maize [14] and tomato [15], due to their important role in various biological, physiological and molecular processes. However, expression and characterization analysis of essential members of these TFs under different abiotic stress in groundnut is yet to be investigated. In the present study, we analyzed 10 WRKY encoding transcripts for gene structure, evolutionary relationship, conserved motifs and expression analysis under high temperature, drought and heavy metal stress in groundnut.

2. MATERIALS AND METHODS

2.1 Identification, Characterization and Sub-cellular Localization of WRKY Genes

We retrieved 10 WRKY genes from Plant Transcription Factor Database (http://planttfdb.cbi.pku.edu.cn/) which shared homology with stress responsive WRKY genes from *Arabidopsis* and *Glycine max.* The length, molecular weight and pI of each deduced polypeptide were calculated using the ExpasyProtParam tool (http://web.expasy.org/protparam/). The putative

WRKY homologs were determined by BLASTP (https://www.arabidopsis.org/Blast/index.jsp). Further, CELLO (http://cello.life.nctu.edu.tw/) and WOLF PSORT (http://www.genscript.com/psort/wolf_psort.html) programs were used to predict the sub- cellular localizations. Multiple sequence alignment of candidate domains from groundnut, *Arabidopsis* and *Glycine max* was performed using clustalw from Bioedit. To study the homology among the 10 WRKY genes, phylogenetic tree was constructed using Neighbour joining method in MEGAv6.0.

2.2 Conserved Motifs and Gene Structure Analysis

The MEME Suite tool v4.9.1 (http://meme.nbcr.net/meme) [16] was used for analysis of the conserved motifs of AhWRKY protein sequences. Gene Structure Display Server from Center for Bioinformatics, Peking University, was used to display the exon- intron junctions (http://gsds.cbi.pku.edu.cn/index.php). The genomic and mRNA sequences of WRKY genes were downloaded and used as query for generating its gene structure. The number of introns and exons were estimated based on this alignment and confirmed by the coordinates given in the sequences.

2.3 Plant Material and Abiotic Stress Treatments

Seeds of groundnut (ICGV1999) were surface-sterilized and grown under controlled conditions at 28°C day/25°C night with a 12-h light/12-h dark photo period. After 10 days of germination, seedlings (shoot, leaf, cotyledon, stem and root) were exposed to high-temperature [42°C for 2 h (induction) followed by 48°C for 6 h]. Drought stress was stimulated by withholding water for 5 days and for heavy metal stress, seedlings were hydroponically exposed to 300 µM $CdCl_2$ for 72 h. After the stress treatment, control and stress exposed tissues were harvested immediately and stored at -80°C for further analysis.

2.4 Gene Expression Analysis by qPCR and Pearson Correlation

Total RNA was isolated from control and stress treated tissues (shoot, leaf, cotyledon, stem and root) using TRiZol (Invitrogen) according to the manufacturer's instructions and then treated with RNAase-free DNAase I (Promega). All RNA samples were quantified by Nanodrop 2000 (Thermo Scientific). cDNA was synthesized by reverse transcription with 500 ng of total RNA using PrimeScript RT Reagent Kit (Takara) according to the manufacturer's instructions. Gene specific primers were designed using Primer3 software. Table 1 depicts the forward and reverse primers for 10 WRKY genes, their amplicon length and melting temperature. qRT-PCR reactions were performed using SYBR Green PCR Master mix (Takara) on Lightcycler96 Real time PCR (Roche). Each PCR reaction (20 µl) included 2 µl cDNA, 1x SYBR Green Master mix, 0.5 µl sequence specific forward primer (10 µM), 0.5 µl reverse primer (10 µM), and 7 µl sterile water. Actin was used as a reference to quantify the expression of *AhWRKY* genes. The reaction conditions were 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 55°C for 30s and 72°C for 15s. Three biological replicates were used. The $\Delta\Delta^{Ct}$ method was used for quantification. To analyse the qPCR results of stress inducible AhWRKY genes for statistical significance, Pearson correlation coefficient was used to calculate R and p values. STRING 10 (http://string.embl.de/), computational tool was used to predict the protein- protein interaction of the stress inducible AhWRKY proteins in *Arabidopsis* with the default parameters.

3. RESULTS

3.1 Characterization of *AhWRKY* Genes and Sub- cellular Localization

We obtained 10 *AhWRKY* genes and their corresponding protein sequences from Plant Transcription Factor database. Basic information like molecular weight and pI are depicted in Table 2. The average polypeptide length was 340.8 residues with the length ranging from 199 aa (AhWRKY44) to 568 aa (AhWRKY3). The pI values range from 5.2559 to 10.2566. The metal chelation zinc finger motif pattern of the three groups were C-X_{4-5}-C-X_{22-23}HxH (C2H2) (I), C-X_5CX_{22-23}-HxH (C2H2) (II) and C-X_7-CX_{23}-HXC (C2HC) (III). The sub-cellular localization of 10 AhWRKY proteins were analyzed using WOLF PSORT (http://www.genscript.com/psort/wolf_psort.html) and CELLO (http://cello.life.nctu.edu.tw/) [17] programs. The results showed 9 of 10 AhWRKY proteins were predicted to be localized in nucleus and 3 of 10 proteins were predicted to be localized in cytoplasm and chloroplast.

Table 1. Primers used for real time PCR

Gene	Forward primer (5'- 3')	Reverse primer (5'- 3')	Amplicon length (bp)	Tm (°C)
Actin	TTGGAATGGGTCAGAAGGATGC	AGTGGTGCCTCAGTAAGAAGC	196	57.13
AhWRKY41	CCTCTGAGGGAGGACAATCA	AAGGCCACTCTCAAAGCTCA	108	60.19
AhWRKY20	AATGCTGGTTGCCCTGTTAG	CCATCCCAAGATCAAGGCTA	205	60.13
AhWRKY4	GGTGGAGAATCCGATGAGAA	ATCAGCCAAATCCTCTCCCT	147	60
AhWRKY40	TGGCTGAAATGCTTTCTGTG	TCTCGGAGTTCCCATTGTTC	180	60
AhWRKY12	GCTACAGCCACAGTCACAGC	TCGGATCCATGCTTCTAACC	106	60
AhWRKY15	TGCAGCAGTGTAAGAGGGTG	CAGCTGCAGTGAGAGAGTGG	115	59.91
AhWRKY69	GAAGCAAGTTGAACGAAGCC	GAGGAGGAAGAGGAGGAGGA	114	59.83
AhWRKY3	AGGGTTGAGTCCTTCTGGGT	GGAGCTGTCGGAGCTGTTAC	177	60
AhWRKY2	GCTAGAGCAGGGTTCAATGC	GGGTGGTAGGACTGAGACCA	124	59.9
AhWRKY44	ATCAGCCATGAAAGATTCGG	GCGGATGTTAAAGCCTTCTG	140	60

Table 2. Features of 10 WRKY proteins in groundnut

Protein	TF ID	GmWRKY homolog	Deduced polypeptide			Subcellular localization		Group	Domain
			L (aa)	pI	MW (kDa)	Psort	Cello		
AhWRKY41	Ahy002014	41	218	9.7727	25.211	Nuclear	Nuclear	GIII	C-X_7-CX_{23}-HXC
AhWRKY20	Ahy003521	20	358	7.9025	39.382	Nuclear	Nuclear	GI	C-X_{4-5}-C-X_{22-23}HxH
AhWRKY22	Ahy008917	22	370	5.2559	40.371	Nuclear	Nuclear	GIIe	C-X5CX22−23-HxH
AhWRKY40	Ahy011981	40	320	8.2094	35.704	Nuclear	Nuclear	GIIa	C-X_5CX_{22-23}-HxH
AhWRKY12	Ahy014145	12	218	8.0863	24.62	Cytoplasm	Nuclear	GIIc	C-X_5CX_{22-23}-HxH
AhWRKY15	Ahy014372	15	363	10.2566	39.276	Nuclear	Nuclear	GIId	C-X_5CX_{22-23}-HxH
AhWRKY69	Ahy014637	69	235	5.3279	24.885	Nuclear, cytoplasm	Nuclear	GIIe	C-X_5CX_{22-23}-HxH
AhWRKY3	Ahy017324	3	568	7.9178	61.9	Nuclear	Nuclear	GI	C-X_{4-5}-C-X_{22-23}HxH
AhWRKY2	Ahy018168	2	559	5.175	60.538	Nuclear	Nuclear	GI	C-X_{4-5}-C-X_{22-23}HxH
AhWRKY44	Ahy020733	44	199	8.57	22.147	Nuclear, choloroplast	Nuclear	GIIe	C-X_5CX_{22-23}-HxH

3.2 Multiple Sequence Alignment and Phylogenetic Analysis

To analyze the features of AhWRKY domain in groundnut, we performed multiple sequence alignment of the conserved domains derived from *Arabidopsis* and *Glycine max*. The sequence alignment reveals that all the 10 AhWRKY proteins share the conserved domain (WRKYGQK) and zinc finger motifs (Fig. 1). To examine their evolutionary relationship, we constructed phylogenetic tree and showed well organized classification of 10 AhWRKYs falls into group I (AhWRKY20, AhWRKY3 and AhWRKY2), group IIa (AhWRKY40), group IIc (AhWRKY12), group IId (AhWRKY15), group IIe (AhWRKY22, AhWRKY44 and AhWRKY69) and group III (AhWRKY41) (Fig. 2).

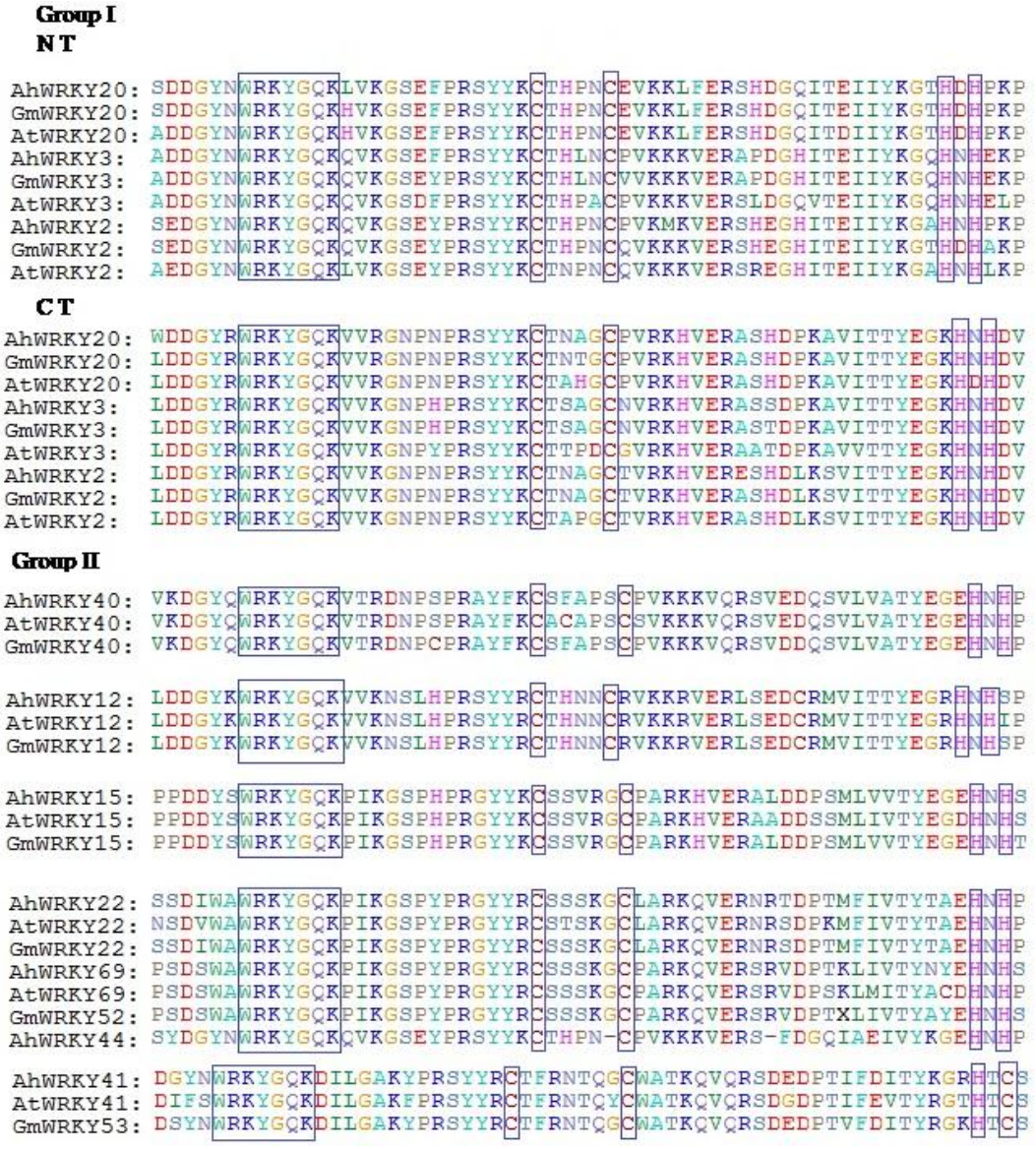

Fig. 1. Multiple sequence alignment of conserved WRKY domains from groundnut, *Arabidopsis* and *Glycine max.* NT and CT represent N termini and C termini of Group I WRKY domains respectively. The highly conserved WRKYGQK domain is indicated in the box. Cystein (C) and histidine (H) are also represented within the box, which indicate the zinc finger motifs

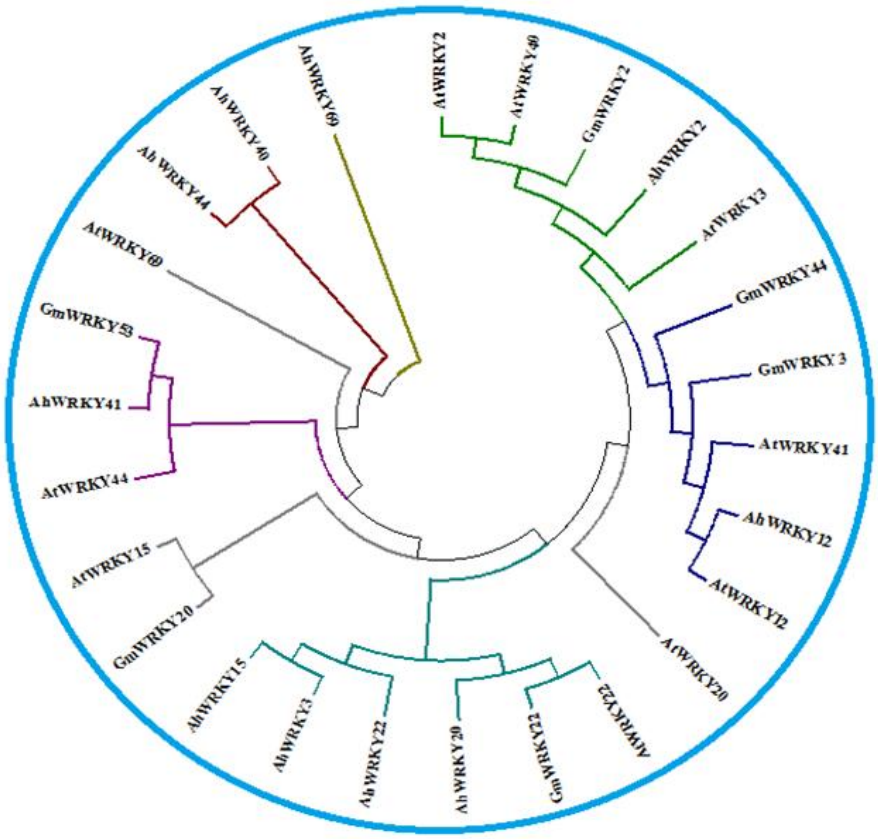

Fig. 2. Unrooted phylogenetic tree of groundnut, *Arabidopsis* and *Glycine max* WRKY sequences. The tree was constructed by the MEGA v6.0 program with the neighbor-joining algorithm. The evolutionary relations were calculated using the p-distance method

3.3 Analysis of Motif Composition and Gene Structure

The WRKY gene family share significant sequence conservation within domain regions and to investigate the homologous sequence and frequency of the most prevalent amino acids, sequence logos were produced using the amino acid sequences of AhWRKY proteins in MEME Suite tool and six motifs were defined (Fig. 3a and b). Group I consist of AhWRKY20 shares all the six conserved motifs while AhWRKY3 and AhWRKY2 share five motifs. Group II members (AhWRKY40, 12, 15, 22, 69, 44) shares motifs 1, 2 and 5. Group III member AhWRKY41 shares motifs 1, 2 and 6.The gene structure analysis revealed that the WRKY genes harbored at least two exons with varying length. In addition, a separate phylogenetic tree was generated from the complete protein sequences of all the WRKY genes. The most closely related members in the same subfamilies shared similar exon/ intron structures in terms of intron number and exon length (Fig. 4).

3.4 Expression Patterns of *AhWRKY* Genes under Different Abiotic Stress Treatments

In order to characterize the relative expression of WRKY genes under different abiotic stress, we conducted qPCR. Under high temperature stress, *AhWRKY2, 40, 41, 69* and *44* were found to be up- regulated by 2.9, 1.3, 4.7, 5.2 and 1.02 folds respectively (Figs. 6, 7 and 8). On the other hand, *AhWRKY20, 22, 12, 15* and *3* were down regulated by 0.26, 0.36, 0.91, 0.73 and 0.59 fold respectively (Figs. 5, 6 and 7). During drought period, *AhWRKY41* and *2* showed 2.4 and 2 fold up regulation while, *AhWRKY40* shows significant down regulation by 0.48 fold (Figs. 6 and 5). Under heavy metal stress, most of the genes were down regulated while *AhWRKY3* is significantly up regulated by 6.85 fold. To

summarize the overall expression patterns, *AhWRKY41* showed induced expression in all three stress conditions (Fig. 8). *AhWRKY2* and *69* showed enhanced expression under high temperature and drought while *AhWRKY15* shows up-regulation during drought and heavy metal stress. *AhWRKY20* gene was repressed in all the three stress conditions. The other genes show differential expression patterns under different abiotic stress which may indicate the involvement of these genes in complex stress regulatory mechanisms. The tissue specific expression (leaf, cotyledon, stem and root) data of 10 *AhWRKY* genes under abiotic stress were depicted in Figs. 5, 6, 7 and 8. Analysis of Pearson correlation co-efficient of qPCR results with R value of 0.997 and p-value of 0.00001, indicating that the results are highly significant. The prediction of protein- protein interaction between stress inducible AhWRKY orthologs in *Arabidopsis* was studied by STRING 10 program. The interaction network shows that out of 10 AhWRKY stress inducible proteins, AhWRKY40, AhWRKY15, AhWRKY22 and AhWRKY44 shows their association with WRKY18, GUN5, WRKY33, STZ, RHL41, DIC2, CZF1 and CML38 (Fig. 9).

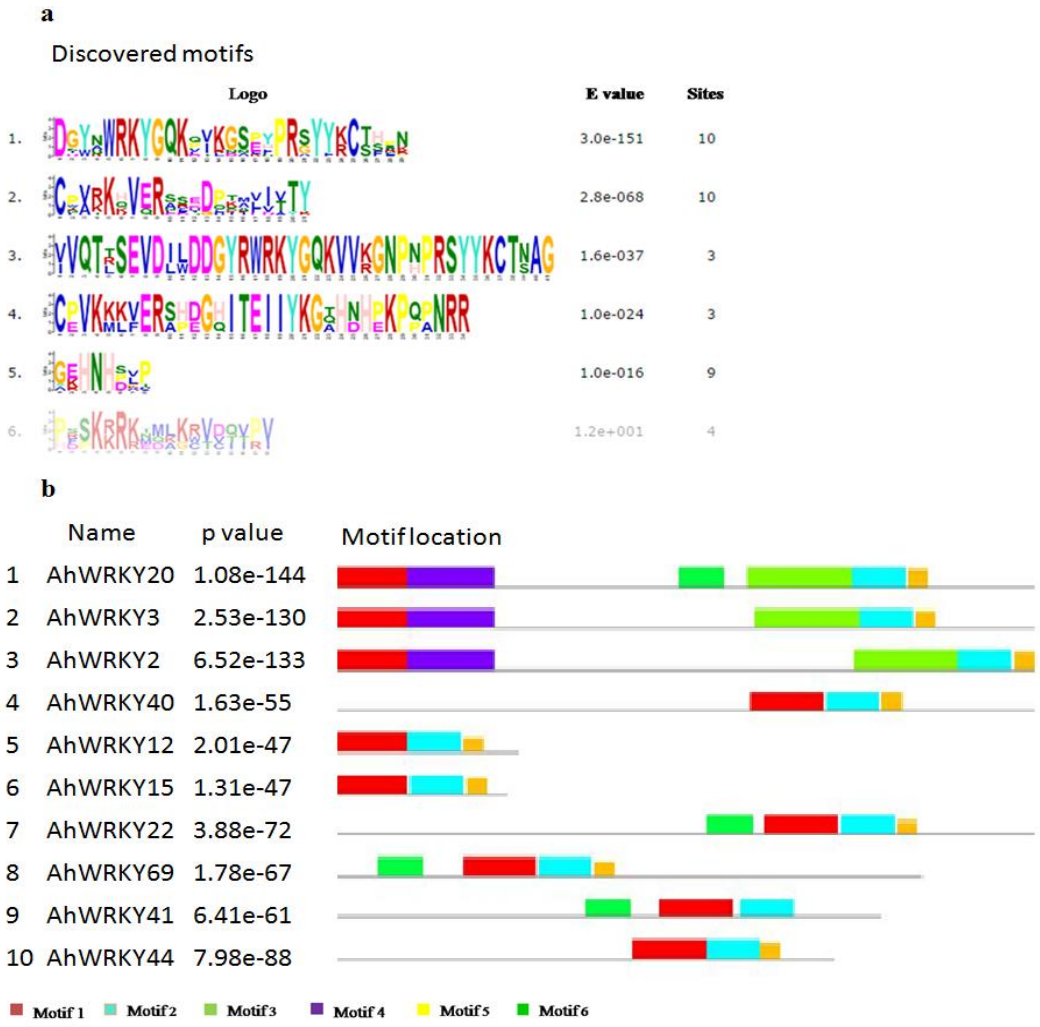

Fig. 3. a) The domain prediction of ten *A. hypogaea* WRKY protein sequences was performed using MEME software, which generated a letter logo to represent the WRKY domain, zinc finger and other motifs. The height of the letters in the y-axis represents the degree of conservation and relative frequency of each amino acid at that position. b) The distribution of conserved motifs among the putative AhWRKY proteins is shown and different motifs are represented by different color blocks as indicated at the bottom of the figure

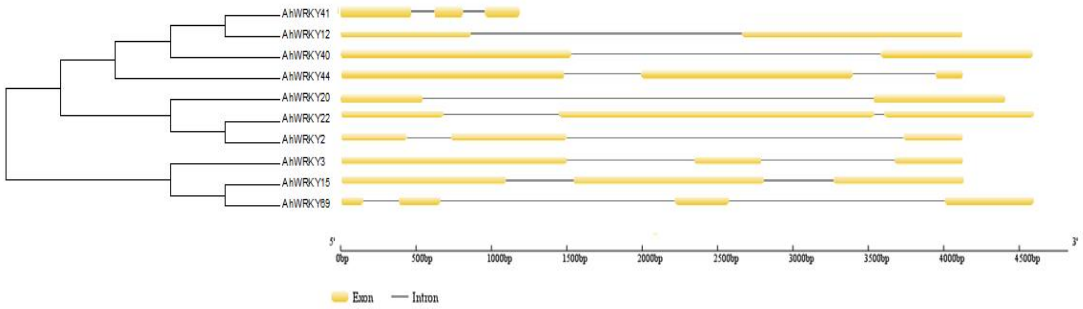

Fig. 4. Phylogenetic relationship and gene structure of the WRKY genes. Yellow box indicate exons and black line indicates introns

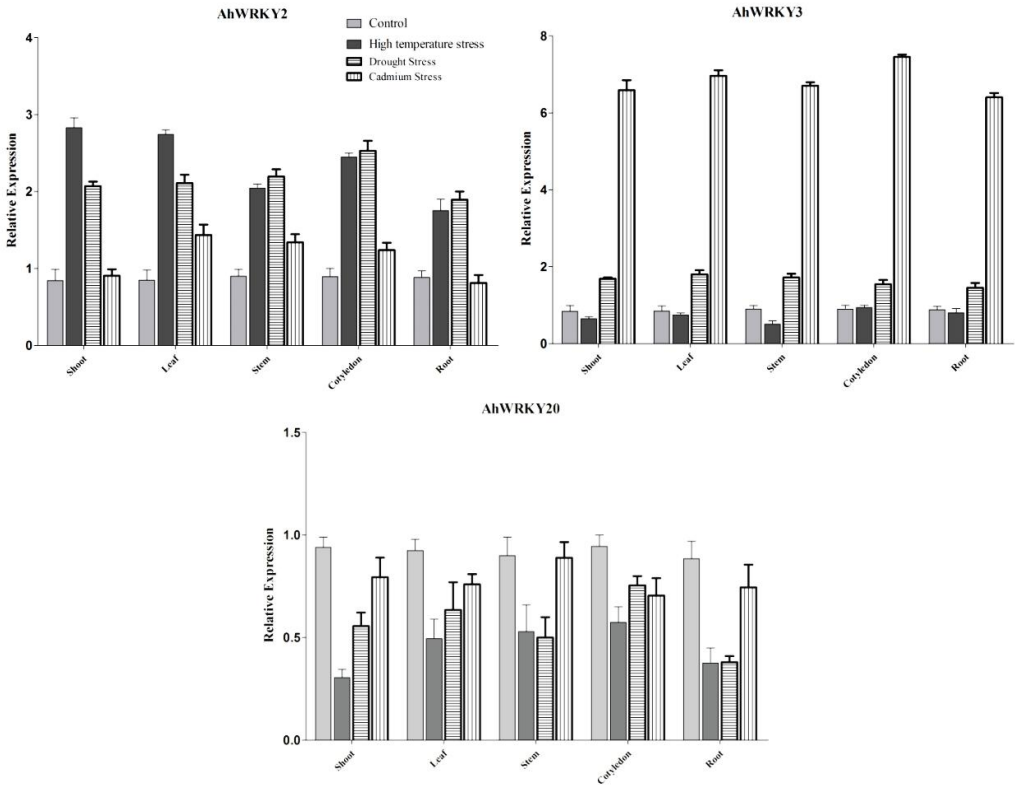

Fig. 5. Expression pattern of group I *AhWRKY* genes under high temperature, drought and heavy metal stress in different tissues

4. DISCUSSION

Plants have developed a great ability to reprogram their transcriptome in a highly dynamic and temporal approach through an integrated network of transcription factors to adapt to the changing environmental stress conditions. Among these, WRKY proteins are important members of transcription factors involved in regulation of plant stress responses [18]. It has been well documented that WRKY TFs were connected with various plant defense mechanisms under abiotic stress conditions [19]. Meanwhile, non-*WRKY* genes that enhance plant drought and salt stress by either efficient ROS elimination through the activation of the cellular antioxidant systems or the activation of stress-associated genes have been extensively reported in *Arabidopsis* [20,21], *Oryza sativa* [22,23], *Poncirus trifoliate* [24,25], and wheat

[26,27]. Functional characterization and deciphering the stress tolerance mechanism by WRKY was elucidated only in model crops. In groundnut, no reports on expression and functional characterization of stress associated WRKYs are found till date. Multiple sequence alignment of the groundnut WRKY domain shows homology with *Arabidopsis* and *Glycine max*. Hence, we predict that the functional annotations of AhWRKY may be fairly similar to AtWRKYs and GmWRKYs. Phylogenetic analysis reveals that 10 WRKYs were classified into three groups. Of the three, the Group II were found to be abundant, which includes *AhWRKY22, AhWRKY40, AhWRKY12, AhWRKY15, AhWRKY69* and *AhWRKY44*, were further assembled into four sub-groups (IIa, IIc, IId and IIe). The WRKY protein belonging to Group I identified in every ancestral organism has two WRKY domains and represents the ancestral form and evolved early [14]. In addition, motifs analysis using MEME tool confirms the presence of WRKY domain and zinc finger motifs. Motifs 1 and 3 are WRKY domain containing motifs and motif 2 and 5 are zinc finger containing domains are present in all the WRKY proteins. Previously, it was reported that, among the two domains only the C- terminal domain belonging to Group I has sequence specific DNA binding activity with W-box while N-terminal domain showed weaker binding activity [28]. However, the N-terminal WRKY domain might alternatively provide an interface for protein-protein interactions that coincide with the function of zinc finger like domains [14]. It was assumed that, due to the variability in the N- terminal domain during evolution, it may be evolved into another pattern to accomplish other regulatory functions or deleted from the sequence. The functions of other motifs are yet to be elucidated. In addition, there is similarity in the distribution of conserved motifs and the number of exons. As WRKY genes are themselves transcriptionally regulated, understanding the regulatory processes governed by these genes is a challenge. However, their distinct expression pattern in various tissues under specific biological condition might unfold the regulatory functions of these transcription factors. Several studies have described the essential roles of WRKY TFs in the regulation of gene expression [29-32].

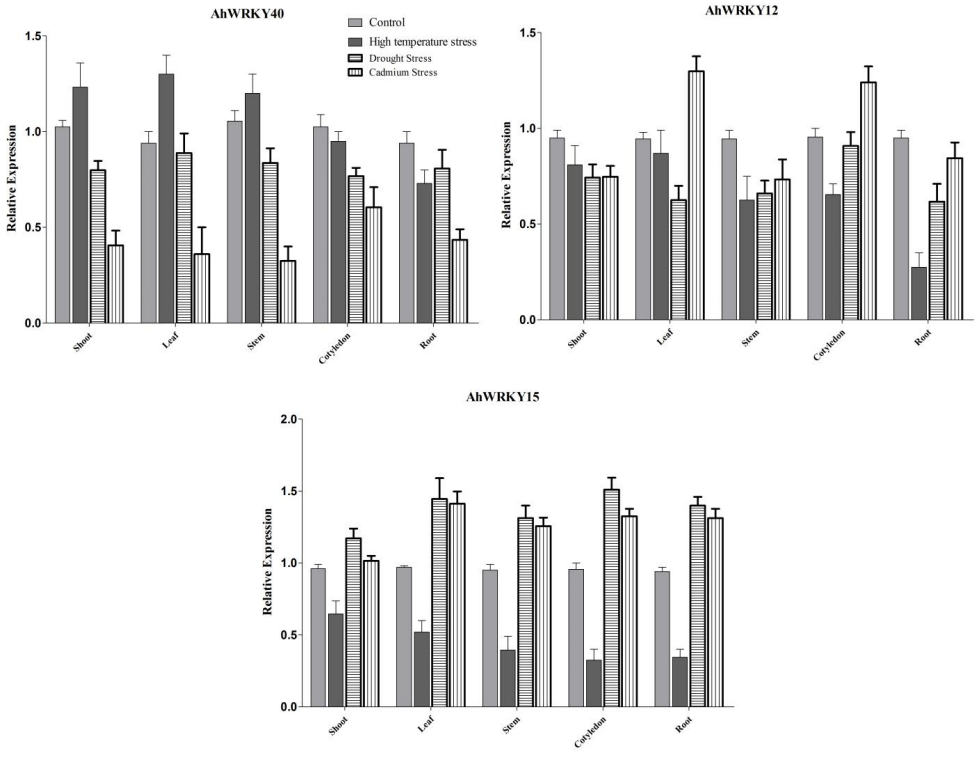

Fig. 6. Expression pattern of group II a, c & d *AhWRKY* genes under high temperature, drought and heavy metal stress in different tissues

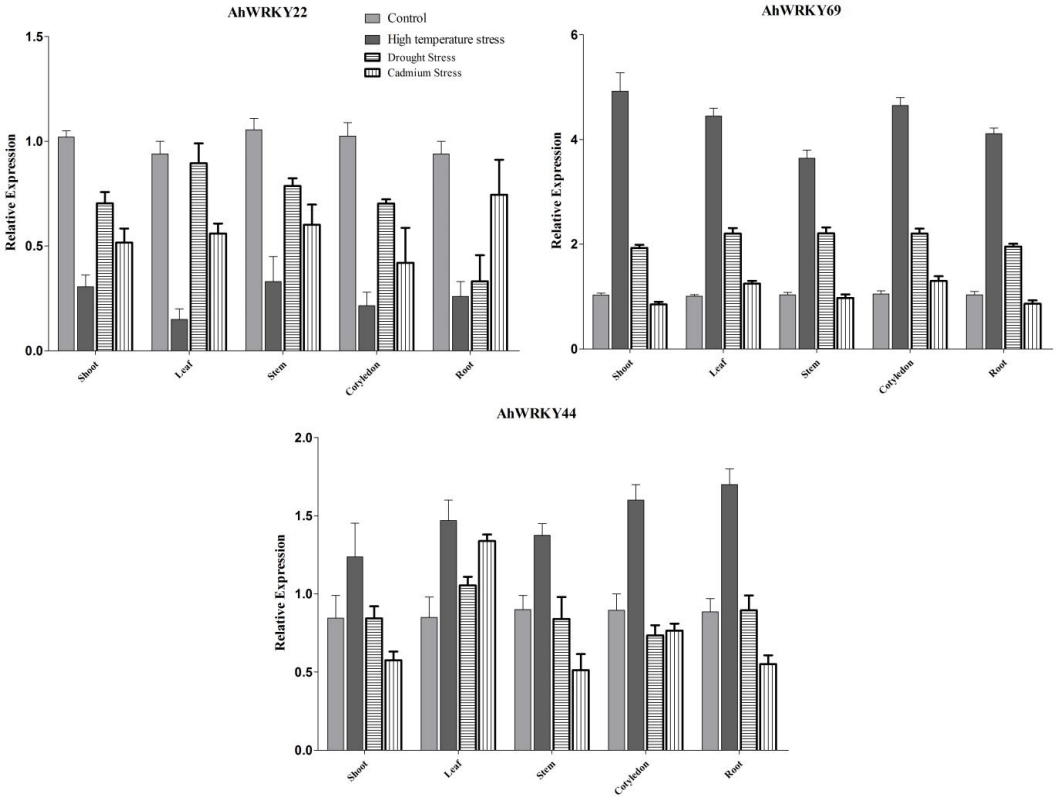

Fig. 7. Expression pattern of group IIe *AhWRKY* genes under high temperature, drought and heavy metal stress in different tissues

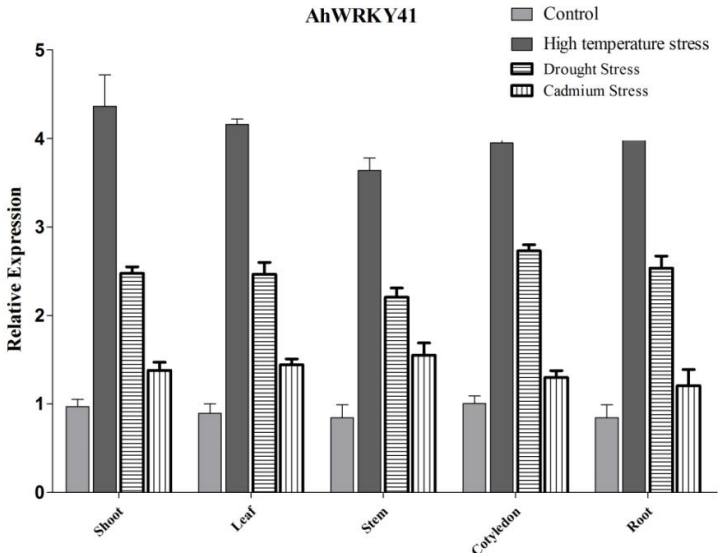

Fig. 8. Expression pattern of group III *AhWRKY41* under high temperature, drought and heavy metal stress in different tissues

In various plants such as Arabidopsis, *Oryza sativa*, Glycine max, wheat, cotton, maize and Populus, a number of WRKY genes are characterized that function as key regulators in signaling pathways for resistance to abiotic stress [33-37,14,11]. In present study, we

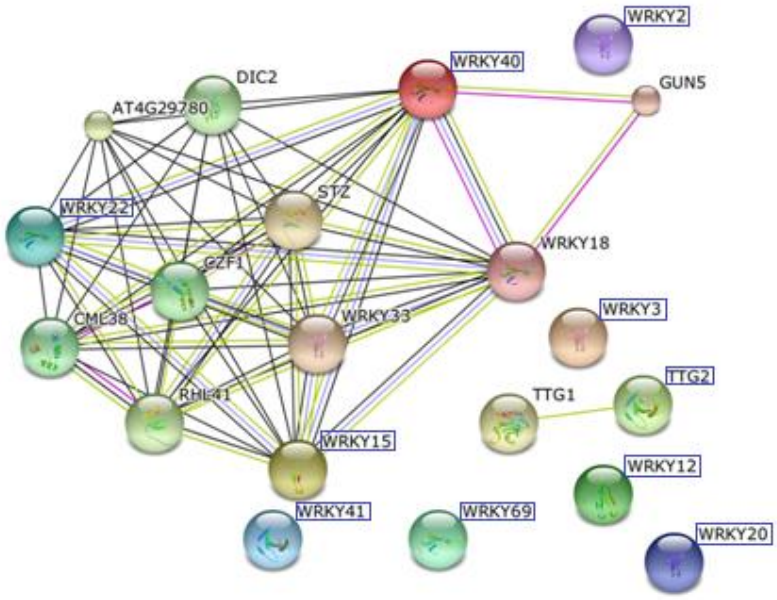

Fig. 9. Protein-protein interaction of AhWRKY proteins from string program. AhWRKY40 represented as WRKY40, AhWRKY15 as WRKY15, AhWRKY22 as WRKY22 and AhWRKY44 as TTG2

imposed three abiotic stress treatments such as high temperature, drought and heavy metal on 10 day old seedlings and studied expression analysis of AhWRKY genes. The results indicated that most of the AhWRKY genes were induced in various tissues. Compared to the drought and heavy metal stress, there are few reports on response of WRKY genes to high temperature stress. *AhWRKY41* is induced in all the three stresses in various tissues including leaf, cotyledon, stem and root indicating that it may be involved in multi abiotic stress response. Zhong et al. [38] reported the regulation of ABI3 expression and seed dormancy by WRKY41 and ABA in *Arabidopsis.* Luo et al. [39] demonstrated the tolerance of *GsWRKY20* over-expression lines to drought which exhibited decreased water loss rate and stomatal density in Arabidopsis and also showed the transgenic lines mediated ABA signaling by selectively promoting the expression of negative regulators and repressing the positive regulators. Our study emphasize that *AhWRKY20* shares homology with *GmWRKY20* is repressed in all three abiotic stress conditions and *AhWRKY40* is repressed in drought and heavy metal stress suggests these genes might play an important role in drought stress response and ABA signaling. Xiang et al. [40] analysed the physiological function of the *Arabidopsis* WRKY22 during dark-induced senescence which was suppressed by light and promoted by darkness, thus evidences the participation of AtWRKY22 in the dark-induced senescence. Our results showed that *AhWRKY22* repressed in all three stress conditions which gives a clue that it could be involved in senescence. Further, *AhWRKY2* was induced in response to high temperature and drought stress and evidences shows its homology in Arabidopsis, WRKY2 transcription factor mediates seed germination and post germination developmental arrest by ABA [41]. Lai et al. [42] demonstrated the positive role of WRKY3 and WRKY4 in plant resistance to necrotrophic pathogens. Interestingly, *AhWRKY3* is highly induced under cadmium which gives evidence for its vital role in heavy metal stress responses in groundnut. WRKY TFs were implicated integrating ROS homeostasis regulation and abiotic stress resistance in many crops. A recent study explored APTEALA 2 is regulated by different members of WRKY and NAC family, in addition, the link between the ROS-response ZAT12 zinc finger protein and iron regulation in cells [43-45]. The protein-protein interaction network identified WRKY18, GUN5, WRKY33, STZ, RHL41, DIC2, CZF1 and CML38 which show association with AhWRKY proteins. WRKY18 and WRKY33 interacting with elicitor- responsive *cis*-acting element, positively modulates defense related gene expression and disease resistance [45,46]. GUN5 (GENOMES UNCOUPLED 5), a

multifunctional protein was involved in chlorophyll synthesis, plastid to nucleus retrograde signalling and ABA perception [47]. STZ (salt tolerance zinc finger), was found to repress the stress responsive genes DREB1A and LTI78 and could be involved in jasmonate (JA) early signaling response. Similarly, RHL41 is involved in light acclimation, cold and oxidative stress responses [48], while DIC2 could be involved in protecting plant cells against oxidative stress. CZF1 (zinc finger CCCH domain-containing protein) is involved in salt stress response. We found complete similarity with defense related proteins like GUN5, STZ, RHL41, DIC2 and other members of WRKY family with the identified AhWRKY41 and we hypothesize that AhWRKY41 positively modulates defense signaling. Further, complete characterization of AhWRKY41 and its counter parts of its modulation during abiotic stress conditions should be ascertained.

5. CONCLUSION

Various abiotic stress such as high temperature, drought and heavy metal adversely affect plant growth and development. WRKY Transcription factors are known to regulate plant stress responses by altering the expression of stress responsive genes. In this study we have characterized the expression of 10 WRKY transcription factors from groundnut. The findings demonstrate that AhWRKY41 would be a positive regulator involved multi abiotic stress tolerance. The tissue specific expression analysis of WRKY TFs under different abiotic stress and their *in silico* characterization may help in complete understanding of regulatory role of these factors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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