



SDI Review Form 1.6

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	Ms_IJBCRR_32784
Title of the Manuscript:	Characterization and expression analysis of WRKY transcription factors in Groundnut (Arachis hypogaea L.,)
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<u>Compulsory</u> REVISION comments	The paper is interesting. It presents novel results on genetic study of Groundnut (<i>A. hypogaea</i>) and could be accepted. I have minor remarks for text improvement and the figures quality. Overall results on motif analysis of WRKY genes could be discussed in more details in relation to molecular mechanism of protection against drought/environmental stress. Gene expression analysis of WRKY genes and bioinformatics are given separately. I'd like see some discussion why presence of binding motif in the transcription factor corresponds to gene expression in different tissues/conditions. Minor English typos are shown in my comments. Please check all the signs"- " across the text, sometimes words should be joined in one without space, sometimes separated without "- " (like, "zinc-finger" or "zinc finger", "qRT- PCR").	We have reframed our manuscript by considering the reviewer comments including the English typos
<u>Minor</u> REVISION comments	Recommend reduce or join redundant histograms, update figure 5. In the text: Abstract: Page 1 - raw 12: "In addition, we conducted expression..." - may remove word "In addition" since the expression profiling is important part of the research. raw 14: AhWRKYs - here abbreviation with prefix 'Ah' is given for the first time in the text. Need explain of avoid it in the abstract. And suffix 's' is redundant.	The figure 5 has been updated by including the group name and the size has been reduced. The corrections are made in the manuscript according to the comments



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	All the abbreviations for gene names like AhWRKY, OsWRKY should be explained in the text at the place of first appearance, indicate organism. Otherwise a may not understand. Raw 15: "revealed that..." - remove word "that" or rephrase English Page 2 - raw 34-35: "OsWRKY45 and 11 in Arabidopsis" - please indicate clearly that it is about rice genes WRKY45 and WRKY11, and remove italic font. raw 37: "TaWRKY2, 10 and19" - again need note that it is Arabidopsis (or what species?) gene, separate numbers. "Recent studies demonstrated WRKY..." - please fix English "Recent studies demonstrated that WRKY..." and possible add more references. "Studies" - the word is in plural, or it is only one study? Raw 40: "TaWRKY79" - again abbreviation without explanation, and might confuse with AtWRKY79 Raw 45: Same about "GmWRKY21" - is it <i>G.max</i> gene? Page 3 - raw 54 - remove "etc." since all the crop plants references were given in text before. Page 4 - raw 78: "exon intron" - should be "exon-intron" Page 5 - raw 97: (Table 1). Should be short description what one can see from the Table 1 - at least one phrase, like "Table 1 presents forward and reverse primers for the genes tested, amplicon length and melting temperature..." raw 101: "...for quantitative the expression.." - please fix English - could be "to quantify the expression.." or like that	
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	raw 105: "...calculate p values at the 0.05 level..." it is wrong sense - either just calculate p-value or estimate significance, "0.05 level" should not be here Page 7 - raw 122: 9/10 and 3/10. It is not clear - maybe change text. 9 of 10 genes are localized in nucleus? Or predicted to be localized? 3 of 10 genes predicted may also be localized in nucleus? raw 127: "...reveals, all" - maybe "...reveals that all"? Page 9 - Figure 1. Please indicate by different types of arrows WRKYGQK motif and other motifs. It seems CT - C termini alignment is redundant here - nothing to show and compare? The arrows are only for N terminal domain. Page 10 - Figure 2 - not discussed in the text. What reader can see from Figure 2? Maybe indicate where are groundnut genes on this phylogenetic tree. Page 11 - raw 160: "to characterize" - fix the word raw 174: "co - efficient" - please fix raw 175: "p-value of 0.00001, ... are significant at <0.05" - it is evident, just remove 0.05 "...p-value of 0.00001 indicating that the results are highly significant". Pge 12 - Figure 3 - Panel a -there are 6 motifs, and first "WRKY" being seen very clear. But please write something about other motifs. Have they any associations with other known protein motifs? Panel b is too small. Please mark also motifs 1-6 on panel a for clarity. Is Motif1 the same as 1. in Panel a? Page 13 - Figure 4 has too small legend, not visible. At least make larger size.	Figure 1 has been updated and the motifs are indicated within the box The resolution of figure 3 has been increased
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	What reader can see from the figure? How exons location corresponds to motifs from previous figure or WRKY genes classification? Need write a phrase in the text. Figure 5 has several panels. It is too large, maybe show it in some more compact form? Again, what reader can see from it except difference in tissue expression? Please add a note to the diagrams about groups of genes (I, II and III) classified in the text. Need some discussion in the text, what is the main observation in relation to presence of motifs, phylogenetic classification. For example, AhWRKY3 gene (page 14) is most responsive to Cadmium stress. Why? Page 19 Figure 6 should be update. STRING web-service allow to remove extra lines (evidence mode). Too many colors. I'd recommend redraw this figure (even manually, in PPT, of course citing STRING as data source) keeping graph structure and indicate WRKY genes by larger font in the figure. What we can say protein interaction of WRKY genes? May just data for such interaction are incomplete for Groundnut? It is for discussion. raw 218: "Although" - please remove the word, and add a reference to that phrase raw 234: "In addition" - word is not need here. IT is better add some discussion and characterization of other MEME motifs (shown in the figure). If new motifs are not similar to known (DNA-binding) motifs - just discuss it. Page 21 raw 259: "...emphasize,AhWRKY20" - fix as	With respect to AhWRKY3 gene were found to be up-regulated significantly and results obtained were consistent with duplicates. However the detailed WRKY3 gene signalling should be deciphered under cadmium toxicity The WRKY TFs from groundnut have been highlighted in the figure.
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	“...emphasize that AhWRKY20” Page 22: Raw 275: “The protein- protein interaction network...” Please fix word “protein-protein” and indicate that it is by STRING database. Or authors used other sources for network reconstruction? The genes in the networks are described by their role in stress response. But what is possible mechanisms of interactions? Are they targets of WRKY as transcription factor? Or is there protein-protein contact?	
<u>Optional/General</u> comments	The manuscript needs some update, including English and figures. I think second review from my side is not necessary. Expression data could be discussed in more details.	The expression data is discussed with fold change and with reference to its expression in other model plants.