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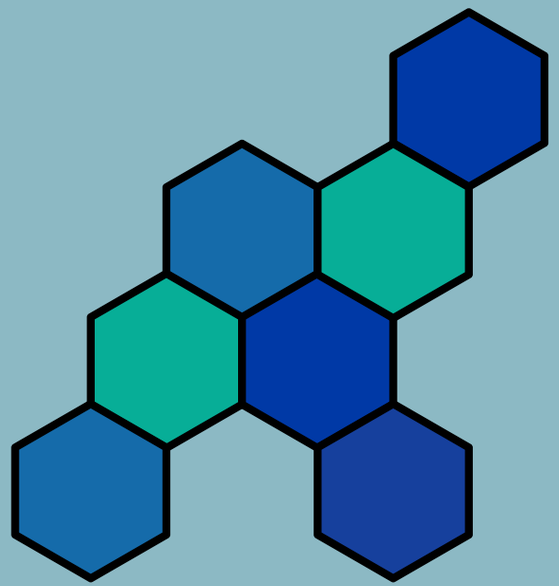

Table of content

abstract	i
Introduction	2
Methods	2
Results	3
Discussion	5
References	5
Appendix	6

Specific WRKY transcription factors in Triticum aestivum (wheat)

Joelle Weir Allisha Saini Lilaa Abdelhadi Sarah Alshammaa

Highlights

- A total of 160 stress-responsive TaWRKY transcription factors were identified across all 21 wheat chromosomes.
- Extensive segmental duplication and elevated Ka/Ks ratios indicate adaptive evolution of TaWRKY genes.
- Conserved WRKY motifs and W-box binding domains underline their central role in stress-responsive gene regulation.
- Heat and drought stress induce distinct TaWRKY expression patterns across tolerant and susceptible wheat cultivars.
- TaWRKY transcription factors represent promising molecular targets for improving wheat abiotic stress tolerance.

ABSTRACT

WRKY transcription factors (TFs) play a critical role in mediating plant responses to abiotic stress, such as heat and drought, in Triticum aestivum (wheat). This study investigates the expression patterns, distribution, and evolutionary characteristics of WRKY TFs in response to these stress conditions, with a focus on their role in enhancing stress tolerance. A total of 160 UDS (undifferentiated stress-responsive) TaWRKY genes were identified, distributed across all 21 wheat chromosomes, with chromosome 3B harboring the highest number. A significant portion of these genes (85) were found to be duplicated, with 80 segmental duplications indicating a history of gene expansion. Phylogenetic analysis revealed evolutionary relationships between TaWRKY and TuWRKY (a close relative from Triticum uniaristatum) genes, suggesting a strong selective pressure on these genes in wheat. A Ka/Ks ratio analysis further indicated positive selection, particularly for divergent genes, pointing to adaptive evolution in these transcription factors. Motif analysis highlighted the conserved structural elements within TaWRKY proteins, crucial for DNA binding at the W-box, underscoring their regulatory role in stress responses. Expression analysis of stress-responsive genes in two wheat cultivars, Capelle Desprez and Plainsman, revealed distinct gene expression patterns under heat and drought stress, with significant upregulation and downregulation of specific genes, emphasizing the differential regulatory mechanisms in response to these stressors. Overall, the findings suggest that TaWRKY TFs are essential for regulating wheat's response to abiotic stress, and their overexpression could enhance stress tolerance by modulating key physiological traits in transgenic plants.

INTRODUCTION

As climate change continues to escalate as one of the most pressing global challenges, its far-reaching impacts are increasingly felt across ecosystems and human livelihoods. Among the most vulnerable are our agricultural systems, with crops standing as the first to bear the burden of shifting weather patterns and rising temperatures. The most vulnerable to this change is Triticum aestivum (wheat) which 21% of the world's food depends on and grows on 100 million hectares of farmland worldwide (Ortiz et al., 2008). Alongside climate change, prolonged drought and unseasonal floods also disrupt traditional farming practices affecting the overall wheat production (Bibi & Rahman, 2023). Due to this increasing threat, understanding the relationship between climate change and crop resilience is critical to developing adaptive strategies that can protect food systems for further generations. The best approach to this is to analyze what can help make wheat more tolerant and resilient to heat and drought. One way of doing so is to understand how wheat adapts to abiotic stress which offers insight for breeding climate-resilient crops (Ortiz et al., 2008). This paper focuses on understanding plant stress biology and underscores the importance of leveraging genomic tools to address the challenges posed by a changing climate. Furthermore, this paper focuses on specific WRKY transcription factors in Triticum aestivum that are expressed differently in response to abiotic stress condition, such as drought and heat. These transcription factors play a crucial role in enhancing stress tolerance, with their overexpression leading to significant changes in stress-related physiological traits in transgenic wheat plants (Gupta et al., 2018). By combining comparative genomics, associative mapping, and transcriptome-wide association studies in Gupta et al. research, they were able to identify 160 UDS – TaWRKY genes and 107 IICS-TaWRKY genes, that show their distribution across wheat chromosomes and their functional roles in stress adaptation. WRKY transcription factors are especially important because they help the wheat with drought stress and are involved in the ABA (abscisic acid) signaling pathway. This is important because ABA is a plant hormone that helps plants response to stress like drought and when its pathway is activated, the plants ability to tolerate drought is increased by it reduces transpiration, it closes the stomate and it slows down the growth to conserve energy and resources (Kumar et al., 2014). 160 UDS-TaWRKY genes and 107 IICS-TaWRKY genes are transcription factors that regulate the expression of stress-responsive genes in wheat (Gupta et al., 2018). They do so by binding to W-box motifs in DNA to activate or repress genes involved in drought tolerance, heat tolerance, osmotic adjustment and antioxidant defense (Kumar et al., 2014). These genes are the focus of this paper as they are critical targets for molecular breeding and biotechnology aimed at improving wheat's resilience and tolerance to climate change.

METHODS (Gupta et al., 2018)

Gupta (et. Al.) 's publication, Deciphering genome-wide WRKY gene family of Triticum aestivum L. and their functional role in response to Abiotic stress, establishes that resistance to drought and heat are influenced by WRKY transcription factors. This was exposed through methodology of mapping and gene expression collation: Associative Mapping, Comparative Genomics, and Transcriptome-Wide Association.

Comparative Genomics

The processing and analysis of UDS-TaWRKY genomic, coding, and transcript sequences for this investigation are covered in this instructional guide. Data downloads, sequence validation, ortholog identification, and gene location visualization are all part of the Comparative Genomics process.

Steps used by Gupta to refine a set of 160 UDS-TaWRKY sequences$_1$ and 107 IICS-TaWRKY sequences$_{1,}$, each validated and examined for WRKY domain presence.

Download UDS-TaWRKY Sequences from Phytozome Database. The researchers first went to the database Phytozome v11.0. Next, in the Phytozome search window, the WRKY domain ID: PF03106 was entered in the search bar. The goal of the researchers here will identify all sequences with the WRKY domain in the Triticum aestivum genome. The resulting set of the of 172 UDS-TaWRKY genomic is downloaded. The coding and transcript sequences is placed in FASTA format. These sequences were then listed in Supplementary Table S1. (Gupta, 2019)

Validate UDS-TaWRKY Sequences through PlantTFDBv4.0 and iTAK Database. PlantTFDBv4.0 is accessed in the Plant Transcription Factor Database. The PlantTFDB is used as search function to input the downloaded UDS-TaWRKY sequences and confirm their transcription factor status. This is to cross-validate each sequence. The researchers then accessed iTAK Database and validated the UDS-TaWRKY sequences again for consistency.

Examine Genomic and CDS Sequences Using GSDS v2.0 . The researchers accessed GSDS (Gene Structure Display Server) v2.0: GSDS. The genomic and CDS sequences of UDS-TaWRKY proteins is input into GSDS v. 2.0. GSDS then visualizes the exon-intron structure of each sequence, helping you identify where introns and exons are located.

Search Against UniProt and NCBI Databases. The UniProt Database: UniProtisused to performa a search for the UDS-TaWRKY sequences to identify IICS-TaWRKY sequences. The NCBI database (https://www.ncbi.nlm.nih.gov/) is used for further sequence validation.

Manual Screening and Sequence Refinement. The researchers manually went through the downloaded sequences to remove any duplicate sequences and those that are incomplete or fragmented. The sequences are removed without WRKY Domain. It is ensured that only sequences containing the WRKY domain are retained for further analysis. After screening, you will be left with 160 UDS-TaWRKY sequences and 107 IICS-TaWRKY sequences suitable for further analysis (the final number of sequences). These can be found in Supplementary Tables S2 and S3. (Gupta, 2019)

Download WRKY Sequences from Triticum urartu. Visit The Ensemble Plant Database is accessed. 83 unique WRKY sequences were then downloaded (67 characterized and 16 uncharacterized) from Triticum urartu (TuWRKY) to search for Triticum uraru (TuWRKY) sequences). UniProt can be used as an alternative to the Ensembl.

Validate Sequences Using HMMER. HMMER is accessed to perform sequence alignment to ensure that your sequences are correctly annotated as WRKY domains.

Confirm WRKY Domains and Locations Using InterPro. InterPro is then used to upload the UDS-TaWRKY sequences to confirm the number and location of WRKY domains within each sequence and perform a domain analysis.

Calculate Physiochemical Properties Using ExPASy ProtParam. ExPASy ProtParam is accessed to analyze the sequences. The UDS-TaWRKY protein sequences in input to calculate physiochemical properties such as molecular weight, isoelectric point (pI), and amino acid composition.

Steps used by Gupta to find the chromosomal location and orthologous genes in Oryza sativa$_2$ and Arabidopsis$_2$ for comparative analysis:

Identify Orthologs Against Oryza sativa and Arabidopsis Using BLASTp (Basic Local Alignment Search Tool) NCBI the orthologous genes of UDS-TaWRKY sequences are identified in Oryza sativa (rice) and Arabidopsis thaliana (a model plant).

Identify Chromosomal Location of UDS-TaWRKY Genes. Using UGRI-BLAST to align the UDS-TaWRKY sequences with all chromosomes of Triticum aestivum are determined in their respective chromosomal locations.
A physical map of the UDS-TaWRKY genes was then generated:

Construct a Physical Map Using PhenoGram. PhenoGram is accessed. The chromosomal location data of UDS-TaWRKY genes obtained in the previous step is input into the program. The data is used to construct a physical map showing the distribution of UDS-TaWRKY genes across the wheat genome.

Associative Mapping

These procedures investigate how TaWRKY transcription factors interact with DNA in response to abiotic stressors like heat and drought by combining molecular modeling with expression data analysis.

Modeling and TaWRKYs 'W-box' of DNA interaction analysis. The first step is the identification of conserved DNA-Binding Domain (DBD) sequences for TaWRKYs. In this case, Triticum aestivum WRKYs are a group of transcription factors, and the DNA-binding domain (DBD) is crucial for their interaction with specific DNA sequences, like the 'W-box' (TTGACT/C). The conserved DBD sequences for each TaWRKY were identified, focusing on TFs that are involved in heat and drought stress responses. (Rushton, 2010)

The second step is the selection of representative TaWRKYs for Molecular Modeling. TaWRKY017, TaWRKY014, and TaWRKY054 were selected for further modeling. These sequences were chosen because of their high sequence identity (ranging from 41.94% to 64.86% based on the sequence alignment analyses using bioinformatics tools such as BLAST) with the WRKY transcription factors from Arabidopsis (PDB ID: 2AYD), which serves as a reference.
Swiss-Modeler was used to create 3D models of the selected TaWRKY transcription factors based on the available templates (PDB ID: 2AYD). This step helps in structurally visualizing how these proteins could interact with DNA, especially focusing on the 'W-box' recognition.
The third step is using the Protein-DNA docking process to model the interaction between the TaWRKY proteins and the DNA helix, particularly focusing on the 'W-box' motif.

The NPDock web server performs Global macromolecular docking (using GRAMM) to predict the initial interactions, then scoring the complex based on how well the protein fits with the DNA. Clustering of best-scored structures is used to identify the most stable complexes. Local refinement using Monte Carlo (MC) simulations to further optimize the docking process, ultimately identifying the best interaction complex. In the Monte Carlo simulation, the temperature ranged from 280 K (initial) to 300 K (final), and the simulation ran for 1000 steps. (Gupta, 2019)

Microarray-based analysis of heat and/or drought-responsive genes. Gene Expression Data was obtained from GSE18205 (Gene Expression Omnibus) to study heat and drought stress responses in wheat. Microarray experiments were, then, conducted using Agilent 15 k wheat seed-specific arrays, with samples taken from two wheat cultivars (Capelle Desprez and Plainsman) grown under heat and drought conditions. The data was normalized using the Robust Multi-Array Analysis (RMA) method, and differentially expressed genes (DEGs) were identified by calculating False Discovery Rate (FDR) and fold change values using R software.A heatmap was created to visualize the expression patterns of TaWRKY genes and other related genes.

Gene ontology annotation and enrichment analysis. GO annotation was done to classify the functions of UDS-TaWRKY sequences. The AgriGO toolkit was used for Singular Enrichment Analysis (SEA) to identify overrepresented functional categories in TaWRKYs. Additionally, GO enrichment of orthologous sequences from Arabidopsis and rice was performed using BiNGO apps in Cytoscape v3.4.0. The significance of these results was tested using the Benjamini and Hochberg false discovery rate (q-value) at 0.05.

Transcriptome-Wide Association

The Transcriptome-Wide Association method is used to find correlations between phenotypes or characteristics, like how a plant reacts to abiotic stress (heat and drought), and the expression of genes in the transcriptome, which is the collection of all RNA molecules in the cell.

Extraction of genomic DNA from young leaves of wheat and target genes primer synthesis. The wheat leaves are then collected. Young wheat leaves (20–25 days old) were collected from 9 different wheat varieties grown in Varanasi, India, in November 2014, collected from research farms at Banaras Hindu University. These wheat varieties were either heat and/or drought-tolerant or susceptible. The wheat varieties include:

- Drought Tolerant: C-306, HD-2888, HD 2733
- Heat Tolerant: DBW-14, HUW 234, K 9107, LOK 1
- Heat Susceptible: HUW 468, PBW 343

The leaves were prepared by mechanically ruptured (broken down) using a sonicator (a device that uses sound waves to break cells open), which helped release the cells' contents, including genomic DNA. After the leaves were ruptured, the genomic DNA was extracted using a standard method called the CTAB protocol with some minor adjustments. The DNA was washed with ethanol to purify it and then stored at –20 °C to keep it safe until further use.

Initial amplification of heat or/and induced TaWRKY genes. The researchers selected 10 heat/drought-responsive TaWRKY genes (from UDS-TaWRKY) and 3 genes (from IICS-TaWRKY) identified through in-silico methods (computational prediction). They then used NCBI Primer-BLAST to design 19 primers (short DNA sequences) that would help amplify (copy) specific parts of the selected genes. Each gene had 2 primers designed. The primers were designed based on how long the amplification product should be and to ensure they would work well for Polymerase Chain Reaction (PCR), a method used to amplify DNA. The designed primers were used to amplify the TaWRKY genes in the genomic DNA from the 9 wheat varieties using PCR (Polymerase Chain Reaction). PCR is like a "DNA photocopier" that makes many copies of a specific DNA segment.

The PCR process involved several steps:

First, the denaturation: The DNA was heated to 95°C to separate the DNA strands.

Second, the annealing process occurs when the temperature was lowered to allow primers to attach to the DNA at 59–61°C. Third, the extension process started when the DNA polymerase enzyme added new DNA bases to make copies at 72°C. These steps were repeated for 40 cycles to get enough copies of the target gene. After PCR, the results (how much DNA was amplified) were checked by running the products on an agarose gel, which separates DNA fragments by size.

Qualitative gene amplification through multiplex PCR. Multiplex PCR is a method that allows the amplification of multiple genes in a single PCR reaction. The 19 primers were grouped based on the size of the PCR product they produced, with groups having similar sizes (differing by at least 50 base pairs). PCR reactions were set up for each group of primers using a special PCR mix (called Qiagen Hotstar PCR Master Mix). The PCR products were then screened on an automated gel electrophoresis system to separate and visualize the different DNA products. The gel was analyzed using QIAxel screen gel software to identify if specific genes were amplified and to determine the alleles (gene variations) present.

RESULTS

Dr. Gupta's research show the following results from the method described above

The results identified the number, locations, and duplication events of UDS TaWRKY genes. These genes are distributed across all 21 wheat chromosomes, with TaWRKY genes present throughout the genome. Gene distribution analysis revealed a total of 160 UDS TaWRKY genes, with chromosome 3B being the most abundant (16 genes) and chromosome 7B the least abundant (2 genes). Additionally, 85 genes were identified as duplicated, including 80 segmental duplications. Genetic and phylogenetic relationships between TaWRKY and TuWRKY transcription factors were also observed. Each gene exhibited variations in exon and intron numbers, though it is unclear whether this aspect is directly relevant to the research thesis. The figure on the left illustrates the evolutionary correlation of WRKY domains through an unrooted phylogenetic tree, constructed using multiple sequence alignment. Meanwhile, the figure on the right maps the genome-wide locations of WRKY genes, indicating their chromosomal placement and approximate positions.

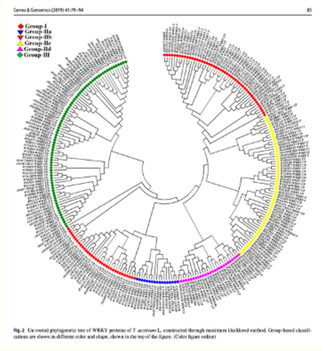

Fig. 2 Un-rooted phylogenetic tree of WRKY proteins of T. aestivum L. constructed through maximum likelihood method. Group-based classifications are shown in different color and shape, shown in the top of the figure. (Color figure online)

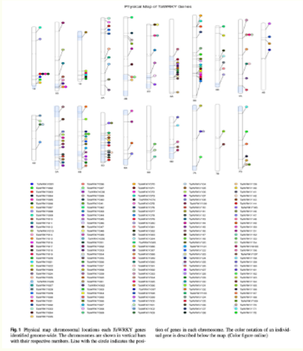

Fig. 1 Physical map chromosomal locations each TaWRKY genes identified genome-wide. The chromosomes are shown in vertical bars with their respective numbers. Lists with the circle indicates the position of genes in each chromosome. The color notation of an individual gene is described below the map. (Color figure online)

The Ka/Ks ratio analysis of TaWRKY and TuWRKY transcription factors was conducted to determine whether these genes are undergoing positive or neutral selection. The Ka/Ks ratio is a key measure in evolutionary biology, comparing the rates of nonsynonymous (Ka) and synonymous (Ks) substitutions in protein-coding genes. Nonsynonymous changes alter the DNA sequence and modify the protein, whereas synonymous changes affect the DNA but do not alter the protein sequence. The analysis revealed that divergent genes exhibit significantly higher Ka/Ks ratios than control genes, suggesting stronger positive selection. This indicates that highly divergent genes may be undergoing adaptive evolution, contributing to their functional specialization. The results provide a detailed classification of TaWRKY and TuWRKY TFs, based on their phylogenetic relationships and structural variations. These findings suggest that selective pressures have shaped the evolution of WRKY genes in wheat, influencing their biological functions.

The following figure presents a comprehensive motif analysis of TaWRKY transcription factors in wheat:

- ·Motif Distribution: Panels a, c, and f depict the distribution of conserved motifs in TaWRKY proteins across Groups I, II, and III. Each color-coded block represents a specific motif present within the protein sequences.
- ·Motif Sequence Logos: Panels b, d, and e visually represent sequence logos for identified motifs, illustrating the conservation of amino acid residues. Larger letters indicate highly conserved residues, emphasizing key structural features of the motifs.

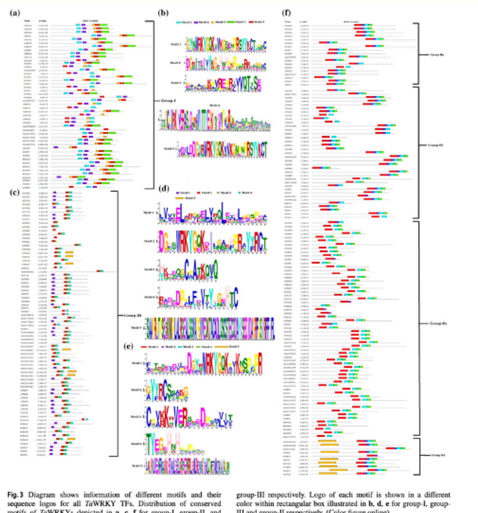

Fig. 3 Diagram shows information of different motifs and their sequence logos for all *Ta*WRKY TFs. Distribution of conserved motifs of *Ta*WRKYs depicted in **a**, **c**, **f** for group-I, group-II, and group-III respectively. Logo of each motif is shown in a different color within rectangular box illustrated in **b**, **d**, **e** for group-I, group-III and group-II respectively. (Color figure online)

The molecular interactions between TaWRKY and the W-box DNA complex were observed, highlighting their role in gene regulation and plant response mechanisms in Triticum aestivum. To support growth and regulate gene expression, TaWRKY transcription factors (TFs) bind to the W-box within the DNA-binding domain, which contains conserved residues essential for strong DNA-protein interactions. This binding process plays a crucial role in regulating gene expression, ensuring an appropriate response to biotic and abiotic stress factors. The beta 2 strand specifically interacts with the W-box, forming hydrogen bonds between the positively charged lysine on beta-2 and the negatively charged phosphate backbone of DNA. The following figure illustrates the binding affinity between DNA and TaWRKY proteins, emphasizing the structural basis of these critical interactions.

Table 2 List different solvent accessible surface area (SASA) for each group representative and their template structure

Name of different surface area calculation	TaWRKY-I	TaWRKY-II	TaWRKY-III	Crystal structure 2AYD and DNA complex
Complex SASA	7965.1	8270.6	8087.5	7651.2
Free protein SASA	5591.2	5717.5	5128.5	4803.4
Free DNA SASA	4066.1	4068.6	4069.1	4069.7
Protein backbone SASA	1277.7	1160	1079.8	1104.5
Protein side chain SASA	4313.5	4557.5	4048.7	3698.9
DNA backbone SASA	2870.6	2871.7	2873.7	2872.6
DNA bases SASA	1195.5	1196.9	1195.4	1197.1
Buried protein surface	793.2	696.3	560.2	611.5
Buried protein backbone surface	138.5	210.7	97.5	91.2
Buried protein side chain surface	654.7	485.5	462.7	520.3
Buried DNA surface	899	819.3	549.9	610.5
Buried DNA backbone surface	682	667.8	445.7	368.7
Buried DNA bases surface	217	151.5	104.2	241.8
DNA major groove surface	766.7	766.5	766.1	767.5
DNA minor groove surface	423.7	425.1	424.4	424.4
Buried DNA major groove surface	200	41.1	0	241.8
Buried DNA minor groove surface	17	110.4	104.2	0
Buried protein polar surface	276.8	302	291.8	302.1
Buried protein apolar surface	516.4	394.2	268.4	309.4
Buried DNA polar surface	513.3	453.2	271.3	392.5
Buried DNA apolar surface	385.7	366.1	278.7	218
Interface area	846.1	757.8	555.1	611

The expression analysis of heat and/or drought-responsive genes during the early grain development of wheat was conducted to identify the function of WRKY transcription factors and monitor gene regulation under stress conditions. The results revealed that 49 genes were expressed when the Capelle Desprez cultivar was subjected to drought, while 88 genes were expressed when exposed to both drought and heat. Similarly, the Plainsman cultivar exhibited 88 expressed genes under heat and drought stress. The following heatmap illustrates gene expression patterns in Capelle Desprez, showing that under heat and drought conditions, 76 genes were downregulated and 11 genes were upregulated. Additionally, under drought-only conditions, 2 genes were downregulated, while 48 genes were upregulated, highlighting the distinct genetic response to environmental stressors. This displays the importance of TaWRKY genes crucial role in plants defense against heat and drought stresses.

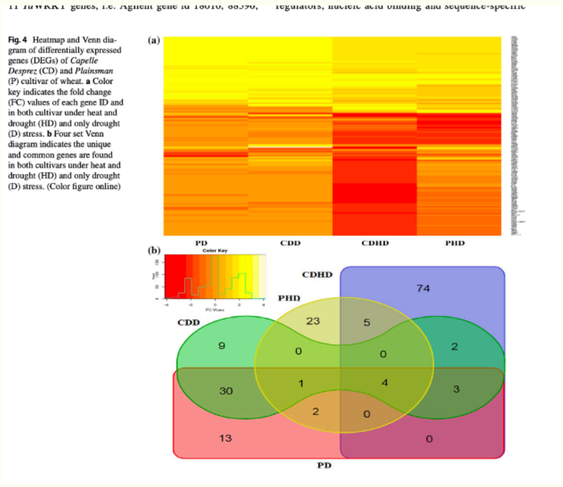

Fig. 4 Heatmap and Venn diagram of differentially expressed genes (DEGs) of *Capelle Desprez* (CD) and *Plainsman* (P) cultivar of wheat. **a** Color key indicates the fold change (FC) values of each gene ID and in both cultivar under heat and drought (HD) and only drought (D) stress. **b** Four set Venn diagram indicates the unique and common genes are found in both cultivars under heat and drought (HD) and only drought (D) stress. (Color figure online)

The GO annotation analysis highlights the molecular functions of TaWRKY transcription factors (TFs) in supporting stress tolerance in wheat. Most TaWRKY proteins function as transcription regulators, binding to nucleic acids with sequence-specific DNA-binding capabilities, which are crucial for activating genes involved in osmotic adjustment and antioxidant defense mechanisms. These TFs play a vital role in regulating stress-response genes, helping wheat adapt to drought and heat stress by modulating gene expression in response to environmental changes. They contribute to key biological processes such as transcription regulation, cellular metabolism, biosynthesis, nitrogen compound metabolism, and RNA metabolic processes, which are essential for plant growth and stress resilience. The TaWRKY TFs primarily function as transcription regulators by binding specifically to DNA sequences that control gene expression, particularly in stress response pathways. These transcription factors are localized in the nucleus, where they interact with target genes to initiate or suppress gene expression in response to environmental stressors. The figure below presents the Gene Ontology (GO) analysis of TaWRKY TFs in wheat, emphasizing their critical role in transcriptional regulation, stress response, and metabolic processes, which support wheat's adaptation to drought and heat stress.

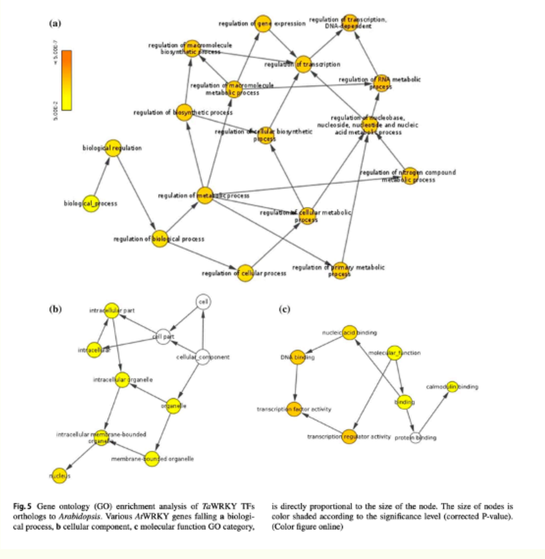

Fig. 5 Gene ontology (GO) enrichment analysis of *Ta*WRKY TFs orthologs to *Arabidopsis*. Various *Ar*WRKY genes falling **a** biological process, **b** cellular component, **c** molecular function GO category, is directly proportional to the size of the node. The size of nodes is color shaded according to the significance level (corrected P-value). (Color figure online)

The in-vitro observation of heat and drought-responsive TaWRKY genes revealed key insights into their expression patterns under stress conditions. Higher band amplification in PCR suggests higher gene expression, and it was observed that heat and drought stress led to increased PCR amplification, indicating greater gene activity in response to these conditions. However, TaWRKY017 and TaWRKY054 showed no amplification in any wheat genotypes, suggesting they may not be expressed under heat or drought stress. Several heat-sensitive genes were identified, including WRKY7, WRKY11, TaWRKY151, TaWRKY103, TaWRKY002, and TaWRKY099, as they were only expressed in heat-susceptible genotypes, indicating their association with heat sensitivity. In contrast, drought-tolerant genes such as TaWRKY014 and TaWRKY090 were amplified in C 306, a well-known drought-resistant wheat cultivar, reinforcing their role in drought response. A limitation of this study was that RNA-based gene expression analysis was not performed due to resource constraints, preventing further validation of expression patterns. The image below presents the PCR Gel Electrophoresis, illustrating the amplification patterns, with each column representing a different wheat cultivar.

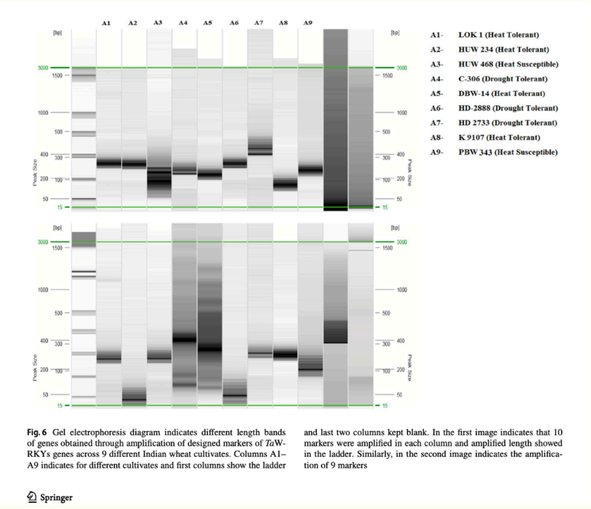

Fig. 6 Gel electrophoresis diagram indicates different length bands of genes obtained through amplification of designed markers of *Ta*W-RKYs genes across 9 different Indian wheat cultivates. Columns A1–A9 indicates for different cultivates and first columns show the ladder and last two columns kept blank. In the first image indicates that 10 markers were amplified in each column and amplified length showed in the ladder. Similarly, in the second image indicates the amplification of 9 markers

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Comparing Gupta's Paper to Khoso:

Similarities – validations

Similarly, Zeng's paper also emphasizes the importance of the W-box in regulating stress response genes, aligning with similar findings. Both studies confirm that WRKY transcription factors (TFs) bind to W-box sequences (TTGACY) in the promoter regions of stress-responsive genes, highlighting the key role of W-box binding in gene regulation. Additionally, both papers support that WRKY genes are highly conserved, particularly the WRKYGQK domain, which is essential for DNA binding. The studies further agree that TaWRKY genes regulate stress responses, particularly in abiotic stress conditions such as heat and drought, by modulating gene expression. The findings also reinforce that W-box binding is a key mechanism in stress gene regulation, as WRKY TFs bind to W-box elements (TTGACC/T) to regulate downstream gene expression. Ultimately, Khoso's paper is validated by Gupta's, as both studies align in their conclusions about the conserved function of WRKY transcription factors in stress adaptation.

Comparing Gupta's Paper to Zeng:

Similarities (validated):

Both papers emphasize the role of WRKY transcription factors (TFs) in abiotic stress responses, particularly in heat and drought resistance. They highlight that specific WRKY genes, such as TaWRKY1, TaWRKY33, and TaWRKY70, are upregulated in response to these stressors, contributing to plant adaptation. Additionally, both studies confirm that WRKY TFs bind to the W-box (TTGAC sequence) in DNA, playing a crucial role in gene regulation and directly influencing plant stress responses. This shared conclusion reinforces the significance of WRKY TFs in regulating stress adaptation mechanisms in plants.

Differences:

The Gupta's paper mentions that TaWRKY33 and TaWRKY1 are upregulated in heat stress. In comparison, the Zeng's paper provides additional insights, stating that certain WRKY genes, such as AtWRY33, are actually inhibited under high temperatures. Moreover, the Gupta's paper suggests that TaWRKY33 and TaWRKY1 are positively involved in stress responses, while the third paper states that WRKY genes can sometimes act as both positive and negative regulators, for instance the GhWRKY33 acting as a negative regulator in drought stress).

DISCUSSION

WRKY transcription factors are crucial in plant resilience to environmental stresses like drought, temperature variations, and salinity (1). They act as molecular switches, regulating the expression of stress-sensitive genes through complex signaling networks. Under heat and drought stress, pathways like the phenylpropanoid and ABA-associated pathways are affected.

WRKY TFs interact with hormonal pathways involving abscisic acid (ABA), ethylene, and jasmonic acid (JA), which regulate processes like stomatal closure and heat shock protein regulation. Combining genes like TaWRKY33 could improve crop resilience to climate change. Research indicates that the overexpression of certain WRKY TFs can enhance tolerance to heat, salinity, and drought stresses in various plants (1). For example, TaWRKY1, TaWRKY33, and TaWRKY70 are upregulated in response to heat and drought. The MAPK cascade is a central regulator of high-temperature stress, leading to the expression of WRKY transcription factors and stress-related genes. TaWRKY proteins function as transcription regulators, bind to nucleic acids, and exhibit sequence-specific DNA binding, which is essential for activating genes involved in osmotic adjustment and antioxidant defense mechanisms (1) . They regulate stress-response genes, helping wheat adapt to drought and heat stress by modulating gene expression.

RNA-based gene expression analysis was not performed due to resource limitations, preventing further validation of expression patterns. Future research could focus on validating these expression patterns and exploring the potential of specific WRKY genes for improving crop resilience to climate change.

Acknowledgements and References

Bibi, F., & Rahman, A. (2023). An Overview of Climate Change Impacts on Agriculture and Their Mitigation Strategies. Agriculture, 13(8), 1508. https://doi.org/10.3390/agriculture13081508

Gupta, S., Mishra, V. K., Kumari, S., Raavi, Chand, R., & Varadwaj, P. K. (2018). Deciphering genome-wide WRKY gene family of Triticum aestivum L. and their functional role in response to Abiotic stress. Genes & Genomics, 41(1), 79–94. https://doi.org/10.1007/s13258-018-0742-9

Khoso, M. A., Hussain, A., Ritonga, F. N., Ali, Q., Channa, M. M., Alshegaihi, R. M., ... & Manghwar, H. (2022). WRKY transcription factors (TFs): Molecular switches to regulate drought, temperature, and salinity stresses in plants. Frontiers in plant science, 13, 1039329.

Kumar, D., Kapoor, A., Singh, D., Lopamudra Satapathy, Singh, A. K., Kumar, M., Prabhu, K. V., & Mukhopadhyay, K. (2014). Functional characterisation of a WRKY transcription factor of wheat and its expression analysis during leaf rust pathogenesis. Functional Plant Biology, 41(12), 1295–1295. https://doi.org/10.1071/fp14077

Li, S., Muneer Ahmed Khoso, Xu, H., Zhang, C., Liu, Z., Sindho Wagan, Khuzin Dinislam, & Liu, L. (2024). WRKY Transcription Factors (TFs) as Key Regulators of Plant Resilience to Environmental Stresses: Current Perspective. Agronomy, 14(10), 2421–2421. https://doi.org/10.3390/agronomy14102421

Ortiz, R., Sayre, K. D., Govaerts, B., Gupta, R., Subbarao, G. V., Ban, T., Hodson, D., Dixon, J. M., Iván Ortiz-Monasterio, J., & Reynolds, M. (2008). Climate change: Can wheat beat the heat? Agriculture, Ecosystems & Environment, 126(1-2), 46–58. https://doi.org/10.1016/j.agee.2008.01.019

Rushton, P. J., Somssich, I. E., Ringler, P., & Shen, Q. J. (2010). WRKY transcription factors. Trends in Plant Science, 15(5), 247–258. https://doi.org/10.1016/j.tplants.2010.02.006

Zeng, Y., He, S., Jing, Y., Nong, Y., Tang, S., Yang, Z., ... & Li, M. (2024). WRKY proteins r egulate the development of plants in response to abiotic stresses. Journal of Plant I nteractions, 19(1), 2299865.

Appendix

1.In Triticum aestivum (wheat), the 160 UDS-TaWRKY and 107 IICS-TaWRKY sequences are significant because they correspond to distinct subgroups of the WRKY gene family. Plant responses to a variety of abiotic stressors, including drought, salt, and temperature extremes, are significantly influenced by the WRKY gene family.

UDS-TaWRKY Sequences: The term "Upstream Domain Sequence," or UDS for short, may refer to a particular subset of WRKY genes that control how the body reacts to abiotic stressors. The 160 UDS-TaWRKY sequences most likely correspond to a specific subset of wheat WRKY transcription factors that are important regulators of the stress response. Their detection and examination can shed light on the genetic mechanisms by which wheat responds to stress.

IICS-TaWRKY Sequences: IICS, which may also stand for "Intron-Exon-Count Sequence," is a method of classifying or recognizing WRKY genes according to their structural characteristics, including exons and introns. A unique set of WRKY genes whose intron-exon structure may be crucial to their role in stress response are represented by the 107 IICS-TaWRKY sequences. Researchers can map out how the genes in wheat's stress tolerance systems work by knowing the precise sequences within these groups.

2.Both rice and Arabidopsis are model plants with comprehensive functional annotations and fully sequenced genomes.

Since rice is a model monocot and Arabidopsis is commonly considered the "model" dicot, they serve as great benchmarks for comparing gene families among different plant species.
Wheat, which has a more complicated genome (hexaploid and big), can benefit greatly from the abundance of knowledge on gene structure, function, and regulatory mechanisms that the well-understood genomes of rice and Arabidopsis offer.

It is a calculated move to take use of the abundance of information available about Oryza sativa and Arabidopsis to investigate WRKY genes in Triticum aestivum. Despite the difficulties of working directly with wheat's more complex genome, this allows researchers to obtain important insights into gene function and stress responses that may be applied to wheat.