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Pradeep Kumar

1. Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India
2. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Telangana, India

Dharavath Hathiram

1. Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India
2. Department of Genetics and Plant Breeding, College of Agriculture, PJTAAU, Hyderabad, Telangana, India

Aayushi Malik

Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India

Preeti Adhana

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Telangana, India

Sagar Agarwal

Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India

Pravesh Kumar

Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India

Geeta Boken

Skill Faculty of Agriculture, Shri Vishwakarma Skill University, Palwal, Haryana, India

Corresponding Author:

Pradeep Kumar

1. Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India
2. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Telangana, India

***In-silico* identification and characterization of biotic and abiotic stress-responsive transcription factors in okra (*Abelmoschus esculentus* L.)**

Pradeep Kumar, Dharavath Hathiram, Aayushi Malik, Preeti Adhana, Sagar Agarwal, Pravesh Kumar and Geeta Boken

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Abstract

Transcription factors (TFs) play a central role in regulating stress-responsive gene networks; however, comprehensive information on stress-associated TFs in okra remains limited. In the present study, an in-silico approach was employed to identify and characterize transcription factors involved in biotic and abiotic stress tolerance in okra using transcriptome-derived unigene sequences. A total of 153,414 unigene sequences obtained from the World Vegetable Center were analyzed using PlantTFDB, leading to the identification of 962 transcription factors. Based on sequence similarity, BLAST E-values, and functional annotation against *Arabidopsis thaliana*, 17 transcription factors belonging to the WRKY, NAC, ERF, and bZIP families were shortlisted as putatively stress-responsive. Among these, WRKY and NAC families were predominantly associated with biotic stress responses, while ERF and bZIP families were mainly linked to abiotic stress tolerance. Further characterization of these transcription factors was performed through conserved protein motif analysis using the MEME Suite (GLAM2). A total of 17 gapped protein motifs were identified, exhibiting family-specific conservation patterns. Comparative motif analysis using TOMTOM revealed strong similarity between the predicted motifs and known transcription factor binding motifs, confirming their regulatory relevance. Overall, this study provides a comprehensive in-silico framework for identifying stress-responsive transcription factors in okra and highlights candidate genes that may be exploited for functional validation and crop improvement strategies aimed at enhancing stress tolerance.

Keywords: Okra, transcription factors, biotic stress, abiotic stress, PlantTFDB, MEME suite

1. Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) is an important vegetable crop belonging to the family Malvaceae and is widely cultivated in tropical and subtropical regions of the world. It is known by different local names, including lady's finger, gumbo, and bhindi. Although its precise origin remains debated, Abyssinia has been suggested as the primary center of origin (KHAJURIA, 2023; Davis, 2022) ^[15, 7]. The genus *Abelmoschus* comprises both cultivated and wild species, among which *A. esculentus* is the most extensively grown. Okra is an allopolyploid species showing considerable variation in chromosome number and ploidy levels, reflecting its complex evolutionary history (Nieuwenhuis *et al.*, 2024; Yadav *et al.*, 2025) ^[24, 38].

Nutritionally, okra is valued for its high content of dietary fiber, proteins, vitamins (A, B, and C), and essential minerals such as calcium, iron, and phosphorus (Gemede *et al.*, 2015; Bawa & Badrie, 2016; Uwiringiyimana *et al.*, 2024) ^[12, 4, 34]. In addition to its culinary importance, okra has industrial and medicinal value, with mature fibers used in paper production and traditional remedies for genitourinary disorders and dysentery (Gaur *et al.*, 2025; Elkhalfi *et al.*, 2021) ^[11, 9]. Globally, okra occupies a significant area under cultivation, with India being the largest producer, contributing over 70% of the world's total production (J. Singh & Nigam, 2023; Tikoo *et al.*, 2025) ^[31, 33]. Despite its economic importance, okra productivity remains limited by multiple biotic and abiotic stresses. Among biotic stresses, viral diseases such as yellow vein mosaic virus and enation leaf curl virus (ELCV) cause severe yield losses ranging from 50% to 90% (Mubeen *et al.*, 2021; Mohapatra, 2023; Sastry *et al.*, 2025) ^[23, 21, 27]. ELCV belongs to the

genus Begomovirus of the family Geminiviridae and is transmitted by the whitefly (*Bemisia tabaci*) in a semi-persistent manner (Idrees *et al.*, 2024; Fiallo-Olivé *et al.*, 2020) [14, 10]. The association of monopartite begomoviruses with betasatellites further enhances disease severity in okra (A. K. Singh *et al.*, 2021; Varma & Singh, 2024) [30, 35]. Abiotic stresses such as drought, high temperature, and poor soil conditions further exacerbate yield instability, collectively posing major challenges to sustainable okra production.

Plant responses to biotic and abiotic stresses are primarily regulated at the transcriptional level through complex gene regulatory networks. Transcription factors (TFs) are key regulatory proteins that activate or repress gene expression by modulating RNA polymerase II activity (Baillio *et al.*, 2019; Chacón-Cerdas *et al.*, 2020; Sharma *et al.*, 2024) [2, 6, 29]. TF-mediated regulation represents the major control point of gene expression, enabling plants to respond dynamically to developmental cues and environmental stimuli (Swift *et al.*, 2022; Alvarez *et al.*, 2021) [32, 1]. In plants, TFs constitute approximately 8% of the total gene pool, highlighting their functional importance. Several TF families, including AP2/ERF, WRKY, NAC, MYB, bZIP, ARF, and B3, play critical roles in regulating stress-responsive pathways and plant development (Liu *et al.*, 2023; Ma *et al.*, 2024; Zheng *et al.*, 2025; Xiong *et al.*, 2025) [39, 37]. These TFs function through sequence-specific DNA binding and protein–protein interactions and often cooperate with chromatin-modifying complexes to regulate transcription (Morgunova & Taipale, 2017; Velthuijs *et al.*, 2021) [22, 36]. Alterations in TF activity and regulatory specificity are recognized as major contributors to evolutionary adaptation and stress tolerance in plants.

Advances in genome sequencing and bioinformatics have enabled large-scale identification and classification of TFs across plant species. Databases such as PlantTFDB provide comprehensive information on TF families, domain architectures, and functional annotations (Blanc-Mathieu *et al.*, 2024) [5]. In addition, motif discovery tools such as the MEME Suite facilitate the identification of conserved protein motifs and DNA-binding domains, providing insights into TF function and regulatory mechanisms (Machanick & Bailey, 2011) [20]. Despite the availability of such resources, systematic information on transcription factors associated with biotic and abiotic stress tolerance in okra remains limited. Therefore, the present study aims to perform an in-silico identification and characterization of transcription factors in okra, with particular emphasis on their potential roles in stress tolerance. The findings are expected to provide a valuable genomic resource for functional studies and to support future breeding and biotechnological efforts aimed at developing stress-resilient okra cultivars.

2. Materials and Methods

2.1 Data Source

Unigene sequences derived from transcriptomic data of okra (*Abelmoschus esculentus* L.) were obtained from the World Vegetable Center (AVRDC). These sequences constituted the primary dataset for identifying transcription factors associated with biotic and abiotic stress responses.

2.2 Identification of Transcription Factors

Transcription factors (TFs) were identified using PlantTFDB version 5.0, an integrative plant transcription factor database that classifies TFs based on conserved DNA-binding domains and family-specific signatures. Unigene sequences were uploaded in FASTA format to the TF prediction module. Prediction was

performed using domain annotation and BLAST searches against curated plant TF datasets, particularly from *Arabidopsis thaliana*.

For each predicted TF, information on TF family, best-hit *A. thaliana* homolog, BLAST E-value, and functional annotation was retrieved. TFs showing significant sequence **similarity** and low E-values were shortlisted. Based on their established roles in stress tolerance, transcription factors belonging to the WRKY, NAC, ERF, and bZIP families were selected for downstream analyses.

2.3 Protein Motif Discovery

Conserved protein motifs within the selected transcription factors were identified using the MEME Suite, employing the GLAM2 (Gapped Local Alignment of Motifs) algorithm. GLAM2 is particularly suited for identifying gapped motifs characteristic of transcription factor DNA-binding domains. Protein sequences corresponding to the selected TFs were used as input, and motif discovery was performed using default parameters with multiple independent replicates to ensure motif consistency.

Motifs were ranked based on GLAM2 scores, with higher scores indicating stronger motif conservation. Information regarding motif length, alignment, positional distribution, and marginal scores was recorded for further analysis.

2.4 Motif Comparison and Validation

To validate the functional relevance of the identified motifs, comparative motif analysis was conducted using the TOMTOM tool within the MEME Suite. Predicted motifs were compared against curated motif databases to identify similarity with known transcription factor binding motifs. Motifs exhibiting significant similarity and alignment scores were considered biologically relevant.

2.5 Data Output and Visualization

Output files, including transcription factor prediction tables, motif alignments, and motif comparison results, were generated in HTML and PNG formats and used for result interpretation and figure preparation.

3. Results and Discussion

3.1 Identification and Classification of Stress-Responsive Transcription Factors

Transcription factors associated with biotic and abiotic stress responses in okra were identified using the PlantTFDB web-based platform. Unigene sequences derived from okra transcriptomic data were screened to predict transcription factors based on conserved DNA-binding domains and family classification. Based on prior evidence of stress-responsive TF families in plants, particularly in *Arabidopsis thaliana*, four major TF families WRKY, NAC, ERF, and bZIP were selected for detailed analysis (S. Li *et al.*, 2024) [19]. A total of 17 transcription factors were identified as putatively involved in stress responses in okra (Table 1). Among these, 10 TFs were associated with biotic stress tolerance, including eight WRKY and two NAC family members. WRKY and NAC transcription factors are well documented as central regulators of plant defense signalling, particularly in pathogen recognition and activation of downstream defense genes (Saha *et al.*, 2024; Dong *et al.*, 2024) [25, 8]. The predominance of WRKY TFs observed in this study supports their conserved and prominent role in regulating biotic stress responses.

In contrast, seven transcription factors were identified as

associated with abiotic stress tolerance, comprising five ERF and two bZIP family members. ERF and bZIP TFs are known to regulate plant responses to drought, salinity, temperature extremes, and hormonal signaling pathways, particularly through abscisic acid-dependent and independent mechanisms (Banerjee & Roychoudhury, 2017; Hu *et al.*, 2022) [3, 13]. The presence of AP2/ERF-related subfamilies, including DREB and RAV, further highlights the importance of these TFs in regulating dehydration, heat shock, cold, and salt stress responses. BLAST analysis against *A. thaliana* revealed that

okra TF unigenes showed significant homology with well-characterized transcription factors, with strong E-values ranging from 1.00E-11 to 1.00E-110. Functional annotations of best hits indicated involvement in hormone signaling, stress-responsive transcriptional regulation, and developmental control, suggesting functional conservation across species. These results demonstrate that okra possesses a diverse and conserved repertoire of transcription factors potentially involved in both biotic and abiotic stress adaptation.

Table 1: seventeen Predicted transcription factors those are responsible for biotic and abiotic stress in okra

S. No	TF ID	Family	Best hit in <i>A. thaliana</i>	Blast e-value	Description for the best hit
1	Unigene102680_TOT5864	WRKY	AT1G50600.1	1.00E-39	scarecrow-like 5
2	Unigene102808_TOT5864	WRKY	AT3G54220.1	1.00E-29	GRAS family protein
3	Unigene10308_TOT5864	WRKY	AT1G75080.2	1.00E-110	BES1 family protein
4	Unigene104561_TOT5864	WRKY	AT4G17880.1	1.00E-39	bHLH family protein
5	Unigene104982_TOT5864	WRKY	AT3G56850.1	1.00E-29	ABA-responsive element binding protein 3
6	Unigene105075_TOT5864	WRKY	AT5G66770.1	1.00E-28	GRAS family protein
7	Unigene106746_TOT5864	WRKY	AT4G28530.1	1.00E-47	NAC domain containing protein 74
8	Unigene113096_TOT5864	WRKY	AT3G22830.1	1.00E-44	heat shock transcription factor A6B
9	Unigene21397_TOT5864	NAC	AT3G19360.1	1.00E-12	C3H family protein
10	Unigene23290_TOT5864	NAC	AT1G26610.1	1.00E-16	C2H2-like zinc finger protein
11	Unigene16076_TOT5864	bZIP	AT4G36930.1	1.00E-15	bHLH family protein
12	Unigene15231_TOT5864	bZIP	AT2G04890.1	1.00E-29	SCARECROW-like 21
13	Unigene43763_TOT5864	ERF	AT5G06510.2	1.00E-11	nuclear factor Y, subunit A10
14	Unigene45594_TOT5864	ERF	AT2G01570.1	1.00E-17	GRAS family protein
15	Unigene46947_TOT5864	ERF	AT2G40950.1	1.00E-15	bZIP family protein
16	Unigene61893_TOT5864	ERF	AT1G14440.1	1.00E-15	homeobox protein 31
17	Unigene64241_TOT5864	ERF	AT2G37120.1	1.00E-15	S1FA-like DNA-binding protein

3.2 Identification and Characterization of Conserved Protein Motifs

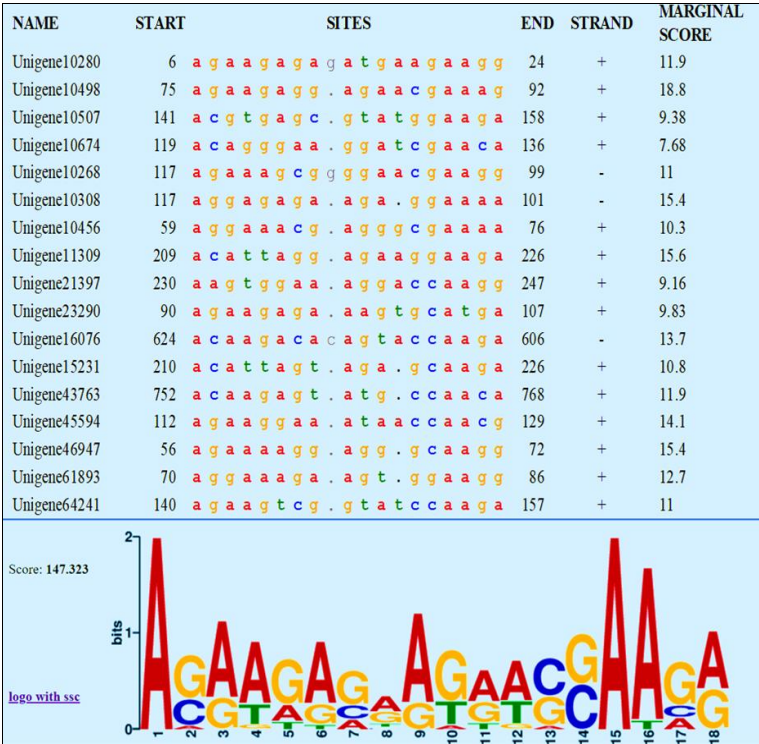


Fig 1: Identification of total 17 gapped protein motif from the unigenes sequences by using MEME Suite (GLAM2)

To further elucidate the functional properties of the identified transcription factors, conserved protein motifs were analyzed using the MEME Suite, specifically the GLAM2 algorithm. The same 17 unigene sequences identified as transcription factors were used for motif discovery. GLAM2 is particularly suitable

for detecting gapped motifs, which are characteristic of transcription factor DNA-binding domains and regulatory regions (Schultheis *et al.*, 2024) [28]. A total of 17 gapped protein motifs were identified across the WRKY, NAC, ERF, and bZIP transcription factor families

(Figure 1). These motifs exhibited conserved aligned columns interspersed with insertions and deletions, reflecting structural flexibility while maintaining functional specificity. Such gapped motifs are known to contribute to DNA-binding affinity and protein–protein interactions, which are critical for transcriptional regulation (Santos, 2025) [26].

GLAM2 assigned motif scores based on alignment strength, with higher scores indicating stronger motif conservation. Site-

specific scores further reflected the degree of similarity between individual sequences and the consensus motif (Figure 2). Family-specific motif patterns were observed, with WRKY motifs showing high conservation consistent with their well-defined WRKY DNA-binding domain, while ERF and bZIP motifs displayed greater variability, likely reflecting their involvement in multiple stress signalling pathways.

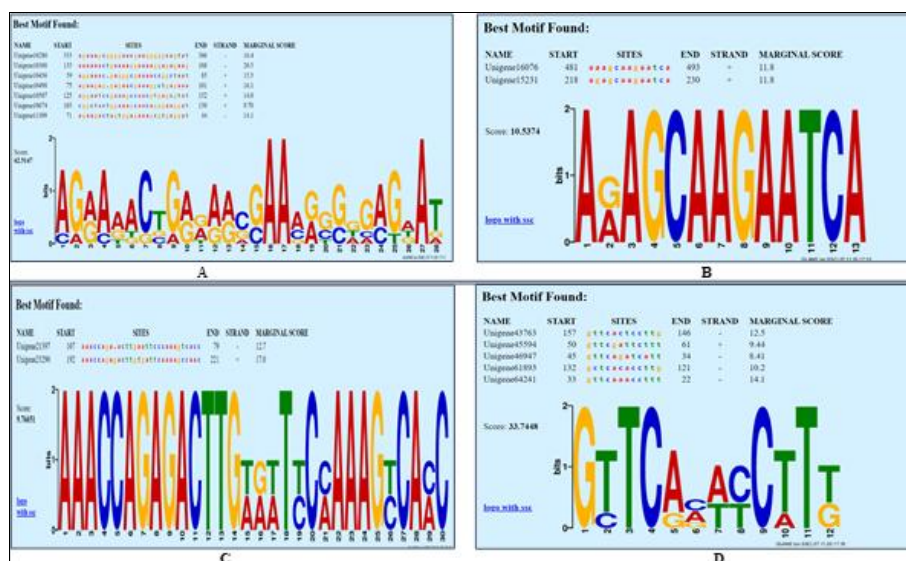


Fig 2: GLAM 2 gapped motif output; A. Wrky family motif, B. NAC family motif, C. bZIP family motif and D. ERF family motif

3.3 Comparative Motif Analysis and Functional Implications

To validate the biological relevance of the identified motifs, motif comparison analysis was performed using the TOMTOM tool. TOMTOM analysis revealed that the predicted motifs showed strong similarity to known transcription factor binding motifs corresponding to WRKY, NAC, ERF, and bZIP families (Figure 3). This confirms that the identified motifs represent authentic regulatory elements involved in transcriptional control.

The conservation of these motifs across TF families suggests that okra transcription factors share regulatory mechanisms similar to those characterized in model plants. Given that transcription factors regulate stress responses by coordinating large gene networks, the presence of conserved motifs supports their potential role in orchestrating defense and stress-adaptive pathways.

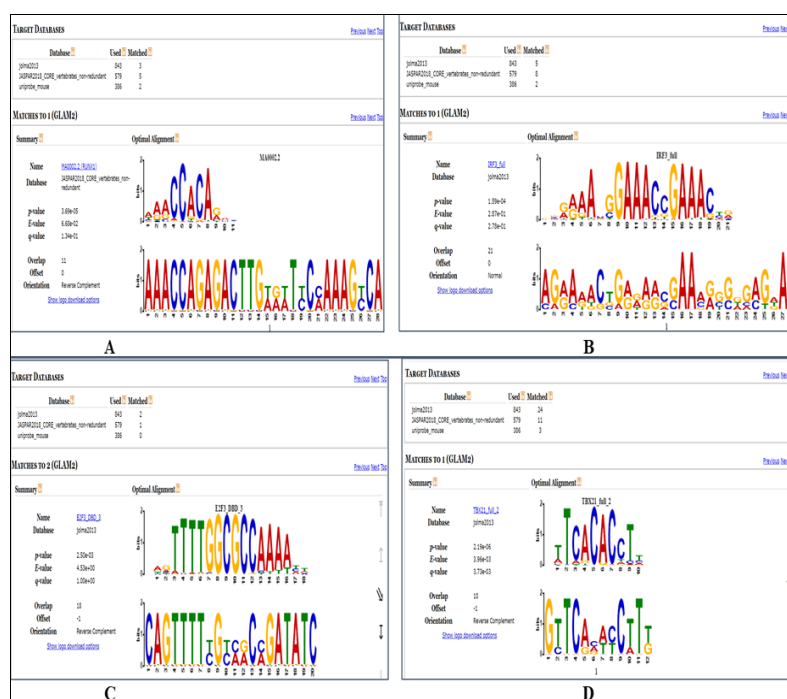


Fig 3: TOMTOM output. The figure shows the TOMTOM output from searching a single DNA motif against a collection of okra transcription factor binding site motifs identified via GLAM 2. TOMTOM shows that the query motif closely resembles the binding motif for transcription factor. A. WRKY, B. NAC, C. bZIP and, D. ERF families of transcription factors

4. Implications for Okra Improvement

The identification of stress-responsive transcription factors and their conserved motifs provides valuable insights into the transcriptional regulation of biotic and abiotic stress tolerance in okra. WRKY and NAC TFs identified in this study may serve as key candidates for improving disease resistance, particularly against viral pathogens. Similarly, ERF and bZIP TFs may be targeted for enhancing tolerance to drought, salinity, and temperature stress.

These findings demonstrate the potential application of transcription factor genes in okra improvement through marker-assisted selection, transgenic approaches, or genome editing strategies. Overall, this in-silico study establishes a foundational framework for future functional validation and utilization of transcription factors to develop stress-resilient okra cultivars.

5. Conclusion

A total of 962 transcription factors were identified from 153,414 okra unigenes through in-silico analysis, of which 17 key transcription factors belonging to WRKY, NAC, ERF, and bZIP families were selected based on significant homology with *Arabidopsis thaliana*. WRKY and NAC families were mainly associated with biotic stress responses, whereas ERF and bZIP families were linked to abiotic stress tolerance. Future functional validation of these transcription factors through expression analysis and genome editing may enable their effective deployment in developing stress-resilient okra cultivars.

6. Disclaimer (Artificial intelligence)

The author (s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

7. Competing Interests

The authors have declared that no competing interests exist.

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