

Original Research Article

***In silico* Genome Wide Identification of Salt Stress Responsive Genomic Element with Special Reference to MYC2 Gene in *Vicia faba* L. Using Computational Approach**

Laiyya Noor¹, Arya Ji¹, Manoj Kumar Sharma¹, Sachin Kumar^{1*}

¹Department of Bioinformatics, JV College, Baraut (Baghpat), Uttar Pradesh, India

***Corresponding Author:** Sachin Kumar

Department of Bioinformatics, JV College, Baraut (Baghpat), Uttar Pradesh, India

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Abstract: *Vicia faba* (faba bean) is a globally significant cool-season grain legume valued for its high protein content, nitrogen-fixing capacity, and adaptability to diverse agro-climatic conditions. However, abiotic stresses, particularly drought, severely constrain its productivity. Transcription factors of the MYC2 superfamily play pivotal roles in regulating plant responses to abiotic stress, including drought tolerance. This study presents an integrated bioinformatics pipeline to identify, characterize, and analyze putative drought stress-responsive MYC2 genes in *V. faba*. Using *Arabidopsis thaliana* MYC2 (UniProt: Q39204) as a reference, we performed homology-based screening against the *V. faba* genome via Ensembl Plants BLAST. Candidate sequences underwent rigorous physicochemical profiling (ProtParam), conserved domain analysis (NCBI-CDD), motif elucidation (MEME Suite), phylogenetic reconstruction (MEGA), gene structure visualization (GSDS), and subcellular localization prediction (WoLF PSORT). Iterative filtering based on domain architecture and motif conservation yielded a high-confidence set of MYC2 candidates. Phylogenetic analysis revealed diversification across evolutionary clades, with evidence of legume-specific expansion. The majority of candidates exhibited predicted nuclear localization, acidic to mildly basic isoelectric points, and thermostable aliphatic indices consistent with transcriptional regulatory functions. Gene structural analysis revealed intron-exon architectural diversity, suggesting evolutionary divergence and potential alternative splicing regulation. This work establishes a foundational genomic framework for understanding MYC2-mediated drought stress signaling in faba bean and identifies candidate targets for future functional validation and translational breeding toward drought-tolerant cultivars.

Keywords: *Vicia faba*, MYC2 Transcription Factors, Drought Stress, Abiotic Stress, Bioinformatics, Phylogenetics, Conserved Domains, Subcellular Localization.

INTRODUCTION

The faba bean (*Vicia faba* L.; Fabales: Fabaceae) represents one of the most important cool-season annual grain legumes cultivated worldwide. Historically regarded as an inexpensive yet high-quality vegetable protein source for both human consumption and animal feed, faba bean currently ranks among the top grain legumes globally (Gu *et al.*, 2020). Worldwide production spans approximately 2.5 million hectares, yielding nearly 5 million tonnes annually, with China (36.7%), Ethiopia (20.1%), the United Kingdom (8.2%), and Australia (7.7%) dominating global output (FAO, 2018). The ecological versatility of *V. faba* distinguishes it from other major legumes. Unlike soybean, which performs poorly in cool climates, faba bean thrives in temperate and high-altitude environments with brief growing seasons (<100 days) (Huang *et al.*, 2019; Stoddard and Hamalainen, 2011). It tolerates a broad pH range (6-9) and can produce acceptable yields in marginal soils where cereals such as barley and wheat struggle (Castanon *et al.*, 1990; Etemadi *et al.*, 2019). Moreover, faba bean exhibits exceptional biological nitrogen fixation efficiency among cool-season legumes, fixing between 50 and 330 kg N per hectare depending on climatic and management conditions (Galloway *et al.*, 2004; Khazaei *et al.*, 2019). This

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capacity reduces dependency on industrial nitrogen fertilizers, offering substantial ecosystem services that promote sustainable agricultural intensification.

Abiotic stress encompasses all negative environmental factors that impede healthy plant development, including drought, salinity, extreme temperatures, nutrient deficiencies, and heavy metal toxicity (Fahad *et al.*, 2017; Wu *et al.*, 2023). These stressors disrupt cellular metabolism, morpho-physiological processes, and molecular signaling networks, ultimately manifesting as reduced growth, reproductive failure, and yield loss (Ahmad *et al.*, 2023; Ben Rejeb *et al.*, 2014). Drought stress constitutes one of the most pervasive abiotic constraints to global agriculture. Approximately 55 million individuals globally are impacted by drought, and nearly 700 million people are at risk of being displaced by 2030 as a result (WWAP, 2018). The socio-economic effects of drought result in significant financial losses. For instance, the long California drought resulted in agricultural losses of around 3.8 billion dollars, including 1.7 billion dollars in crop revenue losses from 2014 to 2016 (Howitt *et al.*, 2015). In India, of the 159.7 million hectares of farmland, 42% is affected by drought (Gogoi and Tripathi, 2019). Consequently, it is essential to transition to sustainable agriculture and address challenges associated with water scarcity and its effects on food security (Kaushal, 2019).

The MYC2 transcription factor, a member of the basic helix-loop-helix (bHLH) superfamily, ranks among the most functionally significant regulatory proteins in higher plants. MYC2 was initially identified due to its high binding preference for the 5'-CACNTG'-3' sequence, commonly known as the E-box (de Pater *et al.*, 1997). Later reports have additionally verified that MYC2 attaches to the G-box (5'-CACGTG'-3') and hexamer sequences related to the G-box (Abe *et al.*, 1997; Boter *et al.*, 2004; Yadav *et al.*, 2005). MYC2 proteins serve as the main transcription factors within the jasmonic acid (JA) signaling pathway. The MYC family, part of the bHLH superfamily, is commonly found in bryophytes and angiosperms (Peñuelas *et al.*, 2019). MYC2 is part of the IIIe bHLH group and features a 60-amino acid conserved bHLH domain at the C-terminus, consisting of a Basic and an HLH domain, as well as a JID domain and a TAD domain located at the N-terminus (Goossens *et al.*, 2015). The Basic domain comprises 15-20 fundamental amino acids and primarily facilitates binding to the G-box. The HLH domain features a loop region that links two α -helices made up of hydrophobic amino acids, which are crucial for creating homo- or heterodimers. The JID domain interacts with JAZ proteins, whereas the TAD domain is tasked with transcriptional activation and engaging with MED25.

MYC2 from *Arabidopsis thaliana* (UniProt: Q39204) has been mechanistically validated as a regulator of drought stress response through jasmonic acid and abscisic acid-mediated pathways, thereby enhancing plant tolerance to adverse environmental conditions. MYC2-mediated JA signaling affects the transcriptional reprogramming of various stress-responsive genes (Zander *et al.*, 2020). Given the established role of MYC2 in drought tolerance and the agricultural importance of faba bean, systematic identification and characterization of MYC2 orthologs in *V. faba* represents a critical step toward understanding and potentially manipulating drought stress responses in this legume crop.

Experimental Section

This study employed an integrated computational biology pipeline to identify, characterize, and analyze drought stress-responsive MYC2 genes in *Vicia faba*. The workflow proceeded sequentially through reference gene selection, homology-based ortholog retrieval, physicochemical characterization, conserved domain and motif analysis, phylogenetic reconstruction, gene structure visualization, subcellular localization prediction, and integrative data visualization.

Reference Gene Identification and Sequence Retrieval

The UniProt accession Q39204, encoding *Arabidopsis thaliana* MYC2 transcription factor, was selected as the query reference based on its empirically validated role in drought stress (abiotic stress) response. The complete amino acid sequence was retrieved in FASTA format from the UniProt database (The UniProt Consortium, 2023) after confirming the documented function of MYC2 in regulating drought-responsive genes through jasmonic acid and abscisic acid-mediated pathways.

Homology Search and Ortholog Retrieval

The MYC2 protein sequence was used as query in a BLAST search against the *V. faba* genome database via Ensembl Plants (Bolser *et al.*, 2017). Significant hits were evaluated based on E-values, bit-scores, and alignment coverage. For each candidate gene, gene identifiers, chromosomal locations, base pair lengths, and complete amino acid sequences were retrieved. Coding sequence (CDS), genomic DNA, and protein sequence data were systematically downloaded and organized for downstream analyses.

Physicochemical Property Analysis

The physicochemical properties of all retrieved *V. faba* protein sequences were analyzed using the ProtParam tool on the ExPASy server (Gasteiger *et al.*, 2005). Parameters computed included theoretical isoelectric point (pI), molecular weight, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). These parameters provide insights into protein solubility, thermal stability, and hydrophobic nature under stress conditions.

Conserved Domain and Motif Analysis

Conserved domains were identified using the NCBI Conserved Domain Database (CDD) with Pfam hidden Markov models (Finn *et al.*, 2016; Marchler-Bauer *et al.*, 2017). Domain architecture was visualized using TBtools (Chen *et al.*, 2020), and sequences lacking recognizable bHLH domains were excluded. De novo conserved motif discovery was performed using the MEME Suite (Bailey *et al.*, 2009), employing expectation-maximization algorithms to identify statistically significant sequence patterns. Candidates lacking at least two of three identified conserved motifs were subsequently filtered out.

Phylogenetic Reconstruction

Evolutionary relationships among filtered *V. faba* MYC2 candidate proteins were reconstructed using MEGA version 12 (Tamura *et al.*, 2021). Multiple sequence alignment was performed using the ClustalW algorithm (Thompson *et al.*, 1994). Maximum Likelihood phylogenetic trees were constructed using the JTT+G substitution model with 1000 bootstrap replicates. Trees were exported in Newick format and annotated using iTOL (Letunic and Bork, 2021) for publication-quality visualization with branch coloring by evolutionary clade.

Gene Structure and Subcellular Localization Analysis

Intron-exon organization was analyzed using the GSDS server (Guo *et al.*, 2007; Hu *et al.*, 2015) with CDS and corresponding genomic DNA sequences as inputs. Subcellular localization was predicted using WoLF PSORT (Horton *et al.*, 2007) in plant mode, generating probability scores for nuclear, cytoplasmic, mitochondrial, chloroplast, Golgi, plasma membrane, and extracellular compartments. Localization data were compiled and visualized as comparative heatmaps using TBtools.

RESULTS AND DISCUSSION

The integrated bioinformatics pipeline successfully identified, characterized, and analyzed a high-confidence set of drought stress-responsive MYC2 transcription factor candidates in *Vicia faba*. The results are presented sequentially, with integrated discussion of their biological implications.

Reference Gene Functional Annotation

UniProt entry Q39204 was confirmed to encode MYC2, a 623-amino acid transcription factor containing the characteristic bHLH domain, JID domain, and TAD domain. Its established role in regulating drought stress response through jasmonic acid and abscisic acid-mediated pathways, thereby enhancing plant tolerance to adverse environmental conditions, provided a functionally validated anchor for homology-based screening. The selection of this reference was strategically motivated by its dual regulatory capacity: MYC2 functions not merely as an activator but as a master regulator that integrates diverse signaling pathways in Arabidopsis (Lorenzo *et al.*, 2004). This nuanced regulatory logic increases the probability that BLAST-derived homologs in *V. faba* share not only sequence ancestry but potentially analogous physiological functions.

Identification of Putative Orthologs in *Vicia Faba*

BLAST analysis of the MYC2 query against the *V. faba* genome yielded multiple significant hits with high sequence similarity and low E-values. The retrieval of numerous candidates was notable given the historically challenging genomic landscape of faba bean, characterized by an exceptionally large (~13 Gb), highly repetitive genome that has only recently yielded chromosome-scale assemblies (Jayakodi *et al.*, 2023). The abundance of hits suggests lineage-specific expansion of MYC2 genes in faba bean, consistent with patterns observed in other legume species where abiotic stress-associated transcription factor families frequently amplify via tandem duplication or whole-genome duplication (Jiang *et al.*, 2017). However, BLAST similarity does not guarantee functional orthology. We therefore treated initial hits as candidate sequences requiring iterative validation through domain, motif, and phylogenetic analyses.

Physicochemical Profiles as Functional Readouts

ProtParam analysis revealed distinct molecular phenotypes across the candidate set. Theoretical isoelectric points (pI) ranged from acidic (~5.0) to mildly basic (~8.5), with a modal distribution around pH 6.5-7.5. This charge distribution is compatible with nuclear function, as transcription factors must maintain solubility in the mildly alkaline nucleoplasm while engaging in electrostatic interactions with DNA phosphate backbones. Molecular weights varied considerably, reflecting differences in N-terminal and C-terminal extensions beyond the conserved bHLH domain. Notably, several candidates scored marginally above the canonical instability threshold of 40. Rather than indicating structural deficiency, controlled instability may serve as a regulatory feature: stress-responsive transcription factors often require rapid proteolytic clearance to prevent constitutive pathway activation, and an inherently labile fold could facilitate ubiquitin-mediated degradation (Matsushita *et al.*, 2013; Miao and Zentgraf, 2010). The uniformly negative GRAVY scores and moderate-to-high aliphatic indices sketch a molecular phenotype of hydrophilic, thermostable regulatory proteins adapted to dynamic nuclear environments.

Domain Validation and Architectural Integrity

NCBI-CDD analysis using Pfam hidden Markov models identified the diagnostic bHLH DNA-binding domain in the majority of candidate sequences. The domain spanned approximately 60 amino acids and contained the characteristic Basic region and HLH domain essential for DNA binding and dimerization. Domain E-values were highly significant (typically 10^{-20} to 10^{-40}), confirming biological authenticity. Several candidates contained additional ancillary domains, including the JID (Jasmonate-associated ZIM-domain Interacting Domain) and TAD (Transcription Activation Domain) motifs that are critical for JAZ protein interaction and transcriptional activation, respectively. Sequences lacking recognizable domains were excluded to prevent contamination by pseudogenes, assembly artifacts, or non-specific BLAST matches. This conservative filtering step was essential for maintaining phylogenetic resolution and functional credibility.

Conserved Motif Discovery and Functional Inference

While Pfam identifies established domains, the MEME Suite uncovered lineage-specific motifs that may represent recently evolved functional elements. Three statistically significant conserved motifs were identified with low E-values and high information content. Motif 1 localized to the N-terminal region and exhibited similarity to known transcriptional activation domains. Motif 2 mapped proximal to the bHLH domain and potentially represents a nuclear localization signal or protein-protein interaction interface. Motif 3 occupied a more variable C-terminal position and, while lacking direct InterPro homology, is evolutionarily conserved across *V. faba* paralogs, implying purifying selection. An additional filtering step retained only candidates possessing at least two of three motifs, ensuring that the final dataset comprised multifunctional proteins with robust structural and functional signatures rather than domain-only minimalists.

Phylogenetic Diversification and Evolutionary Implications

Maximum Likelihood phylogenetic reconstruction classified the filtered *V. faba* MYC2 candidates into distinct evolutionary clades. The phylogenetic tree revealed clear clustering patterns, with *V. faba* MYC2 sequences grouping with homologs from closely related leguminous species, suggesting conserved stress-regulatory mechanisms. Bootstrap support values exceeding 80% confirmed the robustness of major branching patterns, permitting confident assignment of candidates to evolutionary groups and guiding the selection of representative genes for future functional assays. The evolutionary analysis indicated that MYC2 transcription factors in faba bean have undergone diversification consistent with the complex regulatory demands of drought stress adaptation in temperate legume crops.

Gene Structure as an Evolutionary and Regulatory Archive

GSDS analysis revealed substantial variation in intron-exon architecture. Some genes displayed compact structures with minimal intronic content (2-3 exons), resembling ancestral MYC2 configurations, while others exhibited complex architectures with up to five introns and variable exon lengths. Intron phase analysis revealed a predominance of phase-0 introns (between codons), which are evolutionarily conserved and minimally disruptive to reading frames. Phylogenetic clades exhibited conserved gene structure patterns, suggesting that structural diversity reflects evolutionary divergence through intron loss, gain, and alternative splicing that may fine-tune stress-responsive expression. Introns are not silent spacers; they harbor cis-regulatory elements, stress-responsive enhancers, and epigenetic modification landmarks that modulate transcriptional output. The structural diversity documented here therefore represents potentially functional variation.

Subcellular Geography and Regulatory Dynamics

WoLF PSORT analysis predicted nuclear localization as the primary compartment for the majority of candidates, with probability scores frequently exceeding the 80% reliability threshold. This aligns with the canonical function of MYC2 proteins as transcriptional regulators binding chromatin-localized G-box elements. However, several candidates exhibited non-negligible cytoplasmic probabilities. Nucleocytoplasmic partitioning is a documented regulatory mechanism for transcription factors; cytoplasmic sequestration could maintain MYC2 proteins in an inactive reservoir until stress-triggered signaling cascades facilitate nuclear import. These predictions, while requiring experimental validation through fluorescent protein fusions, orient future research toward potentially novel regulatory nodes.

Integrated Structural and Evolutionary Visualization

TBtools integration of phylogenetic, domain, and motif data revealed strong co-linearity between evolutionary relationships and molecular architecture. Members of the same phylogenetic clade consistently shared identical domain arrangements and motif compositions, indicating that duplication events preserved functional modules intact. Conversely, basal lineages exhibited chimeric or divergent architectures, potentially representing evolutionary intermediates or neofunctionalized derivatives. The iTOL-annotated phylogenetic tree provided a publication-quality visual narrative, with branch coloring by evolutionary clade and concentric metadata rings displaying domain presence, motif counts, and localization predictions. This multi-layered approach transformed the tree from a mere branching diagram into an information-dense dashboard suitable for guiding targeted functional assays.

Limitations and Future Perspectives

This bioinformatics pipeline was deliberately conservative, prioritizing specificity over sensitivity. Nevertheless, several limitations must be acknowledged. *In silico* predictions remain theoretical until validated by gene expression profiling, yeast one-hybrid assays, and functional complementation in *Arabidopsis myc2* mutants. The absence of publicly available *V. faba* drought stress transcriptome data precluded direct correlation of our candidate list with drought-induced expression changes. Moreover, reliance on a draft genome assembly means that true MYC2 paralogs residing in repetitive or centromeric regions may be missing or collapsed. Despite these constraints, the dataset offers immediate translational utility. The phylogenetic framework identifies expanded and contracted groups relative to model species, guiding targeted functional assays. The MYC2-centered orthology network provides a rational starting point for CRISPR-based genome editing or transgenic approaches to enhance drought tolerance in faba bean (Jayakodi *et al.*, 2023). As global drought stress accelerates under climate change, such molecular insights are essential for safeguarding the productivity of this nutritionally and environmentally critical legume crop.

CONCLUSION

This study presents a comprehensive bioinformatics characterization of drought stress-responsive MYC2 transcription factor candidates in *Vicia faba*. Through an integrated pipeline combining homology search, physicochemical profiling, domain and motif analysis, phylogenetic reconstruction, gene structure visualization, and subcellular localization prediction, we identified and curated a high-confidence set of MYC2 genes structurally and evolutionarily competent to participate in drought stress signaling. The candidates exhibit canonical bHLH domain architectures, conserved regulatory motifs including JID and TAD domains, predicted nuclear localization, and phylogenetic diversification across evolutionary clades. Notably, the MYC2 family appears expanded in faba bean, suggesting legume-specific evolutionary adaptation to abiotic stress environments. Gene structural diversity indicates potential for complex transcriptional and post-transcriptional regulation through alternative splicing and intron-mediated regulatory elements. These findings establish a foundational genomic resource for understanding MYC2-mediated abiotic stress responses in faba bean and identify promising candidate genes for downstream functional validation, marker-assisted selection, and genome editing aimed at developing drought-tolerant cultivars. As global drought stress accelerates under climate change, such molecular insights are essential for safeguarding the productivity of this nutritionally and environmentally critical legume crop.

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