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Review

Plant Novel Transcription Factors and Their Roles in Gene Regulation

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Abstract: Transcription factors (TFs) are critical regulators of gene expression, playing key roles in various biological processes such as growth, development, and stress responses in plants. While many TF families like MYB, bZIP, and WRKY have been identified and extensively studied in model plants, a substantial number of novel TFs remain uncharacterized, particularly in non-model and economically significant plant species. The discovery of these novel TFs offers a promising avenue for advancing our understanding of plant biology and improving crop traits. Recent advancements in high-throughput sequencing, genomics, and bioinformatics have facilitated the identification of previously overlooked or newly evolved TFs, expanding the repertoire of known plant transcriptional regulators. This review highlights the emergence of novel TF families and their functional roles in regulating plant growth, adaptation to abiotic and biotic stresses, and metabolic pathways. We examine the molecular mechanisms by which these TFs govern gene expression, their interactions with other signaling pathways, and their potential applications in crop improvement. In addition, we explore the challenges and opportunities associated with the functional characterization and validation of novel TFs, as well as the technologies that are enabling these discoveries. Special attention is given to the potential of novel TFs to enhance stress tolerance, disease resistance, and overall productivity in crops, with a focus on their integration into biotechnological approaches, such as genome editing and transgenic crop development. The review concludes by outlining future research directions and the potential impact of novel TFs in addressing global agricultural challenges, including climate change, food security, and sustainability.

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1. Introduction

Transcription factors (TFs) are DNA-binding proteins that interact with other transcriptional regulators, including chromatin remodelling/ modifying proteins, to either attract or prevent RNA polymerases from accessing the DNA template [5,53]. The complexity of transcriptional regulation in plants is demonstrated by the fact that TFs make up around 7% of their coding sequence. As molecular switches, they respond to both internal and external stimuli by turning genes on or off, thereby ensuring precise control over cellular and physiological processes. The

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ability of TFs to modulate complex gene networks makes them indispensable for the proper functioning of plants [35]. Plants are amazing organisms.

In addition to ensuring their growth and success in reproduction, they can construct intricate organic superstructures from basic inorganic molecules while stationary in space and exposed to harsh environmental conditions like water, light, temperature and nutrients, and to biological challenges from competitors, pests, and pathogens. Because of evolution, plants have a flexible developmental program that allows them to adapt reproduction to the environment and produce new vegetative organs. Short-term differentiation is another ability of plant cell to address more pressing environmental issues. The main mechanism governing plant development and differentiation is gene transcription, which is regulated by TFs and other proteins that either attract or prevent RNA polymerases from accessing the DNA template. TFs are usually defined as sequence-specific DNA-binding proteins that are capable of activating and/or repressing transcription. The genomes of plants seem to code many more TFs than those of animals, such as Drosophila melanogaster and Caenorhabditis elegans, which suggests that plants' transcriptional control is at least as intricate as that of animals [48]. Over 1,800 TF genes are found in Arabidopsis thaliana, accounting for over 7% of all protein-coding genes [2].

Over the years, extensive studies has directed towards well-characterized TF families such as MYB, AP2/ERF, bZIP, and WRKY, which regulate various aspects of plant biology, from development and metabolism to stress responses [20]. However, current progresses in genomic, transcriptomic, and proteomic technologies have unveiled a plethora of previously uncharacterized TFs. These novel TFs often exhibit unconventional structures, unique DNA-binding motifs, or distinct regulatory mechanisms, distinguishing them from classical TFs. Understanding these novel TFs is crucial, not only for unravelling the complexities of plant regulatory networks but also for addressing pressing agricultural challenges. With global food security threatened by climate change, resource limitations, and increasing population, identifying and leveraging these emerging TFs can pave the way for developing stress-resilient and high-yield crops. Furthermore, novel TFs hold promise for engineering plants with enhanced adaptability to diverse environments, improved nutritional content, and optimized secondary metabolite production.

In this review, we explore the latest discoveries in novel TFs, emphasizing their roles in plant development, stress responses, and metabolic regulation. We also discuss their impending applications in crop improvement, the challenges associated with their functional characterization, and future directions in this rapidly evolving field.

2. Novel Transcription Factor Families

2.1. GARP Family: New Insights into Photosynthesis Regulation

The GARP (Golden2, ARR-B, and Psr1) family of transcription factors plays an integral role in regulating chloroplast development and nutrient signalling pathways, particularly nitrogen assimilation [7] (Figure1a.). These TFs bind to specific DNA sequences in photosynthesis-related and nutrient-responsive genes, modulating their expression to ensure optimal plant growth and development. Recent studies have proved the dual functionality of GARP TFs in both chloroplast biogenesis and metabolic adaptation. For instance, Golden2-like (GLK) transcription factors, a subgroup of GARP TFs, have been shown to directly regulate genes associated with chlorophyll biosynthesis, light harvesting, and thylakoid membrane assembly. This regulation is essential for efficient photosynthesis, especially under variable light conditions [45]. Additionally, ARR-B proteins, another subgroup within the GARP family, serve as key mediators in the cytokinin signalling pathway. Cytokinins are plant hormones that control cell division, nutrient allocation, and chloroplast differentiation. ARR-B TFs help integrate cytokinin signals into the regulation of nitrogen uptake and assimilation, optimizing resource use efficiency [42] (Figure 1b).

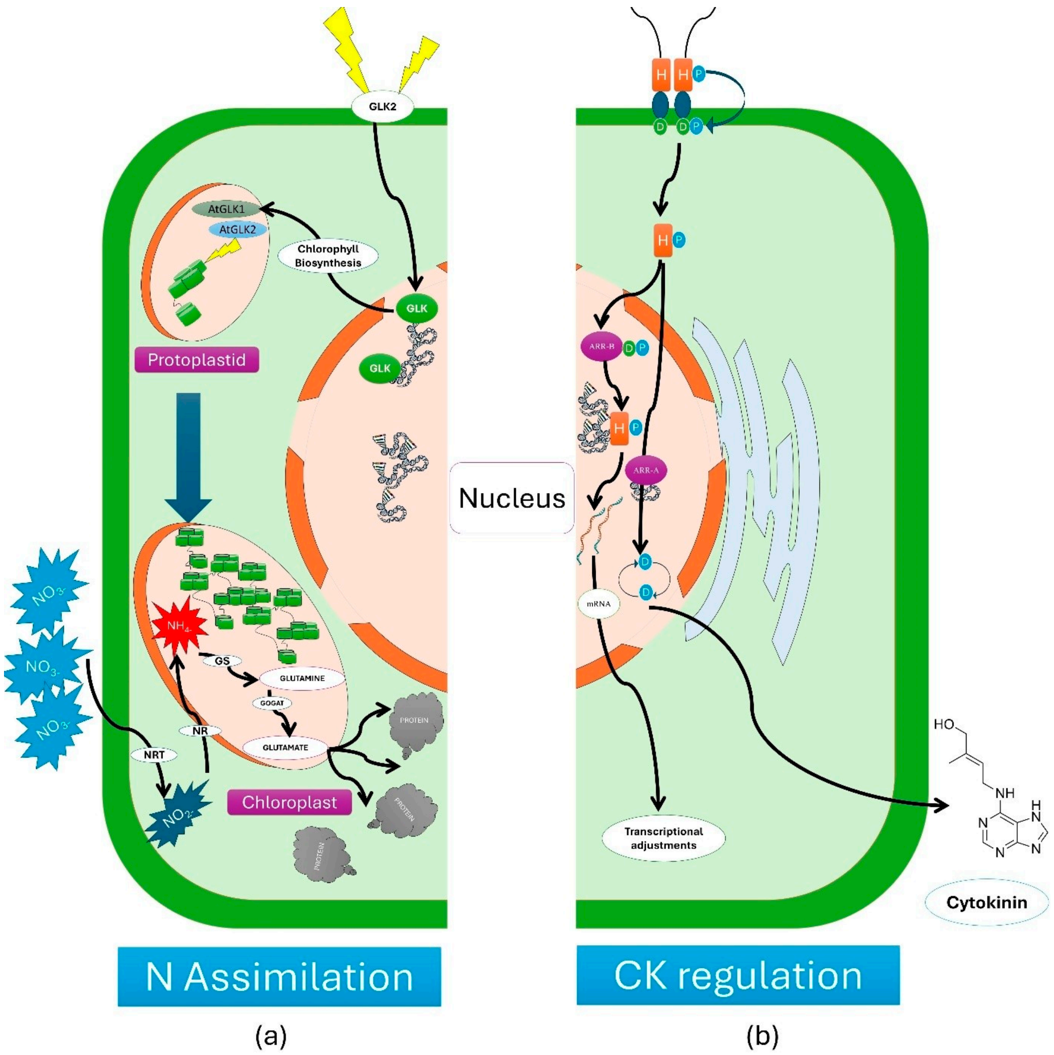

Figure 1. 1a. Regulation of Golden2-like (GLK) transcription factors on nitrogen assimilation a) Golden2-like2 (GLK2 TF) will get triggered through external factors and send a signal for chlorophyll biosynthesis to GLK in the nucleus, b) Arabidopsis thaliana GLK2 (GLK2) in protoplastid will increases the chloroplast for increasing the light use efficiency, c)applied nitrogen will be taken up by the plant as NO_{3-} (Nitrate) form and will be converted as Nitrite (NO_{2-}) through Nitrate reductase (NRT), d) the reduced NO_{2-} will be converted to ammonia (NH_4) through nitrite reductase (NR), e) the accumulated NH_4 with the help of chloroplast will be converted to Glutamine thorough Glutamine synthase (GS) e) Glutamine will be converted to glutamate with the help of glutamine and 2-oxoglutarate (GOGAT), further it will be converted into proteins. Figure 2b. Regulation Cytokinin (CK) and transcriptional adjustments by type-A Arabidopsis response regulators (ARR-A) and type-B Arabidopsis response regulators (ARR-B) a) Activated CK will promote the ARR-A which regulated the proteins required for production of higher amount CK b) Activated CK will promote the ARR-B for synthesis of mRNA for transcriptional adjustments.

The third subgroup, Psr1 (Phosphorus Starvation Response 1), plays a critical role in phosphorus homeostasis. Psr1 TFs activate genes involved in phosphate transport and remobilization under nutrient-deprived conditions, ensuring continued photosynthetic activity despite environmental limitations [9]. Collectively, these findings highlight the versatility of the GARP family in maintaining photosynthetic efficiency and nutrient balance, making them attractive targets for crop improvement. Genetic engineering approaches that enhance GARP TF expression or activity could lead to higher yields and better stress resilience in agricultural crops.

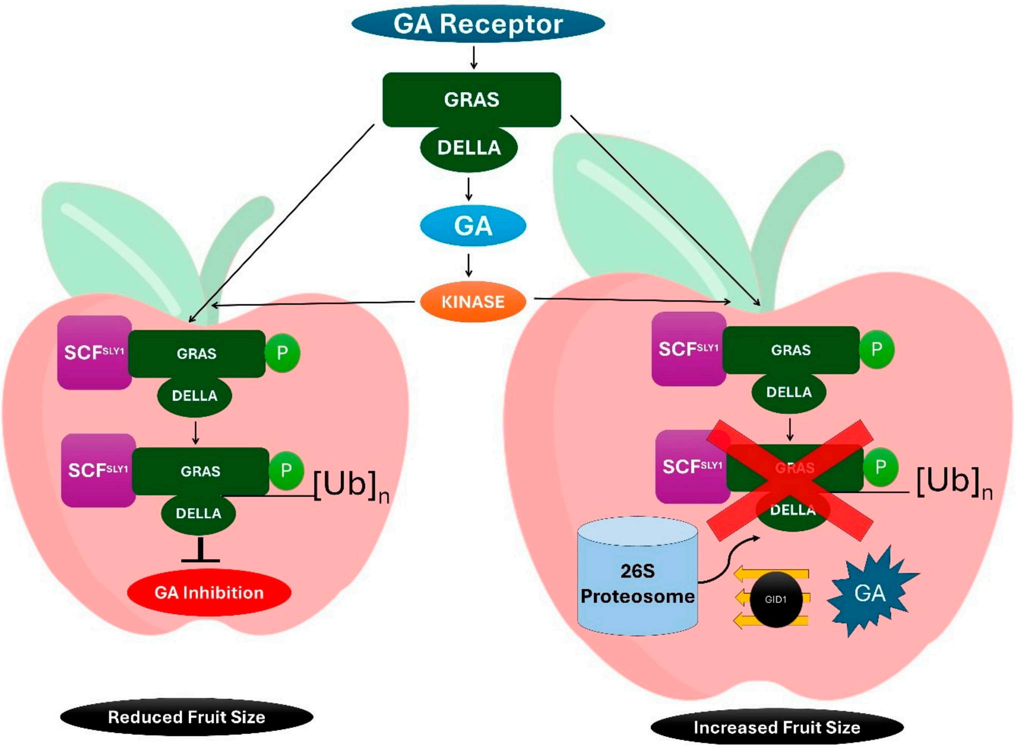

Figure 2. Regulation of GRAS (GAI, RGA, and SCR) in GA (Gibberellic acid) production, a) the activation of ubiquitin ligase complex with GRAS-DELLA will lead to inhibition of GA production b) The GA inhibition will lead to reduced fruit size c) GA binds to GID1 which interacts with DELLA proteins d)26S proteasome degrades the DELLA protein through Ubiquitination e) degradation of DELLA proteins promote cell elongation and growth in response to gibberellin.

2.2. GRAS Proteins: Versatility in Symbiosis and Stress

GRAS (GAI, RGA, and SCR) proteins represent a diverse family of transcription factors involved in numerous plant processes, including gibberellin (GA) signaling, root development, symbiotic interactions, and abiotic stress responses. Initially identified for their roles in GA signaling, GRAS TFs have since been recognized for their multifaceted functions in plant biology [28] (Figure 2.). A key role of GRAS proteins lies in promoting symbiotic associations, particularly arbuscular mycorrhizal (AM) and rhizobial symbioses [36]. For example, NSP1 (Nodulation Signalling Pathway 1) and NSP2 are indispensable for the establishment of nodules in leguminous plants. These nodules facilitate nitrogen fixation, a critical process for plant growth in nutrient-poor soils. NSP1 directly regulates the expression of nodulation genes, while NSP2 interacts with other regulatory components to fine-tune symbiotic responses [44]. Similarly, RAM1 (Required for Arbuscular Mycorrhization 1), another GRAS TF, controls the expression of genes necessary for AM symbiosis, enhancing phosphorus uptake in plants [24] (Figure 2.).

GRAS proteins also play pivotal roles in abiotic stress responses, including drought, salinity, and heat stress. For instance, DELLA proteins, a subgroup of GRAS TFs, act as repressors in the gibberellin signalling pathway. Under stress conditions, reduced gibberellin levels stabilize DELLA proteins, which in turn activate stress-responsive genes (Figure 3). This mechanism allows plants to prioritize survival over growth during adverse conditions [8]. Another example is SCL14 (SCARECROW-like 14), which mediates plant responses to oxidative stress. SCL14 establishes a complex with TGA transcription factors to activate genes involved in detoxification pathways, protecting plants from reactive oxygen species (ROS) damage [39]. In addition to stress and symbiosis, GRAS proteins are crucial for root and shoot development. SCR (SCARECROW) and SHR (SHORT-ROOT) are GRAS TFs that regulate root radial patterning and stem cell maintenance.

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These proteins form a regulatory cascade that ensures proper tissue differentiation and organ development [16].

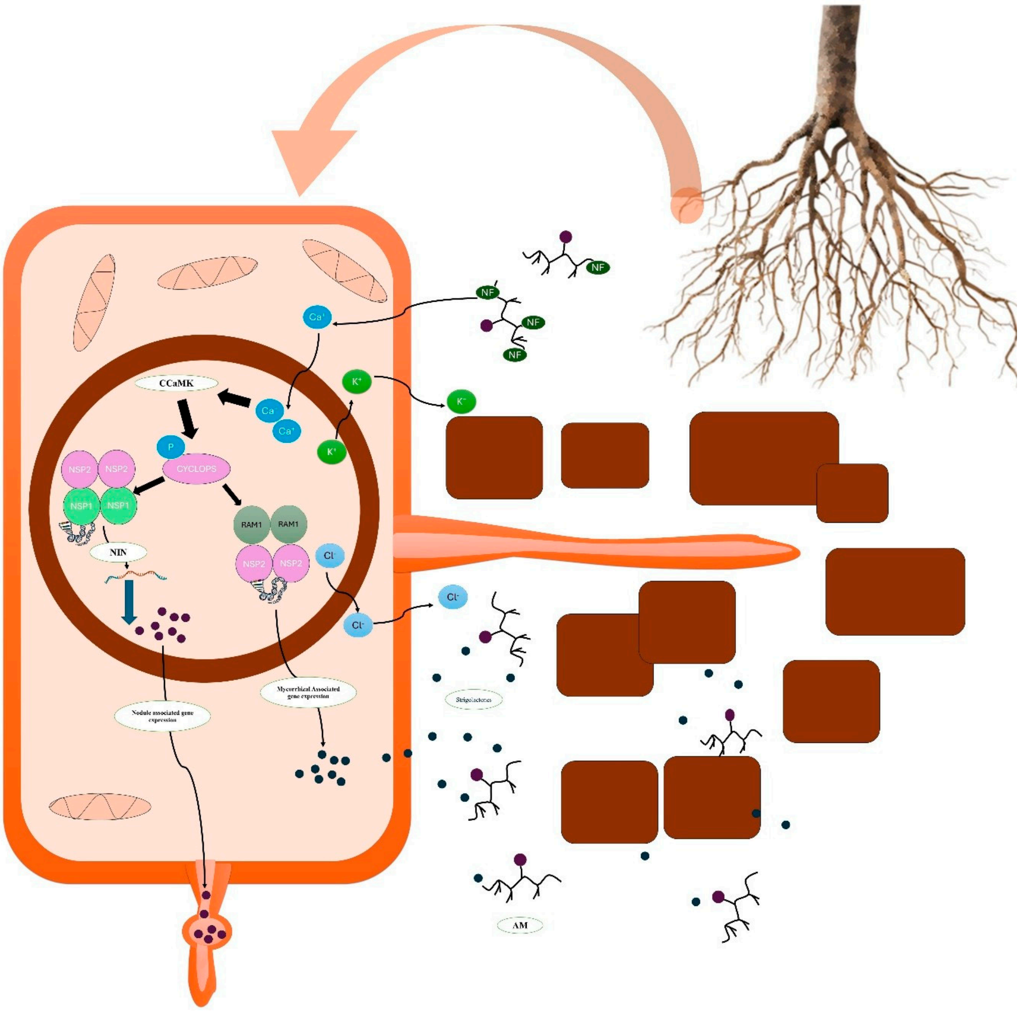

Figure 3. Role of nodulation signalling pathway 1(NSP1) and nodulation signalling pathway 2 (NSP2) in nodule and symbiotic association of mycorrhiza a) Node factor (NF) from mycorrhiza will trigger the calcium oscillation in the cells b) Calcium spiking will activate the nuclear-localized calcium- and calmodulin-dependent protein kinases (CCaMKs), c) CCaMK directly phosphorylates CYCLOPS, enabling its DNA-binding ability and transcriptional activity, d)CYCLOPS activates NPS 1 and NPS 2 leading to transcriptional activity through NIN (NODULE INCEPTION) triggering the nodule development d) CYCLOPS triggers the NSP1 and RAM1, helpful in formation of hyphopodia on the surface of the root and production of strigogalactone.

Given their diverse roles, GRAS proteins offer immense potential for agricultural biotechnology. Genetic modifications that enhance GRAS protein activity, improve nutrient acquisition, stress tolerance, and overall plant resilience. For instance, overexpression of NSP1 and RAM1 enhances nodulation efficiency and phosphorus uptake, respectively, while manipulating DELLA proteins can optimize stress responses without compromising growth.

2.3. NAC with Unusual Regulatory Mechanisms

The NAC (NAM, ATAF, and CUC) family of transcription factors is renowned for its roles in plant development, stress responses, and senescence. However, recent discoveries have identified NAC subgroups with atypical regulatory mechanisms and DNA-binding properties, expanding our understanding of their functional diversity. Certain NAC proteins exhibit unusual DNA-binding motifs that deviate from the conventional NAC recognition sequences. These motifs enable them to target unique gene sets, particularly those involved in specialized secondary metabolism. For example, specific NAC TFs regulate genes responsible for flavonoid and lignin biosynthesis, which are critical for plant defence and structural integrity [5]. Novel NAC TFs have been linked to enhanced drought and heat tolerance. Some of these TFs are capable of binding to promoter regions of abiotic stress-responsive genes and activating their expression under adverse conditions. They also interact with other regulatory proteins and signalling pathways, fine-tuning the plant's adaptive responses [14,15,32].

Recent data indicates that certain NAC TFs participate in chromatin remodelling by recruiting histone modifiers to stress-inducible genes [31]. This epigenetic regulation ensures a rapid and sustained transcriptional response to environmental stimuli. A unique subgroup of NAC TFs has been implicated in regulating Programmed Cell Death (PCD) during senescence and pathogen attack. These TFs activate genes encoding proteases and other enzymes involved in cellular breakdown, contributing to nutrient remobilization and defence [62]. The identification of NAC TFs with unusual regulatory properties opens new avenues for agricultural innovation. By engineering crops to overexpress stress-tolerant or metabolite-enhancing NAC TFs, researchers can develop plants with improved resilience and productivity. Furthermore, understanding their epigenetic roles could inform strategies for modulating gene expression in a stable yet flexible manner.

Beyond these major families, there are several other novel TFs such as bZIP, AITRs, TCP, CPP, NIN-like reported in different plant species (Table 1). These novel TFs have a crucial part in their respective plant development and growth under some favourable or unfavourable conditions.

Table 1. Novel transcription factors reported in various plant species and their functions.

S.N. Tran-Scription Factor (TF)	TF Family	Plant Species	Function	
SUSIBA2	WRKY	Hordeum vulgare cv Pongo	Binds to the Sugar-Responsive Elements of the iso1 Promoter for participation in sugar signalling	Sun et al., 2003
AtVOZ1 and AtVOZ2,		Arabidopsis	bind to the 38-bp cis-acting region of A. thaliana V-PPase gene, AVP1	Mit- suda et al., 2004
NtWRKY12	WRKY	Tobacco (Nicotiana tabacum 'Samsun NN'	NtWRKY12 and TGA1a act synergistically in PR-1a expression induced by salicylic acid and bacterial elicitors.	Van- verk et al., 2008
myb52, myb-like TF, hb5 hb15 showed		Arabidopsis	Hyper lignified SCW Ectopic lignification	Cassan-Wang et al., 2013
JcNAC1	NAC	Jatropha curcas	enhanced tolerance to	Qin et al.,

			drought and increased susceptibility to pathogens	2014
Gb-WRKY2	WRKY	Ginkgo biloba	flowers and strongly induced by methyl jasmonate	Liao et al., 2015
NAC050 and NAC052	NAC	Arabidopsis	involved in transcriptional repression and flowering time control by associating with the histone demethylase JMJ14.	Ning et al., 2015
GIP1	bZIP	Arabidopsis	early stages of Arabidopsis development	Shaikhali, 2015
AITR	ABA-induced transcription repressors (AITRs)	Arabidopsis	6 Arabidopsis AITR genes are induced by exogenous ABA	Tian et al., 2017
OsPCF2 (OsCPP5) OsNIN-like2, OsNIN-like3 and OsNIN-like4	TCP CPP NIN-like	Oryza sativa L.) genotype Hasawi	regulators of OsNHX1 gene expression in a salt tolerant rice genotype	Almeidaet al., 2017
PvBMY1	APETALA2/Ethyle Response Factor	Pnaenicum virgatum L	increase biomass yield in greenhouse-grown switchgrass by regulation of photosynthesis and related metabolism be-like	AmHleyvpaotelidgne-bavaram et al., 2018
PvBMY3	Nuclear-Factor Y			
Bel-like				

3. Functional Insights and Mechanisms

3.1. Unique DNA-Binding Domains

Unique DNA-binding domains (DBDs) in plant novel transcription factors are specialized protein regions that enable precise regulation of gene expression by interacting with specific DNA sequences. These domains often exhibit distinctive structural features beyond classical motifs like homeodomains or zinc fingers, adapting to plant-specific regulatory needs. For instance, some

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plant transcription factors target DNA secondary structures such as G-quadruplexes or interact with cis-regulatory elements unique to plant genomes, enabling fine-tuned control over growth, development, and stress responses [1,19].

The identification of these novel DBDs is motivated by developments in genetics and bioinformatics. Techniques like RNA sequencing, ChIP-seq, and CRISPR-based screens help uncover transcription factors and their DNA-binding preferences [56]. Protein structure 162 prediction tools, such as AlphaFold, provide insights into the structural basis of DNA 163 recognition in novel plant transcription factors [17]. Biochemical methods, including 164 electrophoretic mobility shift assays (EMSAs), further validate DNA-binding specificity 165 and unravel functional interactions [52].

Functionally, plant transcription factors with unique DBDs play critical roles in diverse processes such as seed development, flowering, and response to environmental cues like drought, salinity, and pathogens. They often act as master regulators, integrating signals from phytohormones like auxins, gibberellins, or abscisic acid, to control large networks of target genes. Recent discoveries include TFs with atypical DNA-binding domains. Examples include: TIFY proteins, which interact with jasmonic acid signalling components to regulate stress responses. RWP-RK TFs, pivotal in nitrogen-related signaling pathways.

3.2. Post-Translational Modifications (PTMs)

Novel TFs often exhibit complex regulation through PTMs such as phosphorylation, ubiquitination, and SUMOylation. For example: The ubiquitin-mediated degradation of certain GRAS TFs fine-tunes their activity under fluctuating environmental conditions.

PTMs of plant novel TFs are critical regulatory mechanisms that fine-tune their activity, stability, localization, and interactions, enabling plants to respond dynamically to internal and external stimuli. These chemical modifications alter the functional properties of TFs after protein synthesis, providing an additional layer of control over gene expression. Phosphorylation, mediated by kinases, is among the most common PTMs, modulating the DNA-binding activity, nuclear localization, or protein-protein interactions of TFs in response to environmental signals like light, drought, or pathogens [58]. Ubiquitination often targets TFs for proteasomal degradation, ensuring timely downregulation of transcriptional activity, while sumoylation can stabilize TFs or alter their subcellular distribution [66,67]. Acetylation and methylation affect TF stability and DNA-binding efficiency, linking transcriptional regulation to broader epigenetic contexts [12].

PTMs also enable plant TFs to serve as switches for molecules in signaling cascades. For example, phosphorylation of TFs downstream of hormone signalling pathways, such as those involving auxins, abscisic acid, or jasmonates, fine-tunes gene expression for stress responses, growth, and development. Crosstalk between different PTMs, such as phosphorylation and ubiquitination, further enhances the specificity and adaptability of TF-mediated transcriptional regulation.

3.3. Non-Coding RNA Interactions

Non-coding RNAs (ncRNAs) regulate novel transcription factors (TFs) in plants, influencing their stability, activity, and specificity to control gene expression during growth, development, and stress responses. Among ncRNAs, microRNAs (miRNAs) are well-studied and regulate TFs post-transcriptionally by targeting their mRNAs for degradation or translational repression. For instance, in Arabidopsis thaliana, miR156 targets SPL (SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE) TFs, modulating vegetative phase transitions and flowering. Overexpression of miR156 delays flowering, while its suppression accelerates it [18]. Long non-coding RNAs (lncRNAs) interact with TFs at various levels, often serving as scaffolds, decoys, or guides to regulate transcription [33]. In rice, the lncRNA LAIR interacts with NAC TFs to control leaf senescence during stress, enhancing the expression of stress-responsive genes and promoting survival [43]. Small interfering RNAs (siRNAs) guide chromatin-level regulation by recruiting TFs to specific genomic loci, often silencing transposable elements or stress-responsive

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genes. For example, in maize, siRNAs guide TFs to ensure genome stability by suppressing transposable elements during development [65]. Circular RNAs (circRNAs), though less explored in plants, act as sponges or decoys for miRNAs or TFs, indirectly modulating transcriptional networks [7,67]. These diverse interactions provide plants with flexible regulatory mechanisms to adapt to environmental challenges and developmental cues. Leveraging ncRNA-TF interactions can revolutionize agriculture by enabling precise control of traits. For example, engineering miR156 or lncRNAs like LAIR can optimize flowering time, improve stress tolerance, or enhance productivity, offering sustainable solutions for crop improvement in changing climate conditions [37].

4. Biotechnological Applications

4.1. Enhancing Stress Tolerance

Novel TFs have been harnessed to develop stress-resilient crops. These TFs activate or repress stress-responsive pathways, enabling plants to adapt to environmental challenges such as drought, salinity, cold, and pathogen attacks. For instance, overexpression of GARP TFs enhances photosynthetic efficiency under nutrient-limited conditions, while manipulation of TIFY proteins confers drought and pest resistance [50]. The DREB (Dehydration-Responsive Element Binding) family of TFs, which belong to the AP2/ERF superfamily. These TFs bind to dehydration-responsive elements (DREs) in the promoters of stress-responsive genes. Overexpression of DREB2A in crops like rice and wheat has been shown to improve drought and heat tolerance by activating genes involved in osmo-protection and water-use efficiency [49].

Another example is the NAC (ATAF1/2, CUC2, NAM,) family of TFs, which regulate multiple stress-response pathways. For instance, the overexpression of SNAC1 in rice enhances tolerance to drought and salinity by improving root architecture and osmotic balance, resulting in better water absorption and retention [41]. MYB transcription factors also play a significant role in stress tolerance. AtMYB44, for example, enhances tolerance to drought and salinity in Arabidopsis by modulating abscisic acid (ABA)-responsive pathways [29]. WRKY transcription factors, such as WRKY28 in Arabidopsis, are key regulators in pathogen defense by modulating salicylic acid and jasmonic acid signaling pathways [27].

4.2. Boosting Yield and Quality

By targeting GRAS and NAC TFs, researchers have achieved improvements in biomass, seed quality, and secondary metabolite production. The integration of synthetic biology approaches with novel TFs has accelerated trait optimization. GRAS TFs, named after GAI, RGA, and SCR, are involved in hormonal signaling, particularly gibberellins (GA), and root development [21]. For example, the GRAS TF DRO1 in rice regulates root angle, enhancing deep rooting and improving drought tolerance, which directly contributes to higher yields under water-limited conditions (Batool et al., 2024). Similarly, SlGRAS40 in tomatoes influences fruit ripening and quality by regulating sugar accumulation and stress resistance [47].

NAC TFs (NAM, ATAF, and CUC) are known for their role in stress tolerance and senescence, which are crucial for crop productivity and quality. The rice NAC TF OsNAC67 improves grain yield and quality under drought by enhancing photosynthesis and reducing oxidative damage [57]. AtNAP, a NAC TF in Arabidopsis, regulates senescence, ensuring nutrient remobilization to seeds, thereby improving seed quality [30].

4.3. Precision Breeding Tools

CRISPR-Cas9 technology has been employed to modify novel TFs and their target genes, enabling precise control over regulatory networks. This approach holds promise for tailoring crops to specific environmental challenges.

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5. Challenges and Future Directions

Despite notable advancements in describing novel transcription factors (TFs), several challenges remain. Functional redundancy among TF families complicates the identification of distinct roles, and limited knowledge of TF interactomes hinders a comprehensive understanding of their involvement in complex regulatory networks. Additionally, ethical and regulatory challenges surrounding the deployment of genetically modified crops need to be addressed. Future research should focus on utilizing high-throughput functional screens to discover more novel TFs, integrating multi-omics approaches to map TF-mediated regulatory networks, and developing robust biotechnological applications that can be effectively deployed in the field to increase the yield of crops and resilience.

6. Conclusion

The discovery of novel transcription factors has revolutionized our understanding of plant gene regulation. These TFs offer unprecedented opportunities to address global challenges such as food security and climate resilience. By leveraging advanced genetic and biotechnological tools, we are able to realize the maximum potential of these molecular regulators to create sustainable agricultural systems.

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