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FROM *ARABIDOPSIS* TO CROPS: THREE DECADES OF WRKY TRANSCRIPTION FACTOR NETWORKS UNDER BIOTIC AND ABIOTIC STRESS

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Abstract *Over the past three decades, WRKY transcription factors have been identified as central regulators of plant stress-responsive translational networks. Early Studies in the mid-1990s identified WRKY proteins as pathogen-responsive transcriptional factors in Arabidopsis thaliana, and their domain structure, DNA-binding specificity, and role in salicylic acid-mediated defense signaling pathways were revealed. The further expansion of genomic resources following the year 2010 enabled the identification of expanded WRKY gene families in major crops, which exhibited significant levels of gene duplication, divergence, and stress-responsive expression. Despite the present rapid advancement, the use of WRKY-based genetic manipulation strategies to improve crop performance has been inconsistent. This review integrates the previous thirty years of WRKY research from a translational standpoint. This review is organized around three interconnected themes: the bibliometric data, which reveal changes in methodology, species bias, and stress imbalance in the literature. Next, we look at the conserved regulatory features revealed in Arabidopsis, such as autoregulation, WRKY gene cross-regulation, and hormone regulation. The proliferation of WRKY gene families in polyploidy agricultural species is also discussed from the standpoint of gene duplication and redundancy. Finally, we examine the structural hurdles to translational success by contrasting successful and unsuccessful translation initiatives. Finally, we propose a network-centric approach to future WRKY research that focuses on combinatorial perturbation, quantitative modulation, and field validation. Future progress will depend on understanding how WRKY regulatory logic can be harnessed to enhance stress resilience while maintaining productivity in stress responses; the essential question now is how WRKY's regulatory logic might be used to improve stress tolerance while preserving plant productivity.*

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Introduction: From Discovery to Translational Dilemma

Plant responses to environmental stress are not governed by isolated genes but by layered regulatory hierarchies in which transcription factors coordinate developmental priorities with survival demands (Rushton et al., 2010; Singh et al., 2002). Over the past thirty years, WRKY transcription factors have taken center stage among these regulators (Eulgem et al., 2000; Rushton et al., 2010). WRKYs are now widely recognized to function at critical junctures of biotic and abiotic stress signaling, which started with the discovery of a small family of pathogen-responsive DNA-binding proteins (Eulgem et al., 2000; Rushton et al., 1996). They have an impact on feedback control, transcriptional reprogramming, and hormonal integration (Chen et al., 2012; Pandey and Somssich, 2009). However, the path of discovery has not always been directly translated into agronomic implementation. The inconsistent performance of WRKY-based crop strategies stands in stark contrast to the intellectual maturity of WRKY biology (Chen et al., 2017).

The initial characterization of WRKY proteins in the mid-1990s originated from attempts to analyze pathogen-induced transcriptional activation in *Arabidopsis thaliana* (Rushton et al., 1996). The WRKYGQK motif and its related zinc-finger structure created a stable DNA-binding framework based on W-box promoter elements (Eulgem et al., 2000; Rushton et al., 2010). Rapid functional exploration was made possible by this structural

clarity. Salicylic acid-dependent defense pathways were implicated in loss- and gain-of-function analyses, and later research revealed responsiveness to jasmonate, ethylene, and abscisic acid signaling (Chen et al., 2012; Dong et al., 2003; Pandey and Somssich, 2009). It soon became apparent that WRKY proteins rarely function as straightforward on/off switches. Rather, they are a component of regulatory loops that frequently attach to the promoters of other WRKY genes, including their own (Eulgem and Somssich, 2007; Pandey and Somssich, 2009). Arabidopsis provided an experimentally viable system for the accurate genetic dissection of these principles by generating feedback architectures that have the ability to either amplify or weaken. By employing successive waves of transcriptomic profiling, chromatin immunoprecipitation, and mutant characterization, researchers were able to define networks rather than linear pathways (Chen et al., 2012; Rushton et al., 2010). WRKY factors were shown to help pathogen defense and abiotic stress tolerance talk to each other, sometimes making responses better and other times requiring trade-offs (Bakshi and Oelmüller, 2014). These findings contributed to a conceptual framework of WRKYs as regulatory nodes embedded within interconnected circuits (Pandey and Somssich, 2009; Rushton et al., 2010). Importantly, this understanding emerged within a diploid genome of relatively modest size, where gene redundancy, although present, was manageable and experimentally resolvable (Eulgem et al., 2000).

The landscape changed when high-quality genome assemblies for important crop species such as Rice, wheat, maize, and soybean became available (Brenchley et al., 2012; Project, 2005; Schmutz et al., 2010; Schnable et al., 2009). Rice, wheat, maize, soybean, and other agronomically significant plants exhibited significantly enlarged WRKY repertoires (Hu et al., 2021; Nan et al., 2020; Wu et al., 2005). Whole-genome duplication, segmental duplication, and lineage-specific expansion led to the formation of large paralogous clusters whose functional differentiation was not immediately apparent (Vision et al., 2000). Expression studies consistently exhibited significant induction in response to drought, salinity, heat, or pathogen invasion, thereby substantiating the notion that WRKYs are pivotal to stress responsiveness (Phukan et al., 2016). Nonetheless, the prevalence of responsive transcripts did not consistently align with unequivocal phenotypic causality (Chen et al., 2017). In polyploid crops, redundancy can help protect against changes, and differences in promoters can change the regulatory output to target specific lineages (Panchy et al., 2016). The disconnect between molecular understanding and agronomic achievement is the defining feature of the present-day tension in translation. Altering individual WRKY genes in crops may have quantifiable results in the controlled environment of the lab (Phukan et al., 2016). Increased tolerance to a particular pathogen or abiotic stress may be evident in the greenhouse. Yet such modifications do not consistently produce stable yield advantages across variable field environments (Mittler, 2006). In certain instances, constitutive activation of defense-associated WRKY pathways reduces growth rate or reproductive output under non-stress conditions (Huot et al., 2014). In the latter, seemingly promising expression patterns fail to translate into strong phenotypes as environmental complexity increases. Overall, the results suggest that the problem is not in detecting WRKY involvement, but in predicting the effects at the network level in complex genetic backgrounds(Chen et al., 2017). This cumulative body of evidence thus suggests that the problem should be reframed. The problem is no longer whether WRKY transcription factors regulate stress responses, as it has already been shown that they do (Chen et al., 2012). The problem now is what components of the WRKY regulatory architecture are trans-species and trans-ecological (Pandey and Somssich, 2009). The architectural features such as autoregulation, cross-family interactions, and hormone interactions appear to be generally conserved. By contrast, dosage sensitivity, paralog compensation, and promoter rewiring may alter network behavior in ways that limit direct translational inference (Panchy et al., 2016).

To understand this difference, we need to go beyond just listing gene induction patterns. It requires an understanding of evolutionary history, genomic context, and environmental realism. Crop plants often endure concurrent and successive stresses that diverge from regulated experimental settings (Suzuki et al., 2014). The combinatorial stress landscape present in agricultural systems may reveal latent trade-offs inherent in WRKY-centered networks. The challenge facing the field is not a lack of molecular detail, but rather the difficulty of applying regulatory logic from a simple organism to more complex agricultural systems(Bailey-Serres et al., 2019). This review revisits three decades of WRKY research through that translational lens. To shed light on changes in methodological focus and species representation, we first examine the field's bibliometric evolution. Next, we look at regulatory principles that were established in Arabidopsis and assess how they are modified in crop genomes that exhibit diversification and duplication. We determine the structural and ecological limitations that influence phenotypic outcome by comparing reported translational successes with frequent failures. Lastly, we suggest that a network-aware framework that combines combinatorial genetic perturbation with validation under multi-stress, field-relevant conditions will be essential to future advancements. The broader implication extends beyond WRKY biology. It concerns how mechanistic understanding derived from tractable model systems can be responsibly and effectively translated into crop improvement

strategies. WRKY transcription factors provide a revealing case study in this ongoing effort to bridge molecular insight and agricultural resilience.

Bibliometric Landscape of WRKY Research (1995–2025)

Understanding the context in which a given field of study evolved can considerably aid a mechanistic interpretation. Bibliometric trends reveal not only growth patterns, but also shifts in conceptual and methodological advances.

2.1 Growth Trajectory of WRKY Research

The late 1990s and early 2000s were a period of foundational discoveries for WRKY research. The number of articles was limited, but they focused on gene discovery, domain analysis, and *Arabidopsis* defence signaling(Dong et al., 2003; Eulgem et al., 2000; Rushton et al., 1996). The introduction of high-throughput sequencing methods around 2010 resulted in a considerable increase in the number of publications, which mirrored genome-wide identification assessments across multiple species(Bakshi and Oelmüller, 2014; Chen et al., 2017; Rushton et al., 2010). Later technology advancements in RNA sequencing, chromatin immunoprecipitation sequencing, and CRISPR editing are linked to increased publication growth(Doudna and Charpentier, 2014; Jinek et al., 2012; Park, 2009; Wang et al., 2009). The annual growth publication is shown in Figure 1 panel A**.**

Species Bias in WRKY Studies

A species distribution analysis reveals that Arabidopsis is over-represented in relation to its agricultural relevance(Eulgem et al., 2000). Rice and maize come next because of their value as model crops with well-developed genomic resources(Hu et al., 2021; Project, 2005; Schnable et al., 2009; Wu et al., 2005). Wheat and soybean genome analysis have shown a considerable rise following genome assembly advances(Brenchley et al., 2012; Consortium et al., 2018; Schmutz et al., 2010). Despite its spread into crop species, the model system maintains its supremacy. Functional validation in *Arabidopsis* is still more widespread than in polyploid crops(Chen et al., 2017; Phukan et al., 2016; Rushton et al., 2010). This has ramifications for our understanding of WRKY gene function, as shown in Figure 1 panel B.

Stress Focus Imbalance

The WRKY gene family has long been researched in the context of biotic stress, particularly pathogen-triggered defence(Eulgem et al., 2000). As the relevance of climate change became more widely recognized, the number of studies on abiotic stress expanded significantly(Bakshi and Oelmüller, 2014; Chen et al., 2012). However, research on coupled stresses is very uncommon because plants are subjected to pressures both simultaneously and sequentially in agricultural contexts, the lack of combined stress studies is a significant gap(Suzuki et al., 2014; Zandalinas et al., 2021). Single-stress experiments do not provide information about regulatory integration. The stress focus imbalance distribution has been shown in graph panel C in Figure 1.

Keyword Evolution and Thematic Shifts

Keyword analysis depicts the evolution of topics. The initial research concentrates on "defence," "pathogen," and "salicylic acid." The study stages include "abiotic stress," "ABA," and "reactive oxygen species." Recent publications emphasize "yield," "genome editing," and "CRISPR." The pattern reflects a shift from the subject's molecular description to a more translational purpose. The topic of whether or not this goal has been met in the field will be addressed in the sections that follow, and the evolution timeline has been shown in Panel D in Figure 1.

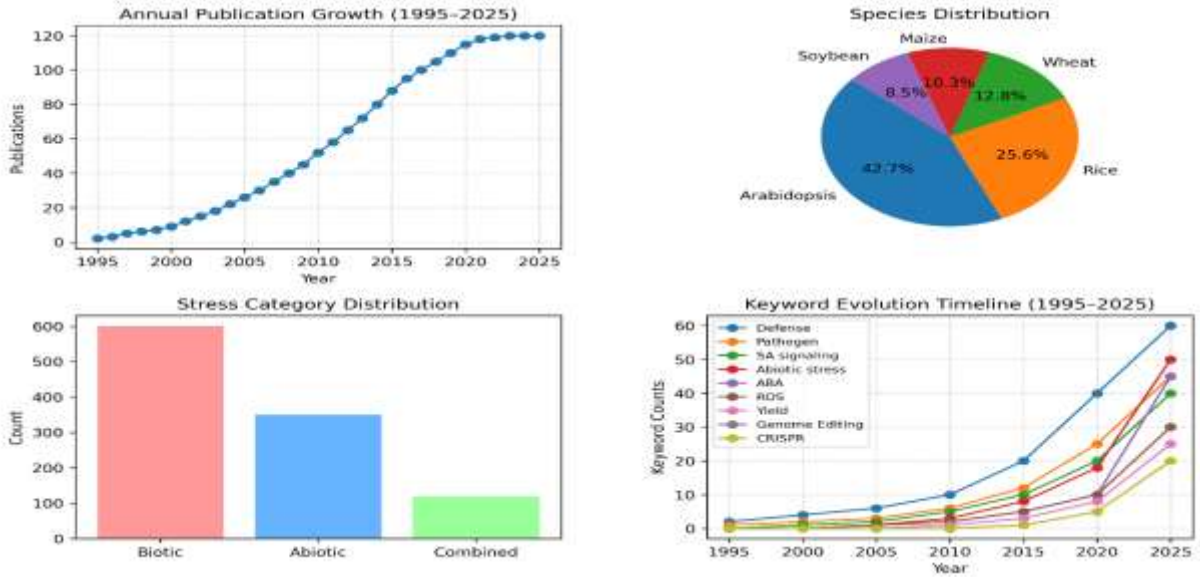

Figure 1: Bibliometric signature of WRKY research (1995–2025).
Panel A: Annual publication growth.

Panel B: Species distribution.
Panel C: Stress category distribution.
Panel D: Keyword evolution timeline.

The upward trajectory indicates sustained interest rather than transient enthusiasm. However, growth alone does not equate to balanced knowledge.

Conserved WRKY Regulatory Principles Revealed by *Arabidopsis*

If the translational gap symbolizes the modern dilemma of WRKY biology, then *A. thaliana* serves as its intellectual underpinning. This model organism has contributed significantly to our understanding of WRKY transcription factors; nonetheless, the potential of *Arabidopsis* resides not in its ability to provide ready-made agricultural solutions, but rather in its ability to discover regulatory organization principles.

Structural Organization and DNA Recognition

The WRKY domain, which contains the conserved WRKYGQK sequence and a zinc-finger domain, is the distinguishing structural element of WRKY proteins, consisting of around 60 amino acids(Rushton et al., 1996). Numerous changes that affect the heptapeptide core have been noted in agricultural species, despite the domain being well preserved(Ross et al., 2007). However, the consensus sequence is more prevalent in Arabidopsis, providing a more stable background for biochemical studies(Eulgem et al., 2000; Rushton et al., 2010). The preferred binding sites for WRKY proteins are the W-box elements (TTGACC/T) found in the promoter regions of stress-responsive genes(Eulgem et al., 2000). Nevertheless, early models were unable to comprehend how context-dependent this relationship is. In addition to the core W-box sequence, the surrounding nucleotides, chromatin structure, and the presence of other cis-elements in the promoter all influence binding affinity(Brand et al., 2013; Ciolkowski et al., 2008).

The phenomenon of combinatorial binding adds another layer of complexity. A promoter often has a large number of W-boxes arranged in tandem or overlapping patterns. WRKY proteins bind cooperatively in certain circumstances and competitively in others(Birkenbihl et al., 2017b). This shows that it is important not only to have a W-box, but also to know where it fits within the wider cis-regulation framework. W-boxes can be found in the promoters of many WRKY genes (Eulgem and Somssich, 2007; Pandey and Somssich, 2009). This topological feature facilitates autoregulatory circuits and crosstalk among members of the WRKY gene family. The ultimate result is a regulatory mechanism in which WRKY proteins activate both downstream stress genes and each other in a cascade fashion (Pandey and Somssich, 2009; Rushton et al., 2010).

Autoregulation and Network Feedback

One of the most striking discoveries in Arabidopsis research is the role of WRKY transcription factors in feedback regulation(Eulgem and Somssich, 2007). Some WRKY genes are activated promptly upon pathogen infection and then down-regulate their own promoters, preventing future activation. Others cause the transcription of more WRKY genes, increasing the signal(Eulgem and Somssich, 2007; Pandey and Somssich, 2009).

As a result, rather than prolonged activation, the system alternates between oscillatory and short transcriptional activation. From a biological aspect, this feedback regulation would prevent the system from overactivating defence genes, which would be detrimental to growth (Huot et al., 2014; Pandey and Somssich, 2009). From a systems perspective, this yields a self-regulating WRKY network rather than a purely linear one (Eulgem and Somssich, 2007; Rushton et al., 2010).

Cross-regulation among members of the WRKY family complicates matters further. Some WRKY proteins are known to promote transcription, whereas others serve as repressors in a partner-dependent manner(Pandey and Somssich, 2009). Protein-protein interactions with VQ motif-containing proteins, MAP kinases, and other transcription factors increase regulatory complexity (Jing and Lin, 2015). This creates a highly interconnected system in which perturbations in one component can be minimized or amplified by another. This is very crucial when trying to do translational modifications in plants(Chen et al., 2017).

Hormonal Integration as a Defining Feature

Possibly the most important discovery from the study of *Arabidopsis thaliana* is that WRKYs are vital in plant hormone signal transduction pathways(Dong et al., 2003). In particular, the signal pathway of salicylic acid is closely related to the regulation of WRKYs(Yu et al., 2001). It should be noted that WRKYs do have interactions with other signal pathways of jasmonic acid and ethylene hormones(Li et al., 2004). In such cases, they are often involved in counteracting interactions between the signal pathways of SA and JA hormones(Li et al., 2004). In addition, another level of integration of signal pathways comes from abscisic acid-related stress responses, particularly in drought and osmotic stresses(Chen et al., 2012). It appears that WRKYs are not just involved in stress responses but are actually centers of hormonal interactions(Pandey and Somssich, 2009). This can be seen from a broader perspective by considering that a given WRKY gene may actually be induced by different types of pathogen and abiotic stress signals. The translation of all these aspects indicates that the role of WRKYs cannot be seen from a perspective of "stress tolerance genes" even in *Arabidopsis thaliana* because of hormonal interactions.

Post-Translational Control and Signal Dynamics

Though the regulation of transcriptional induction is of primary interest, post-translational regulation also plays a role in the regulation of the activities of the WRKY proteins(Ishihama and Yoshioka, 2012). The mitogen-activated protein kinases have the potential to phosphorylate the DNA or modulate the strength of the trans-activating domains, but proteasomal degradation limits the levels of the WRKY proteins(Ishihama and Yoshioka, 2012). This variety of regulation highlights the necessity of realizing that the expression of the WRKY genes alone is not adequate to understand the functions of the gene products(Chen et al., 2012).

Crop Expansion Era: Duplication, Redundancy, and Expression Inflation

While Arabidopsis provided insights into genetic simplicity, crop genomes evolved throughout time to add scale, redundancy, and complexity. Rather than just doubling the number of WRKY genes, the switch from a diploid model organism to polyploid cereals and legumes changed the environment in which they functioned dramatically.

The spread of WRKY gene families in crops is neither accidental nor insignificant. Rather, it is a complicated interaction of whole-genome duplication, segmental duplication, tandem duplications, and lineage-specific gene retention. In wheat, for example, polyploidization has resulted in the amplification of ancestral gene sets in subgenomes. Palaeopolyploidization has also helped to expand WRKY gene families in soybean and maize. This suggests that many crop species have WRKY gene families that are much larger than those observed in *Arabidopsis*, as shown in Figure 2. This alone makes translation difficult. When an Arabidopsis WRKY gene has many paralogs in crops with partially or wholly different functions, orthology becomes muddled.

WRKY Family Expansion and Evolutionary Retention

Functional conservation is not ensured following gene duplication. Following gene duplication, paralogs can take very diverse evolutionary routes. Some paralogs retain their ancestral function. Others have common expression domains. Others accumulate mutations that cause minor shifts in regulatory specificity (Blanc and Wolfe, 2004).

In crops, comparative analyses often reveal:

- Tissue-specific divergence among paralogs(Wu et al., 2005)
- Differential responsiveness to distinct stress signals(Chen et al., 2012)
- Altered promoter architecture relative to *Arabidopsis* counterparts(Chen et al., 2017)

These findings show that sequence similarity is insufficient to assure functional conservation. Furthermore, the existence of duplicated WRKY genes suggests their functional significance. Redundant genes can protect against environmental changes. Buffering network output stabilizes it while reducing the predictability of experimental manipulation of individual genes.

Expression Profiling Versus Functional Validation

The genomic age has made it possible to quickly isolate WRKY genes that are induced by drought, salt, heat, pathogens, and oxidative stress. Transcriptome analyses frequently indicate that a given circumstance induces transcription of dozens of WRKY genes. In some ways, the sheer quantity of possible inducible genes has outpaced functional validation. Responsiveness to expression is used to assess functional relevance. However, transcriptional induction does not suggest a causal role in phenotype. There could be redundant WRKY genes, or secondary genes that aren't key regulators(Chen et al., 2012; Phukan et al., 2016). Overexpression analyses in plants may result in greater stress tolerance. However, such enhancements are not always uniform in terms of stability throughout different stages of development and environmental conditions. In some cases, excessive levels of constitutive expression might affect plant shape, cause late flowering, or impair biomass production(Huot et al., 2014). The mismatch between expression analysis and phenotypic observation raises concerns about methodology. Transcript levels are a snapshot of regulatory activity, not a representation of network requirements. Without a thorough knockout or precision regulation study, it is difficult to discern between central and peripheral regulatory sites(Chen et al., 2012; Huot et al., 2014).

Redundancy as Network Insurance

Functional redundancy is viewed as an impediment to translation efficiency. However, in evolutionary terms, redundancy serves as insurance. In plants subjected to natural selection and uncertain stress conditions, scattered regulatory competence is favorable. In polyploid crop plants, numerous WRKY gene paralogs can provide redundancy if a single gene is deleted or downregulated. This serves as a stress inhibitor, mitigating the impact of genetically changed phenotypes. This explains how single-gene knockouts may cause modest or context-dependent problems. This explains why the WRKY mutation, which causes different phenotypes in Arabidopsis, can result in diminished phenotypes in crop plants. The difference is that the network is not just larger, but also more resilient to disturbance(Conant and Wolfe, 2008; Panchy et al., 2016).

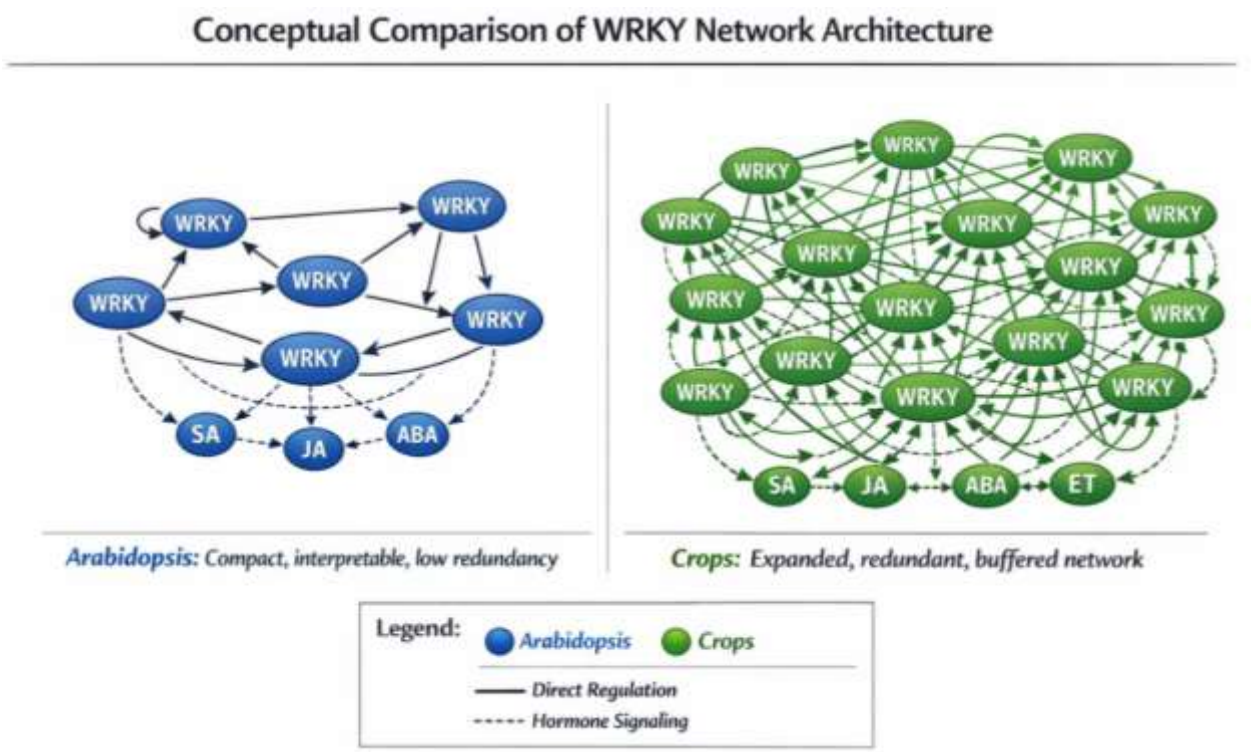

Figure 2: Conceptual comparison of WRKY network architecture in *Arabidopsis* and crops. The figure should depict a relatively compact, interpretable WRKY interaction network in *Arabidopsis*, contrasted with an expanded, highly interconnected, and partially redundant network in crops. The visual emphasis should highlight duplication-derived buffering and overlapping regulatory connections.

Regulatory Rewiring and Promoter Divergence

Even where orthography is obvious, regulatory regulation can differ. Crop promoter regions for WRKY genes commonly differ in their cis-element composition from Arabidopsis promoter regions. The distribution of W-boxes, hormone-responsive elements, and stress-response motifs can all affect the thresholds of induction and interaction. The downstream goals can also differ. A WRKY protein that regulates a specific collection of defence genes in Arabidopsis may interact with a transcriptional landscape that is quite different in crops. This rewiring does not always require significant sequence differences; modest changes in promoter context or chromatin accessibility might redirect regulatory signals(Eulgem and Somssich, 2007; Rushton et al., 2010). This level of complexity makes straight translation challenging. Sequence similarity may not always indicate network similarity. The combined effect of duplication, redundancy, and regulatory variances results in a system that behaves differently despite having similar components. This realization sets the stage for an important distinction: while translation is frequently inconsistent, it is not always unsuccessful. We now provide examples of Arabidopsis-based WRKY information that has been successfully translated(Panchy et al., 2016).

Translation Successes: When *Arabidopsis* WRKY Knowledge Works

Whereas issues in translational research are being debated, it would be incorrect to claim that research on WRKY has been universally ineffective in crops. There are instances where conserved orthology corresponds with phenotypic gain. These samples share some common qualities. First, the WRKY of interest serves as a hub in stress response networks in Arabidopsis and other crop species. Second, functional validation extends beyond expression investigations to encompass knockouts and genome editing. Third, phenotyping is performed under a variety of stress and developmental situations. In these cases, the conservation of regulatory logic appears to be strong enough to support interspecies inference(Rushton et al., 2010).

Table 1: Conserved WRKY modules between *Arabidopsis* and major crops

***Arabidopsis* WRKY**	**Crop Ortholog / Functionally Related Paralog***	**Stress Context Evaluated**	**Evidence Type in Crop**	**Translational Assessment**
AtWRKY33	OsWRKY45 (rice); TaWRKY33-like genes (wheat, predicted ortholog based on phylogeny)	Necrotrophic fungal pathogens; blast disease	Overexpression; pathogen inoculation assays; transcriptional profiling	Generally positive for disease resistance; field-level durability

				requires further validation
AtWRKY70	OsWRKY13 (rice; functionally linked to SA–JA balance)	SA/JA signaling; bacterial and fungal infection	Overexpression; hormone-response assays; disease scoring	Context-dependent; resistance enhancement accompanied by altered defense balance
AtWRKY18/40/60 cluster	OsWRKY24/53-like paralogs (rice; ABA-responsive clade members)	Drought and ABA-mediated stress	Transgenic overexpression; physiological stress measurements	Mixed outcomes; improved stress indices under controlled conditions; growth penalties reported in some lines
AtWRKY25/26	Heat-inducible ZmWRKY paralogs (maize; clade IIe members)	Heat stress	Expression analysis; transgenic validation in heterologous systems	Partial functional similarity; limited direct field validation in maize backgrounds
AtWRKY53	HvWRKY ortholog candidates (barley; senescence-associated clade)	Senescence; stress–development interaction	Expression profiling; comparative developmental analysis	Mechanistic parallels in senescence regulation; limited agronomic translation
AtWRKY6	OsWRKY and ZmWRKY paralogs implicated in nutrient signaling	Phosphate signaling; nutrient stress	Mutant characterization (Arabidopsis); expression and functional assays in crops	Conserved regulatory theme; limited evidence for yield-level impact
AtWRKY46/54/70 module	GmWRKY paralogs (soybean; drought-responsive group III members)	Drought and osmotic stress	Overexpression; physiological and biochemical stress metrics	Improved stress tolerance under greenhouse conditions; long-term productivity effects unclear
AtWRKY28	TaWRKY28-like genes (wheat; defense-associated clade)	Stripe rust; fungal pathogens	Overexpression; pathogen assays	Enhanced resistance in controlled infection assays; multi-environment stability not fully assessed
AtWRKY8	SlWRKY family members (tomato; salt-responsive paralogs)	Salinity and osmotic stress	Transgenic overexpression; ion leakage and survival assays	Increased tolerance in controlled salinity trials; developmental modulation observed
AtWRKY30	GmWRKY and OsWRKY stress-inducible paralogs (phylogenetically related)	Abiotic stress (drought/salinity)	Expression profiling; functional overexpression	Inducible stress responsiveness conserved; quantitative phenotypic impact variable.

The purpose of Table 1 is not to catalog every reported study, but to highlight high-confidence examples where orthology corresponds with functional reproducibility.

Conserved Stress-Responsive Hubs

Some WRKY genes appear to function as evolutionarily conserved hubs. When these genes are altered, the effects on defence signaling and abiotic

stress responses are identical. In such cases, redundancy may be reduced, or paralog expression may be partially partitioned, making the phenotypic impact more predictable(Qiu and Yu, 2009; Yokotani et al., 2013). Even when there is success, the influence is more likely to be quantitative than qualitative. The degree to which tolerance can be raised may not be significant. This suggests that WRKY gene alteration focuses on fine-tuning rather than modifying stress responses(Birkenbihl et al., 2017a; Huot et al., 2014).

Hormonal Integration as a Shared Mechanism

Translation success is likely to result in the conservation of hormone integration pathways. If the SA and JA signalling pathways in Arabidopsis communicate via a WRKY protein, and the same hormonal equilibrium is retained in a crop plant, translation predictability will improve(Birkenbihl et al., 2017b; Pandey and Somssich, 2009). However, the degree of conservation varies between agricultural plants. Hormonal equilibria differ between monocots and dicots, annuals and perennials, and stress-tolerant versus high-yield agricultural plants(Verma et al., 2016). As a result, even common regulatory components might act in distinct physiological circumstances(Verma et al., 2016).

Conditions Enabling Translational Robustness

Successful cases often share experimental rigor:

- Use of gene editing rather than constitutive overexpression
- Evaluation under both controlled and semi-field conditions
- Assessment of growth penalties alongside stress tolerance
- Multi-season validation where feasible

These methodological elements suggest that translational robustness is as much about experimental design as about gene choice.

Translation Failures: Structural Barriers in Crop Contexts

While recorded achievements demonstrate the viability of cross-species transmission, inconsistencies indicate that success is not guaranteed(Chen et al., 2017). The essential question is not whether WRKY proteins play a role in stress response regulation, as this has been established, but whether manipulating individual WRKY genes may be utilized to successfully alter large plant systems. The information gained over the previous decade suggests that translation frequently fails, not due to incorrect gene selection, but due to network-level restrictions that were not completely grasped during early translation attempts. This process is aided by four mechanisms: redundancy caused by gene family expansion, trade-offs in stress signaling, evolutionary regulatory rewiring, and the gap between laboratory simplification and agricultural complexity(Birkenbihl et al., 2017a; Huot et al., 2014; Panchy et al., 2016; Zandalinas et al., 2021)

Functional Redundancy and Genetic Buffering

Despite the presence of a multigene family in WRKY genes, Arabidopsis has a low level of redundancy when compared to polyploid crop plants. Mutants with a single gene typically exhibit distinct phenotypes, particularly when genes play main regulatory functions. Such visibility encourages a gene-based approach to function. In crop plants, the situation is quite different. Polyploidization and segmental duplication resulted in the production of several paralogs within the same lineage. Paralogs frequently have similar expression patterns and partially redundant biological roles. If one gene is inactivated, others can compensate by transcriptional activation(Lynch and Conery, 2000; Panchy et al., 2016; Wendel, 2015). This buffering may hide phenotypic changes. A deletion that produces noticeable disease vulnerability in Arabidopsis may result in relatively minor alterations in crops. Overexpression of a single paralog, on the other hand, may be ineffective in overcoming the regulatory bottleneck imposed by co-existing family members(Conant and Wolfe, 2008). Redundancy should not be confused with weakness. According to evolutionary biologists, redundancy improves resilience in the face of environmental change. However, for the translational biologist, redundancy complicates projections. The notion that manipulating a single ortholog can reproduce results in a model system becomes increasingly questionable as the size of the gene family grows. Combinatorial editing methods have begun to address this issue, but other challenges emerge, such as cumulative growth costs and regulatory imbalances(Gao, 2021; Zhang et al., 2018).

Growth–Defense Trade-Offs as Embedded Constraints

Stress tolerance is never a separate process from development. Defence resources are frequently allocated at the expense of growth, reproduction, and resource acquisition. WRKY transcription factors frequently play a role in the interaction of these trade-off decisions(Huot et al., 2014; Karasov et al., 2017). In regulated environments, higher resistance is a sign of success. However, when measured over the entire growth cycle, the constitutive activation of defence signaling can result in reduced biomass, delayed blooming, or poor grain fill. These are not artefacts of experimental design, but rather inherent trade-offs (Karasov et al., 2017). The problem isn't just that WRKY overexpression activates defence mechanisms. Rather, the balance of hormone signaling pathways, particularly the interconnections between the salicylic acid, jasmonic acid, and abscisic acid signaling pathways, is impacted. Changes to this equilibrium have an impact on developmental timing and metabolic prioritization(Berens et al., 2017; Pandey and Somssich, 2009). In *Arabidopsis*, the life cycle can conceal minor yield costs. Small modifications can have a significant impact on productivity in plants, particularly grains, where

development is complicated. Translation is therefore impossible because the WRKY proteins are not irrelevant. Their importance extends beyond stress tolerance to include a larger regulatory function. A network hub is invariably a site of redistribution(Zandalinas et al., 2021).

Regulatory Rewiring Across Evolutionary Distance

Similarity in sequences frequently leads to hypotheses regarding functional similarity. Nonetheless, regulatory networks evolve through progressive changes to promoter shape, chromatin organization, and interaction partners. Even if the coding areas are conserved, upstream and downstream interactions may differ. In crop species with large genomes, promoter regions frequently contain expanded arrays of cis-elements. The distribution patterns of W-box motifs, hormone response elements, and stress-related binding sites can differ significantly from those in Arabidopsis(Weirauch and Hughes, 2011). Downstream targets may also differ. A WRKY protein that directly regulates a collection of defence genes in Arabidopsis may encounter a target list that has been partially changed in rice or wheat due to variations in promoter composition(Birkenbihl et al., 2017b). This could imply that the sequence is conserved, but the network is not. Translation attempts that assume the wiring is steady may encounter unanticipated phenotypic reactions because the circuitry surrounding it has been updated(Panchy et al., 2016).

Environmental Realism Gap

The majority of functional analysis is conducted under controlled conditions to test certain variables in isolation. Plants are tested with a particular pathogen strain, a specific drought treatment, or a consistent salt concentration. This is a scientifically viable technique. Stresses, however, are rarely seen in isolation in natural environments(Suzuki et al., 2014). Temperature, humidity, soil type, microbial communities, and stress timing vary among natural habitats. Drought and pathogen exposure can coexist, as can heat stress and reproduction, as well as nutrient constraint. WRKY transcription factors, which are found at nodes of hormonal convergence, interact dynamically with this complicated input. A genotype that is highly adapted to one stress coordinate may exhibit unpredictable behavior when numerous signals are supplied at the same time. Failure to translate may thus imply oversimplification of the biological environment rather than an error in molecular interpretation(Pandey and Somssich, 2009; Zandalinas et al., 2021). The conceptual decision tree of translational failure is shown in Figure 3.

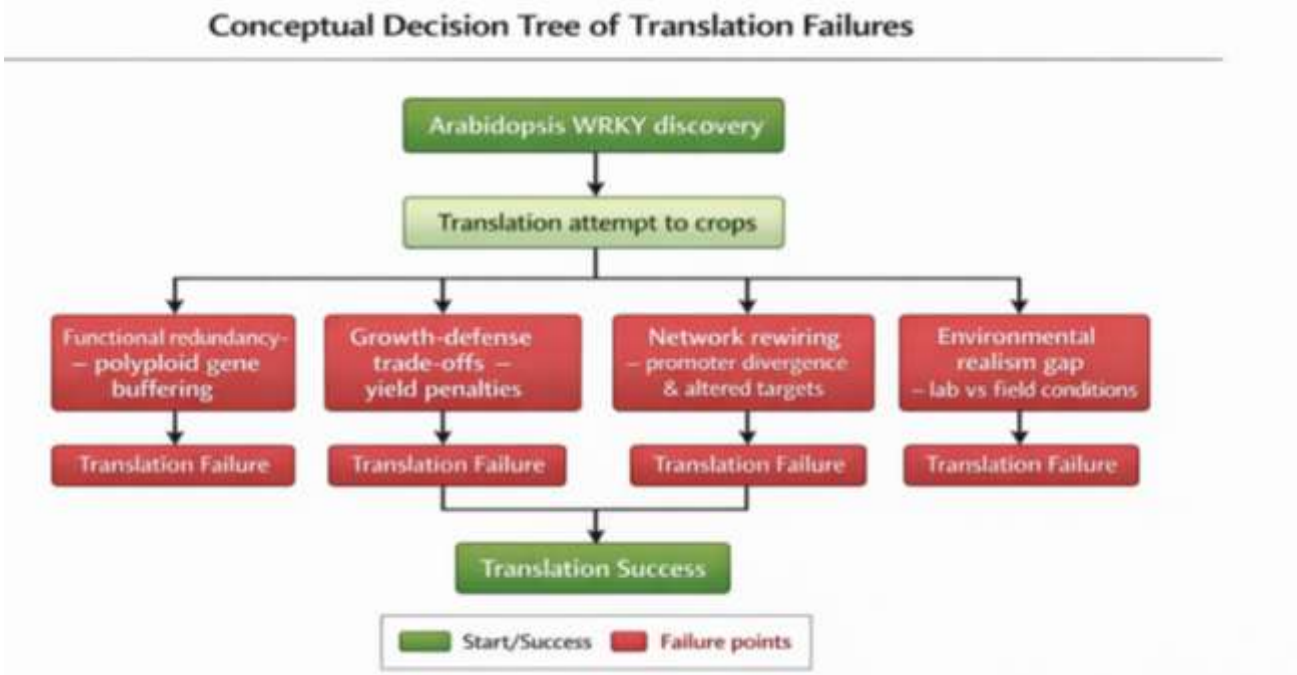

Figure 3: Conceptual decision tree illustrating barriers to translation of WRKY-based strategies from *Arabidopsis* to crops

The figure should depict progression from gene discovery to translational attempt, branching into failure nodes associated with redundancy, trade-offs, regulatory rewiring, and environmental complexity

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Dosage Sensitivity and Quantitative Imbalance

Dosage sensitivity is another factor that is often overlooked. Transcription factors have a nonlinear influence on gene expression. A tiny amount of this factor has a significant effect on gene expression, but overexpression causes disruption. Constitutive promoters, which are often utilized in transformation research, increase expression beyond physiological limitations. This impact produces phenotypes that are not biologically augmented, but rather saturated(Gao, 2021; Huot et al., 2014). However, a partial loss-of-function mutation may result in compensatory effects in paralogs or upstream regulators. This behavior is

difficult to understand without quantitative regulation. Genome editing approaches that fine-tune promoter activity or change individual amino acid residues can provide a more precise picture of a biological system than a simple knockdown or overexpression strategy(Gao, 2021; Rodríguez-Leal et al., 2017).

Synthesis of Failure Mechanisms

When these mechanisms are viewed together, it appears that translational inconsistency is not random. Rather, it is the product of the structural similarities between crop genomes and regulatory networks. Redundancy reduces the impact of disturbances. Hormonal integration involves trade-offs. Evolutionary divergences cause changes to network design. Environmental variability contradicts lab results. Dosage effects complicate the results of investigations(Huot et al., 2014; Panchy et al., 2016; Zandalinas et al., 2021). Knowing these boundary conditions alters the translation of model system information into a network engineering challenge with ecological restrictions. This knowledge foreshadows a more general conceptual question: What is the right conceptual placement for WRKY proteins, given that they are involved in a variety of stress responses?

WRKY Transcription Factors as Hubs of Biotic–Abiotic Stress Integration

The traditional classification of WRKY transcription factors as "defence regulators" or "abiotic stress regulators" is becoming increasingly ineffective. This is consistent with the finding that numerous WRKY proteins appear to act at "convergence points" rather than in distinct pathways. WRKY proteins are biologically significant because they can integrate competing physiological demands rather than only activating specific pathways(Birkenbihl et al., 2017b; Chen et al., 2017). This viewpoint explains both their scientific usefulness and the translational issue raised in the prior section.

Hormonal Crosstalk and Signal Convergence

Plant stress signaling is also not "modular" in the engineering sense, as the signal transduction pathways for salicylic acid, jasmonic acid, ethylene, and abscisic acid are interconnected, often antagonistically. This complicated interplay also includes WRKYs. SA-related WRKYs boost pathogen response gene expression during biotic stress, but they may also have a negative effect on JA-mediated responses, altering resistance spectra. ABA-related WRKYs participate in drought stress responses, including stomatal regulation, osmo-protectant accumulation, and oxidative status, but ABA also affects defense-related pathways, resulting in a complicated interaction(Berens et al., 2017; Chen et al., 2012). Instead of acting as on/off switches, WRKY proteins have been discovered to behave as context-sensitive amplifiers, which means that their activity is regulated by promoter elements, partners, phosphorylation status, and chromatin accessibility. For example, a single WRKY gene can execute different functions under pathogen attack and water restriction. This adaptability supports the hypothesis that WRKY proteins act as a network rather than a linear pathway(Rushton et al., 2010).

Integration Under Combined Stress Conditions

Stressors are rarely utilized alone in agricultural systems; instead, they are typically combined to achieve a non-additive physiological effect. For example, the combination of drought stress and pathogen infection may have an unexpected effect on hormone regulation, which would not be expected if either stress was administered alone. WRKY transcription factors are highly sensitive to these complex conditions. Several WRKY transcription factors are upregulated in response to both abiotic and biotic stresses, according to expression studies conducted in a variety of taxa. However, transcription factor activation may not necessarily predict regulatory effects. The main question is how WRKY factors govern transcriptional changes in complex settings(Suzuki et al., 2014; Zandalinas et al., 2021). Under combined stress, the WRKY protein may prioritize survival over growth, shifting transcriptional resources to protective metabolites and defence proteins. Alternatively, the WRKY may change signaling thresholds to avoid costly pathways from being activated too frequently. This decision-making mechanism is included in the network's design. Understanding the function of WRKY proteins in this way involves a departure from traditional experimental approaches that concentrate on single variable changes(Huot et al., 2014).

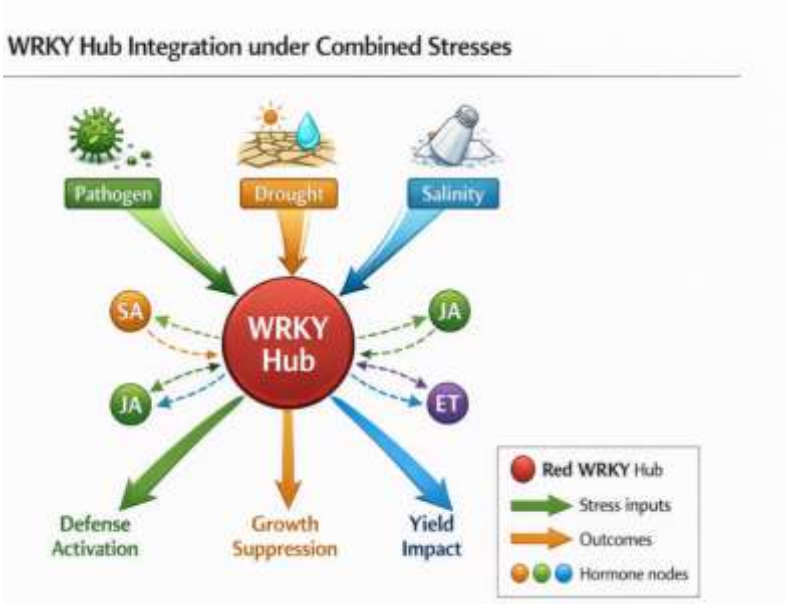

Figure 4: Conceptual model of WRKY-mediated integration under combined stresses. The figure should depict a central WRKY node receiving inputs from pathogen signaling, drought/salinity stress, and hormonal pathways (SA, JA, ABA, ET). Arrows should extend toward outputs

such as defense activation, growth modulation, oxidative balance, and yield impact.

Conflict Resolution Between Growth and Survival

Integration necessarily entails arbitration. When competing signals emerge, regulatory hubs facilitate trade-offs. WRKY proteins can control genes involved in secondary metabolism, cell wall reconfiguration, reactive oxygen detoxification, and developmental alterations. These processes require the spending of resources. The biological question is not whether WRKY activation enhances stress tolerance—it often does—but whether the resulting transcriptional state optimizes long-term fitness under fluctuating conditions(Birkenbihl et al., 2017a; Huot et al., 2014). The contrast between the two types of genes is crucial in crops, since reproductive fitness is judged not only by survival, but also by production. A gene that allows the crop to withstand stress but does not contribute to the continuation of the reproductive process, such as grain filling, will be of limited use. As a result, the efficacy of WRKY gene-based approaches must be assessed not only in terms of stress markers but also in terms of growth(Gao, 2021).

Network Position and Systemic Influence

Transcription factors at peripheral positions in the network are frequently targeted for modification, with negligible side effects. Hubs, on the other hand, provide regulatory functions for several modules. WRKY proteins usually have hub regulator-like features, such as large expression domains, several upstream activators, and a varied set of downstream targets. The hub position gives both regulatory authority and sensitivity. Changing a hub could have far-reaching effects for the whole network. This is one among the reasons why WRKY overexpression can produce phenotypes unrelated to the desired stress response target(Huot et al., 2014; Rushton et al., 2010). Understanding WRKYs as network hubs changes one's perspective on experimental approaches. Rather than determining whether a particular change improves stress response, investigate how the network responds to interruption(Gao, 2021).

Implications for Translational Strategy

If WRKY transcription factors manage the integration of various signals, resulting in growth-defense arbitration, simple gene-centric techniques will be insufficient to comprehend the complexities of their roles. Quantitative modulation, tissue specificity, and environmental verification will be critical to the translation process. While overexpression efforts will likely be insufficient, promoter engineering may be employed to create inducible activation. In addition, numerous gene knockouts may be required to change redundant modules. It will be critical to confirm the roles of these transcription factors not only during early vegetative growth but also during reproductive growth stages(Gao, 2021; Rodríguez-Leal et al., 2017). Furthermore, the WRKY transcription factors' integrative role lends credence to the argument that a network-centric research paradigm is required. Translation of WRKY functions will no longer be about transferring genes, but about comprehending the regulatory landscape.

Lessons from Three Decades and Future Directions

Significantly, during the last 30 years, WRKY transcription factors have provided unprecedented insights into the molecular logic of plant stress responses. However, as noted in previous sections, the transfer of these discoveries from model organisms to crops has not always been successful. The analysis of these results reveals several crucial lessons for future crop enhancement initiatives.

Limitations of Gene-Centric Strategies

Several factors underlie the limitations of gene-centric approaches:

Network Complexity: WRKYs operate within highly interconnected networks. Modifying a single gene has a limited impact on stress responses. There are redundant paralogs, feedback, and cross-regulatory interactions that work to reduce the impact of single-gene alterations, particularly in polyploid agricultural species(Kitano, 2004; Panchy et al., 2016).

Context-Dependent Activity: The WRKY function responds significantly to developmental stages, tissue types, and environmental circumstances. It is difficult to replicate all of the interactions that occur in the wild in a controlled laboratory environment. This is where the discrepancy lies, and it is what accounts for the lack of correlation between lab and field data(Mittler, 2006; Zandalinas et al., 2021).

Trade-Offs and Dosage Sensitivity: Constitutive overexpression/knockout can disrupt delicate balances of growth, reproduction, and stress tolerance. The phenotypes may be beneficial in one stress environment but detrimental to fitness in others. These limitations underscore the need to shift focus away from genes and toward the global regulation in which WRKYs operate(Gao, 2021; Huot et al., 2014).

Network-Centric Strategies for Next-Generation Research

To improve translational potential, future WRKY research should use network-oriented approaches that take into consideration redundancy, combinatorial control, and environmental variability.

Gene Stacking and Combinatorial Perturbation

Dealing with multiple paralogs or WRKYs that interact with each other can assist in the removal of functional redundancy. Additionally, by using combinatorial techniques, it is conceivable to enhance stress tolerance without sacrificing too much. Combinatorial techniques must be guided by both phylogeny and co-expression approaches, choosing genes that work well together(Gao, 2021; Panchy et al., 2016).

Promoter Engineering and Fine-Tuning

Instead of constitutive overexpression, stress-inducible or tissue-specific promoters can be used to modulate WRKY activity in a time/space-appropriate

way. This decreases the likelihood of pleiotropic effects while preserving key functions(Rodríguez-Leal et al., 2017).

CRISPR-Based Quantitative Modulation

Genome editing techniques, which are still in the early phases of development, enable precise adjustments in regulatory components, allowing for modest changes in transcription factor levels. This may increase network stability at the field level(Rodríguez-Leal et al., 2017; Zhang et al., 2018).

Combined Stress and Field-Relevant Screening

Experimental validation should take into account the complexities of agricultural circumstances. To provide a more accurate estimate of translation potential, the evaluation process should take into account multiple developmental stages and simultaneous pressures. Variations in water availability, pathogen levels, and nutrition levels should also be considered in the environmental circumstances(Mittler, 2006; Suzuki et al., 2014).

From Mechanistic Insight to Agronomic Relevance

Collectively, these network-centric techniques connect mechanistic insight with application. The debate shifts from "Which WRKY gene can improve resistance?" down to "How does the WRKY network regulate integrated stress responses in a crop context?"

This perspective emphasizes:

- The need for multi-layered evidence, combining transcriptomic, functional genomics, and phenotypic analysis(Zhang et al., 2018).
- The importance of ortholog validation and evolutionary context, ensuring that functional parallels are accurately interpreted(Panchy et al., 2016).
- Recognition of trade-offs inherent in regulatory hubs, guiding realistic expectations for crop improvement(Huot et al., 2014).

By situating WRKY research within this broader framework, the community can move beyond incremental gene discovery and toward strategies capable of producing robust, field-ready crops.

Conceptual Framework: Linking Discovery to Translation

Three decades of research have yielded a systematic framework for future WRKY research:

Discovery Phase: Identify stress-responsive WRKYs in model systems; determine network location and regulatory logic.

Network Analysis Phase: Map interactions among paralogs and with hormone signaling; evaluate potential for redundancy and crosstalk.

Targeted Modulation Phase: Apply combinatorial gene editing and promoter engineering to adjust activity in crops.

Field Validation Phase: Test under multi-stress, real-world conditions; monitor yield, growth, and physiological resilience.

Table 2: From Gene Discovery to Field Translation formalizes this approach, contrasting current gene-centric practices with recommended network-based strategies and expected gains

Current Practice	Limitation	Recommended Network-Centric Approach	Expected Gain
Single-gene overexpression in *Arabidopsis*	Often fails in crops due to functional redundancy and buffering by paralogs.	Identify key network hubs and target multiple paralogs simultaneously using combinatorial editing	Greater likelihood of observable phenotypic improvement; mitigates redundancy masking
Constitutive promoters driving WRKY expression	Growth-defense trade-offs lead to yield penalties under non-stress conditions	Use stress-inducible or tissue-specific promoters for precise temporal and spatial regulation	Minimized fitness cost, balanced growth and stress tolerance
Knockout of single WRKY ortholog	Partial phenotypic effect or no observable change due to paralog compensation	Employ multiplex CRISPR editing of redundant WRKY family members based on co-expression networks	Enhanced functional impact; clearer linkage between gene perturbation and phenotypic outcome
Laboratory assays under single-stress conditions	Poor translation to field due to environmental complexity and combined stresses	Screen edited lines under multi-stress, field-relevant conditions including pathogen, drought, and salinity combinations	Reliable prediction of performance under realistic agricultural conditions
Transcriptomic-only functional inference	Expression changes do not always correlate with phenotypic outcomes	Integrate transcriptomic, proteomic, and metabolomic data to assess network impact	Stronger mechanistic understanding; better prediction of biological relevance
Relying solely on *Arabidopsis* orthology	Evolutionary divergence leads to promoter and network rewiring;	Validate ortholog function in crop species; assess promoter architecture and downstream targets before manipulation	Reduced translational failure; higher confidence in applied strategies

	unexpected regulatory outputs		
Single-time point measurement of stress response	Ignores temporal dynamics; may miss late-stage effects	Conduct longitudinal analyses across developmental stages and multiple stress periods	Capture comprehensive stress-response dynamics; improved field applicability
Overemphasis on gene discovery	Generates large gene lists with limited translational impact	Shift focus to network topology, hub identification, and integrated pathway modulation	Increases probability of achieving agronomically meaningful improvements
Functional validation in controlled environment only	Environmental realism gap; may not predict yield outcomes	Combine greenhouse and multi-location field trials; incorporate variable soil, water, and pathogen conditions	Predictable and robust stress tolerance and yield stability under real-world conditions
Binary knockout or overexpression strategies	Nonlinear network responses; dosage sensitivity ignored	Fine-tune gene expression quantitatively via promoter editing or CRISPR-based modulation	Avoids pleiotropic effects; maintains physiological balance while enhancing stress resilience

Conclusions

Over the last three decades, significant progress has been achieved in the field of plant stress biology through studies on WRKY transcription factors. WRKY transcription factors have emerged as significant regulators of both biotic and abiotic stress responses in plants, beginning with their identification in the model plant *Arabidopsis thaliana* and progressing to their characterization in important crop plants. However, the route to translation has been fraught with difficulties, particularly in dealing with the complexity of the WRKY gene family.

Our review underscores several key points:

Arabidopsis Provides Foundational Mechanistic Insights

Arabidopsis research has discovered the WRKY network's fundamental properties, which include autoregulation, feedback, and interaction with hormonal signaling. This information is important, but it does not always translate into a phenotype.

Translation Requires Network Awareness

WRKY genes rarely function in isolation. Because of their status as network hubs, attempts to change WRKY genes separately may produce unanticipated or even counterintuitive results in the context of the polyploidy agricultural plant genome. Network-centric techniques are more likely to provide consistent increases in stress resilience.

Environmental Realism is Critical

Laboratory testing conducted under single-stress circumstances frequently fail to account for the complexity present in agricultural conditions. The key to translational success is to examine WRKY-modulated responses under a variety of stressors, developmental stages, and field situations.

Fine-Tuned, Context-Sensitive Modulation Outperforms Binary Approaches

Constitutive overexpression or deletion of WRKY genes frequently results in growth-defense trade-offs. Inducible, tissue-specific, or quantitatively regulated expression techniques provide more delicate control, reducing secondary effects while increasing functional impact.

Integration of Multi-Layered Data Enhances Predictive Power

Instead of only studying gene expression, it is feasible to examine network effects using transcriptomic, proteomics, and metabolomics. Such a strategy is required to discover candidate WRKYs with translational potential.

Future Perspective

Moving forward, WRKY research should embrace a comprehensive, network-focused, and agriculturally informed framework:

- **Gene discovery must be complemented by network mapping**, hub identification, and combinatorial functional analysis.
- **Ortholog validation in crops** should precede translational attempts to account for promoter divergence, downstream target variation, and evolutionary rewiring.
- **Field-oriented testing under combined stresses** should replace single-factor laboratory assays to ensure relevance to agricultural productivity.
- **Quantitative modulation** using promoter engineering and CRISPR-based approaches can achieve balanced growth-defense outcomes without penalizing yield.

By accepting these ideas, the community can build on three decades of mechanistic knowledge to provide demonstrable advances in crop resilience, productivity, and adaptation.

Final Remarks

WRKY transcription factors represent the opportunities and challenges that exist when transferring plant biology to agriculture. It is not sufficient to regard these genes as part of the response to environmental stress. Instead, we should think of

these genes as arbiters of complicated signaling and resource allocation processes. We should address the context in which these genes function, the combinatorial nature of their activities, and the design of studies that reflect the field's complexity. In conclusion, while Arabidopsis will remain an important discovery engine, crop improvement success will be determined by a network-centric approach that is context-aware and experimentally informed. Research on WRKY genes must shift away from the traditional approach to gene discovery and toward an integrative approach that is appropriate for crop development.

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Statements and Declarations

Data Availability statement

All relevant data are within the manuscript file.

Author's Contribution Statement

MH, SHUHS and AS collected data and wrote manuscript equally. MS, and HA make final editing. MS supervised the whole research work. All authors have read the final manuscript and approve its submission.

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Declaration of Generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work, the author(s) have not used any ChatGPT (OpenAI) tool.