The role of WRKY transcription factors in plant abiotic stresses

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ABSTRACT

The WRKY gene family has been suggested to play important roles in the regulation of transcriptional reprogramming associated with plant stress responses. Modification of the expression patterns of WRKY genes and/or changes in their activity contribute to the elaboration of various signaling pathways and regulatory networks. Furthermore, a single WRKY gene often responds to several stress factors, and then their proteins may participate in the regulation of several seemingly disparate processes as negative or positive regulators. WRKY proteins also function via protein–protein interaction and autoregulation or cross-regulation is extensively recorded among WRKY genes, which help us understand the complex mechanisms of signaling and transcriptional reprogramming controlled by WRKY proteins. Here, we review recent progress made in starting to reveal the role of WRKY transcription factors in plant abiotic stresses. This article is part of a Special Issue entitled: Plant gene regulation in response to abiotic stress.

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1. Introduction

Being unable to move, plants constantly encounter various fluctuating abiotic environmental factors, such as water deficiency (drought), excessive salt (salinity), threshold temperatures (from freezing to scorching), decreased availability of essential nutrients (nutrient starvation) and variable light conditions. These factors can occur at multiple stages of plant development and often more than one stress simultaneously affects the plant, potentially restricting plant growth, plant development or even determining plant species distribution across different types of environments. Much attention had been paid to these abiotic factors because of their potential impact on agricultural production and quality.

Plants have evolved intricate mechanisms at multiple levels that increase tolerance in order to adapt to adverse conditions. For example, at the cellular level, closure of stomata and inhibition of vegetative growth help the plants survive in water-limited conditions [1]. At the molecular level, the induction of stress-responsive and stress-tolerance genes also contribute to the plants to adapt to unfavorable environmental conditions [2]. Recognition of stress cues and transduction of the signals to activate adaptive responses and regulation of stress-related genes are the key steps leading to plant stress tolerance [3]. Induction of stress-related genes occurs mainly at the transcriptional level, and modification of the temporal and spatial expression patterns of specific stress-related genes is an important part of the plant stress response [4]. Plants devote a large portion of their genome capacity to transcription, with the Arabidopsis (Arabidopsis thaliana) and rice (Oryza sativa) genome coding for more than 2100 and 2300 transcription factors respectively [5]. These transcription factor genes often belong to large gene families, which in some cases are plant-specific. Among them, the WRKY transcription factors compose one large family of regulatory proteins in plants.

Although discovered relatively recently, the WRKY transcription factors are becoming one of the best-characterized classes of plant transcription factors. Previous studies have demonstrated that WRKY transcription factors participated in various biotic stress responses [6, 7] and several developmental and physiological processes, including embryogenesis, seed coat and trichome development, leaf senescence, regulation of biosynthetic pathways, and hormone signaling [8–15]. Currently, some researchers have focused on the functional analysis of WRKY factors in plant responses to abiotic stress such as drought, cold and nutrient deficiency. Moreover, test materials were no longer restricted to model plants such as Arabidopsis (A. thaliana) but also applied to non-model plants, in particular crop species. In this review, we will emphasize on the roles of WRKY transcription factor genes in plant abiotic stresses.

2. Structure characterization and classification

WRKY proteins belong to the WRKY-GCM1 superfamily of zinc finger transcription factors that evolved from Mutator or Mutator-like (Mule) transposases [16, 17]. Although WRKY transcription factors are reported in some non plant species [18–20], they constitute a large family of transcription factors in plants [20]. A large number of WRKY genes have been identified from Arabidopsis (74) [20], rice (＞100) [10], soybean (197) [21], papaya (66) [7], poplar (104) [7].
The WRKY protein family owes its name to the highly conserved 60 amino acid long WRKY domains, which contain a conserved amino acid sequence motif WRKYQK at N-termini and a novel zinc-finger-like motif at C-termini [6]. The heptapeptide WRKYQK motif showed slight variations in a few WRKY proteins [24–27]. Both of these two motifs are vital for the high binding affinity of WRKY transcription factors to the consensus cis-acting elements termed the W box (TTGACT/C), although alternative binding sites have been identified [28–31]. Almost all WRKY transcription factors show binding preference to the cognate cis-acting element, however, the binding site preferences are also partly determined by additional adjacent DNA sequences outside of the TTGACY-core motif [29]. Initially, based on both the number of WRKY domains and the features of their zinc-finger motif, the WRKY protein family can be categorized into three distinct groups [6]. The first group (I) has two WRKY domains; group II has one WRKY domain containing the same Cys2-His2 zinc-finger motif, and group III has one WRKY domain containing the different Cys2-His/Cys Cys2-His2 zinc-finger motif. The group II WRKY proteins are further divided into subgroups α–ε based on additional conserved structural motifs outside the WRKY domain [6]. Later, based on more accurate phylogenetic analysis, Zhang and Wang [25] classified the WRKY factors into Groups I, IIα + IIβ, IIc, IID + IId, and IIe with the Group IIα genes not being monophyletic. In addition to both the highly conserved WRKY domain and the Cys2-His2/Cys Cys2-His2 zinc-finger motif, the WRKY proteins still contain the following structures: putative basic nuclear localization signals, leucine zippers, serine-threonine-rich region, glutamine-rich region, proline-rich region, kinase domains and TIR-NBS-LRRs. Thereby, owing to such structure characterizations, the WRKY proteins can play their appropriate roles in regulation of gene expression.

3. Function in abiotic stresses

WRKY transcription factors function as important components in the complex signaling progresses during plant stress responses. However, compared with the research progress in biotic stresses, far less information is available to understand the function of WRKY proteins in abiotic stresses. Considering the relative large number of WRKY transcription factors from different plants and their unknown and diverse roles under complex environmental stimulations, it remains a big challenge to uncover their roles in abiotic stresses. Until recently, the possible involvement of WRKY proteins in the abiotic stress responses was deduced indirectly from transcription profiling; however, recent functional analyses have provided some direct evidence. The recent data presented here mainly summarized the function of most of WRKY transcription factors in regulating transcriptional reprogramming associated with plant abiotic responses (Table 1). The tight regulation and fine-tuning of WRKY proteins during plant stress responses contribute to the establishment of complex signaling webs and the important roles of WRKY proteins in plant abiotic stress responses make them potential candidates for imparting stress tolerance.

3.1. Expression pattern of WRKY genes under abiotic stresses

Numerous studies have demonstrated that many WRKY genes behave strongly and rapidly induced expression when respond to certain abiotic stresses, such as wounding, drought or salinity, indicating their regulatory function in these signaling pathways. Northern blotting analysis revealed that 10 of 13 OsWRKY genes differentially respond to NaCl, PEG, cold or heat treatment [32]. In wheat, 8 of 15 WRKY genes were also responsive to low temperature, high temperature, NaCl or PEG treatment [33]. Microarray profiling of NaCl-treated Arabidopsis roots revealed that there are 18 AtWRKY genes differentially responding to salinity, and the corresponding expression levels were UV, oxidative stress, cold, salt-stress, drought, osmotic stress, and ABA signaling.

Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Induced by abiotic factors</th>
<th>Function in abiotic stress</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AtWRKY2</td>
<td>At5g56270</td>
<td>NaCl, mannitol</td>
<td>Negative regulator in ABA signaling</td>
<td>[57,58]</td>
</tr>
<tr>
<td>AtWRKY6</td>
<td>At1g6200</td>
<td>H$_2$O$_2$, ethylene viologen, Pi and B starvation</td>
<td>Negative regulator in low Pi stress and positive regulator in low B stress</td>
<td>[35,65,66]</td>
</tr>
<tr>
<td>AtWRKY8</td>
<td>At4g31800</td>
<td>ABA</td>
<td>ABA signaling, NaCl and mannitioao tolerancc</td>
<td>[60,61]</td>
</tr>
<tr>
<td>AtWRKY12</td>
<td>At4g01250</td>
<td>H$_2$O$_2$, dark</td>
<td>Enhanced dark-induced senescence</td>
<td>[13]</td>
</tr>
<tr>
<td>AtWRKY25</td>
<td>At2g30250</td>
<td>Ethylene, NO, NaCl, mannitol, cold, heat, ABA, cold</td>
<td>Tolerance to heat and NaCl, increased sensitivity to oxidative stress and ABA</td>
<td>[40,41,48]</td>
</tr>
<tr>
<td>AtWRKY26</td>
<td>At5g07100</td>
<td>Heat</td>
<td>Tolerance to heat</td>
<td>[41]</td>
</tr>
<tr>
<td>AtWRKY33</td>
<td>At2g38470</td>
<td>NaCl, mannitol, cold, H$_2$O$_2$, ozone oxidative stress, UV</td>
<td>Tolerance to heat and NaCl, increased sensitivity to oxidative stress and ABA</td>
<td>[41,48]</td>
</tr>
<tr>
<td>AtWRKY34</td>
<td>At4g26440</td>
<td>Cold</td>
<td>Negative regulator in pollen specific cold response</td>
<td>[44]</td>
</tr>
<tr>
<td>AtWRKY39</td>
<td>At3g04670</td>
<td>Heat</td>
<td>Tolerance to heat</td>
<td>[64]</td>
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<td>ABA</td>
<td>ABA signaling</td>
<td>[60,61]</td>
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<td>AtWRKY60</td>
<td>At2g25000</td>
<td>ABA</td>
<td>ABA signaling, NaCl and mannitioao tolerancc</td>
<td>[60,61]</td>
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<td>AtWRKY63</td>
<td>At1g66000</td>
<td>ABA</td>
<td>Negative regulator in ABA signaling while positive regulator in drought tolerance</td>
<td>[59]</td>
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<td>AtWRKY75</td>
<td>At5g13080</td>
<td>Pi deprivation</td>
<td>Positive regulator in Pi starvation</td>
<td>[36]</td>
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<td>OsWRKY98</td>
<td>05g05610</td>
<td>Drought, salinity, H$_2$O$_2$, ABA, NAA</td>
<td>Tolerance to osmotic stress</td>
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<td>OsWRKY72</td>
<td>11g20870</td>
<td>Salinity, heat, ABA, NAA, osmotic stress, sugar starvation</td>
<td>Negative regulator in ABA signaling and sugar starvation</td>
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<td>OsWRKY89</td>
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<td>Salinity, ABA, UV-B, wounding</td>
<td>Tolerance to UV-B radiation</td>
<td>[81]</td>
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<td>GmWRKY13</td>
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<td>Increased sensitivity to salt and mannitol while decreased sensitivity to ABA</td>
<td>[55]</td>
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<td>Cold tolerance</td>
<td>[55]</td>
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<td>DQ322608</td>
<td>Salt, drought</td>
<td>Salt and drought tolerance</td>
<td>[55]</td>
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<tr>
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<td>EF03036</td>
<td>Salinity, cold, drought</td>
<td>Negative regulator in osmotic stress</td>
<td>[49]</td>
</tr>
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<td>DQ831118</td>
<td>Sugar</td>
<td>Sugar signaling</td>
<td>[70]</td>
</tr>
<tr>
<td>HvWRKY41</td>
<td>DQ83124</td>
<td>Sugar</td>
<td>Sugar signaling</td>
<td>[70]</td>
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<tr>
<td>HvWRKY46 (SUSIBA2)</td>
<td>AA323206</td>
<td>Sugar</td>
<td>Sugar signaling</td>
<td>[71-73]</td>
</tr>
<tr>
<td>NwWRKY3</td>
<td>AY456271</td>
<td>Wounding</td>
<td>JA signaling</td>
<td>[80]</td>
</tr>
<tr>
<td>NwWRKY6</td>
<td>AY456272</td>
<td>Wounding</td>
<td>JA signaling</td>
<td>[80]</td>
</tr>
</tbody>
</table>

genes that were induced by 150 mM NaCl treatment [34]. Transcript levels of both AtWRKY6 and AtWRKY7 are enhanced during phosphate deprivation [35,36]. There are many additional studies to show that numerous WRKY genes respond to wounding, drought, heat, cold or heat pre-treated chilling [10,37–48]. Interestingly, the induced expression of WRKY genes is often extremely rapid and transient, and appears independent of de novo synthesis of regulatory factors [6,37]. The immediate-early expression behavior of WRKY genes assure the successful transduction of the signals to activate adaptive responses and regulation of stress-related genes, and finally result in plant stress tolerance. Furthermore, a single WRKY gene often simultaneously responds to several stress factors, indicating its diverse regulatory function during plant stress responses. AtWRKY25 and AtWRKY33 respond to both heat and salt treatments [40,41,48]. TcWRKY53 is also simultaneously induced by cold, salt and PEG treatments [49]. Thus, WRKY genes appear to be expressed under different abiotic stresses and could therefore participate in the control of signaling processes associated with transcriptional reprogramming when plants encounter various adversities. Based on their expression pattern under different abiotic stresses, a researcher may obtain some clues as to their regulatory functions toward particular stress conditions.

3.2. Drought, salinity, osmotic stress and ABA signaling

Drought is often associated with salinity, important abiotic stress factors, usually affecting plant growth, development, survival and crop productivity. Thus, understanding the complex mechanism of drought and salinity tolerance is important for agriculture production. Interestingly, several WRKY proteins were shown to be involved in plant drought and salinity stress responses [50]. For example, over-expression of OsWRKY11 under the control of HSP101 promoter led to enhanced drought tolerance, as showed by the slower leaf-wilting and increased survival rate of green plant parts [51]. Similarly, the altered salt and drought tolerance of 35S:OsWRK45 and 35S:OsWRK72 Arabidopsis plants may be attributed to induction of ABA/stress-related genes [52,53]. OsWRKY08, whose transcripts was enhanced by PEG, NaCl or abscisic acid (ABA) treatment, improves the osmotic stress tolerance of transgenic Arabidopsis through positive regulation of the expression of two ABA-independent abiotic stress responsive genes, AtCOR47 and AtRD21 [54]. Plants which have over-expressed GmWRKY54 showed enhanced salt and drought tolerance, possibly through the regulation of transcription factor STZ/Zat10, while over-expression of GmWRKY13 led to increased sensitivity to salt and mannitol stresses [55]. Similarly, the expression of TcWRKY53 was strongly induced by NaCl and drought stresses and transgenic tobacco plants overexpressing TcWRKY53 showed depressed expression of two ERF family genes, NtERF5 and NtEREBP-1 [49]. In Arabidopsis, the transcripts of two closely related WRKY transcription factors (AtWRKY25 and AtWRKY33) were increased by ABA, drought or NaCl treatment and both the Atwrky33 null mutants and Atwrky25Atwrky33 double mutants showed moderately increased NaCl-sensitivity; however, overexpression of either AtWRKY25 or AtWRKY33 led to increased Arabidopsis NaCl tolerance [41]. These data provide evidence that different WRKY proteins play differential roles in specific abiotic stress responses.

ABA is a stress hormone and plays essential roles in plant responses to abiotic stresses. Previous research had demonstrated that WRKY proteins may act as activators or repressors in ABA signaling. LiWRKY21 was shown to function as an activator to control ABA-regulated expression of genes [15]. Transient expression analysis showed that OsWRKY24 and OsWRKY45 act as repressors while OsWRKY72 and OsWRKY77 act as activators to the same ABA-inducible promoter [56]. AtWRKY2 was induced by NaCl and mannitol treatments [57], and studies using Atwrky2 T-DNA insertion mutants indicated that AtWRKY2 possibly functioned as a negative feedback regulator of ABA-mediated arrest of seed germination and post-germination growth [58]. Analysis of T-DNA insertion mutant of AtWRKY63 (ABO3) indicated that AtWRKY63 play an important role in plant responses to ABA and drought stress. AtWRKY63 was induced by ABA treatment and mutation of AtWRKY63 rendered the mutants more sensitive to ABA in both seedling establishment and seedling growth and less drought tolerant. EMSA showed that the W-box sequence upstream of the AtABF2 promoter could be bound by AtABO3, supporting its repressed expression in the Abo3 mutant plants. However, overexpression of AtABO3 did not result in drought tolerance, thus AtABO3 need either co-factors or some post-translational modifications to activate the downstream genes for stress tolerance [59]. Recently, two research groups reported their results about the function of a group of structurally related WRKY proteins, AtWRKY18, AtWRKY40 and AtWRKY60, in ABA signaling. Shang et al. [60] showed that the three WRKY proteins function as negative regulators of ABA signaling in seed germination and postgermination growth. Further genetics analysis showed that AtWRKY40 acts as a central negative regulator among the three WRKY proteins and could directly inhibit the expression of several important ABA-responsive genes, such as AtABF4, AtABF4, AtABF5, AtDREB1A, AtMYB2 and AtABI1B, by directly binding to the W-Box sequences upstream of their promoters. High levels of ABA recruits AtWRKY40 from the nucleus to the cytosol and promotes ABA–WRKY40 interaction, thereby expression of ABA-responsive genes is relieved and finally ABA responses occur (Fig. 1). Another work on the three WRKY genes using both single, double, and triple mutants and overexpression lines showed that AtWRKY18 and AtWRKY60 have a positive effect on plant ABA sensitivity for inhibition of seed germination and root growth. They also increase plant sensitivity to salt and osmotic stress tolerance.
stresses. While AtWRKY40, on the other hand, antagonizes AtWRKY18 and AtWRKY60 in the effect on plant sensitivity to ABA and abiotic stress in germination and growth assays [61]. These results indicate that WRKY proteins function as key components during ABA signaling.

3.3. Temperature stress

Temperature that exceeds an organism’s optimal tolerance range is considered as an important abiotic stress factor. In agriculture, high or low temperature acts as a major negative factor limiting crop production. Thus, finding an effective strategy to enhance plant’s adaptability to rapid and/or drastic changes in temperature is of particular importance for agricultural production. Tremendous work has been done in the past two decades to reveal the complex molecular mechanism in plants’ responses to extreme temperature and there is increasing evidence that WRKY proteins are involved in responses to both heat and cold stresses. For example, a WRKY transcription factor in tobacco (Nicotiana tabacum L.) responds to a combination of drought and heat stress [38]. Another example is that transgenic Arabidopsis plants overexpressing GmWRKY21 showed increased tolerance to cold stress when compared with wild-type plants [55]. Moreover, overexpression of OsWRKY11 under the control of HSP101 promoter led to enhanced heat tolerance [51]. The expression of AtWRKY18, AtWRKY33, AtWRKY40, and AtWRKY46 is elevated in Arabidopsis over-expressing plants, which possess enhanced thermotolerance compared with wild-type plants [62]. Microarray analysis of A. thaliana hsfla/hslb double knockout mutants has revealed that nine of 60 analyzed AtWRKY genes are regulated by heat stress and, among these nine genes, AtWRKY7 is a Hsfla1/1b-dependent heat stress gene [63]. Our recent studies have shown that AtWRKY25, AtWRKY26, and AtWRKY33, three types of I WRKY proteins, were involved in the regulation of resistance to heat stress. They show distinct expression patterns upon high temperature treatment, with induced expression of AtWRKY25 and AtWRKY26 and repressed expression of AtWRKY43. Mutation of these three genes render the mutant plants more sensitive to heat stress, as can be seen from reduced germination, decreased survival, and elevated electrolyte leakage. In contrast, transgenic plants overexpressing AtWRKY25, AtWRKY26, or AtWRKY33 showed enhanced resistance to heat stress. These three WRKY transcription factors participated in the heat response through modulating transcriptional reprogramming of heat-inducible genes (Fig. 2A). Interestingly, AtWRKY25, AtWRKY26, and AtWRKY33 were also involved in regulation of the heat-induced ethylene-dependent response. Thus, these three proteins play overlapping and synergetic roles in plant thermotolerance through positively regulating the cooperation between the ethylene-activated and heat shock protein-related signaling pathways [41]. Our previous research also exhibited that heat stress-induced AtWRKY39 positively regulates the cooperation between the SA- and JA-activated signaling pathways that mediate responses to heat stress [64]. Contrary to AtWRKY25, AtWRKY26, AtWRKY33, and AtWRKY39, AtWRKY34 participates in the pollen-specific cold stress response. Promoter-GUS analysis revealed that AtWRKY34 expression is pollen-specific and its expression is enhanced by cold treatment. Mutation of AtWRKY34 make the pollen more insensitive to cold stress compared with that of wild type, while the pollen of AtWRKY34 overexpressing plants is sterile even under normal growth conditions. The AtWRKY34 transcription factor negatively mediates cold sensitivity of mature Arabidopsis pollen through regulating the expression of transcriptional activator CBFs (Fig. 2B) [44]. Taken together, WRKY proteins function in plants’ adaption to temperature variations through transcriptional reprogramming of downstream stress-related genes.

3.4. Nutrient deficiency

Various nutrient elements are required for plant’s normal growth and development, and deficiency in any necessary element will have a significant effect on plant architecture formation or even its adaptability to various adversities. Several studies have indicated that the WRKY transcription factors also participate in the nutrient deficiency response signaling pathways. AtWRKY75 was the first WRKY member reported to be involved in regulating phosphate starvation. AtWRKY75 was strongly induced in plant during Pi-deficiency and

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suppression of the AtWRKY75 expression conferred the plant more susceptible to Pi stress and decreased Pi uptake during Pi starvation. Expression of several Pi-starvation associated genes, such as phosphatases, Mt4/TPS1-like genes and high affinity Pi transporters was decreased in AtWRKY75 RNAi plants [36]. However, these genes were AtWRKY75’s direct target or not should be further determined. Thus identifying the gene(s) whose expression is specifically and directly regulated by AtWRKY75 as well as its interacting partners will help us to clarify the complex mechanisms of plant responses to low Pi stress. Recently, another member, AtWRKY6 was also determined to function in plant responses to low Pi stress through negatively regulating Arabidopsis PHOSPHATE1 (PHO1) expression. Transgenic plants overexpressing AtWRKY6 had a phenotype similar to the Atpho1 mutant plants and were more sensitive to low Pi stress and accumulated less Pi in shoots when compared with wild-type or Atwrky6-1 mutant plants. ChIP-qPCR analysis showed that AtWRKY6 negatively regulated AtPHO1 expression through directly binding to the two W-boxes upstream of the AtPHO1 promoter in a Pi dependent manner. Furthermore, low Pi-induced release of AtPHO1 repression may result from 26S proteosome-mediated proteolysis of AtWRKY6 protein (Fig. 3). In addition, as an interacting partner of AtWRKY6, AtWRKY42 could also inhibit AtPHO1 expression through directly binding to W-boxes of the AtPHO1 promoter [35]. Taken together, both AtWRKY75 and AtWRKY6 participated in the Pi-deficiency response; however, they regulated the expression of different types of downstream target genes, thus they may function in different regulatory pathways during low Pi stress. AtWRKY6 was also represented as the first transcription factor involved in the response to boron deficiency. However, opposite to low Pi stress, AtWRKY6 functions as a positive regulator in low-boron response [65,66]. Thus, AtWRKY6 seems to respond to several aspects of nutrient deficiency induced stresses, implicating its diverse functions in these signaling pathways.

In Arabidopsis, WRKY45 and WRKY65 are involved in the regulation of gene expression during carbon starvation [67]; and the 35S: OsWRKY72 transgenic Arabidopsis also revealed increased sensitivity to sugar-starvation stress [53]. Similarly, there are also three OsWRKY genes whose expression showed significant alternation in sucrose-starved rice suspension cells [68], AtNDPK3a, which is induced by sucrose and glucose, was possibly regulated by AtWRKY4 and AtWRKY34 [69]. Interestingly, three HvWRKY genes involved in plant sugar signaling [70] and HvWRKY46 (SUSIBA2) participated in sugar signaling by mediating the expression of ISO1 and SBEL1b [71–73]. Thus, WRKY proteins may also be involved in the regulation of the many metabolic and stress-related proteins that were involved in sugar signaling.

3.5. ROS signaling

Various abiotic stresses always aggravate the production of reactive oxygen species (ROS) in mitochondria, via consumption of oxygen in a so-called oxidative burst in plants [74]. ROS like H2O2 act as important signal transduction molecules, mediating the acquisition of tolerance to various stresses [75]. In Arabidopsis, the expression of AtWRKY30, AtWRKY75, AtWRKY48, AtWRKY39, AtWRKY6, AtWRKY53, AtWRKY22, and AtWRKY8 were significantly induced by H2O2 treatment [12,13,43,76]. Several important enzymes such as zinc finger proteins, ascorbate peroxidases (APX), and NADPH oxidases have revealed the key nodes in the ROS signal network. In zinc finger protein gene Atzat12 mutant plants, the expression of AtWRKY25 was unable to be enhanced after H2O2 treatment, suggesting that the induction of AtWRKY25 during oxidative stress was dependent on AtZat12 [77]. Also AtWRKY70 was constitutively expressed in ROS-scavenging enzyme gene Atapx1 mutant plants, suggesting its possible role in ROS signaling [78]. Under light stress, the expression of several other WRKY transcription factor genes including AtWRKY6, AtWRKY18, AtWRKY25, AtWRKY33, AtWRKY40, AtWRKY46, AtWRKY54, and AtWRKY60, was also elevated in Atapx1 mutant plants when compared with wild type plants, implying the possible involvement of these WRKY proteins in ROS signaling [79]. Hence, WRKY transcription factors appear to play an important role in ROS signaling web.

3.6. Other abiotic stresses

Besides of the involvement of abiotic stresses mentioned above, WRKY proteins also participated in other abiotic stress responses, such as wounding and UV radiation. Numerous studies showed that a number of WRKY genes were induced by wounding treatment [37,43]. Both NoWRKY3 and NoWRKY6 respond to wounding, and interestingly, NaWRKY3 is required for NaWRKY6 elicitation by fatty acid–amino acid conjugates (FACs) in larval oral secretions that are introduced into wounds during feeding [80]. Silencing either or both genes make plants highly vulnerable to herbivores and reduce M. sexta-elicited JA and JA-Ile/-Leu levels (Fig. 4). The response to wounding and herbivore-specific signals (FACS) imply that the two WRKYs help plants to differentiate mechanical wounding from herbivore attack. In Arabidopsis, three WRKY genes were strongly induced by UV-B light treatment [45]. Similarly, OsWRKY89 was strongly

![Fig. 3. The role of AtWRKY6 transcription factor in signaling pathways during Pi starvation. The AtPHO1 promoter contains six W-boxes and four of them were shown (Q, X, Y, and Z). Under normal conditions, the expression of AtPHO1 was repressed by AtWRKY6 through directly binding to the W-box motifs Wx and Wy within the AtPHO1 promoter (left); while under low Pi conditions, AtWRKY6 protein was degraded via a 26S proteosome-mediated proteolysis and then repression of AtPHO1 transcription by AtWRKY6 is relieved (right) [35].](image-url)
Fig. 4. Wounding induced NaWRKY3 and NaWRKY6 coordinate responses to herbivory. NaWRKY3 is strongly induced by wounding treatment while NaWRKY6 is only moderately induced. NaWRKY3 is required for NaWRKY6 elicitation by herbivore feeding which introduces fatty acid–amino conjugates (FACs) in Manduca sexta larval oral secretions into wounds. Both of them regulate expression of jasmonic acid (JA) biosynthesis genes (LOX, AOS, AOC and OPE1), and NaWRKY3 may also regulate JAR4 or unknown JA conjugating genes, thereby increasing the levels of JA and JA-Ile. This is turn enhances defense gene expression, such as TPI and DTO, and finally regulate plant defenses against herbivores [86]. The symbol “?” represents JAR4 or unknown JA conjugating enzymes.

induced by UV-B radiation and over-expression of OsWRKY89 enhanced tolerance to UV-B irradiation through increasing the wax deposition on leaf surfaces of transgenic plants [81]. In addition, we found that over-expressing AtWRKY22 and OsWRKY23 in Arabidopsis accelerated leaf senescence in darkness [13,82].

3.7. One WRKY toward multiple processes

Numerous studies have demonstrated that a single transcription factor may function in several seemingly disparate signaling pathways, as can be deduced from their induced expression profile by various stress factors. Recent research suggest that the three structurally related WRKY proteins, AtWRKY18, AtWRKY40 and AtWRKY60, participate in at least three phytohormone-mediated signaling pathways (SA, JA and ABA) [60,61,83,84]. Pandey et al. demonstrate that WRKY18/40 negatively modulate EDS1 signaling but positively regulate JA-signaling when responding to G. orontii infection [84]. Two closely related WRKY transcription factors (AtWRKY25 and AtWRKY33) respond to both biotic and abiotic stresses, e.g., P. syringae, NaCl, cold and heat [41,48,85,86]. Both of them act as a negative regulator of the defense response to P. syringae while functioning as a positive regulator in NaCl and heat stress response. Studies on AtWRKY6 showed that it at least functions in three different processes, including pathogen defense, senescence and phosphate (Pi) and boron (B) deficient responses [35,65,66,87]. These data demonstrated that a single WRKY gene can function as regulator of several different processes and may also mediate the crosstalk between different signaling pathways.

4. Defining direct target genes

In order to better understand WRKY transcription factors’ biological functions and their possible signaling pathways, it is necessary to identify their downstream target genes. Through comparing the expression pattern of different genotypes using the microarray technology, certain potential targets of the WRKY gene could be obtained on the genome scale. For example, a set of 12 genes showed marked differences in mature pollen’s expression between Atwrky34-1 mutant and wild-type plants after treatment at 4 °C for 48 h [44]. Microarray analysis also confirmed that the expression of several ABA signaling pathway genes, such as AtABI5, AtABI3, was significantly enhanced in Atwrky2 mutants compared with wild type [58]. cDNA-AFLP analysis provide us another method to identify putative target genes. For example, several downstream genes, including FRK1/SIRK, were identified to be AtWRKY6’s target gene, and these genes work together to play important roles in the plant leaf senescence [87]. This method could also be used to identify other WRKY gene’s target gene involved in plant abiotic stresses. However, the methods shown above only provide us the candidate target genes, and whether these genes are WRKY proteins’ direct target genes or not need to be further determined. Thus other advanced methods were needed to determine their direct target genes. One of them is the chromatin immunoprecipitation (ChIP) technique. The ChIP technique has been suggested to be an effective strategy in monitoring DNA–protein and protein–protein interactions in vivo under various conditions and in a dynamic manner [88]. Using this method, an increasing number of single WRKY gene’s targets have been identified under certain abiotic stresses. For example, several important ABA responsive genes, such as AtABI4, AtABI4, AtABI5, AtDREB1A, AtMYB2 and AtRAB18, have been confirmed to be bound by AtAD1A (AtWRKY40) in vivo through direct interaction with the W-box sequence upstream of their promoters [60]. Both AtWRKY6 and AtWRKY42 could also inhibit AtPHO1 expression through directly binding to W-boxes of the AtPHO1 promoter [35]. In B. hygrometrica, chromatin immunoprecipitation showed that four W boxes upstream of the BhGols1 gene promoter was directly bound in vivo by the early dehydration and ABA-inducible BhWRKY1 [89]. Identification of important components that are directly regulated by WRKY transcription factors will add to our knowledge on the understanding of stress-induced signaling pathways. Considering on the large number of WRKY proteins and their distinct roles in specific stress responses, much work is still needed to find out their target genes in corresponding stress pathways.

Interestingly, consistent with the substantial enrichment of W boxes in promoters of numerous WRKY genes, the WRKY transcription factors can directly bind to both their own and other WRKY transcription factors’ promoters (autoregulation or crossregulation). Chromatin immunoprecipitation (ChIP) studies showed that PcWRKY1 protein binds to the W boxes of its native promoter as well as to that of PcWRKY3 [90]. Electrophoretic Mobility Shift Assays (EMSA) showed that AtWRKY18 and AtWRKY40 recognize the W-box sequences upstream of the AtWRKY60 gene promoter and both of them activate AtWRKY60 expression in protoplasts, indicating that AtWRKY60 might be a direct target gene of AtWRKY18 and AtWRKY40 in ABA signaling [61]. Recently, ChIP-qPCR assay demonstrated that AtWRKY33 could regulate its own expression through directly binding its own promoter [91]. These results imply that extensive auto-regulation and cross-regulation among WRKY genes facilitate transcriptional reprogramming during plant stress responses.

5. Identifying Interacting partners

To understand how the WRKY proteins participated in various plant stress responses, it is necessary and urgent to identify their interacting proteins using yeast two-hybrid screens or other technologies. Many WRKY proteins were shown to be important components of specific signal pathways, however their interacting partners remain to be identified. Up to now, several reports have demonstrated that WRKY transcription factors carry out their diverse functions in various stress signaling pathways through physical interaction with different proteins, such as MAP kinases, MAP kinase kinases, histone
deacetylases, calmodulin, etc. [22,92]. During stress response and signal transduction, WRKY proteins were phosphorylated by various MAPks [93], ultimately regulating plant stress response gene activation. AtWRKY38 and AtWRKY62 interact with Histone Deacetylase 19 (HDAC19) and finally fine-tune plant basal defense responses [94]. Thus, histone deacetylases may also play essential roles in plant stress responses through maintaining the appropriate acetylation state of histones. WRKY proteins can also form functional homo- or heterodimers among some WRKY proteins to perform their function. Interestingly, the heterodimer formation between different WRKY proteins may have positive or negative effects on their DNA binding activities [61,83]. In the abiotic area, some research also showed that WRKY proteins play their roles through protein–protein interaction. AtWRKY66 interacts with at least a dozen proteins including its closest homolog AtWRKY42 and co-overexpression of both resulted in stronger repression on ProPHO1:GUS expression. Furthermore, AtWRKY66 protein was degraded by the 26S proteasome through polyubiquitination by interaction with unknown proteins under Pi-deficient conditions [35]. Besides their interaction, AtWRKY18, AtWRKY 40 and AtWRKY 60 can also form complexes with the magnesium-protoporphyrin IX chelatase H subunit (CHLH/ABAR), a receptor for ABA in A. thaliana [60,83]. The identification of WRKY proteins’ interacting partners contribute to the reconstrucion of signaling webs that involve WRKY proteins.

6. Conclusions and future prospects

Thanks largely to the use of diverse technologies and approaches, including physiology, chemical genetics, molecular computational and informational biology, the field of plant signal transduction and gene regulation has shown rapid progress, which helps us to understand the complex mechanisms underlying various aspects of plant responses to abiotic stresses. Much progress in WRKY transcription factors’ functional research has been obtained over the past 15 years. However, most of advances are related with the involve ment in biotic stresses, and there are few examples of functional research into abiotic stresses. Furthermore, considering the size of this gene family, the identification of WRKY function in abiotic stresses will remain a big challenge in the coming years. In order to achieve a better understanding of their role during abiotic stresses, it is of vital importance to identify the interacting partner of WRKY proteins under a certain condition which cooperate in regulating the trans cription of downstream target genes. This is also important to determine the key components of signal transduction pathways with which they physically cooperate. Second, combined with microarrays and chromatin immuno precipitation assays or even massive parallel sequencing, we could directly determine the specific WRKY DNA-binding sites on a global scale under certain abiotic stresses. In this way, we then may gradually come to understand the complex mechanisms of signaling and transcriptional reprogramming controlled by WRKY proteins and the plant processes in which they participate. One can expect that further molecular studies of WRKY transcription factors under abiotic stress will clarify the fine-tuning mechanisms that are controlled by WRKY proteins in plants, with significant benefits to agricultural production.

Acknowledgments

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References

[2] A. Matsui, J. Ishida, T. Morosawa, Y. Mochizuki, E. Kaminuma, T.A. Endo, M. Okamoto, E. Nambara, M. Nakajima, M. Kawashima, M. Satou, J.M. Kim, N. Kobayashi, T. Toyoda, K. Shirozaki, M. Seki, Arabidopsis DNA binding activities [61,83]. In the abiotic area, some research also showed that WRKY proteins play their roles through protein–protein interaction. AtWRKY66 interacts with at least a dozen proteins including its closest homolog AtWRKY42 and co-overexpression of both resulted in stronger repression on ProPHO1:GUS expression. Furthermore, AtWRKY66 protein was degraded by the 26S proteasome through polyubiquitination by interaction with unknown proteins under Pi-deficient conditions [35]. Besides their interaction, AtWRKY18, AtWRKY 40 and AtWRKY 60 can also form complexes with the magnesium-protoporphyrin IX chelatase H subunit (CHLH/ABAR), a receptor for ABA in A. thaliana [60,83]. The identification of WRKY proteins’ interacting partners contribute to the reconstrucion of signaling webs that involve WRKY proteins.

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