Critical Reviews in Plant Sciences

ISSN: 0735-2689 (Print) 1549-7836 (Online) Journal homepage: http://www.tandfonline.com/loi/bpts20

The WRKY Transcription Factor Family in Model Plants and Crops

Fei Chen, Yue Hu, Alessandro Vannozzi, Kangcheng Wu, Hanyang Cai, Yuan Qin, Alison Mullis, Zhenguo Lin & Liangsheng Zhang

To cite this article: Fei Chen, Yue Hu, Alessandro Vannozzi, Kangcheng Wu, Hanyang Cai, Yuan Qin, Alison Mullis, Zhenguo Lin & Liangsheng Zhang (2018): The WRKY Transcription Factor Family in Model Plants and Crops, Critical Reviews in Plant Sciences, DOI: 10.1080/07352689.2018.1441103

To link to this article: https://doi.org/10.1080/07352689.2018.1441103

Published online: 05 Mar 2018.

CRITICAL REVIEWS IN PLANT SCIENCES
https://doi.org/10.1080/07352689.2018.1441103

Taylor & Francis
Taylor & Francis Group

The WRKY Transcription Factor Family in Model Plants and Crops

Fei Chen[a], Yue Hu[a], Alessandro Vannozzi[b], Kangcheng Wu[a], Hanyang Cai[a], Yuan Qin[a], Alison Mullis[c], Zhenguo Lin[c], and Liangsheng Zhang[a]

[a]State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops; Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops; Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology; Fujian Agriculture and Forestry University, Fuzhou, China; [b]Department of Agronomy, Food, Natural Resources, Animals, and Environment (DAFNAE), University of Padova, Legnaro, Italy; [c]Department of Biology, Saint Louis University, St Louis, Missouri, USA

ABSTRACT

The WRKY gene family in flowering plants encodes a large group of transcription factors (TFs) that play essential roles in diverse stress responses, developmental, and physiological processes. In this review, we provided a comprehensive screenshot about the studies on WRKY TFs in model plants and in crops of economical relevance. Specifically, we discussed the history of discovery and functional characterization, classification, and evolutionary history, 3D structure and physiological functions of WRKY transcription factors. Based on the previous functional studies of WRKY genes in model plants such as *Arabidopsis* and rice, we summarized various roles of WRKY TFs in a broad range of biological processes as well as their degradation process. We also discussed the characterization and functional studies of WRKY TFs in important crops. Considering the rapid progress of high-throughput techniques, especially genomics and transcriptomics, which have been instrumental in advancing our understanding of the crop genomes, we comment one-by-one on the applications of a suite of new and high-throughput techniques to accelerate the studies of WRKY genes in crops.

KEYWORDS

environmental stress; gene family; growth and development; transcription factor; WRKY

I. Studies on WRKY transcription factor family in model plants

A. A brief discovery history of the wrky genes

Transcription factors (TFs) play essential roles in plants, as well as in all other living organisms, by controlling the expression of genes involved in various cellular processes (Riechmann and Ratcliffe, 2000; Amor *et al.*, 2004; Han *et al.*, 2014). TFs also play a central role in the process of crop domestication and are targets of molecular breeding of crops (Doebley *et al.*, 2006; Century *et al.*, 2008). For example, five of the six major genes controlling morphological and structural changes during crop domestication are TFs (Doebley *et al.*, 2006). The accumulation of completely sequenced plant genomes and the development of bioinformatics tools have largely facilitated the identification, functional characterization, and evolutionary studies of TF families in plants.

Angiosperm genomes are predicted to contain more than 1,000 TF genes, which were classified into 58 families based on their DNA binding domains (Zhang *et al.*, 2011). The WRKY gene family is the 7th largest TF family in flowering plants following basic helix-loop-helix (bHLH), myeloblastosis (MYB), Ethylene responsive factor (ERF), NAM (no apical meristem), ATAF1/2 and CUC2 (cup-shaped cotyledon) (NAC), basic leucine Zipper (bZIP), and C2H2 families (Jin *et al.*, 2014). WRKYs have attracted a lot of attention because they are involved in a broad range of biological processes, including diverse biotic/abiotic stress responses, developmental, and physiological processes (Birkenbihl *et al.*, 2017b; Jiang *et al.*, 2017). The WRKY TFs are defined by the presence of a WRKY domain, a ~60-residue DNA-binding domain containing a highly conserved heptapeptide motif WRKYGQK. The first WRKY gene was identified in 1994 from eudicot crop sweet potato (*Ipomoea batatas*), encoding a 549 amino acid protein called *SPF1* (SWEET POTATO FACTOR1) (Ishiguro and Nakamura, 1994). The SPF1 protein binds to the promoter of two genes coding for sporamin (protease inhibitor) and

CONTACT Liangsheng Zhang fafuzhang@163.com State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops; Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops; Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology; Fujian Agriculture and Forestry University, Fuzhou, China 350002; Zhenguo Lin zhenguo.lin@slu.edu Department of Biology, Saint Louis University, St Louis, MO 63103, USA.

Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/bpts.

one beta-amylase gene in tuberous roots (Ishiguro and Nakamura, 1994). In 1995, two WRKY proteins, ABF1 and ABF2, were isolated from a monocot plant wild oat, *Avena fatua*. Both proteins have a zinc finger structure (C-X_{4-5}-C-X_{22-23}-HXH) within the DNA binding domain following the WRKYGQK sequence, and are involved in the regulation of seed germination (Rushton *et al.*, 1995). In 1996, three WRKY members, WRKY1, WRKY2, and WRKY3 were identified in parsley (*Petroselinum crispum*). The three WRKY genes can be induced by elicitors, and all the three WRKYs regulate ribosomal protein gene expression (Rushton *et al.*, 1996).

B. Updates of research tools

The first WRKY gene was cloned by means of southwestern screening of the cDNA library (Ishiguro and Nakamura, 1994). The same method was used to identify and clone WRKY genes in the 1990s (Rushton *et al.*, 1995; Pater *et al.*, 1996; Eulgem *et al.*, 1999; Yang *et al.*, 1999). After completion of the first sequenced plant genome *Arabidopsis thaliana*, 68 WRKY genes were identified based on homology search using BLAST (Altschul *et al.*, 1990; The Arabidopsis Genome Initiative, 2000). In another study, 75 WRKY genes were identified from the *Arabidopsis* genome (Riechmann and Ratcliffe, 2000). In the rice genome, 83 WRKYs were identified using BLAST-based search (Goff *et al.*, 2002). Protein homolog searches have been greatly facilitated by (i) the development of the hidden Markov model (HMM), (ii) the implementation of HMMER software (Eddy, 2009), and (iii) the availability of HMM seeds for various gene families (Eddy, 1996), which were designed to increase the sensitivity of homology searches. HMM method was first employed to identify the WRKY genes in the rice genome (Xie, 2005). The rapid accumulation of WRKY gene sequences (6,320 sequences from 89 species) in the Pfam database (Finn *et al.*, 2014) makes it possible to screen genomes for WRKY sequences by means of HMM-based searches without using BLAST or other computationally expensive bioinformatics tools.

The implementation of other bioinformatics tools has strongly accelerated researches on WRKYs. A plant transcription factor database, PlantTFDB, recorded 58 TF gene families from 165 plant species, including 14,549 WRKY genes (Jin *et al.*, 2017). An online database (www.mpipz.mpg.de/20985/WRKY_References) has been developed reporting all the WRKY-related publications. Phylogenetic tree reconstruction tool MEGA (Tamura *et al.*, 2013; Kumar *et al.*, 2018) and multiple sequence alignment tools have been extensively used for phylogenetic analyses. The Gene Structure Displayer Server (http://gsds.cbi.pku.edu.cn/) is useful for WRKY structure display (Li *et al.*, 2017a). Various tools, such as Trinity (github.com/trinityrnaseq) and SOAPdenovo (Li *et al.*, 2010), are very popular in expressional quantification of WRKY genes. BLAST, Jbrowse, and complicated search systems (such as Phytomine and Biomart) have been developed and integrated into comprehensive databases such as Phytozome (phytozome.jgi.doe.gov) and EsemblPlants (plants.ensembl.org). Many gene family-specific databases have also been constructed, including those for rice kinase genes (http://kinase.com/web/current/#) (Dardick *et al.*, 2006) and the homeobox gene family (Zhong *et al.*, 2008). However, a WKRY-specific database is still not yet available. We believe that a WKRY-specific database would facilitate the studies of WKRY genes through providing access to the sequence, structure, expression patterns of WRKY genes, and related publications for WRKY genes.

C. Classification of the wrky gene family

The current and widely accepted system of WRKY classification was established in 2000 based on the genomic characterization of this gene family in *Arabidopsis* (Eulgem *et al.*, 2000). According to this classification, WRKY genes in plants were hitherto classified into the following three groups: I, II, and III based on the number of WRKY domains and the features of their zinc-finger-like motif (Eulgem *et al.*, 2000), with group II WRKYs further divided into the following five subgroups: IIa, IIb, IIc, IId, and IIe based on their phylogenetic relationships (Eulgem *et al.*, 2000). Group I WRKY proteins harbor two WRKY domains, whereas groups II and III WRKY proteins contain only one WRKY domain. Group II and III WRKY proteins differed by the type of zinc finger motif. The zinc finger motif in group II is same as group I, which is C-X_{4-5}-C-X_{22-23}-H-X_1-H, whereas the zinc finger in group III is C-X_7-C-X_{23}-H-X_1-C (Bakshi and Oelmüller, 2014). To illustrate evolutionary relationships of WRKY proteins, we built a phylogenetic tree based on members from four representative land plants, including *Arabidopsis thaliana, Vitis vinifera, Oryza sativa*, and *Selaginella moellendorffii* (Figure 1). The phylogenetic tree supports the previous classifications. The groupings of WRKY genes are further supported by their conserved intron–exon structures (Figure 1). Each of the subgroup or subfamilies contains a characteristic intron insertion in the coding region. A highly conserved phase-2 intron is present between the WRKYGQK motif and the zinc finger motif in the subfamily I's C-terminal WRKY domain, and subfamily IIc, IId+IIe, and III (Figure 1). In contrast, both subfamilies IIa and IIb contain the same conserved intron within the zinc finger motif (Zhang and Wang,

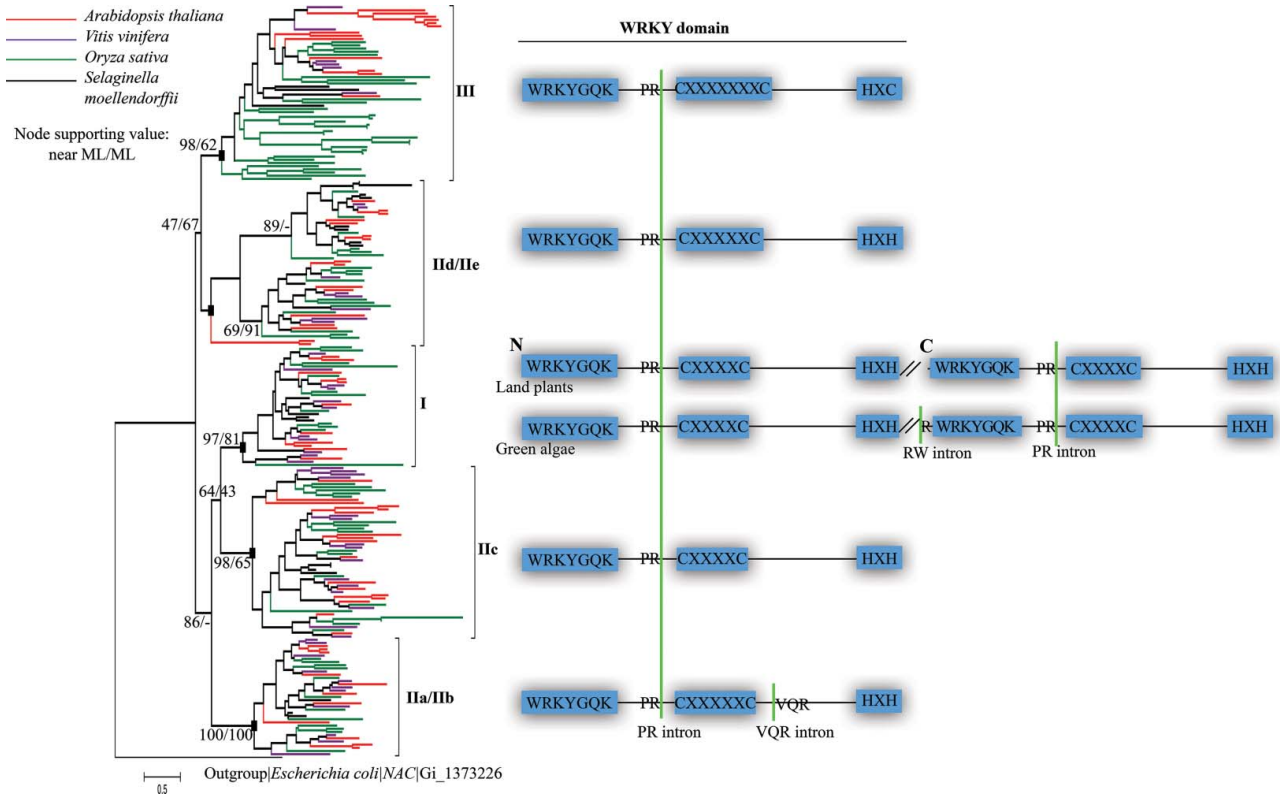

Figure 1. Classification of land plant WRKY gene family. The phylogenetic tree was reconstructed using both near maximum-likelihood (ML) and ML method based on the WRKY amino acid sequences from four representative land plants: *A. thaliana, V. vinifera, Oryza sativa*, and *S. moellendorffii*. The WRKY gene family is classified into I, IIa, IIb, IIc, IId, IIe, and III subfamilies with evidence of tree topology and conserved intron insertion patterns.

2005). This pattern suggests that the phase-2 intron between WRKYGQK and the zinc finger motifs was probably present in the common ancestor of WRKY family, but was subsequently lost in subfamilies IIa and IIb. Because both the split of IIa and IIb and split of IId and IIe have occurred in the ancestor of land plants (Zhang and Wang, 2005), much later than other groups, we thereby propose that IIa and IIb should be merged as a single subfamily, and merge the IId and IIe subfamilies (Figure 1).

Challenges are arising since rapid accumulation of genome sequencing data will lead to discovery of many new members and even novel WRKY subfamily. For example, four *Arabidopsis* WRKYs have been found fused with additional domains, (i) Leucine-rich repeat (LRR) domains at the N-terminal (At4G12020, At5g45050, At5G45260), and (ii) BDP1 domain at the N-terminal (At1G55600). Although the tandem duplicated gene pair *At5g45050* and *At5G45260* were grouped into the IIe subfamily, phylogenetic places of At4G12020 and At1G55600 were uncertain (Figure 1). Recruitment of novel domains/genes will lead to the birth of novel subfamilies, such as the subfamily I with two WRKY domains. The future of WRKY classification will obviously be challenged in facing the tremendous WRKYs from various plants.

D. Origin and diversification of WRKY genes

WRKY genes were initially believed to be plant-specific (Eulgem *et al.*, 2000). Sampling from broader taxonomic groups revealed that WRKY genes are also present in other eukaryotic lineages, such as fungi, Amoebozoa, diplomonads, and slime molds (Zhang and Wang, 2005; Rinerson *et al.*, 2015). Based on the distribution patterns of WRKYs, it was speculated that nonplant WRKYs originated from multiple ancient gene transfer events (Rinerson *et al.*, 2015). However, it seems large-scale sampling will be required to elucidate the gene transfer details.

Zhang and Wang (2005) showed that only subfamily I WRKYs are present in green algae, whereas subfamilies IIa+IIb, IIc, and IId+IIe evolved in the common ancestor of land plants, and subfamily III emerged in the common ancestor of seed plants. Based on a larger number of sequences from more algal species and more detailed analyses, it was found that subfamily IId could be traced back to an ancestral sequence in Charophyte alga *Klebsormidium flaccidum* (aka *K. nitens*) (Rinerson *et al.*, 2015). Also, subfamily III members were found in moss, but IIa were originated in seed plants. In the latter study, two alternative hypotheses regarding the evolution of the WRKY gene family were proposed (Rinerson *et al.*,

2015): (i) all WRKY genes were originated from Group I C-terminal WRKY domains; (ii) subfamilies IIa and IIb evolved directly from an ancestral algal WRKY gene with a single domain that was separated from the subfamily I-derived lineage.

The classification of the WRKY gene family is supposed to change as far as a higher number of sequences from different organisms are considered. Currently, the most comprehensive study of the WRKY gene family only includes 43 plant species and other eukaryotes (Mohanta *et al.*, 2016), without considering any species from glaucophyta, rhodophyta, pteridophyta, chlorophyta, and charophyta, which are important for evolutionary inferences. This study (Mohanta *et al.*, 2016) refreshed and challenged the current classification, introducing a differential classification of WRKYs in dicots and monocots. However, such classification will probably change again as far as more genomes are be available.

E. Structure of WRKY genes and proteins

Although WRKY proteins can greatly vary in size, all of them harbor a conserved WRKY domain consisting of two parts, the DNA-binding heptapeptide WRKYGQK, and the zinc-finger binding motif. The two motifs together span approximately 60 amino acids in length at the N-terminus and at the C-terminus in the WRKY domains (Figure 2A) (Eulgem *et al.*, 2000). Some WRKY genes encode triple or tetrad WRKY domains in which some are fused genes encoding novel domains such as ZF-SBP, CBS, kinase, PAH, ULP_PROTEASE, TIR, NAC, LRR, ATP_GRASP, B3 (Figure 2A) (Mohanta *et al.*, 2016). According to the current version of Pfam database (Version 31), 69 domain architectures have been identified based on 6320 WRKY proteins from 89 species (http://pfam.xfam.org/family/PF03106#tabview = tab1). Subfamily I of WRKY proteins contain two WRKY domains. The C-terminal WRKY domain functions in DNA binding, but the function of the N-terminal WRKY domain remains unclear (Duan *et al.*, 2007). Only the C-terminal WRKY domain is present in the members of subfamily II and III.

The WRKY domain includes a positively charged β strand that binds to the *cis*-acting element designed as W-box (C/T)TGAC(C/T). In a genome-wide investigation, W-box is the predominant binding motif for three *Arabidopsis* WRKYs, namely WRKY18, WRKY33, and WRKY40 (Birkenbihl *et al.*, 2017a). W-box elements are prevalent in plant genomes. For example, 32,162 TTGACY, 60,612 TTGAC, and 14,857 TTTGACY were identified in *Arabidopsis*. Recent work (Brand *et al.*, 2013) suggests that the W-box has a degenerated/core TGAC

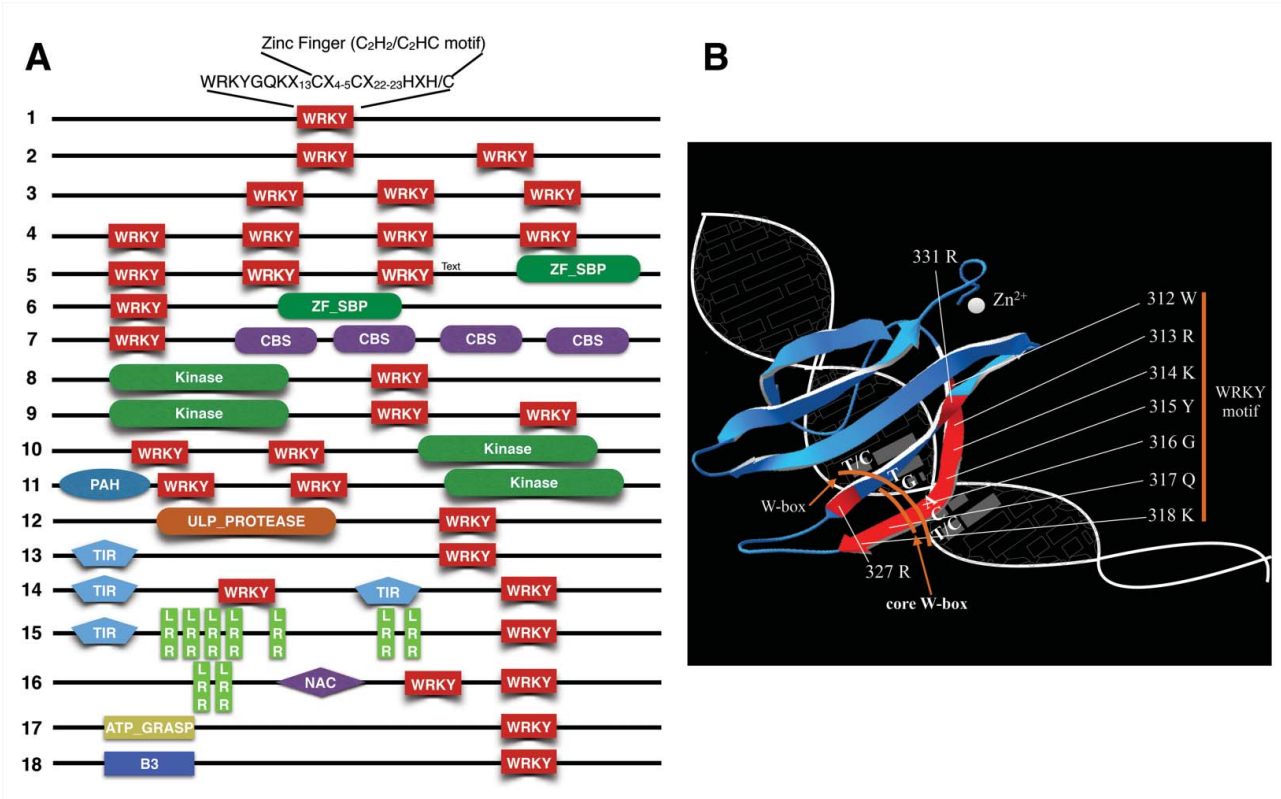

Figure 2. Domain structure of WRKY family and the working mechanism of a typical WRKY protein. (A) Representative domain organizations of WRKY proteins. (B) 3D structure of a WRKY protein and its binding to a W-box on the gene promoter.

motif, composed of an ultra-conserved GAC core and the upstream thymine and downstream pyrimidine (C/T). The GAC core interacts with the WRKY proteins whereas the flanking residues help dictate recognition by specific WRKY factors. These motifs are widely found in genes with various functions (Jiang *et al.*, 2017). Multiple W-box elements could form a cluster in promoter regions, for example, the barley WRKY38 requires two neighboring W-boxes for efficient binding (Marè *et al.*, 2004).

WRKY proteins also bind to non-W-box DNA *cis* elements. For example, the WT-box (GGACTTTC) is required for binding of *Arabidopsis* WRKY26 and WRKY41 (Kanofsky *et al.*, 2017) and WRKY70 (Machens *et al.*, 2014). However, not all WT-box can be bound by WRKYs based on yeast one-hybrid screens (Kanofsky *et al.*, 2017). In rice, the OsWRKY13 protein binds to both W-box and PRE4 element (Cai *et al.*, 2008). NtWRKY12 from tobacco binds specifically to the WK box (TTTTCCAC) (van Verk *et al.*, 2008). Future chromatin immunoprecipitation (ChIP)-seq studies are needed to unravel the diversity of sequences that are recognized by WRKYs.

The three-dimensional (3D) structure of a TF is valuable for studying its binding and activation mechanism. Currently, three 3D structure models related to *Arabidopsis* WRKY proteins are available in the PDB database (www.rcsb.org), namely 1WJ2 and 2LEX for the C-terminal WRKY domain of AtWRKY1, 2AYD for the C-terminal WRKY domain of AtWRKY1. 2AYD has the best resolution at 1.6 Å. A typical WRKY domain comprises five parallel β-strands (Figure 2B). The core WRKYGQK motif locates on the second, and the outermost β-strand, enabling its interaction with the major DNA groove. The WRKY domain is similar to the glial cell missing (GCM) and the NAC domains in terms of sequence and structure (Babu *et al.*, 2006). The WRKY and GCM domains share the zinc finger domain, but the WRKY domain contains a conserved DNA-binding motif WRKYGQK.

Variants of the WRKYGQK motif have been found in various plant lineages (Mohanta *et al.*, 2016), including WRKYGEK, WRKYGKK, WSKYEQK, WRKYSEK. Some variants have differences only in the WRKY pattern, such as WRRY, WSKY, WKRY, WVKY, WKKY, WRIC, WRMC, WIKY, and WKRY (Jiang *et al.*, 2017). Because changes in WRKYGQK pattern could alter their DNA binding affinity, some of these variants might lack DNA-binding affinity and even ability.

F. Functions and regulatory network of WRKYs

WRKY genes have been extensively studied in the eudicot model plant *A. thaliana* and monocot model plant *O. sativa*. Therefore, the roles of WRKYs in diverse cell signaling and physiological processes discussed here are based mainly on studies from the two model organisms.

1. Abiotic stress

Harsh environmental factors such as drought, flooding, salinity, heat, low temperature, and strong ultraviolet (UV) radiation, adversely affect the growth and development of plants. It was projected that elevated global CO_2 would also bring more unexpected abiotic stresses for plant growth (Feng *et al.*, 2014). Therefore, it is important to study the molecular mechanism of abiotic stresses and to identify important genes responsible for stress-tolerance (Chen et al., 2013a). In plant cell signaling, WRKY TFs have been regarded as a jack of many trades (Bakshi and Oelmüller, 2014) from plant growth and various stress responses, providing an important basis for genetic improvement of crops.

Drought and salt stresses. Drought stress and salt stress both cause cellular dehydration and are key environmental factors influencing plant yield and spatial distribution (Bartels and Sunkar, 2005). At the molecular level, responses to drought and salt stresses usually share the same signal transduction pathways, causing reactive oxygen species (ROS) and abscisic acid (ABA) accumulation (Bartels and Sunkar, 2005; Miller *et al.*, 2010). All WRKY subfamilies have members involved in response to drought and salt stresses (Figure 3). AtWRKY18, AtWRKY40, and AtWRKY60, which are subfamily IIa/IIb members, negatively regulate the transcription of receptor-like kinase CRK5. AtWRKY18 and AtWRKY40, but not AtWRKY60, directly bind to the promoter of *CRK5*. Knock-out of all the three genes led to the significantly increased expression of *CRK5*, but no change of *CRK5* expression was observed if only one or two of them were knocked out, suggesting a close interaction between the three WRKY genes (Lu *et al.*, 2016).

Several WRKY genes involved in the drought stress response are ABA-dependent. In *Arabidopsis, AtWRKY1* is involved in the stomatal closure via the regulation of membrane transporters to maintain moisture (Qiao *et al.*, 2016). Also, AtWRKY1 TF binds to the promoter of *MYB2, ABCG40, DREB1A*, and *ABI5*, thus regulating the drought response. *AtWRKY1* knockout mutant showed a higher sensitivity to ABA and lower drought resistance than wild type, suggesting a negative regulatory role of AtWRKY1 in ABA signaling pathway of guard cells (Qiao *et al.*, 2016). Compared with wild-type, the *AtWRKY63* knockout mutant had decreased tolerance to drought stress (Ren *et al.*, 2010). AtWRKY46, AtWRKY54, and AtWRKY70 all belong to the group III and are engaged in BR signaling to regulate both growth

and osmotic stress (Chen *et al.*, 2017). Drought leads to high expression of ABA, which induces high expression of *AtWRKY57* that binds to W-box in the promoter region of the downstream response genes (*RD29A, NCED3* are both VQ motif-containing genes). This activates gene expression, resulting in a high seed germination rate in drought environment (Jiang *et al.*, 2012). In addition, AtWRKY63 binds to the *ABF2* promoter and activates expression of *RD29A* and *COR47* (Ren *et al.*, 2010).

In rice, overexpression of *OsWRKY30* dramatically enhances drought resistance, which is a signal hub to downstream of proteins OsMAK3, OsMPK4, OsMPK7, OsMPK14, OsMPK20-4, and OsMPK20-5 (Shen *et al.*, 2012). Drought-induced senescence could increase the expression level of *OsWRKY80*, which is putatively regulated by ABA (Ricachenevsky *et al.*, 2010). OsWRKY47 binds to the W-box in the promoters of *Cys Rich Repeat Secretory Protein 55 Precursor* and *Calmodulin-Binding Protein*. Knockout mutants of *OsWRKY47* are highly susceptible to drought and have reduced yield, whereas mutants with overexpression of *OsWRKY47* are more resistant to drought (Raineri *et al.*, 2015). Furthermore, OsWRKY45 was also found involved in ABA signaling and salt stress in rice (Tao *et al.*, 2011).

Salt stress is another important negative factor of plant growth and development. Salt stress usually produces ROS, which are one of the primary signal transduction signals. At*WRKY8* is highly expressed in plant roots and is significantly upregulated under salt stress. The knockout mutant of *AtWRKY8* is more sensitive to salt with seed germination and subsequent inhibited growth. AtWRKY8 regulates Na^+ / K^+ balance by binding to a VQ motif in the promoter of *RD29A* (Chen *et al.*, 2010; Hu *et al.*, 2013). AtWRKY28 acts synergistically with AtHBH17 (AtAIB), a member of the bHLH family, to increase tolerance to salt stress and oxidative stress in the presence of high salt concentrations. Under the high mannitol concentration, AtWRKY28 causes the plant roots to elongate, and effectively enhances plant tolerance to drought stress (Babitha *et al.*, 2013; Chen *et al.*, 2013b). Another WRKY family member, *AtWRKY75*, is also found involved in the response of plants to salt stress (Chen et al., 2013b; Hossain et al., 2016). *AtWRKY15* is induced by ROS. The increased expression of *AtWRKY15* makes *Arabidopsis* more susceptible to osmotic stress and oxidative stress (Vanderauwera *et al.*, 2012). *OsWRKY30* and *OsWRKY72* from subfamily III are activated by ROS, and overexpression of them makes plants more susceptible to salt stress (Yu *et al.*, 2010; Scarpeci *et al.*, 2013). In general, as shown in Figure 3, five members of the IIc population (AtWRKY57, AtWRKY28, AtWRKY8, AtWRKY75, and OsWRKY72) are involved in osmotic stress. Thus, the IIc members may be the key TFs involved in response to drought and salt stress.

Temperature-induced stresses. Temperature changes have broad effects on plant physiology. WRKY transcription factors play an important role in the response to temperature stress. *AtWRKY25, AtWRKY26*, and *AtWRKY33* participated in heat-induced signal transduction. The heat shock transcription factors, *HsfA2, HsfB1, heat shock protein 101 (Hsp101)*, and *zinc finger protein 10 (Zat10)*, are the master regulators in the activation of transcriptional networks (Ohama *et al.*, 2016). These proteins contained W-box sequences that were recognized by the three WRKY proteins (Li *et al.*, 2011). *AtWRKY39* is induced by heat stress, positively regulating the crosstalk of jasmonate (JA) and salicylic acid (SA) pathways that mediate the heat response (Jqw *et al.*, 2010). Under the control of the *HSP101* promoter, overexpressing *OsWRKY11* enhances heat and drought tolerance in transgenic rice seedlings (Wu *et al.*, 2009).

AtWRKY34, a pollen-specific gene, receives cold signals and transmits to the *C-repeat/DRE-Binding Factor* , effectively alleviating the pollen's cold stress (Zou *et al.*, 2010). *OsWRKY71* is controlled by hypothermia, which activates the expression of downstream genes *OsTGFR, OsDREB1A, TPP1*, and *WSI76*, thereby enhancing plant cold tolerance (Kim *et al.*, 2016). In addition, *OsWRKY76* leads to the increased expression of abiotic stress-associated genes such as peroxidase and lipid metabolism genes to alleviate cold stress (Yokotani *et al.*, 2013).

Waterlogging stress. WRKY transcription factors were involved in the response of waterlogging stress. The expression of *AtWRKY22* was rapidly and strongly induced upon submergence (Hsu *et al.*, 2013). *AtWRKY22* protein binds to *TRE1's* promoter and represses its expression, affecting the plant's resistance to flooding by influencing plant stomatal activity. In addition, *AtWRKY22* also regulates the expression of *MYB15, PUB24*, and *ACS7*, which are related to the plant immune response (Cai *et al.*, 2013). Thus, waterlogging induced the expression of *AtWRKY22* that triggered the immune response in *Arabidopsis*, and contributed to plant resistance to pathogen infection during waterlogging (Hsu *et al.*, 2013).

Ultraviolet stress. Visible light is an essential factor for plant growth, whereas the relationship between UV and plant growth and development is still insufficient. UV-B is a wavelength of 280–315 nm moderate wavelength UV light that may damage DNA bases. Overexpression of

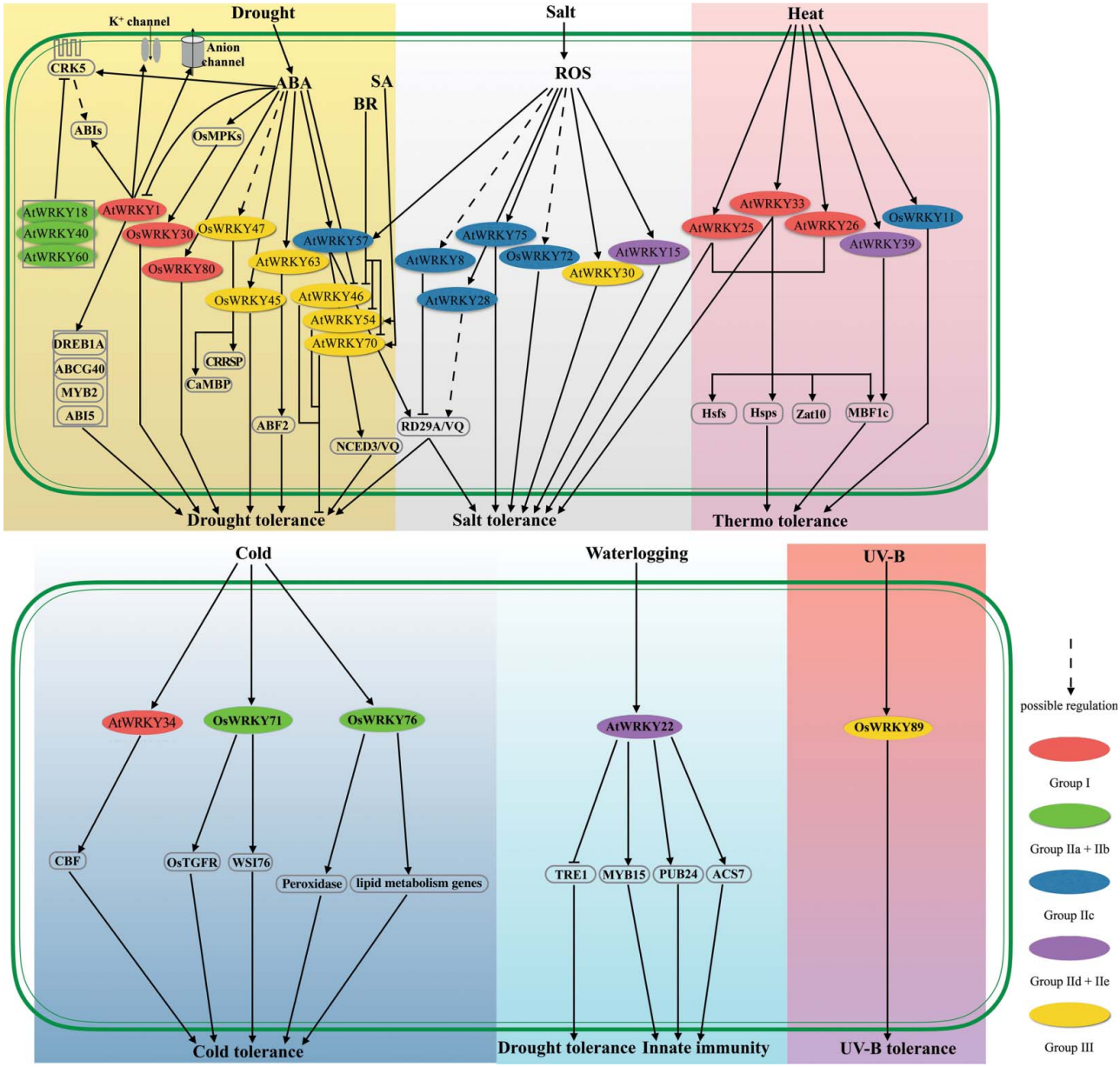

Figure 3. WRKYs in plant abiotic stress signaling network. References were mentioned in the main text.

OsWRKY89 significantly increased the resistance of plants to UV-B (Wang *et al.*, 2007). However, whether or not other WRKYs are involved in response to UV-induced stress or how WRKYs regulate UV-induced signal transduction remains to be elucidated.

In summary, molecular studies have identified many WRKY genes that are involved in various abiotic stress responses. However, there is a need in the future for more extensive field studies of WRKY genes to test the applications of these genes in agriculture.

2. Biotic stress

WRKYs are known to play important roles in plant immune responses to various biotic stresses. Summarized from various publications, members from all subfamilies of WRKYs have been found to be involved in the microbe-associated molecular pattern-triggered immunity, PAMP-triggered immunity, effector-triggered immunity, or system acquired resistance (SAR).

AtWRKY33 and AtWRKY25 proteins (members of subfamily I) bind to the activated MKS1p. This pathway is required for both repression of SA-dependent resistance as well as activation of JA-dependent defense (Andreasson *et al.*, 2005). AtWRKY3 enhances the resistance to the necrotrophic pathogen, whereas AtWRKY4 enhances the resistance to both necrotrophic pathogen and biotrophic pathogen. Overexpression of *AtWRKY3* and *AtWRKY4* inhibit pathogen-induced PR1 (Lai *et al.*, 2008). OsWRKY3 is light-dependent and binds to the upstream sequence of *OsNPR1* and is involved in

immune regulation through SA or JA-induced immune signaling cascade systems. Overexpression of *OsWRKY3* upregulated *OsPR1b, phenylalanine ammonialyase ZB8* and peroxidase *POX22.3* (Liu *et al.*, 2005). *OsWRKY71* is upregulated by SA, methyl jasmonate (MeJA), and pathogen infection. *OsWRKY71* overexpression mutant showed enhanced resistance to *Xanthomonas oryzae* (Xoo), and *OsPR1b* and *OsNPR1*were also upregulated in the mutant, indicating that they may be regulated by OsWRKY71 (Liu *et al.*, 2007).

Ten WRKY genes of subfamily IIc are involved in plant immunity (Figure 4). *AtWRKY28* and *AtWRKY75* are detected to be upregulated by oxalic acid and *Sclerotinia sclerotiorum* infection using microarray screening. These genes are TFs involved in SA and JA/ET-dependent defense signaling pathways, suggesting that *AtWRKY28* and *AtWRKY75* may enhance plant resistance to oxalic acid and fungal infection through the JA/ET pathway (Chen et al., 2013b). In the *AtWRKY48* overexpression mutant, the expression of *PR1* is downregulated, indicating that *AtWRKY48* could regulate plant immunity by negative regulation of Pathogenesis-Related (PR) genes (Xing *et al.*, 2008). In the *AtWRKY50* and *AtWRKY51* double knockout mutant, both the SA content and the JA pathway-related *PDF1.2* gene expression levels are reduced, suggesting that AtWRKY50 and AtWRKY51 mediate SA- and low-oleic acid-dependent repression of JA signaling (Gao *et al.*, 2011). *AtWRKY57* also plays a regulatory role in the process of plant immune response by increasing the susceptibility of plants to *Botrytis cinerea*. AtWRKY57 competes with AtWRKY33 for binding to the promoters of *SIGMA FACTOR BINDING PROTEIN1 (SIB1)*, *SIB2, JASMONATE ZIM-DOMAIN 1 (JAZ1)*, and *JAZ5*, thus affecting the JA-mediated defense signal pathway (Jiang and Yu, 2016). Overexpression of *OsWRKY13* enhances the resistance of rice to bacterial blight and rice blast. *OsWRKY13* plays a negative regulatory role in the JA-induced defense signaling pathway, and plays a positive role in the SA-induced defense signaling pathway (Qiu *et al.*, 2007). *OsWRKY89* could be induced by MeJA to enhance the resistance to *Magnaporthe grisea* and *Sogatella furcifera* (Wang *et al.*, 2007).

In *Arabidopsis* and rice, ten members from the IIa+IIb group are involved in the plant immune response (Figure 4). *AtWRKY18, AtWRKY40*, and *AtWRKY60* are partially functionally redundant. In the *AtWRKY18* and *AtWRKY40* double knockout mutant, a series of immune-related genes such as *camalexin* are detected and showed higher tolerance to the powdery mildew organism, *Golovinomyces orontii* (Schön *et al.*, 2013). *In vitro* experiments, AtWRKY40 regulates immune responses through binding to the promoter of *ENHANCED DISEASE SUSCEPTIBILITY1*, AP2-type TF *redox-responsive transcription factor 1*, and a JA-signaling repressor gene *JAZ8* (Pandey *et al.*, 2010). Overexpression of *AtWRKY18-AtWRKY40* and *AtWRKY18-AtWRKY60* leads to a higher susceptibility to

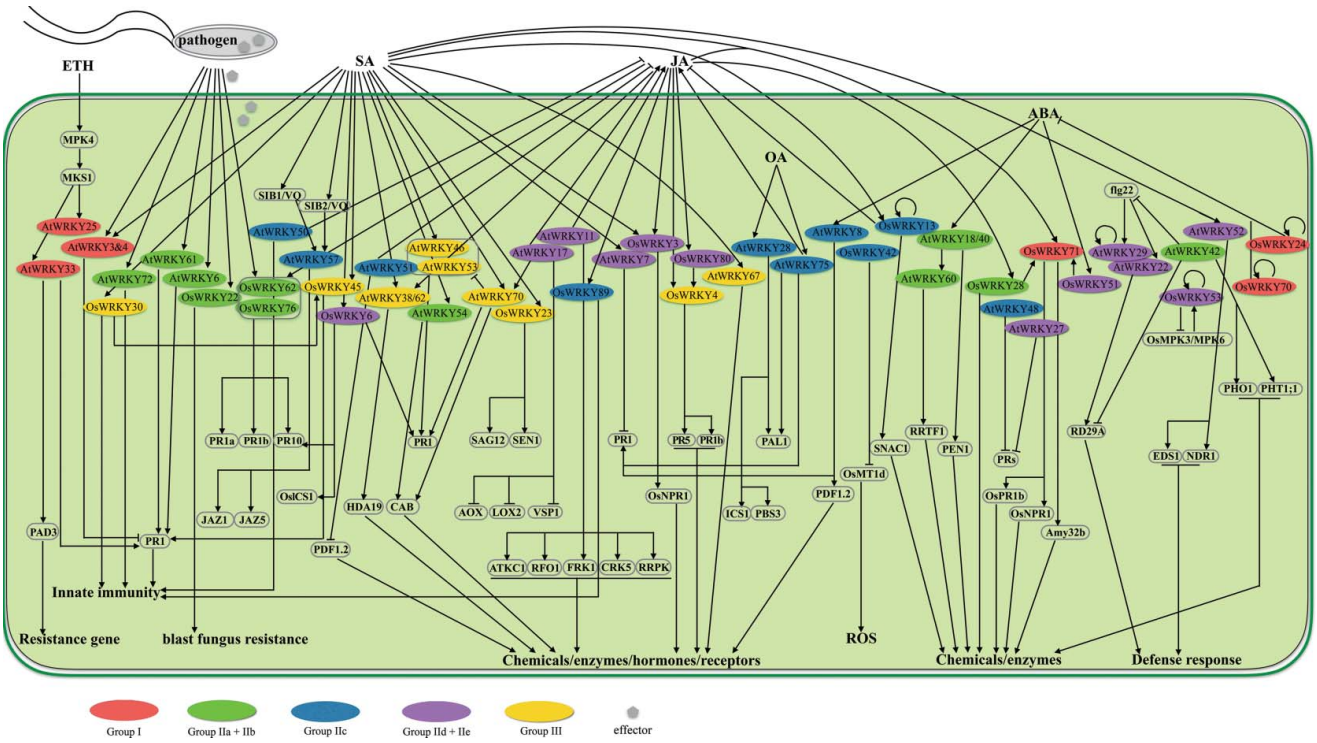

Figure 4. WRKYs in plant abiotic stress signaling network.

Pseudomonas syringae and *Botrytis cinerea*, suggesting that they have functional or physical interactions in the regulation of plant immunity, and this effect is mediated by regulation of the JA and SA pathways (Xu *et al.*, 2006). The *AtWRKY6* knockdown mutant has a greater leaf infection area than the wild type, suggesting that AtWRKY6 might be involved in the regulation in a specific cell layer in the region surrounding the infected area (Robatzek and Somssich, 2002). *AtWRKY61* enhances plant resistance to Turnip Crimp Virus, and *AtWRKY61* may have similar regulatory effects on SAR and PR gene regulation (Gao *et al.*, 2016). AtWRKY72 has a positive regulatory effect on the induction of root-knot nematode, Meloidogyne species and the downy mildew, *Hyaloperonospora arabidopsidis*. This process is not related to the SA signaling pathway, but may be related to the expression of R gene *Mi-1* (Bhattarai *et al.*, 2010). OsWRKY62 and OsWRKY76 proteins form homodimers and heterodimers, and overexpression of the two genes increases susceptibility to *Magnaporthe oryzae* and *Xoo*. In the double-knockout mutant of *OsWRKY62* and *OsWRKY76*, phytoalexin and the expression levels of many resistance genes increases, suggesting that the two genes have a negative regulatiory effect on plant disease resistance (Liu *et al.*, 2016). Overexpression of *OsWRKY28* could enhance the susceptibility of *M. oryzae* to rice blast, and OsWRKY28 could negatively regulate the resistance gene to maintain the dynamic balance (Chujo *et al.*, 2013). *OsWRKY22* is found to be involved in defense of *M. oryzae, M. grisea*, and *Blumeria graminis*. Interestingly, *OsWRKY22* does not show any interaction with other OsWRKYs in coregulatory assays, suggesting that this gene may have a unique role in plant defense (Abbruscato *et al.*, 2012).

Eleven members of IId+IIe subfamily are involved in immune regulation (Figure 4). *AtWRKY27* has a negative effect on plant immunity, and its knockout mutant shows symptoms of *Ralstonia solanacearum* infection. The expression levels of *Nitrate Reductase 2* (NR2/NIA2) and *Asparagine Synthase 2* (ASN2) increase in the *AtWRKY27* mutant. As the promoter region of these two genes contains W-box, they may be regulated by AtWRKY27 (Mukhtar *et al.*, 2008). *AtWRKY52* (aka *Resistance to Ralstonia solanacearum 1, RRS1*) is a receptor of the nucleotide-binding, LRR (NB-LRR). *AtWRKY52* interacts with resistance gene *RPS4 (Resistance to Pseudomonas syringae 4*, a member of *NB-LRR*), bacterial response factors AvrRps4 and PopP2 to form complexes to coactivate immune response. AvrRps4 and PopP2 bind directly to the WRKY motif of *AtWRKY52*. AtWRKY52 together with RPS4 form a bait region that allows the bacterial effector to more easily detect the WRKY motif to which it binds (Sarris *et al.*, 2015). The flagellin receptor FLS2 is a LRR receptor kinase. It activates the Mitogen-activated protein kinase (MAPK) signaling cascade and thereby activates the AtWRKY22 and AtWRKY29, involved in regulating the immune responses to bacteria and fungi (Asai *et al.*, 2002). Knockout experiments showed that *AtWRKY11* and *AtWRKY17* are partially functionally redundant. The double knockout mutant of *AtWRKY11* and *AtWRKY17*, genes activated by the JA signaling pathway, is more resistant to *P. syringae* than any of the single knockout mutants (Journot-Catalino *et al.*, 2006). *AtWRKY7* plays a negative regulatory role in the immunization of *P. syringae* infection and is upregulated by SA induction. Expression of the *PR1* gene regulated by SA is increased in the knockout mutant, whereas in the overexpressed mutant PR-related gene expression it is significantly lower, suggesting that *PR1* may be the target of AtWRKY7 (Kim *et al.*, 2006). OsMKK4 activates *OsMPK3/OsMPK6*, then the latter recognizes SP cluster located in *OsWRKY53*. Plants overexpressing phosphorylated *OsWRKY53* show higher resistance to rice blast than plants overexpressing unphosphorylated *OsWRKY53* itself, indicating that the modified status of the WRKY gene is responsible for its function (Chujo *et al.*, 2014). By binding to the *cis*-element W-box and WLE1 on the promoter of the defense gene *OsPR10a*, OsWRKY51 enhances plant resistance to *Xoo* by activating the expression of this gene (Hwang *et al.*, 2016). In addition, OsWRKY51 has a negative regulatory role in the GA signaling pathway (Zhang *et al.*, 2009). *OsWRKY31* could be induced by *M. grisea*, and lots of defense genes such as *PBZ1* and *OsSci2* could be upregulated in the overexpression mutant of *OsWRKY31*. At the same time, the sensitivity of this mutant to indolebutyric acid (IBA), 1-naphthaleneacetic acid (NAA), and 2,4-D decreased, indicating that *OsWRKY31* may be involved in multiple signal transduction systems (Zhang *et al.*, 2008). OsWRKY68 binds to the W-boxes of the *PR1b* promoter region and, by activating the gene, participates in *Xa21*-regulated *Xoo*-related resistance expression (Shuo *et al.*, 2016).

Nine WRKYs have been reported to participate in the immune response to subfamily III, including five *AtWRKY*s and four *OsWRKY*s (Figure 4). *AtWRKY38* and *AtWRKY62* are induced by *P. syringae* or SA; they then negatively control the expression of the defense gene *Nonexpressor of PR Gene1 (NPR1)*. In a single *WRKY* knockout mutant, the expression of the *PR1* and disease resistance are both enhanced and to a greater extent in the double knockout mutants.

AtWRKY38 and AtWRKY62 interact with *Histone Deacetylase 19*, which plays a positive role in plant immunity and inhibits the activity of *AtWRKY38* and *AtWRKY62* (Kc *et al.*, 2008). In general, *AtWRKY38* and *AtWRKY62* play negative roles in plant defense. *AtWRKY46, AtWRKY53*, and *AtWRKY70* are functionally redundant and play a synergistic role in the immune process. *AtWRKY46* can be induced by SA and *P. syringae*. *AtWRKY46-AtWRKY53* or *AtWRKY46-AtWRKY70* double-knockout mutants, as well as *AtWRKY46-AtWRKY53-AtWRKY70* three-gene knockout mutants increase sensitivity to *P. syringae*, and show lower *PR1* gene expression. The expression profiles show that *AtWRKY46, AtWRKY53*, and *AtWRKY70* may play a role in SA signaling pathway (Hu *et al.*, 2012). In rice, *OsWRKY4* and *OsWRKY45* are involved in controlling rice sheath blight resistance. The *OsWRKY4* expression level is rapidly upregulated in plants infected with the fungal pathogen *Rhizoctonia solani*. Additional experiments show that overexpression of *OsWRKY4* increases the resistance of plants to *R.a solani* infection. In the overexpression mutants, the expression levels of the resistance genes *PR1a, PR1b, PR5*, and *PR10/PBZ1* are enhanced. As these downstream genes are involved in JA and ET-mediated response pathway, *OsWRKY4* may regulate JA and ET signaling pathway in immune regulation. Furthermore, W-box and TG-like (TGAC [C/T]) *cis*-elements are found in the promoter regions of *PR1b* and *PR5*, suggesting that they may be target genes for OsWRKY4 (Wang *et al.*, 2015). *OsWRKY45-1* (from japonica) and *OsWRKY45-2* (from indica) are two alleles of *OsWRKY45*, and they have opposite effects in plant immunoregulation. *OsWRKY45-1* and *OsWRKY45-2* have negative and positive regulatory effects on the infection of *Xoo* and *Xoo* pv oryzicola (*Xoc*), respectively. The expression of *OsWRKY45-1* could lead to the increase of JA and SA content in tissues and defense-related genes. In plants overexpressing *OsWRKY45-2*, the expression of JA is also upregulated, while the expression of SA is downregulated, accompanied by the increased expression of downstream defense genes. In addition, overexpression of both WRKYs can enhance plant resistance to *M. grisea* (Tao *et al.*, 2009; Cheng *et al.*, 2015). OsWRKY77 could regulate the expression level of *PR1, PR2*, and *PR5* in rice, and thus enhance the resistance of plants to *P. syringae* (Lan et al., 2013). Overexpression of *OsWRKY23* activates a series of *PR* genes, thereby increasing plant resistance to *P. syringae* (Jing *et al.*, 2009).

In summary, each subfamily of WRKY has been shown to be involved in the biotic stress response, suggesting that (i) the ancestor of WRKYs might already have evolved the functions in plant immunity, and (ii) a dosage of WRKYs is a critical element for the environmental adaptation of plants.

3. Growth and development

The WRKY genes are involved in a wide-range of plant growth and developmental processes (Table 1). Six *Arabidopsis WRKY* genes and one rice *WRKY* gene have been reported to participate in the process of seed growth and maturation (Table 1). *AtWRKY28* participates in the megasporocyte cell fate (Zhao *et al.*, 2017). *AtWRKY2* and *AtWRKY34* are redundantly involved in pollen formation, pollen tube elongation, seed germination, and early growth after germination. *AtWRKY2* knockout mutant showed high sensitivity to ABA, suggesting that *AtWRKY2* regulates seed germination (Jiang and Yu, 2009). In the *AtMPK3-AtMPK6* double knockout mutant, AtWRKY34 cannot be phosphorylated; thereby, its function is inhibited (Guan et al., 2014). The homozygous *AtWRKY10* (also called as *MINISEED3, MINI3*) knockout mutants have a smaller seed size, are slower in development, and have early cellularization of the endosperm (Luo et al., 2005). The *OsWRKY78* knockout mutant showed semidwarf and small kernel phenotype and produced smaller seeds, suggesting that *OsWRKY78* plays an important role in stem elongation and seed development regulator in rice (Zhang et al., 2011). The AtWRKY41 protein binds to three adjacent W-boxes in the promoter of the *ABSCISIC ACID INSENSITIVE 3 (ABI3)*. Knockout of *AtWRKY41* m significantly downregulated *ABI3* and influenced the seed dormancy (Ding et al., 2014).

In addition to regulation of seed growth, the WRKY genes are involved in regulation of seed coloration (Table 1). *AtWRKY44* (also known as *transparent testa glabra, TTG2*) regulates the epidermal color of *Arabidopsis* seeds by participating in transcriptional regulation. AtWRKY44 binds directly to the upstream regulatory region of *TT12. TTG1, TT2*, and *TT8* are involved in the biosynthesis of proanthocyanidins in *Arabidopsis* and the pigmentation, thus, making *Arabidopsis* seeds brown-colored skin (Gonzalez et al., 2016; Johnson et al., 2002).

The WRKY genes are also involved in plant root development (Table 1). Auxin induces the expression of *AtWRKY23, AUXIN RESPONSE FACTOR7 (ARF7)*, and *ARF19*, serving as part of the auxin feedback loop, to regulate the proper growth of plant roots and the local synthesis of flavonoids (Grunewald et al., 2012). *AtWRKY44* and *AtWRKY75* both regulate the development of root hairs. *AtWRKY44* is the downstream gene of *TTG1* and *GLABROUS1*. It expresses continuously in the root hairs and can cooperate with *GLABRA2* to control the growth of root hairs on plants (Johnson et al., 2002). In the

Table 1. WRKY as key regulators in plant growth and development.

Name	Gene Locus ID	Function	References
AtWRKY28	At4G18170	Ovule development	Zhao *et al.*, 2017
AtWRKY2	AT5G56270	Seed germination, postgermination growth	Jiang *et al.*, 2009
AtWRKY10	AT1G55600	Seed size	Luo *et al.*, 2005
AtWRKY34	AT4G26440	Seed germination, postgermination growth	Guan *et al.*, 2014
AtWRKY41	AT4G11070	Seed dormancy	Ding *et al.*, 2014
AtWRKY44	AT2G37260	Seed coat tannins in the proanthocy	Gonzalez *et al.*, 2016
OsWRKY78	LOC_Os01g54600	Seed development; stem elongation	Zhang *et al.*, 2011 Planta
AtWRKY23	AT2G47260	Root growth; biosynthesis of flavonols	Grunewald *et al.*, 2011
AtWRKY44	AT2G37260	Root hair growth	Verweij *et al.*, 2016
AtWRKY75	AT5G13080	Root hair growth	Devaiah *et al.*, 2007
OsWRKY31	LOC_Os06g30860	Root formation and elongation	Zhang *et al.*, 2008
AtWRKY75	AT5G13080	Leaf senescence	Li *et al.*, 2012
AtWRKY6	AT1G62300	Leaf senescence	Robatzek *et al.*, 2016
AtWRKY54	AT2G40750	Leaf senescence	Besseau *et al.*, 2012
AtWRKY70	AT3G56400	Leaf senescence	Besseau *et al.*, 2012
AtWRKY53	AT4G23810	Leaf senescence	Zentgraf *et al.*, 2009; Miao and Zentgraf, 2010
AtWRKY57	AT1G69310	Leaf senescence	Jiang *et al.*, 2014
AtWRKY22	AT4G01250	Leaf senescence	Zhou *et al.*, 2011
AtWRKY26	AT5G07100	Leaf senescence	Li *et al.*, 2017
OsWRKY42	LOC_Os05g46020	Leaf senescence	Liu *et al.*, 2016
OsWRKY23	LOC_Os01g53260	Leaf senescence	Jing *et al.*, 2009
OsWRKY80	LOC_Os09g30400	Leaf senescence	Ricachenevsky *et al.*, 2010
OsWRKY14	LOC_Os01g53040	Leaf senescence	Kang *et al.*, 2011
AtWRKY12	AT2G44745	Flowering time	Li *et al.*, 2016
AtWRKY13	AT4G39410	Flowering time	Li *et al.*, 2016
AtWRKY71	AT1G29860	Flowering time	Yu *et al.*, 2016
OsWRKY11	LOC_Os01g43650	Flowering time; plant height	Cai *et al.*, 2014
AtWRKY45	AT3G01970	Phosphate uptake	Wang et al., 2014
AtWRKY42	AT4G04450	Phosphate uptake	Su *et al.*, 2015
AtWRKY75	AT5G13080	Phosphate uptake	Devaiah *et al.*, 2007
AtWRKY6	AT1G62300	Phosphate uptake; Boron uptake	Chen *et al.*, 2009; Kawajima *et al.*, 2010
OsWRKY74	LOC_Os09g16510	Phosphate uptake	Dai *et al.*, 2016

AtWRKY75 knockout mutant, the number and length of the root hairs show an increase compared with the wild-type, suggesting that *AtWRKY75* is a negative regulator of root hair development (Devaiah et al., 2007). *OsWRKY31* was also found induced by auxin. Compared with the wild-type, plant lateral root formation and elongation are inhibited in the *OsWRKY31* overexpression mutant. This mutant also shows tolerance to high concentrations of plant growth regulators IBA, NAA, and 2,4-D, suggesting that overexpression of *OsWRKY31* may affect the transport process of auxin (Zhang et al., 2008).

Twelve WRKY genes from rice and *Arabidopsis* have been reported to participate in senescence (Table 1). AtWRKY6 binds to a receptor-like kinase *Senescence-induced receptor-like serine/threonine-protein kinase* and regulates the leaf senescence process (Robatzek and Somssich, 2002). *AtWRKY75* mutant showed leaf senescence inhibition, suggesting that *AtWRKY75* has a positive effect on leaf senescence (Li et al., 2012). *AtWRKY53* has a positive effect on plant senescence (Miao and Zentgraf, 2010), whereas *AtWRKY54* and *AtWRKY70* function redundantly, and potentially interact with *AtWRKY30*, negatively regulating the plant senescence (Besseau et al., 2012). AtWRKY57 acts as a node in the crosstalk of JA and auxin, and mediates the leaf senescence (Jiang *et al.*, 2014). AtWRKY22 is promoted by darkness and suppressed by light and involved in darkness-induced leaf senescence (Zhou *et al.*, 2011). AtWRKY26 is also a positive regulator of leaf senescence (Li *et al.*, 2017b). In rice, *OsWRKY14* is involved in methanol-induced tryptophan biosynthesis as well as tryptophan-induced secondary metabolites (Kang et al., 2011). Overexpression of *OsWRKY23* could accelerate leaf senescence under dark induction (Jing et al., 2009). Using an overexpression mutant, *OsWRKY42* shows early leaf senescence, accumulation of ROS, and decreased chlorophyll content (Han et al., 2014). *OsWRKY80* showed a high level of expression in dark-induced senescent plant leaves, which was induced by 6-Benzylaminopurine and ABA, suggesting that it is a typical senescence-related gene (Ricachenevsky et al., 2010).

Control of flowering time is an important part of the development process of angiosperm plants (Table 1). *AtWRKY12, AtWRKY13*, and *AtWRKY71* are involved in this process (Table 1). *AtWRKY12* and *AtWRKY13* have opposite regulatory effects on the flowering time under short daylight conditions. The flowering time of the AtWRKY12 knockout mutant is delayed compared with wild-type, whereas AtWRKY13 induces flowering.

FRUITFULL (FUL), a direct downstream target gene of *AtWRKY12* and *AtWRKY13*, is the signaling pathway hub of these two WRKY genes. In addition, *AtWRKY12* and *AtWRKY13* can also affect plant flowering by partially regulating *GA3* (Li et al., 2016). *AtWRKY71* has a positive effect on plant flowering, both the active target mutant and the overexpression mutant has earlier flowering time than the wild type. In specific, promoter sequences of *FT, LFY, AP1*, and *CAL* (but not *FUL*) harbor W-boxes (TTTGACT/C), *AtWRKY71* affects the flowering time of plants by directly regulating these genes (Yu et al., 2016). OsWRKY11 acts as a *trans*-regulatory factor, delaying the flowering time of plants by downregulating gene expression of *Early Heading date Ehd2/ROOT INITIATION DEFECTIVE RID1//Indeterminate 1 (Osld1)*; also, its downstream genes include *Heading date1 (Hd1)*, *Ehd1*, and *Hd3a* (Cai et al., 2014).

Four WRKYs and one rice WRKY are involved in plant nutrient utilization in *Arabidopsis* (Table 1). Plant growth and development process require a large amount of phosphorus and boron, and lacking these elements will significantly impact gene regulations. *AtWRKY42, AtWRKY45*, and *AtWRKY75* participate in the regulation of phosphorus deficiency signaling, in which *AtWRKY42* knockout mutant is more sensitive to low-phosphorus stress, and their shoots contained less phosphorus than wild-type (Su et al., 2015). AtWRKY45 can bind to two W-boxes in the promoter region of the *PHOSPHATE TRANSPORTER 1; 1 (PHT1; 1)*, and upregulates the taking up of phosphorus (Wang et al., 2014). *AtWRKY75* has a positive regulatory effect on plant tolerance to phosphorus deficiency, and *AtWRKY75* is significantly upregulated in the condition of insufficient phosphorus in the environment (Devaiah et al., 2007). Besides its role in regulating leaf senescence, AtWRKY6 is involved in responses to low-phosphorus stress via regulating *PHOSPHATE1 (PHO1)* expression (Chen *et al.*, 2009). AtWRKY6 is the first characterized TF that is involved in response to boron deficiency (Kasajima *et al.*, 2010). OsWRKY74 modulates the phosphorus homeostasis and the potential crosstalk between ion and phosphorus starvation (Dai *et al.*, 2017). OsWRKY80 responds to Fe-excess in rice leaves, stems and roots, suggesting a role in Fe signaling (Klein *et al.*, 2010).

G. Degradation of WRKY Proteins

Similar to other eukaryotes, the ubiquitin-proteosome system (UPS) mediated degradation of TFs plays an important role in the regulation of gene expression (Jakoby *et al.*, 2002; Allen *et al.*, 2008). The degradation of a WRKY transcription factor in Japanese goldthread, *Coptis japonica,* CjWRKY, was regulated by UPS (Yamada and Sato, 2016). In *Arabidopsis*, AtWRKY6 is ubiquitinated through interacting with Really Interesting New Gene (RING)-type finger E3 ubiquitin ligase (At1g74410), and this degradation process could be terminated by the 26S proteosome inhibitor MG132 (Chen *et al.*, 2009). Similar to AtWRKY6, AtWRKY53 is also degraded by the action of a Homologous to the E6-AP Carboxyl Terminus (HECT) E3 ubiquitin ligase, which can accelerate the senescence of plant leaf (Miao and Zentgraf, 2010). In Chinese wild grapevine (*Vitis pseudoreticulata*), VpWRKY11 interacts with *Erysiphe necator*-induced RING finger protein 1 (EIRP1) through the RING domain, and degraded by the latter. Through this way, EIRP1 can enhance plant resistance to pathogens (Yu *et al.*, 2013). In rice, OsWRKY53 was able to bind to the leucine zipper domain of the ubiquitin ligase OsUPL5, negatively affecting leaf senescence (Miao and Zentgraf, 2010). OsWRKY45 was also degraded through ubiquitination, playing an important role in rice defense responses (Matsushita et al., 2013).

II. Studies of WRKY genes in crops

Most crops originated from seed plants including gymnosperms and angiosperms (Feuillet et al., 2011). Unlike the model plant *Arabidopsis*, crops usually have large and complex genomes. For example, maize (*Zea mays*) has a genome of 2,106 Mb, the wheat variety Chinese Spring has an allooctoploid genome of 10.2 Gb (www.wheatgenome.org), and sugarcane (*Saccharum officinarum*) has a basic ploidy unit of 40 chromosomes (http://ccdb.tau.ac.il). As of December 31, 2017, the genomes of 270 angiosperm species have been released (www.angiosperms.org). Seventy percent or 168 of the sequenced angiosperm plants are crops and most of the other sequenced plants are the wild relatives of crops with important evolutionary positions.

A. Genome-wide identification of WRKY genes in crops

Genome-wide identification and characterizations of WRKY genes have been carried-out in several crop plants (Table 2). For example, sized duckweed *Spirodela polyrhiza*, which has a small genome, has 43 WRKY genes (Table 2). More than 100 WRKY genes have been identified from crops with large genomes, such as soybean, cotton, and napa (Table 2). Compared to other plants, the Poaceae plants (*Oryza sativa, Zea mays,* and *Sorghum bicolor*) are enriched with subfamily III members. Subfamily IIc and subfamily IIa + IIb were specifically amplified in cruciferous and legumes, respectively (Table 2). Since the first release of *Arabidopsis* genome

Table 2. WRKY gene family characterizations in representative crops.

Type	Species	I	IIa + IIb	IIc	IId + IIe	III	Undefined	Total Number	Reported total number/Reference
Vegetable	*Solanum tuberosum*	35	19	18	33	20	0	80	75 / Schluttenhofer *et al.*, 2014
Vegetable	*Solanum lycopersicum*	17	13	16	23	11	1	81	81 / Huang *et al.*, 2012
Vegetable	*Capsicum annuum*	15	10	13	14	9	1	62	71 / Diao *et al.*, 2016
Drink	*Coffea arabica*	10	9	14	10	5	1	49	49 / Schluttenhofer *et al.*, 2015
Fruit	*Fragaria vesca*	9	10	10	12	15	1	58	62 / Wei *et al.*, 2016
Fruit	*Prunus persica*	10	11	14	14	8	1	56	58 / Chen *et al.*, 2016
Fruit	*Malus domestica*	31	24	33	31	17	5	141	127 / Meng *et al.*, 2016
Vegetable	*Cucumis sativus*	15	9	19	16	7	1	62	55 / Xu *et al.*, 2015
Economic	*Glycine max*	37	45	37	38	24	4	182	197 / Rushton *et al.*, 2010
Vegetable	*Phaseolus vulgaris*	19	21	19	18	14	1	90	90 / Wang *et al.*, 2016
Economic	*Populus trichocarpa*	22	14	25	30	10	2	103	104 / He *et al.*, 2012
Drink	*Theobroma cacao*	12	11	15	12	6	6	59	18 / Borrone *et al.*, 2007
Economic	*Gossypium raimondii*	18	23	35	31	12	2	120	116 / Dou *et al.*, 2014
Fruit	*Carica papaya*	10	10	11	11	7	1	49	52 / Pan *et al.*, 2014
Vegetable	*Brassica rapa*	28	22	37	26	24	4	141	145 / Kayum *et al.*, 2015
Model	*Arabidopsis thaliana*	15	11	17	16	13	2	72	72 / Rushton *et al.*, 2010
Fruit	*Vitis vinifera*	12	11	15	14	6	1	59	59 / Wang *et al.*, 2014
Fruit	*Musa acuminata*	24	34	30	41	14	0	152	147 / Goel et al., 2016
Food	*Oryza sativa*	19	13	17	18	27	7	103	103 / Ramamoorthy *et al.*, 2008
Food	*Zea mays*	28	15	22	28	30	0	132	116 / Wei *et al.*, 2012
Food	*Sorghum bicolor*	18	14	17	17	24	7	97	68 / Pandey *et al.*, 2009
Economic	*Spirodela polyrhiza*	11	8	6	13	3	2	43	34 / Yang *et al.*, 2015
Wild	*Amborella trichopoda*	7	6	7	7	4	1	32	29 / Yang *et al.*, 2015

in 2000, 205 crop genome sequences have been released, whereas only 79 crops have their WRKY genes reported (Figure 5 and Table 3). Considering the importance of crop plants in global economy and human life, further characterizations of the functions of WRKY genes using newly developed techniques will become a necessity.

B. Functional characterization of WRKYs in crops

Although the studies of WRKY in most crops are not as extensive as in those model plants, the WRKY mediated signaling pathway/network has been studied in some crops. Sun *et al.* (2003) identified a WRKY gene *SUSIBA2* in barley that interacts with cis element SURE (sugar-responsive) and W-box in the promoter of *iso1*, involved in the sugar signaling and the biosynthesis of starch. In the chili pepper, *Capsicum annuum*, *CaWRKY1* is a negative regulator influencing pathogen infections, and expression was detected after only one-half hour after the infection by *Pseudomonas syringe*. CaWRKY1 is an ortholog to the *Arabidopsis* WRKY50 and WRKY51 TFs, and therefore might target the same kinds of genes (Oh et al., 2007). In cotton (*Gossypium hirsutum*), plants with overexpression of *GhWRKY44* were more resistant to fungal pathogen *R. solanacearum* and *R. solani*. The expression of *PR-1, PR-2, PR-5, NPR1*, and *PR-4* was also upregulated in overexpressed plants, suggesting that these genes may be involved in the

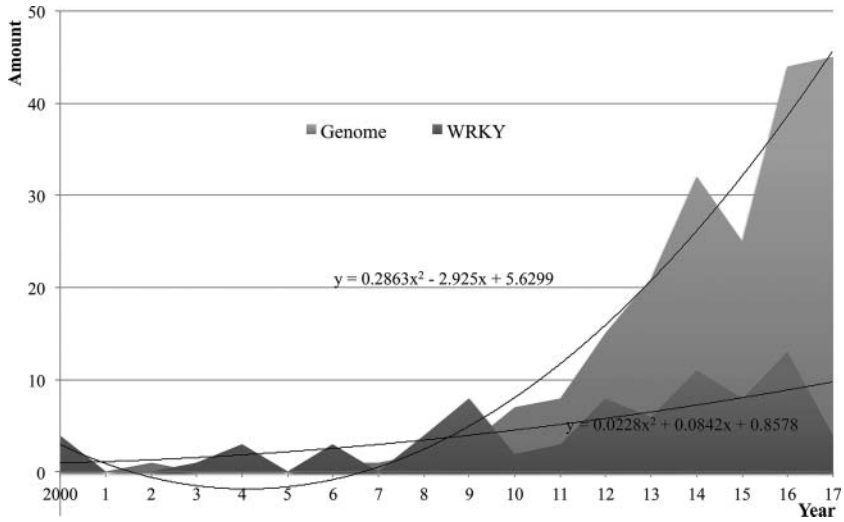

Figure 5. Crop genome decoding progress and WRKY gene family research advances. Statistic resource data could be found at www.angiosperms.org.

Table 3. WRKY research in 205 crop species genomes.

Species	Genome_release	WRKY report	Taxonomy	Crop_attribute
Capsella bursa-pastoris	2017	—	Brassicales	Vegetable
Barbarea vulgaris	2017	—	Brassicales	Vegetable
Momordica charantia	2016	—	Cucurbitales	Vegetable
Brassica juncea	2016	—	Brassicales	Vegetable
Vigna unguiculata	2016	—	Fabales	Vegetable
Zizania latifolia	2015	—	Poales	Vegetable
Moringa oleifera	2015	—	Brassicales	Vegetable
Vicia faba	2015	—	Fabales	Vegetable
Vigna angularis	2015	—	Fabales	Vegetable
Thlaspi arvense	2015	—	Brassicales	Vegetable
Vigna radiata	2014	—	Fabales	Vegetable
Phaseolus vulgaris	2014	—	Fabales	Vegetable
Spinacia oleracea	2013	—	Caryophyllales	Vegetable
Lagenaria siceraria	2013	—	Cucurbitales	Vegetable
Capsella rubella	2013	—	Brassicales	Vegetable
Beta vulgaris	2013	—	Caryophyllales	Vegetable
Cajanus cajan	2011	—	Fabales	Vegetable
Lactuca sativa	2011	—	Asterales	Vegetable
Raphanus sativus	2014	2016	Brassicales	Vegetable
Daucus carota	2016	2015	Apiales	Vegetable
Solanum melongena	2014	2015	Solanales	Vegetable
Brassica oleracea	2014	2015	Brassicales	Vegetable
Solanum pimpinellifolium	2012	2014	Solanales	Vegetable
Cicer arietinum	2013	2013	Fabales	Vegetable
Cucumis sativus	2009	2011	Cucurbitales	Vegetable
Brassica napus	2014	2009	Brassicales	Vegetable
Solanum lycopersicum	2012	2008	Solanales	Vegetable
Medicago truncatula	2011	2008	Fabales	Vegetable
Glycine max	2010	2008	Fabales	Vegetable
Capsicum annuum	2014	2006	Solanales	Vegetable
Brassica rapa	2011	2006	Brassicales	Vegetable
Solanum tuberosum	2011	2000	Solanales	Vegetable
Capsella bursa-pastoris	2017	—	Brassicales	Vegetable
Asparagus officinalis	2017	2017	Asparagales	Vegetable
Momordica charantia	2017	—	Cucurbitales	Vegetable
Cephalotus follicularis	2017	—	Oxalidales	Ornamental
Zoysia pacifica	2016	—	Poales	Ornamental
Fraxinus excelsior	2016	—	Lamiales	Ornamental
Hibiscus syriacus	2016	—	Malvales	Ornamental
Drosera capensis	2016	—	Caryophyllales	Ornamental
Rosa x damascena	2016	—	Rosales	Ornamental
Petunia inflata	2016	—	Solanales	Ornamental
Zoysia japonica	2016	—	Poales	Ornamental
Rosa roxburghii	2016	—	Rosales	Ornamental
Cynara cardunculus	2016	—	Asterales	Ornamental
Nymphaea colorata	2016	—	Nymphaeales	Ornamental
Kalanchoe marnieriana	2016	—	Saxifragales	Ornamental
Kalanchoe laxiflora	2016	—	Saxifragales	Ornamental
Lolium perenne	2015	—	Poales	Ornamental
Phalaenopsis equestris	2014	—	Asparagales	Ornamental
Amaranthus hypochondriacus	2014	—	Caryophyllales	Ornamental
Erythranthe guttata	2014	—	Lamiales	Ornamental
Ensete ventricosum	2014	—	Zingiberales	Ornamental
Petunia integrifolia	2014	—	Solanales	Ornamental
Dianthus caryophyllus	2013	—	Caryophyllales	Ornamental
Tarenaya hassleriana	2013	—	Brassicales	Ornamental
Nicotiana sylvestris	2013	—	Solanales	Ornamental
Lupinus angustifolius	2013	—	Fabales	Ornamental
Nelumbo nucifera	2013	—	Proteales	Ornamental
Mimulus guttatus	2013	—	Lamiales	Ornamental
Kalanchoe fedtschenkoi	2013	—	Saxifragales	Ornamental
Prunus mume	2012	—	Rosales	Ornamental
Aquilegia caerulea	2012	—	Ranunculales	Ornamental
Musa acuminata	2012	2016	Zingiberales	Ornamental
Dendrobium catenatum	2016	2015	Asparagales	Ornamental
Zoysia matrella	2016	2013	Poales	Ornamental
Ipomoea nil	2016	2000	Solanales	Ornamental
Carnegiea gigantea	2017	—	Caryophyllales	Ornamental
Rhododendron delavayi	2017	—	Ericales	Ornamental

(*Continued on next page*)

Table 3. (*Continued*)

Species	Genome_release	WRKY report	Taxonomy	Crop_attribute
Fraxinus excelsior	2017	—	Lamiales	Ornamental
Panax ginseng	2017	2016	Apiales	Medical
Calotropis gigantea	2017	—	Gentianales	Medical
Camptotheca acuminata	2017	—	Cornales	Medical
Rhodiola crenulata	2017	—	Saxifragales	Medicinal
Panax notoginseng	2017	—	Apiales	Medicinal
Erigeron breviscapus	2017	—	Asterales	Medicinal
Citrus medica	2017	—	Sapindales	Medicinal
Mentha longifolia	2016	—	Lamiales	Medicinal
Glycyrrhiza uralensis	2016	—	Fabales	Medicinal
Rhazya stricta	2016	—	Gentianales	Medicinal
Pogostemon cablin	2016	—	Lamiales	Medicinal
Lepidium meyenii	2016	—	Brassicales	Medicinal
Silybum marianum	2016	—	Asterales	Medicinal
Dorcoceras hygrometricum	2016	—	Coleoptera	Medicinal
Ocimum tenuiflorum	2015	—	Lamiales	Medicinal
Ocimum sanctum	2015	—	Lamiales	Medicinal
Azadirachta indica	2012	—	Sapindales	Medicinal
Salix suchowensis	2014	2016	Malpighiales	Medicinal
Salvia miltiorrhiza	2015	2014	Lamiales	Medicinal
Catharanthus roseus	2015	2011	Gentianales	Medicinal
Ficus carica	2017	—	Rosales	Fruit
Dimocarpus longan	2017	—	Sapindales	Fruit
Durio zibethinus	2017	—	Malvales	Fruit
Punica granatum L	2017	2017	Myrtales	Fruit
Fagopyrum tataricum	2017	—	Caryophyllales	Fruit
Ficus carica L.	2017	2017	Rosales	Fruit
Citrus ichangensis	2017	—	Sapindales	Fruit
Citrus grandis	2017	—	Sapindales	Fruit
Macadamia integrifolia	2016	—	Proteales	Fruit
Siraitia grosvenorii	2016	—	Cucurbitales	Fruit
Musa itinerans	2016	—	Zingiberales	Fruit
Olea europaea	2016	—	Lamiales	Fruit
Vitis aestivalis	2016	—	Vitales	Fruit
Artocarpus camansi	2016	—	Rosales	Fruit
Ananas comosus	2015	—	Poales	Fruit
Vaccinium corymbosum	2015	—	Ericales	Fruit
Fragaria orientalis	2015	—	Rosales	Fruit
Fragaria nipponica	2015	—	Rosales	Fruit
Castanea mollissima	2015	—	Fagales	Fruit
Diospyros lotus	2014	—	Ericales	Fruit
Ziziphus jujuba	2014	—	Rosales	Fruit
Vaccinium macrocarpon	2014	—	Ericales	Fruit
Citrus clementina	2014	—	Sapindales	Fruit
Pyrus communis	2014	—	Rosales	Fruit
Actinidia chinensis	2013	—	Ericales	Fruit
Prunus persica	2013	—	Rosales	Fruit
Citrus sinensis	2012	—	Sapindales	Fruit
Cucumis melo	2012	—	Cucurbitales	Fruit
Phoenix dactylifera	2011	—	Arecales	Fruit
Juglans regia	2016	2016	Fagales	Fruit
Musa balbisiana	2013	2016	Zingiberales	Fruit
Morus notabilis	2013	2016	Rosales	Fruit
Juglans regia	2012	2016	Fagales	Fruit
Ginkgo biloba	2016	2015	Gymnosperm	Fruit
Pyrus bretschneideri	2012	2015	Rosales	Fruit
Juglans sigillata	2016	2014	Fagales	Fruit
Malus domestica	2010	2014	Rosales	Fruit
Carica papaya	2008	2014	Brassicales	Fruit
Citrullus lanatus	2012	2012	Cucurbitales	Fruit
Fragaria vesca	2010	2012	Rosales	Fruit
Fragaria x ananassa	2015	2010	Rosales	Fruit
Vitis vinifera	2007	2006	Vitales	Fruit
Citrus parasisi x Poncirus trifoliata	2016	2003	Sapindales	Fruit
Rubus occidentalis	2016		Rosales	Fruit
Secale cereale	2017	—	Poales	Food
Chenopodium pallidicaule	2016	—	Caryophyllales	Food
Fagopyrum esculentum	2016	—	Caryophyllales	Food

(*Continued on next page*)

Table 3. (*Continued*)

Species	Genome_release	WRKY report	Taxonomy	Crop_attribute
Manihot esculenta ssp.*flabellirolia*	2014	2016	Malpighiales	Food
Chenopodium quinoa	2016	2015	Caryophyllales	Food
Setaria italica	2012	2015	Poales	Food
Glycine soja	2014	2013	Fabales	Food
Zea mays	2009	2012	Poales	Food
Sorghum bicolor	2009	2009	Poales	Food
Triticum aestivum	2014	2008	Poales	Food
Oryza sativa	2002	2004	Poales	Food
Hordeum vulgare	2015	2000	Poales	Food
Cucurbita pepo	2017	2012	Cucurbitales	Food
Capsicum baccatum	2017	—	Solanales	Food
Ipomoea batatas	2017	1994	Solanales	Food
Cucurbita moschata	2017	—	Cucurbitales	Food
Cenchrus americanus	2017	—	Poales	Food
Dioscorea rotundata	2017	—	Dioscoreales	Food
Secale cereale L.	2017	—	Poales	Food
Corchorus capsularis	2017	—	Malvales	Economic
Corchirus olitorius	2017	—	Malvales	Economic
Betula pendula	2017	—	Fagales	Economic
Atalantia buxifolia	2017	—	Sapindales	Economic
Quercus lobata	2016	—	Fagales	Economic
Brassica nigra	2016	—	Brassicales	Economic
Eichhornia paniculata	2016	—	Commelinales	Economic
Carthamus tinctorius	2016	—	Asterales	Economic
Pseudotsuga menziesii	2015	—		Economic
Lemna minor	2015	—	Alismatales	Economic
Vitis cinerea x Vitis riparia	2015	—	Vitales	Economic
Aquilaria agallocha	2014	—	Malvales	Economic
Eucalyptus grandis	2014	—	Myrtales	Economic
Camelina sativa	2014	—	Brassicales	Economic
Eragrostis tef	2014	—	Poales	Economic
Picea abies	2013	—		Economic
Picea glauca	2013	—	Pinales	Economic
Phyllostachys heterocycla	2013	2017	Poales	Economic
Linum usitatissimum	2012	—	Malpighiales	Economic
Eucalyptus camaldulensis	2011	—	Myrtales	Economic
Sesamum indicum	2014	2016	Lamiales	Economic
Cannabis sativa	2011	2016	Brassicales	Economic
Spirodela polyrhiza	2014	2014	Alismatales	Economic
Hevea brasiliensis	2013	2014	Malpighiales	Economic
Gossypium raimondii	2012	2014	Malvales	Economic
Lotus japonicus	2008	2014	Fabales	Economic
Jatropha curcas	2010	2013	Malpighiales	Economic
Helianthus annuus	2017	2012	Asterales	Economic
Gossypium barbadense	2015	2012	Malvales	Economic
Brachypodium distachyon	2010	2012	Poales	Economic
Populus trichocarpa	2006	2012	Malpighiales	Economic
Gossypium hirsutum	2015	2011	Malvales	Economic
Populus tremulaxPopulus tremuloides-T89x	2015	2009	Malpighiales	Economic
Populus tremula	2015	2009	Malpighiales	Economic
Pinus taeda	2014	2009		Economic
Elaeis guineensis	2013	2009	Arecales	Economic
Nicotiana benthamiana	2012	2009	Solanales	Economic
Ricinus communis	2010	2009	Malpighiales	Economic
Gossypium arboreum	2014	2004	Malvales	Economic
Nicotiana tabacum	2014	2000	Solanales	Economic
Boehmeria nivea	2017	2013	Rosales	Economic
Eucommia ulmoides	2017	—	Garryales	Economic
Handroanthus impetiginosus	2017	—	Lamiales	Economic
Populus pruinosa	2017	2014	Malpighiales	Economic
Corchorus olitorius	2017	—	Malvales	Economic
Corchorus capsularis	2017	2014	Malvales	Economic
Trifolium pratense	2015	—	Fabales	Drink
Camellia sinensis	2017	2016	Ericales	Drink
Humulus lupulus	2014	2016	Rosales	Drink
Coffea arabica	2017	2013	Gentianales	Drink
Coffea canephora	2014	2010	Gentianales	Drink
Theobroma cacao	2010	2004	Malvales	Drink

immune process as GhWRKY44 target genes (Li *et al.*, 2015). Summer waterlogging can seriously affect the quality of grape fruit, and grape WRKY11 has been confirmed to improve the resistance of grapes to waterlogging. This process is through the regulation of *Atrisne 29A* and *AtRD29B*, two stress response genes (Liu *et al.*, 2011). In maize, ZmWRKY33 can be induced in high salt and drought conditions. Transgenic experiments show that *ZmWRKY33* overexpression can activate multiple stress response genes, including *RD29A*, thereby enhancing plant tolerance to salt stress (Li *et al.*, 2013). In apple (*Malus domestica*), *MdWRKY13* overexpressing plants showed a higher susceptibility to drought, suggesting that this gene may be a negative regulator of apple stress on drought stress, and further testing suggests that this regulation may be related to the proline degradation gene *p5cs1* (Duan *et al.*, 2014).

C. WRKYs in crop domestication and breeding

TFs are suitable candidates for plant domestication and molecular breeding, because they are linked to the recognition of domestication gene to affect spatial and temporal gene expression (Swinnen *et al.*, 2016). Gu *et al.* (2017) reported that a WRKY gene from soybean, *SoyWRKY15a,* was related to seed size and weight variation in wild soybean. The diverged expression levels of *SoyWRKY15a* could distinguish wild soybeans from cultivated soybeans, suggesting a critical role of WRKY genes in the domestication processes of soybean. Because of their critical roles in various signaling pathways, WRKY genes have a very promising potential in plant breeding. Silencing or knockout WRKY genes in feedback inhibition of stress signaling pathways or immune pathways are potential potent targets in molecular breeding of novel crops.

III. Applications of high throughput technologies to accelerate the exploration of crop WRKY genes

Researches based on model plants are instrumental to advance our understandings of the functional roles of the WRKY genes, but it also has limitations. For example, *Arabidopsis* and rice are not ideal study systems for the study of color, floral, nitrogen fixation, perennial, fruit development. The new and improved techniques that have been used in model plants, especially *Arabidopsis*, would significantly facilitate the studies of WRKY genes in crops.

A. Evo-devo based functional inference

Newly duplicated genes usually retain similar functions (Guth and Wegner, 2008). Therefore, phylogenetic analysis of the plant WRKY genes is an effective way to infer the functions of uncharacterized WRKY members based on their evolutionary history and sequence similarity. Such studies could be a convenient way to infer the functions of WRKY genes in crops. Figure 6 shows a phylogenetic tree constructed using WRKYs from the 23 plants listed in Table 2, in which the genes designated by the color dots present those which have been identified, and the hollow circles represent the functional-unknown genes in *Arabidopsis* and *O. sativa*. Most of the subfamily IIa genes are involved in both biotic and abiotic processes, suggesting that this subfamily is largely engaged in stress signaling and those IIa genes in crop may share similar functions. Similarly, the subfamily IId genes are not involved in the regulation of growth and development, suggesting that this subfamily may have evolved to become stress-specific genes. Although genes in subfamily I cover the three functions with most stress-related, when further divided, the small branches of these genes have only one or two functions. Compared to other well-characterized subfamilies, the functions of most subfamily III genes are unknown.

Whole genome sequencing data make it possible for rapid prediction and retrieval of WRKY genes in a species. Although the numbers of WRKY genes and their

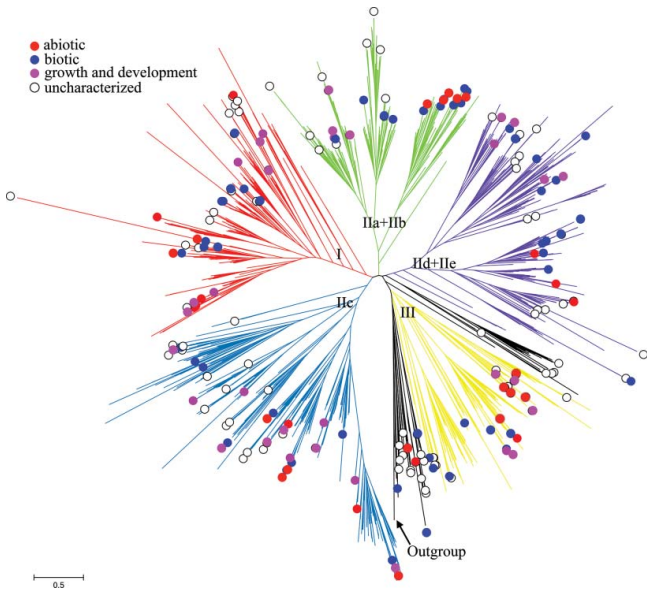

Figure 6. Functional similarity in the phylogenetic view of WRKY gene family in Arabidopsis and rice. The phylogenetic tree using by Arabidopsis and rice WRKY gene family. The genes designated by the color dots have been identified, and the hollow circles represent the function-unknown genes, which came from *Arabidopsis* and *O. sativa*.

classification in many species have been studied, comparative analysis between multiple species will be helpful to understand the evolutionary patterns of WRKY subfamilies and members. Because there are more than 100 copies in many crops, detailed structural and functional studies of every WRKY gene of each crop species is challenging and time-consuming. It is necessary to establish a public database to include all WRKY genes found in crops that have completed genome sequencing. Second, analytical tools such as sequence retrieval, gene structure and expression analysis, and gene phylogenetic tree construction should be integrated.

B. The temporal and spatial expression patterns of WRKY genes

RNA-seq is a powerful tool to study the temporal and spatial expression patterns of the whole WRKY gene family of a plant. Based on RNA-seq data of samples of mock and pathogen inoculated plants, the expression pattern of all the WRKY family members in plant immune responses have been determined (Okay et al., 2014). Similar researches were conducted for WRKYs in wheat drought stress responses (Okay *et al.*, 2014; Satapathy *et al.*, 2014). The expression atlas of WRKYs in the American cotton G. *aridum* under drought stress treatment have also been generated (Fan *et al.*, 2015). The newly developed third generation sequencing platforms, such as Pacific Biosciences and Oxford Nanopore Technologies, are able to generate full-length transcriptome, which offers an opportunity to identify the members of WRKY gene family from highly polyploid crops, such as the sugarcane (Hoang *et al.*, 2017), providing unprecedented knowledge of WRKY gene evolution.

C. Functional characterization of WRKY genes using CRISPR

Gene editing tools such as CRISPR (Clustered Regularly Interspaced Palindromic Repeats)-Cas9 (Songstad *et al.*, 2017; Zhang *et al.*, 2017) have been rapidly developed in the past years. The CRISPR/Cas9 system becomes an important tool in plant molecular biology research due to the precise editing or excision of genes. Liu *et al.* 2016b reported the introduction of a special carrier containing CRISPR/Cas9 into tobacco, which successfully knocked out a tobacco's *NbWRKY70* gene. Many WRKYs can be served as good targets because of their roles in the signaling pathways in model plants.

The CRISPR/Cas9 system could create a mutant library in a fast and convenient way, making functional genomics possible for various crops. Recently, the CRISPR/Cas9 system can create small insertions and deletions (indels) in specific target genes and has been applied to many organisms. Relying on these convenient characters, some CRISPR/Cas9 mutant libraries have been developed for genome-wide mutation screens in cultured eukaryotic cells (Shalem et al., 2015). In rice, the CRISPR/Cas9 system has been successfully applied in the construction of a genome-wide mutant library (Meng *et al.*, 2017). The future application of the CRISPR/Cas9 system in other crops, such as soybean or oilseed plant or other crops, would significantly accelerate the identification and characterization of WRKY genes and have potential use for genetic improvement.

D. Identification of WRKY target genes using ChIP-seq

ChIP-sequencing, also known as ChIP-seq, a powerful tool for studying the interaction between chromatin and DNA, is widely used to determine how TFs influence phenotype-affecting mechanisms. In *Arabidopsis*, AtWRKY18, AtWRKY33, and AtWRKY40 have been demonstrated to modulate pathogen-triggered cellular responses (Walker, 2011). Chip-seq study on *Arabidopsis* revealed that each of the three WRKY proteins bind to more than 1,000 W-box elements, which mainly locate in the 500 bp promoter region. Bioinformatics analyses of these genes identified not only the genes involved in defense signal perception and transduction, but also numerous TFs encoding ethylene response factors. The detailed protocol of WRKY-oriented Chip-seq has been developed to study its genome-wide targets (Walker, 2011). ChIP-seq has also been applied in research of the related TF genes, such as *NAC* and *YABBY* (Walker, 2011). Therefore, ChIP-seq techniques will be instrumental for global identification of WRKY targets, contributing to a better understanding of the WRKY signaling network.

E. Online data analysis and visualization

Large-scale sequencing of genomes, transcriptomes, epigenomes, and specific sequencing such as ChIP-seq, has produced a large amount of heterogeneous data. How to integrate and analyze different types of ome data becomes the focus of bioinformatics research. For a large gene family such as WRKY, a database/webserver can share and update the latest -ome data, providing powerful and fast computational resources, making it possible to analyze the basic features of the WRKY gene family online. On the other hand, scientists have accumulated 1,001 *Arabidopsis* genome sequences (Weigel and Mott, 2009), 3,000 rice genome sequences (The 3,000 rice genomes project, 2014), and will sequence even more

crop genomes. A main challenge is how to compare the difference of WRKYs as fast as possible.

Although there is no WRKY-centered database at present, we propose the conceptional structure of such database. The database should (i) include as many plant genomes as possible to facilitate WRKY gene prediction, (ii) provide sequence search and comparison, (iii) display the genetic structure, (iv) compare gene expression and pathway, and (v) link to related literature.

IV. Conclusions and perspectives

Since the discovery of WRKY and W-box genes in sweet potato and parsley crops in the 1990s, WRKY research has shifted to the model plant *Arabidopsis*. Many important discoveries about the WRKY transcription factors have been reported, from gene structural evolution to functional network. More in-depth studies focusing on WRKY genes in crops are needed, considering their important economic value and nonlaboratory cultivation that faces broader stresses. Although the study of WRKY genes in crops has become more extensive in the past year, it still falls behind crop genome studies. The genome sequencing data have been rapidly accumulated in plants, particularly in crops. The studies of WRKY genes in the model plant *Arabidopsis* have generated rich functional characterization data, which will be valuable for functional prediction of their orthologous genes in crops. The application of various new and improved technologies will also greatly facilitate the functional characterization of crop WRKY genes. Therefore, we propose that future studies should focus on identification and functional analysis of WRKY genes in crops, which will have promising potential for improving yield and quality of crops and reducing pesticide use.

Conflict of interest

No conflict of interest was declared.

Author contributions

L.Z. and F.C. designed the research. F.C. and Y.H. did the data analysis and wrote the draft manuscript. F.C., Y.Q, K.W., A.V., A.M., Y.Q., H.C., Z.L., and L.Z. discussed and improved the review and wrote the final MS. All authors approved the final version of the manuscript.

Funding

F.C. is supported by a grant from State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops (SKB2017004). L.Z. is supported by the National Natural Science Foundation of China (81502437), and a start-up fund from Fujian Agriculture and Forestry University.

References

Abbruscato, P., Nepusz, T., Mizzi, L., Corvo, M. Del, Morandini, P., Fumasoni, I., Michel, C., Paccanaro, A., Guiderdoni, E., Schaffrath, U., Morel, J., Piffanelli, P., and Faivre-Rampant, O. 2012. *OsWRKY22*, a monocot wrky gene, plays a role in the resistance response to blast. *Mol. Plant Pathol.* **13**: 828–841. doi:10.1111/j.1364-3703.2012.00795.x.

Allen, T., Duek, P.D., Whitelam, G.C., Fankhauser, C., and Building, G. 2008. Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. *Plant J.* **53**: 312–323.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**: 403–410. doi:10.1016/S0022-2836(05)80360-2.

Amor, B. Ben, Hohnjec, N., Pu, A., Becker, A., Vernie, T., Gough, C., Niebel, A., Godiard, L., and Gamas, P. 2004. Expression profiling in *Medicago truncatula* identifies more than 750 genes differentially expressed during nodulation, including many potential regulators of the symbiotic program. *Plant Physiol.* **136**: 3159–3176. doi:10.1104/pp.104.043612.

Andreasson, E., Jenkins, T., Brodersen, P., Thorgrimsen, S., Petersen, N.H.T., Zhu, S., Qiu, J.-L., Micheelsen, P., Rocher, A., Petersen, M., Newman, A., Nielsen, H. B., Hirt, H., Somssich, I., Mattson, O., Mundy, J. 2005. The MAP kinase substrate MKS1 is a regulator of plant defense responses. *EMBO J.* **24**: 2579–89. doi:10.1038/sj.emboj.7600737.

Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W.-L., Gomez-Gomez, L., Boller, T., Ausubel, F. M., and Sheen, J. 2002. MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* **415**: 977–983. doi:10.1038/415977a.

Babitha, K. C., Ramu, S. V., Pruthvi, V., Mahesh, P., Nataraja, K. N., and Udayakumar, M. 2013. Co-expression of At*bHLH17* and At*WRKY28* confers resistance to abiotic stress in *Arabidopsis*. *Transgenic Res.* **22**: 327–341. doi:10.1007/s11248-012-9645-8.

Babu, M.M., Iyer, L., Balaji, S., and Aravind, L. 2006. The natural history of the WRKY-GCM1 zinc fingers and the relationship between transcription factors and transposons. *Nucleic Acids Res.* **34**: 6505–6520. doi:10.1093/nar/gkl888.

Bakshi, M., and Oelmüller, R. 2014. WRKY transcription factors Jack of many trades in plants. *Plant Signal. Behav.* **9**: e27700. doi:10.4161/psb.27700.

Bartels, D., and Sunkar, R. 2005. Drought and salt tolerance in plants. *Crit. Rev. Plant. Sci.* **24**: 23–58. doi:10.1080/07352680590910410.

Besseau, S., Li, J., and Palva, E. T. 2012. WRKY54 and WRKY70 co-operate as negative regulators of leaf senescence in. *Arabidopsis thaliana. J. Exp. Bot.* **63**(7): 2667–79. doi:10.1093/jxb/err450.

Bhattarai, K. K., Atamian, H. S., Kaloshian, I., and Eulgem, T. 2010. WRKY72-type transcription factors contribute to basal immunity in tomato and *Arabidopsis* as well as gene-for-gene resistance mediated by the tomato R gene Mi-1. *Plant J.* **63**: 229–240. doi:10.1111/j.1365-313X.2010.04232.x.

Birkenbihl, R. P., Kracher, B., and Somssich, I. E. 2017a. Induced genome-wide binding of three *Arabidopsis* WRKY transcription factors during early MAMP-triggered immunity. *Plant Cell* **29**: 20–38. doi:10.1105/tpc.16.00681.

Birkenbihl, R. P., Liu, S., and Somssich, I. E. 2017b. Transcriptional events defining plant immune responses. *Curr. Opin. Plant Biol.* **38**: 1–9. doi:10.1016/j.pbi.2017.04.004.

Brand, L. H., Fischer, N. M., Harter, K., Kohlbacher, O., and Wanke, D. 2013. Elucidating the evolutionary conserved DNA-binding specificities of WRKY transcription factors by molecular dynamics and in vitro binding assays. *Nucleic Acids Res.* **41**: 9764–9778. doi:10.1093/nar/gkt732.

Cai, M., Qiu, D., Yuan, T., Ding, X., Li, H., Duan, L. I. U., Xu, C., Li, X., and Wang, S. 2008. Identification of novel pathogen-responsive cis -elements and their binding proteins in the promoter of *OsWRKY13*, a gene regulating rice disease resistance. *Plant Cell Env.* **3**: 86–96.

Cai, T., Ye, X., Ma, Y., Wang, X., Chen, F., and Cheng, Z. 2013. Over-expression of 1-aminocyclopropane-1-carboxylic acid oxidase genes (ACO) from *Vitis vinifera* in tomato (*Solanum locopersicum*) and its effects on ethylene release rates. *J. Agri. Biotech.* **21**: 1037–1044.

Cai, Y., Chen, X., Xie, K., Xing, Q., Wu, Y., Li, J., Du, C., Sun, Z., and Guo, Z. 2014. Dlf1, a WRKY transcription factor, is involved in the control of flowering time and plant height in rice. *PLoS ONE* **9**(7): e102529. doi:journal.pone.0102529.

Century, K., Reuber, T. L., and Ratcliffe, O. J. 2008. Regulating the regulators: the future prospects for transcription-factor-based agricultural biotechnology products. *Plant Physiol.* **147**: 20–29. doi:10.1104/pp.108.117887.

Chen, F., Fasoli, M., Tornielli, G. B., Santo, S. D., Pezzotti, M., Zhang, L., Cai, B., and Cheng, Z. M. 2013a. The evolutionary history and diverse physiological roles of the grapevine calcium-dependent protein kinase gene family. *PLoS One* **8**: e80818. doi:10.1371/journal.pone.0080818.

Chen, J., Nolan, T., Ye, H., Zhang, M., Tong, H., Xin, P., Chu, J., Chu, C., Li, Z., and Yin, Y. 2017. Arabidopsis WRKY46, WRKY54 and WRKY70 transcription factors are involved in brassinosteroid-regulated plant growth and drought response. *Plant Cell* **29**: 1425–1439.

Chen, L., Zhang, L., and Yu, D. 2010. Wounding-induced WRKY8 is involved in basal defense in *Arabidopsis*. *Mol. Plant. Microbe. Interact.* **23**: 558–565. doi:10.1094/MPMI-23-5-0558.

Chen, X., Liu, J., Lin, G., Wang, A., Wang, Z., and Lu, G. 2013b. Overexpression of AtWRKY28 and AtWRKY75 in Arabidopsis enhances resistance to oxalic acid and Sclerotinia sclerotiorum. *Plant Cell Rep.* **32**: 1589–1599. doi:10.1007/s00299-013-1469-3.

Chen, Y.-F., Li, L.-Q., Xu, Q., Kong, Y.-H., Wang, H., and Wu, W.-H. 2009. The WRKY6 transcription factor modulates PHOSPHATE1 expression in response to low Pi stress in Arabidopsis. *Plant Cell* **21**: 3554–3566. doi:10.1105/tpc.108.064980.

Cheng, H., Li, H., Deng, Y., Xiao, J., Li, X., and Wang, S. 2015. The WRKY45-2 WRKY13 WRKY42 transcriptional regulatory cascade is required for rice resistance to fungal pathogen. *Plant Physiol.* **167**: 1087–1099. doi:10.1104/pp.114.256016.

Chujo, T., Miyamoto, K., Ogawa, S., Masuda, Y., Shimizu, T., Kishi-Kaboshi, M., Takahashi, A., Nishizawa, Y., Minami, E., Nojiri, H., Yamane, H., and Okada, K. 2014. Overexpression of phosphomimic mutated OsWRKY53 leads to enhanced blast resistance in rice. *PLoS One* **9**: e98737. doi:10.1371/journal.pone.0098737.

Chujo, T., Miyamoto, K., Shimogawa, T., Shimizu, T., Otake, Y., Yokotani, N., Nishizawa, Y., Shibuya, N., Nojiri, H., Yamane, H., Minami, E., and Okada, K. 2013. OsWRKY28, a PAMP-responsive transrepressor, negatively regulates innate immune responses in rice against rice blast fungus. *Plant Mol. Biol.* **82**: 23–37. doi:10.1007/s11103-013-0032-5.

Dai, X., Wang, Y., and Zhang, W. 2017. OsWRKY74, a WRKY transcription factor, modulates tolerance to phosphate starvation in rice. *J. Exp. Bot.* **67**: 947–960. doi:10.1093/jxb/erv515.

Dardick, C., Chen, J., Richter, T., Ouyang, S., and Ronald, P. 2006. The rice kinase database. A phylogenomic database for the rice kinome. *Plant Physiol.* **143**: 579–586. doi:10.1104/pp.106.087270.

Devaiah, B. N., Karthikeyan, A. S., and Raghothama, K. G. 2007. WRKY75 transcription factor Is a modulator of phosphate acquisition and root development in Arabidopsis. *Plant Physiol* **143**: 1789–1801. doi:10.1104/pp.106.093971.

Ding, Z., Yan, J., Li, G., Wu, Z., Zhang, S., and Zheng, S. 2014. WRKY41 controls Arabidopsis seed dormancy via direct regulation of ABI3 transcript levels not downstream of ABA. *Plant J.* **79**: 810–823. doi:10.1111/tpj.12597.

Doebley, J. F., Gaut, B. S., and Smith, B. D. 2006. The molecular genetics of crop domestication. *Cell* **127**: 1309–1321. doi:10.1016/j.cell.2006.12.006.

Duan, G., Li, L., and Liu, Q. 2014. A WRKY transcription factor from Malus domestica negatively regulates dehydration stress in transgenic Arabidopsis. *Acta Physiol. Plant.* **36**: 541–548. doi:10.1007/s11738-013-1434-3.

Duan, M., Nan, J., Liang, Y., Mao, P., Lu, L., Li, L., Wei, C., Lai, L., Li, Y., and Su, X. 2007. DNA binding mechanism revealed by high resolution crystal structure of Arabidopsis thaliana WRKY1 protein. *Nucleic Acids Res.* **35**: 1145–1154. doi:10.1093/nar/gkm001.

Eddy, S. R. 1996. Hidden Markov models. *Curr Opin Struc Biol.* **6**: 361–365. doi:10.1016/S0959-440X(96)80056-X.

Eddy, S. R. 2009. A new generation of homology search tools based on probabilistic inference. *Genome Inform.* **23**: 205–211.

Eulgem, T., Rushton, P. J., Robatzek, S., and Somssich, I. E. 2000. The WRKY superfamily of plant transcription factors. *Trends Plant Sci.* **5**: 199–206. doi:10.1016/S1360-1385(00)01600-9.

Eulgem, T., Rushton, P.J., Schmelzer, E., Hahlbrock, K., and Somssich, I.E. 1999. Early nuclear events in plant defence signalling: rapid gene activation by WRKY transcription factors. *EMBO J.* **18**: 4689–4699. doi:10.1093/emboj/18.17.4689.

Fan, X., Guo, Q., Xu, P., Gong, Y. Y., Shu, H., Yang, Y., Ni, W., Zhang, X., and Shen, X. 2015. Transcriptome-wide identification of salt-responsive members of the WRKY gene family in *Gossypium aridum*. *PLoS One* **10**: e0126148. doi:10.1371/journal.pone.0126148.

Feng, G., Li, Y., and Cheng, Z. M. 2014. Plant molecular and genomic responses to stresses in projected future CO_2 environment. *Crit. Rev. Plant. Sci.* **33**: 238–249. doi:10.1080/07352689.2014.870421.

Feuillet, C., Leach, J. E., Rogers, J., Schnable, P. S., and Eversole, K. 2011. Crop genome sequencing: lessons and rationales. *Trends Plant Sci.* **16**: 77–88. doi:10.1016/j.tplants.2010.10.005.

Finn, R. D., Bateman, A., Clements, J., Coggill, P., Eberhardt, Y., Eddy, S. R., Heger, A., Hetherington, K., Holm, L., Mistry, J., Sonnhammer, E. L. L., Tate, J., and Punta, M. 2014. Pfam: the protein families database. *Nucleic Acids Res.* **42**: 222–230. doi:10.1093/nar/gkt1223.

Gao, Q.-M., Venugopal, S., Navarre, D., and Kachroo, A. 2011. Low oleic acid-derived repression of jasmonic acid-inducible defense responses requires the WRKY50 and WRKY51 proteins. *Plant Physiol.* **155**: 464–476. doi:10.1104/pp.110.166876.

Gao, R., Liu, P., Yong, Y., and Wong, S.-M. 2016. Genome-wide transcriptomic analysis reveals correlation between higher WRKY61 expression and reduced symptom severity in Turnip crinkle virus infected Arabidopsis thaliana. *Sci. Rep.* **6**: srep24604. doi:10.1038/srep24604.

Goff, S. A., Ricke, D., Lan, T., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., Hadley, D., Hutchison, D., Martin, C., Katagiri, F., Lange, B, Moughamer, T., Xia, Y., Budworth, P., Zhong, J., Miguel, T., Paszkowski, U., Zhang, S., Colbert, M., Sun, W., Chen, L., Cooper, B., Park, S., Wood, T., Mao, L., Quail, P., Wing, R., Dean, R., Yu, Y., Zharkikin, A., Shen, R., Sahasrabudhe, S., Thomas, A., Cannings, R., Gutin, A., Pruss, D., Reid, J., Tavtigian, S., Mitchell, J., Eldredge, G., Scholl, T., Miller, R., Bhatnagar, S., Adey, N., Rubano, T., Tusneem, N., Robinson, R., Feldhaus, J., Macalma, T., Oliphant, A., and Briggs, S. 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science* **92**: 92–101. doi:10.1126/science.1068275.

Gonzalez, A., Brown, M., Hatlestad, G., Akhavan, N., Smith, T., Hembd, A., Moore, J., Montes, D., Mosley, T., Resendez, J., Nguyen, H., Wilson, L., Campbell, A., Sudarshan, D., and Lloyd, A. 2016. TTG2 controls the developmental regulation of seed coat tannins in Arabidopsis by regulating vacuolar transport steps in the proanthocyanidin pathway. *Dev. Biol.* **419**(1): 54–63. doi.org/10.1016/j.ydbio.2016.03.031.

Grunewald, W., de Smet, I., Lewis, D. R., Löfke, C., Jansen, L., Goeminne, G., Bossche, R. V., Karimi, M., De Rybel B., Vanholme, B., Teichmann, T., Boerjan, W., Van Montagu M. C. E., Gheysen. G., Muday, G. K., Friml, J., and Beeckman, T. 2012. Transcription factor WRKY23 assists auxin distribution patterns during Arabidopsis root development through local control on flavonol biosynthesis. *Proc. Natl. Acad. Sci. USA* **109**: 1554–1559. doi.org/10.1073/pnas.1121134109.

Guan, Y., Meng, X., Khanna, R., LaMontagne, E., Liu, Y., and Zhang, S. 2014. Phosphorylation of a WRKY transcription factor by MAPKs is required for pollen development and function in. *Arabidopsis. PLoS Genet* **10**(5): e1004384. doi:10.1371/journal.pgen.1004384.

Gu, Y., Li, W., Jiang, H., Wang, Y., Gao, H., Liu, M., Chen, Q., Lai, Y., and He, C. 2017. Differential expression of a *WRKY* gene between wild and cultivated soybeans correlates to seed size. *J. Exp. Bot.* **33**: 408–414.

Guth, S. I. E., and Wegner, M. 2008. Having it both ways: Sox protein function between conservation and innovation. *Cell. Mol. Life Sci.* **65**: 3000–3018. doi:10.1007/s00018-008-8138-7.

Han, X., Kumar, D., Chen, H., Wu, S., and Kim, J. Y. 2014. Transcription factor-mediated cell-to-cell signalling in plants. *J. Exp. Bot.* **65**: 1737–1749. doi:10.1093/jxb/ert422.

Hoang, N. V., Furtado, A., Mason, P.J., Marquardt, A., Kasirajan, L., Thirugnanasambandam, P. P., Botha, F. C., and Henry, R. J. 2017. A survey of the complex transcriptome from the highly polyploid sugarcane genome using full-length isoform sequencing and de novo assembly from short read sequencing. *BMC Genomics* **18**: 395. doi:10.1186/s12864-017-3757-8.

Hossain, M. A., Henríquez-Valencia, C., Gómez-Páez, M., Medina, J., Orellana, A., Vicente-Carbajosa, J., and Zouhar, J. 2016. Identification of novel components of the unfolded protein response in Arabidopsis. *Front. Plant. Sci.* **7**: 650. doi:10.3389/fpls.2016.00650.

Hsu, F.-C., Chou, M.-Y., Chou, S.-J., Li, Y.-R., Peng, H.-P., and Shih, M.-C. 2013. Submergence confers immunity mediated by the WRKY22 transcription factor in Arabidopsis. *Plant Cell* **25**: 2699–2713. doi:10.1105/tpc.113.114447.

Hu, Y., Chen, L., Wang, H., Zhang, L., Wang, F., and Yu, D. 2013. *Arabidopsis* transcription factor WRKY8 functions antagonistically with its interacting partner VQ9 to modulate salinity stress tolerance. *Plant J.* **74**: 730–745. doi:10.1111/tpj.12159.

Hu, Y., Dong, Q., and Yu, D. 2012. *Arabidopsis* WRKY46 coordinates with WRKY70 and WRKY53 in basal resistance against pathogen *Pseudomonas syringae*. *Plant Sci.* **185–186**: 288–297. doi:10.1016/j.plantsci.2011.12.003.

Hwang, S. H., Kwon, S. Il, Jang, J. Y., Fang, I. L., Lee, H., Choi, C., Park, S., Ahn, I., Bae, S. Chul, and Hwang, D. J. 2016. OsWRKY51, a rice transcription factor, functions as a positive regulator in defense response against *Xanthomonas oryzae* pv. *oryzae*. *Plant Cell Rep.* **35**: 1975–1985. doi:10.1007/s00299-016-2012-0.

Ishiguro, S., and Nakamura, K. 1994. Characterization of a cDNA encoding a novel DNA binding protein, SPF1, that recognizes SP8 sequences in the 5′ upstream regions of genes coding for sporamin and β-amylase from sweet potato. *Mol. Gen. Genet.* **244**: 563–571. doi:10.1007/BF00282746.

Jakoby, M., Wolfgang, D.-L., Jesus, V.-C., Jens, T., and Kroj, T. F. P. 2002. bZIP transcription factors in *Arabidopsis*. *Trends Plant Sci.* **7**: 106–111. doi:10.1016/S1360-1385(01)02223-3.

Jiang, J., Ma, S., Ye, N., Jiang, M., Cao, J., and Zhang, J. 2017. WRKY transcription factors in plant responses to stresses. *J. Integr. Plant Biol.* **59**: 86–101. doi:10.1111/jipb.12513.

Jiang, W., and Yu, D. 2009. *Arabidopsis WRKY2* transcription factor mediates seed germination and postgermination arrest of development by abscisic acid. *BMC Plant Biol* **9**: 96. doi:10.1186/1471-2229-9-96.

Jiang, Y., Liang, G., Yang, S., and Yu, D. 2014. Arabidopsis WRKY57 functions as a node of convergence for jasmonic acid- and auxin- mediated signaling in jasmonic acid induced leaf senescence. *Plant Cell* **26**: 230–245. doi:10.1105/tpc.113.117838.

Jiang, Y., Liang, G., and Yu, D. 2012. Activated expression of WRKY57 confers drought tolerance in arabidopsis. *Mol. Plant* **5**: 1375–1388. doi:10.1093/mp/sss080.

Jiang, Y., and Yu, D. 2016. WRKY57 regulates JAZ genes transcriptionally to compromise *Botrytis cinerea* resistance in Arabidopsis thaliana. *Plant Physiol.* **171**(4): 2771–2782. doi:10.1104/pp.16.00747.

Jin, J., Tian, F., Yang, D. C., Meng, Y. Q., Kong, L., Luo, J., and Gao, G. 2017. PlantTFDB 4.0: Toward a central hub for

transcription factors and regulatory interactions in plants. *Nucleic Acids Res.* **45**: D1040–D1045. doi:10.1093/nar/gkw982.

Jin, J., Zhang, H., Kong, L., Gao, G., and Luo, J. 2014. PlantTFDB 3.0: A portal for the functional and evolutionary study of plant transcription factors. *Nucleic Acids Res.* **42**: 1182–1187. doi:10.1093/nar/gkt1016.

Jing, S., Zhou, X., Song, Y., and Yu, D. 2009. Heterologous expression of OsWRKY23 gene enhances pathogen defense and dark-induced leaf senescence in Arabidopsis. *Plant Growth Regu.* **58**: 181–190. doi:10.1007/s10725-009-9366-z.

Johnson, C. S., Kolevski, B., and Smyth, D. R. 2002. *TRANSPARENT TESTA GLABRA2*, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor. *Plant Cell* **14**: 1359–1375. doi:10.1105/tpc.001404.

Journot-Catalino, N., Somssich, I. E., Roby, D., and Kroj, T. 2006. The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in Arabidopsis thaliana. *Plant Cell* **18**: 3289–3302. doi:10.1105/tpc.106.044149.

Kang, K., Park, S., Natsagdorj, U., Kim, Y. S., and Back, K. 2011. Methanol is an endogenous elicitor molecule for the synthesis of tryptophan and tryptophan-derived secondary metabolites upon senescence of detached rice leaves. *Plant J.* **66**: 247–257. doi:10.1111/j.1365-313X.2011.04486.x.

Kanofsky, K., Bahlmann, A. K., Hehl, R., and Dong, D. X. 2017. Combinatorial requirement of W- and WT-boxes in microbe-associated molecular pattern-responsive synthetic promoters. *Plant Cell Rep.* **36**: 971–986. doi:10.1007/s00299-017-2130-3.

Kasajima, I., Ide, Y., Yokota Hirai, M., and Fujiwara, T. 2010. WRKY6 is involved in the response to boron deficiency in *Arabidopsis thaliana*. *Physiol. Plant.* **139**: 80–92. doi:10.1111/j.1399-3054.2010.01349.x.

Kc, K., Lai, Z., Fan, B., Chen, Z., and Kim, K.-C. 2008. *Arabidopsis* WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. *Plant Cell* **20**: 2357–2371. doi:10.1105/tpc.107.055566.

Kim, C. Y., Vo, K. T. X., Nguyen, C. D., Jeong, D. H., Lee, S. K., Kumar, M., Kim, S. R., Park, S. H., Kim, J. K., and Jeon, J. S. 2016. Functional analysis of a cold-responsive rice WRKY gene, OsWRKY71. *Plant Biotechnol. Rep.* **10**: 13–23. doi:10.1007/s11816-015-0383-2.

Kim, K.-C., Fan, B., and Chen, Z. 2006. Pathogen-induced *Arabidopsis* WRKY7 is a transcriptional repressor and enhances plant susceptibility to *Pseudomonas syringae*. *Plant Physiol.* **142**: 1180–1192. doi:10.1104/pp.106.082487.

Klein, F., Raul, R., Sperotto, A., Koprovski, P., Janette, M., and Fett, P. 2010. Identification of Fe-excess-induced genes in rice shoots reveals a WRKY transcription factor responsive to Fe, drought and senescence. *Mol. Biol. Rep.* **37**: 3735–3745.

Kumar, S., Stecher, G., and Tamura, K. 2018. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger Datasets. *Mol. Biol. Evol.* **33**: 1870–1874. doi:10.1093/molbev/msw054.

Lai, Z., Vinod, K., Zheng, Z., Fan, B., and Chen, Z. 2008. Roles of *Arabidopsis* WRKY3 and WRKY4 transcription factors in plant responses to pathogens. *BMC Plant Biol.* **8**: 68. doi:10.1186/1471-2229-8-68.

Lan, A., Huang, J., Zhao, W., Peng, Y., Chen, Z., and Kang, D. 2013. A salicylic acid-induced rice (*Oryza sativa* L.) transcription factor OsWRKY77 is involved in disease resistance of Arabidopsis thaliana. *Plant Biol.* **15**: 452–461. doi:10.1111/j.1438-8677.2012.00664.x.

Li, D., Liu, P., Yu, J., Wang, L., Dossa, K., Zhang, Y., Zhou, R., and Wei, X. 2017a. Genome-wide analysis of WRKY gene family in the sesame genome and identification of the WRKY genes involved in responses to abiotic stresses. *BMC Plant Biol.* **17**: 152. doi:10.1186/s12870-017-1099-y.

Li, H., Gao, Y., Xu, H., Dai, Y., Deng, D., and Chen, J. 2013. ZmWRKY33, a WRKY maize transcription factor conferring enhanced salt stress tolerances in Arabidopsis. *Plant Growth Regul.* **70**: 207–216. doi:10.1007/s10725-013-9792-9.

Li, J., Wang, J., Wang, N., Guo, X., and Gao, Z. 2015. GhWRKY44, a WRKY transcription factor of cotton, mediates defense responses to pathogen infection in transgenic *Nicotiana benthamiana*. *Plant Cell, Tissue Organ Cult.* **121**: 127–140. doi:10.1007/s11240-014-0688-9.

Li, R., Zhu, H., Ruan, J., Qian, W., Fang, X., Shi, Z., Li, Y., Li, S., Shan, G., Kristiansen, K., Li, S., Ynag, H., Wang, J., and Wang, J. 2010. De novo assembly of human genomes with massively parallel short read sequencing. *Genome Res.* **20**: 265–272. doi:10.1101/gr.097261.109.

Li, S., Fu, Q., Chen, L., Huang, W., and Yu, D. 2011. *Arabidopsis thaliana* WRKY25, WRKY26, and WRKY33 coordinate induction of plant thermotolerance. *Planta.* **233**: 1237–1252. doi:10.1007/s00425-011-1375-2.

Li, S., Zhou, X., Chen, L., Huang, W., and Yu, D. 2010. Functional Characterization of *Arabidopsis thaliana* WRKY39 in Heat Stress. *Mol. Cell* **29**: 475–483. doi:10.1007/s10059-010-0059-2.

Li, Z., Peng, J., Wen, X., and Guo, H. 2012. Gene network analysis and functional studies of senescence-associated genes reveal novel regulators of *Arabidopsis* leaf senescence. *J. Integr. Plant Biol.* **54**(8): 526–539 doi:10.1111/j.1744-7909.2012.01136.x.

Li, Z., Zhao, Y., Liu, X., Jiang, Z., Peng, J., Jin, J., Guo, H., and Luo, J. 2017b. Construction of the leaf senescence database and functional assessment of senescence associated genes. *Methods Mol. Biol.* **1533**: 315–333 doi:10.1007/978-1-4939-6658-5_19.

Liu, H., Yang, W., Liu, D., Han, Y., Zhang, A., and Li, S. 2011. Ectopic expression of a grapevine transcription factor VvWRKY11 contributes to osmotic stress tolerance in Arabidopsis. *Mol. Biol. Rep.* **38**: 417–427. doi:10.1007/s11033-010-0124-0.

Liu, J., Chen, X., Liang, X., Zhou, X., Yang, F., Liu, J., He, S. Y., and Guo, Z. 2016. Alternative splicing of rice WRKY62 and WRKY76 transcription factor genes in pathogen defense. *Plant Physiol.* **171**: 1427–1442.

Li, W., Wang, H., and Yu, D. 2016. *Arabidopsis* WRKY transcription factors WRKY12 and WRKY13 oppositely regulate flowering under short-day conditions. *Mol. Plant* **9**(11): 1492–1503. doi:10.1016/j.molp.2016.08.003.

Liu, X. Q., Bai, X. Q., Qian, Q., Wang, X. J., Chen, M. S., and Chu, C. C. 2005. OsWRKY03, a rice transcriptional activator that functions in defense signaling pathway upstream of OsNPR1. *Cell Res.* **15**: 593–603. doi:10.1038/sj.cr.7290329.

Liu, X., Bai, X., Wang, X., and Chu, C. 2007. OsWRKY71, a rice transcription factor, is involved in rice defense

response. *J. Plant Physiol.* **164**: 969–979. doi:10.1016/j.jplph.2006.07.006.

Lu, K., Liang, S., Wu, Z., Bi, C., Yu, Y.-T., Ma, Y., Wang, X.-F., and Zhang, D.-P. 2016. Overexpression of an *Arabidopsis* cysteine-rich receptor-like protein kinase, CRK5, enhances abscisic acid sensitivity and confers drought tolerance. *J. Exp. Bot.* **67**: 5009–5027. doi:10.1093/jxb/erw266.

Luo, M., Dennis, E. S., Berger, F., Peacock, W. J., and Chaudhury, A. 2005. *MINISEED3 (MINI3), a WRKY* family gene, and *HAIKU2 (IKU2)*, a leucine-rich repeat *(LRR) KINASE* gene, are regulators of seed size in Arabidopsis. *Proc. Natl. Acad. Sci. USA.* **102**(48): 17531–17536. doi:10.1073pnas.0508418102.

Machens, F., Becker, M., Umrath, F., and Hehl, R. 2014. Identification of a novel type of WRKY transcription factor binding site in elicitor-responsive cis-sequences from Arabidopsis thaliana. *Plant Mol. Biol.* **84**: 371–385. doi:10.1007/s11103-013-0136-y.

Marè, C., Mazzucotelli, E., Crosatti, C., Francia, E., Stanca, A. M., and Cattivelli, L. 2004. Hv-WRKY38: A new transcription factor involved in cold- and drought-response in barley. *Plant Mol. Biol.* **55**: 399–416. doi:10.1007/s11103-004-0906-7.

Matsushita, A., Inoue, H., Goto, S., Nakayama, A., Sugano, S., Hayashi, N., and Takatsuji, H. 2013. Nuclear ubiquitin proteasome degradation affects WRKY45 function in the rice defense program. *Plant J.* **73**: 302–313. doi:10.1111/tpj.12035.

Meng, X., Yu, H., Zhang, Y., Zhuang, F., Song, X., Gao, S., Gao, C., and Li, J. 2017. Construction of a genome-wide mutant library in rice using CRISPR/Cas9. *Mol. Plant.* doi:10.1016/j.molp.2017.06.006.

Miao, Y., and Zentgraf, U. 2010. A HECT E3 ubiquitin ligase negatively regulates Arabidopsis leaf senescence through degradation of the transcription factor WRKY53. *Plant J.* **63**: 179–188. doi:10.1111/j.1365-313X.2010.04233.x.

Miller, G., Suzuki, N., Ciftci-Yilmaz, S., and Mittler, R. 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell Environ.* **33**: 453–467. doi:10.1111/j.1365-3040.2009.02041.x.

Mohanta, T.K., Park, Y.-H., and Bae, H. 2016. Novel genomic and evolutionary insight of WRKY transcription factors in plant lineage. *Sci. Rep.* **6**: 37309. doi:10.1038/srep37309.

Mukhtar, M. S., Deslandes, L., Auriac, M. C., Marco, Y., and Somssich, I. E. 2008. The Arabidopsis transcription factor WRKY27 influences wilt disease symptom development caused by Ralstonia solanacearum. *Plant J.* **56**: 935–947. doi:10.1111/j.1365-313X.2008.03651.x.

Oh, S. K., Baek, K. H., Park, J. M., Yi, S. Y., Yu, S. H., Kamoun, S., and Choi, D. 2007. Capsicum annuum WRKY protein CaWRKY1 is a negative regulator of pathogen defense. *New Phytol.* **177**: 977–989. doi:10.1111/j.1469-8137.2007.02310.x.

Ohama, N., Sato, H., Shinozaki, K., and Yamaguchi-Shinozaki, K. 2016. Transcriptional regulatory network of plant heat stress response. *Trends Plant Sci.* **22**(1): 1–13. doi:10.1016/j.tplants.2016.08.015.

Okay, S., Derelli, E., and Unver, T. 2014. Transcriptome-wide identification of bread wheat WRKY transcription factors in response to drought stress. *Mol. Genet. Genomics* **289**: 765–781. doi:10.1007/s00438-014-0849-x.

Pandey, S. P., Roccaro, M., Schön, M., Logemann, E., and Somssich, I. E. 2010. Transcriptional reprogramming regulated by WRKY18 and WRKY40 facilitates powdery mildew infection of Arabidopsis. *Plant J.* **64**: 912–923. doi:10.1111/j.1365-313X.2010.04387.x.

Pater, S. De, Greco, V., Pham, K., Memelink, J., and Kijne, J. 1996. Characterization of a zinc-dependent transcriptional activator from *Arabidopsis*. *Nucleic Acids Res.* **24**: 4624–4631 doi:10.1093/nar/24.23.4624.

Qiao, Z., Li, C. L., and Zhang, W. 2016. WRKY1 regulates stomatal movement in drought-stressed *Arabidopsis thaliana*. *Plant Mol. Biol.* **91**: 53–65. doi:10.1007/s11103-016-0441-3.

Qiu, D., Xiao, J., Ding, X., Xiong, M., Cai, M., Cao, Y., Li, X., and Xu, C. 2007. OsWRKY13 mediates rice disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signaling. *Mol. Plant. Microbe. Interact.* **20**: 492–499. doi:10.1094/MPMI-20-5-0492.

Raineri, J., Wang, S., Peleg, Z., Blumwald, E., and Chan, R.L. 2015. The rice transcription factor OsWRKY47 is a positive regulator of the response to water deficit stress. *Plant Mol. Biol.* **88**: 401–413. doi:10.1007/s11103-015-0329-7.

Ren, X., Chen, Z., Liu, Y., Zhang, H., Zhang, M., Liu, Q., Hong, X., Zhu, J., and Gong, Z. 2010. ABO3, a WRKY transcription factor, mediates plant response to abscisic acid and drought tolerance in Arabidopsis. *Plant J.* **63**: 417–429. doi:10.1111/j.1365-313X.2010.04248.x.

Ricachenevsky, F. K., Sperotto, R. A., Menguer, P. K., and Fett, J. P. 2010. Identification of Fe-excess-induced genes in rice shoots reveals a WRKY transcription factor responsive to Fe, drought and senescence. *Mol. Biol. Rep.* **37**: 3735–3745. doi:10.1007/s11033-010-0027-0.

Riechmann, J. L., and Ratcliffe, O. J. 2000. A genomic perspective on plant transcription factors. *Curr. Opin. Plant Biol.* **3**: 423–434. doi:10.1016/S1369-5266(00)00107-2.

Rinerson, C. I., Rabara, R. C., Tripathi, P., Shen, Q. J., and Rushton, P. J. 2015. The evolution of WRKY transcription factors. *BMC Plant Biol.* **15**: 66. doi:10.1186/s12870-015-0456-y.

Robatzek, S., and Somssich, I. E. 2002. Targets of AtWRKY6 regulation during plant senescence and pathogen defense. *Gene. Dev.* **16**: 1139–1149. doi:10.1101/gad.222702.

Rushton, P. J., Macdonald, H., Huttly, A. K., Lazarus, C. M., and Hooley, R. 1995. Members of a new family of DNA-binding proteins bind to a conserved cis-element in the promoters of a-Amy2 genes. *Plant Mol. Biol.* **48429**: 691–702. doi:10.1007/BF00041160.

Rushton, P. J., Torres, J. T., Parniske, M., Wernert, P., Hahlbrock, K., and Somssich, E. 1996. Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of. **15**: 5690–5700.

Sarris, P. F., Duxbury, Z., Huh, S. U., Ma, Y., Segonzac, C., Sklenar, J., Derbyshire, P., Cevik, V., Rallapalli, G., Saucet, S. B., Wirthmueller, L., Menke, F. L. H., Sohn, K. H., and Jones, J. D. G. 2015. A plant immune receptor detects pathogen effectors that target WRKY transcription factors. *Cell* **161**: 1089–1100. doi:10.1016/j.cell.2015.04.024.

Satapathy, L., Singh, D., Ranjan, P., Kumar, D., Kumar, M., Prabhu, K. V., and Mukhopadhyay, K. 2014. Transcriptome-wide analysis of WRKY transcription factors in wheat and their leaf rust responsive expression profiling. *Mol. Genet. Genomics* **289**: 1289–1306. doi:10.1007/s00438-014-0890-9.

Scarpeci, T. E., Zanor, M. I., Mueller-Roeber, B., and Valle, E. M. 2013. Overexpression of AtWRKY30 enhances abiotic stress tolerance during early growth stages in Arabidopsis thaliana. *Plant Mol. Biol.* **83**: 265–277. doi:10.1007/s11103-013-0090-8.

Schön, M., Töller, A., Diezel, C., Roth, C., Westphal, L., Wiermer, M., and Somssich, I. E. 2013. Analyses of wrky18 wrky40 plants reveal critical roles of SA/EDS1 signaling and indole-glucosinolate biosynthesis for Golovinomyces orontii resistance and a loss-of resistance towards Pseudomonas syringae pv. tomato AvrRPS4. *Mol. Plant. Microbe. Interact.* **26**: 758–67. doi:10.1094/MPMI-11-12-0265-R.

Shalem, O., Sanjana, N. E., and Zhang, F. 2015. High-throughput functional genomics using CRISPR-Cas9. *Nat. Rev. Genet.* **16**: 299–311. doi:10.1038/nrg3899.

Shen, H., Liu, C., Zhang, Y., Meng, X., Zhou, X., Chu, C., and Wang, X. 2012. OsWRKY30 is activated by MAP kinases to confer drought tolerance in rice. *Plant Mol. Biol.* **80**: 241–253. doi:10.1007/s11103-012-9941-y.

Shuo, Y., Liang, Z., Liu-yang, M., Jia-nan, S., Cai-qiang, S., Wei, F., and Jin-ping, L. 2016. The expression and binding properties of the rice WRKY68 protein in the Xa21-mediated resistance response to *Xanthomonas* oryzae pv. Oryzae. *J. Integr. Agr.* **3119**: 61265–5.

Songstad, D. D., Petolino, J. F., Voytas, D. F., Reichert, N. A., Petolino, J. F., Voytas, D. F., and Reichert, N. A. 2017. Genome editing of plants. *Crit. Rev. Plant Sci.* **36**: 1–23. doi:10.1080/07352689.2017.1281663.

Sun, C., Palmqvist, S., Olsson, H., Boren, M., Ahlandsberg, S., and Jansson, C. 2003. A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the iso1 promoter. *Plant Cell* **15**: 2076–2092. doi:10.1105/tpc.014597.

Su, T., Xu Q., Zhang F., Chen Y., Li L., Wu W., and Chen Y. 2015. WRKY42 modulates phosphate homeostasis through regulating phosphate translocation and acquisition in Arabidopsis. *Plant Physiol.* **167**: 1579–1591. doi:10.1104/pp.114.253799.

Swinnen, G., Goossens, A., and Pauwels, L. 2016. Lessons from domestication: targeting cis -regulatory elements for crop improvement. *Trends Plant Sci.* **21**: 506–515. doi:10.1016/j.tplants.2016.01.014.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**: 2725–2729. doi:10.1093/molbev/mst197.

Tao, Z., Kou, Y., Liu, H., Li, X., Xiao, J., and Wang, S. 2011. OsWRKY45 alleles play different roles in abscisic acid signalling and salt stress tolerance but similar roles in drought and cold tolerance in rice. *J. Exp. Bot.* **62**: 4863–4874. doi:10.1093/jxb/err144.

Tao, Z., Liu, H., Qiu, D., Zhou, Y., Li, X., Xu, C., and Wang, S. 2009. A pair of allelic WRKY genes play opposite roles in rice-bacteria interactions. *Plant Physiol.* **151**: 936–948. doi:10.1104/pp.109.145623.

The 3, 000 rice genomes project. 2014. The 3,000 rice genomes project. *Gigascience* **3**: 7. doi:10.1186/2047-217X-3-7.

The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **408**: 796–815. doi:10.1038/35048692.

Vanderauwera, S., Vandenbroucke, K., Inzé, A., Cotte, B. Van De, and Mühlenbock, P. 2012. AtWRKY15 perturbation abolishes the mitochondrial stress response that steers osmotic stress tolerance in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **109**: 20113–20118. doi:10.1073/pnas.1217516109.

Verk, M. C. van, Pappaioannou, D., Neeleman, L., Bol, J. F., and Linthorst, H. J. M. 2008. A novel WRKY transcription factor Is required for induction of PR-1a gene expression by salicylic acid and bacterial elicitors. *Plant Physiol.* **146**: 1983–1995. doi:10.1104/pp.107.112789.

Walker, J. M. 2011. *Plant Immunity Methods and Protocols*. Springer, New York. ISBN 978-1-61737-997-0. doi:10.1007/978-1-61737-998-7.

Wang, H., Hao, J., Chen, X., Hao, Z., Wang, X., Lou, Y., Peng, Y., and Guo, Z. 2007. Overexpression of rice WRKY89 enhances ultraviolet B tolerance and disease resistance in rice plants. *Plant Mol. Biol.* **65**: 799–815. doi:10.1007/s11103-007-9244-x.

Wang, H., Meng, J., Peng, X., Tang, X., Zhou, P., Xiang, J., and Deng, X. 2015. Rice WRKY4 acts as a transcriptional activator mediating defense responses toward Rhizoctonia solani, the causing agent of rice sheath blight. *Plant Mol. Biol.* **89**: 157–171. doi:10.1007/s11103-015-0360-8.

Wang, H., Xu, Q., Kong, Y., Chen, Y., Duan, J., Wu, W., and Chen, Y. 2014. Arabidopsis WRKY45 transcription factor activates *PHOSPHATE TRANSPORTER1;1* expression in response to phosphate starvation. *Plant Physiol.* **164**: 2020–2029. doi:10.1104/pp.113.235077.

Weigel, D., and Mott, R. 2009. The 1001 Genomes Project for *Arabidopsis thaliana*. *Genome Biol.* **10**: 107. doi:10.1186/gb-2009-10-5-107.

Wu, X., Shiroto, Y., Kishitani, S., Ito, Y., and Toriyama, K. 2009. Enhanced heat and drought tolerance in transgenic rice seedlings overexpressing OsWRKY11 under the control of HSP101 promoter. *Plant Cell Rep.* **28**: 21–30. doi:10.1007/s00299-008-0614-x.

Xie, Z. 2005. Annotations and functional analyses of the rice WRKY gene superfamily reveal positive and negative regulators of abscisic acid signaling in aleurone cells. *Plant Physiol.* **137**: 176–189. doi:10.1104/pp.104.054312.

Xing, D. H., Lai, Z. B., Zheng, Z. Y., Vinod, K. M., Fan, B. F., and Chen, Z. X. 2008. Stress- and pathogen-induced *Arabidopsis* WRKY48 is a transcriptional activator that represses plant basal defense. *Mol. Plant* **1**: 459–470. doi:10.1093/mp/ssn020.

Xu, X., Chen, C., Fan, B., and Chen, Z. 2006. Physical and functional interactions between and WRKY60 transcription factors. *Plant Cell* **18**: 1310–1326. doi:10.1105/tpc.105.037523.

Yamada, Y., and Sato, F. 2016. Tyrosine phosphorylation and protein degradation control the transcriptional activity of WRKY involved in benzylisoquinoline alkaloid biosynthesis. *Sci. Rep.* **6**: srep31988. doi:10.1038/srep31988.

Yang, P., Chen, C., Wang, Z., Fan, B., and Chen, Z. 1999. A pathogen- and salicylic acid-induced WRKY DNA-binding activity recognizes the elicitor response element of the tobacco class I chitinase gene promoter. *Plant J.* **18**: 141–149. doi:10.1046/j.1365-313X.1999.00437.x.

Yokotani, N., Sato, Y., Tanabe, S., Chujo, T., Shimizu, T., Okada, K., Yamane, H., Shimono, M., Sugano, S. Takatsuji, H., Kaku, H., Minami, E., and Nishizawa, Y., 2013. WRKY76 is a rice transcriptional repressor playing opposite roles in blast disease resistance and cold stress tolerance. *J. Exp. Bot.* **64**: 5085–5097. doi:10.1093/jxb/ert298.

Yu, S., Ligang, C., Liping, Z., and Diqiu, Y. 2010. Overexpression of OsWRKY72 gene interferes in the abscisic acid signal and auxin transport pathway of *Arabidopsis*. *J. Biosci.* **35**: 459–471. doi:10.1007/s12038-010-0051-1.

Yu, Y., Liu, S., Wang, L., Kim, S., Seo, P., Qiao, M., Wang, N., Li, S., Cao, X., Park, C., and Xiang, F. 2016. WRKY71 accelerates flowering via the direct activation of FLOWERING LOCUS T and LEAFY in. *Arabidopsis thaliana*. *Plant J.* **85**: 96–106. doi:10.1111/tpj.13092.

Yu, Y., Xu, W., Wang, J., Wang, L., Yao, W., Yang, Y., Xu, Y., Ma, F., Du, Y., and Wang, Y. 2013. The Chinese wild grapevine (*Vitis pseudoreticulata*) E3 ubiquitin ligase *Erysiphe necator*-induced RING finger protein 1 (EIRP1) activates plant defense responses by inducing proteolysis of the VpWRKY11 transcription factor. *New Phytol.* **1**: 834–846. doi:10.1111/nph.12418.

Zhang, H., Jin, J., Tang, L., Zhao, Y., Gu, X., Gao, G., and Luo, J. 2011. PlantTFDB 2.0 : update and improvement of the comprehensive plant transcription factor database. **39**: 1114–1117.

Zhang, H., Zhang, J., Lang, Z., Botella, J. R., and Zhu, J. 2017. Genome editing-principles and applications for functional genomics research and crop improvement. *Crit. Rev. Plant Sci.* **36**: 291–309. doi:10.1080/07352689.2017.1402989.

Zhang, J., Peng, Y., and Guo, Z. 2008. Constitutive expression of pathogen-inducible OsWRKY31 enhances disease resistance and affects root growth and auxin response in transgenic rice plants. *Cell Res.* **18**: 508–521. doi:10.1038/cr.2007.104.

Zhang, Y., and Wang, L. 2005. The WRKY transcription factor superfamily: its origin in eukaryotes and expansion in plants. *BMC Evol. Biol.* **5**: 1. doi:10.1186/1471-2148-5-1.

Zhang, Z. L., Shin, M., Zou, X., Huang, J., Ho, T. H. D., and Shen, Q. J. 2009. A negative regulator encoded by a rice WRKY gene represses both abscisic acid and gibberellins signaling in aleurone cells. *Plant Mol. Biol.* **70**: 139–151. doi:10.1007/s11103-009-9463-4.

Zhao, L., Cai, H., Su, Z., Wang, L., Huang, X., Zhang, M., and Chen, P. 2017. KLU suppresses megasporocyte cell fate through SWR1-mediated activation of WRKY28 expression in Arabidopsis. *Proc. Natl Acad. Sci. USA* **115**(3): E526–E535. doi:10.1073/pnas.1716054115.

Zhong, Y. F., Butts, T., and Holland, P. W. H. 2008. HomeoDB: A database of homeobox gene diversity. *Evol. Dev.* **10**: 516–518. doi:10.1111/j.1525-142X.2008.00266.x.

Zhou, X., Jiang, Y., and Yu, D. 2011. WRKY22 transcription factor mediates dark-induced leaf senescence in Arabidopsis. *Mol. Cells* **31**: 303–313. doi:10.1007/s10059-011-0047-1.

Zou, C., Jiang, W., and Yu, D. 2010. Male gametophyte-specific WRKY34 transcription factor mediates cold sensitivity of mature pollen in Arabidopsis. *J. Exp. Bot.* **61**: 3901–3914. doi:10.1093/jxb/erq204.