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The role of WRKY transcription factors in plants

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Abstract

The WRKY proteins are one of the largest families of transcriptional regulators in plants. Their biosynthesis is induced during certain stages of plant development and upon pathogen infection. A single WRKY transcription factor may be involved in the regulation of several apparently disparate processes. Their hallmark is strong conservation of the DNA binding domain which contains an invariant WRKYGQK amino acid sequence and a zinc binding motif. However, the overall sequences of individual representatives are highly divergent. Little is known about the 3D-structure of the WRKY proteins. Up to date there have only been results of structural strudies of DNA binding domain available. In this review, the biological function as well the structural studies of the WRKY proteins have been recapitulated.

Key words: WRKY transcription factors, biotic stress, abiotic stress, phytohormones

Introduction

The WRKY transcription factors have been broadly investigated in plants for more than 20 years. The first report on the WRKY transcription factor SPF1 from the sweet potato (*Ipomoea batatas*) has revealed its role in the induction of gene expression by sucrose (Ishiguro and Nakamura, 1994). The initial reports on WRKYs also defined their potential involvement in the regulation of ABF1 and ABF2 genes expression during germination (Rushton et al., 1995). In one of the first reports on the regulation of parsley response to pathogen, the name WRKY (pronounced "worky") family was created, together with the identification of other WRKY proteins: WRKY1, WRKY2 and WRKY3 (Rushton et al., 1996). Since then, an enormous progress in this field has been made. Recently, an access to genome sequencing programs allowed identifying putative WRKY proteins in different plant species. Many members of this family have been cloned and characterized. Moreover, using system biology approaches such as transcriptomic and promoter analyses, allows defining the WRKY's function in a signaling network. Last years have brought subsequent progress in the understanding of WRKYs function in many distant physiological and developmental processes that have revealed a complex network of their relationships.

Distribution among species

Since their first discovery in sweet potato (*Ipomea batata*), multiple genes for WRKY transcription factors have been experimentally identified from more than 80 other plant species (Jin et al., 2014), including *Arabidopsis thaliana*, tobacco (*Nicotiana tabacum*), wild oats (*Avena fatua*), rice (*Oryza sativa*), parsley (*Petroselinum crispum*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), soybean (*Glycine max*), potato (*Solanum tuberosum*), orchardgrass (*Dactylis glomerata*), chamomile (*Matricaria chamomilla*), sugarcane (*Saccharum*), cotton (*Gossypium arboreum*), grape (*Vitis vinifera*), poplar (*Populus trichocarpa*), sorghum (*Sorghum bicolor*) and coconut (*Cocos nucifera*). Most reports refer to angiosperm plants but WRKY have also been reported from gymnosperm *Pinus monticola* (Liu and Ekramoddoullah, 2009). Recently, some members of the WRKY family were identified in the course of searching all available sequence data from lower plants such as ferns (*Ceratopteris richardii*) and mosses (*Physcomitrella patens*). Homologues of WRKY genes have been found only in two non-photosynthetic species: in the slime mold *Dictyostelium discoideum* closely related to the lineage of animals and fungi, and in the unicellular protist *Giardia lamblia,* a primitive eukaryote and a green algae *Chlamy-*

domonas reinhardtii, an early branching of plants. WRKY proteins are a large superfamily of transcription factors. WRKY genes have also been identified in various plants mentioned above. They range from a single WRKY gene copy in a unicellular green alga *Chlamydomonas reinhardtii,* through 37 genes in the moss *Physcomitrella patens*, 74 in *Arabidopsis thaliana*, to at least 109 in rice *Oryza sativa* (Wu et al., 2005) and over 230 genes in soybean *Glycine max* (Zhang et al., 2011). Individual WRKY genes identified in the Arabidopsis genome by sequence similarity comparisons are a single copy of randomly distributed over five chromosomes. WRKY proteins vary in molecular weight from 14.3 kDa (AtWRKY43) to 210.3 kDa (AtWRKY19) (http://www.arabidopsis.org/browse/genefamily/WRKY.jsp). The number of WRKY genes varies in different species and increased during the evolution of plants. The WRKY family evolved from simpler to more complex multicellular organisms, demonstrating the ancient origin of the gene family. Comparing to fern, moss and pine, evolutionary expansion of WRKY gene family occurs in flowering plant genomes. The ancestral WRKY gene seems to be duplicated many times, resulting in a large family among evolutionarily advanced flowering plants. It has been proposed that this expansion is associated with the increasing complexity of plants and the development of highly sophisticated defense mechanisms adapted against pathogens.

To date the WRKY genes have been cloned only from plant species although genome sequence data for species representing several major eukaryotic lineages are already available. There is still no evidence for the presence of WRKY transcription factors (TF) in the animal kingdom. The absence of WRKY homologues in animal genomes i.e. *Caenorhabditis elegans* and *Drosophila melanogaster* and *Saccharomyces cerevisiae* may suggest that WRKY transcription regulators are restricted to the plant kingdom.

Structural features and classification of WRKY proteins

The characteristic feature of WRKY transcription factors is their DNA binding domain known as the WRKY domain. It is region of about 60 amino acids with a characteristic almost invariant to the amino acid sequence Trp-Arg-Lys-Tyr-Gly-Glu-Lys (WRKYGQK) at its amino-terminal end and with a putative zinc-finger motif at its carboxy-terminal end. In a few representatives of WRKY proteins from rice (*Oryza sativa*), the consensus WRKY amino acid sequences have been replaced by WRRY, WSKY, WKRY, WVKY or WKKY suggesting that W(R/K)(K/R)Y might be considered as a new consensus WRKY motif (Xie et al., 2005).

All known WRKY proteins contain either one or two WRKY domains and unique among all already described zinc-finger-like motifs. Despite the strong conservation of their DNA-binding domain, the overall sequence homology of the WRKY proteins outside this conserved region is low. Some WRKY transcription factors can be large and have a number of additional domains, others are slightly larger than the highly conserved DNA-binding domain, which is common in all WRKY transcription factors. Therefore the 74 *Arabidopsis thaliana* WRKY proteins were initially classified into three main groups and five subgroups on the basis of the number and type of their WRKY domains, differences within their zinc-finger motif and the presence of additional characteristic features (Fig. 1). Members of group I contain two WRKY domains, while most proteins which possess a single WRKY domain that belongs to group II or III. Generally, the WRKY domains of group I and group II members have the same type C2–H2 of the zinc-finger motif with a sequence pattern $C-X_{4-5}-C-X_{22-23}-H-X1-H$. In members assigned to group III, the WRKY domains contain a C2–HC zinc finger motif with sequence pattern $C-X_{5-8}-C-X_{25-28}-H-X_{1-2}-C$. Additionally, group II splits up into five distinct subgroups (IIa–e). This classification is based on the presence of ten additional structural motifs that are conserved among the different subsets of the AtWRKY family members. Each of these motifs is unique for a certain subgroup. In some cases, these motifs can reveal clues about their potential functions. They seem to be nuclear localization signals, phosphorylation or calmodulin binding sites or protein dimerization initiators, characteristic for leucine zippers (LZs). A few AtWRKY proteins (AtWRKY10 and AtWRKY38, and AtWRKY52) do not fit precisely to any of the previously established groups. For example AtWRKY10 possesses only one WRKY domain more related to group I. It might be a result of the secondary loss of the N-terminal WRKY domain. Moreover, when we take into consideration a pattern of Cys and His residues within WRKY domains, two other AtWRKY38 and AtWRKY52 seem to belong either to group III or represent members of a novel group. AtWRKY52 also posseses leucine-rich repeat (LRR) characteristic for resistance (R) proteins.

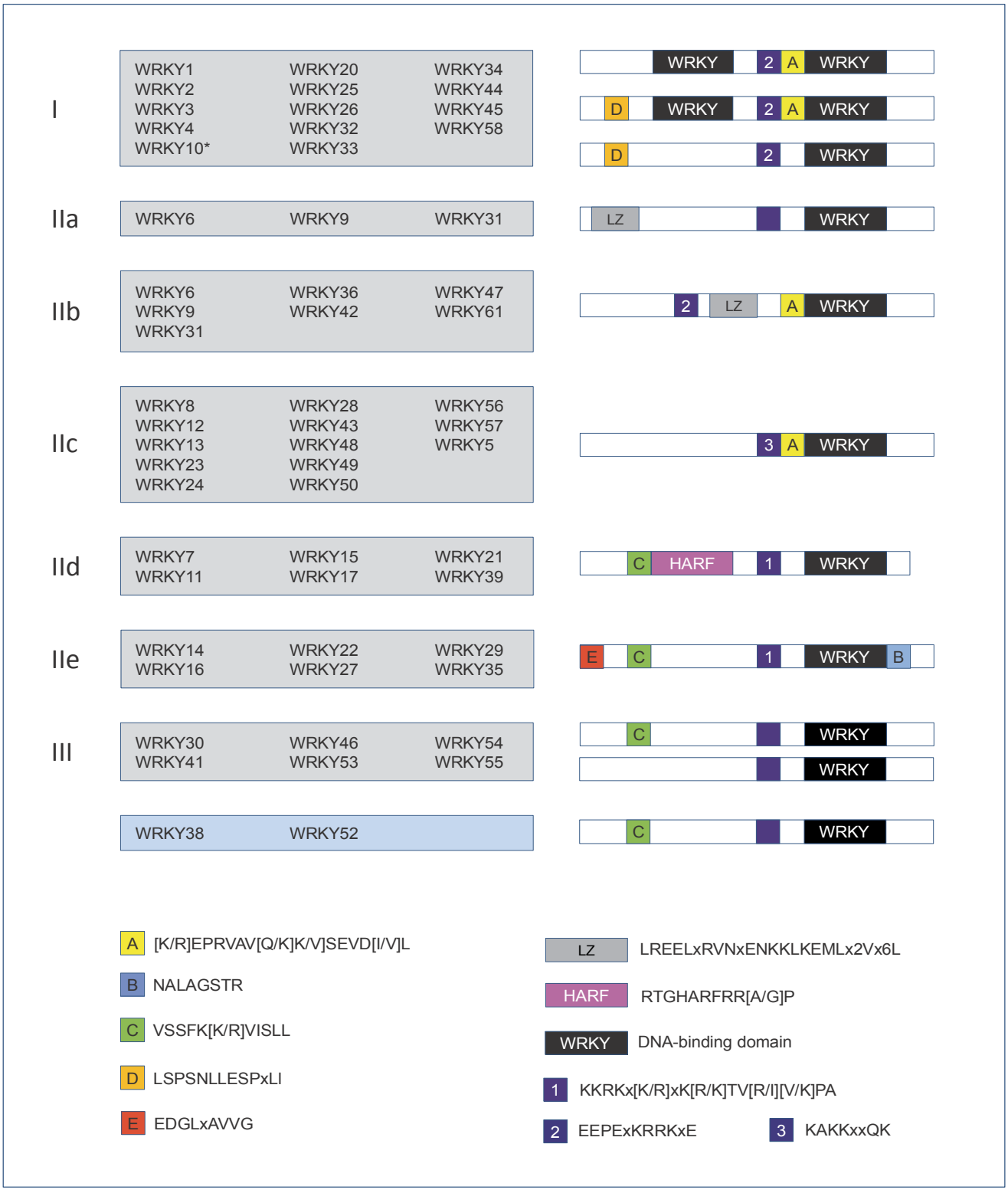

Fig. 1. Classification of AtWRKY Transcription Factors Family members, according to Eulgen et al. (2000)

Nevertheless, *in vivo* and *in vitro* experiments proved that members of all three groups of WRKY proteins possess clear binding preference for the same DNA sequence termed "W-box element" (TTGACY, where Y is C or T) found in the promoter regions of a large number of plant target genes (Ciolkowski et al., 2008; de Pater et al., 1996; Eulgem et al., 1999; Rushton et al., 1995; Rushton et al., 1996; Wang et al., 1998; Yang et al., 1999). The W-box elements contain invariant TGAC core, which is essential for the function and the binding by WRKY (Eulgem et al., 2000; Maeo et al., 2001). Functional W boxes frequently cluster in short promoter regions and act synergistically (Eulgem et al., 1999). Both the WRKY domains as well as the zinc finger motifs are required for proper DNA-protein binding (Maeo et al., 2001). The two WRKY domains of group I appear to be functionally distinct and, interestingly, the C-terminal WRKY domain, although not the N-terminal domain in I group representatives, is responsible for the DNA-binding activity (de Pater et al., 1996; Eulgem et al., 1999; Ishiguro and Nakamura, 1994). The function of the N-terminal WRKY domain remains unclear. Presumably, it might participate in the binding process increasing the affinity or specificity of these proteins for their target sites, or it might provide an interface for protein-protein interactions. Unexpectedly, the single WRKY domains of group II and III family members are more similar in sequence to the C-terminal than to the N-terminal WRKY domain of group I proteins (Fig. 2), which suggests that C-terminal and single WRKY domains are functionally equivalent and constitute the major DNA-binding activity. Moreover, the C-terminal WRKY domain sequence is supposed to be the ancestral type of WRKY gene because of its presence in primitive organisms such as protists or mosses.

Despite differences in zinc finger motives between groups I, II and III, experimental evidences have shown that members of all three groups bind specifically to various W-box elements. Experiments with the use of metal-chelators such as o-phenantroline and EDTA revealed that DNA binding was abolished and the inhibitory effect was relieved when Zn^{2+} was further added to the protein. Other metal cations such as Mg^{2+}, Cu^{2+}, Fe^{2+} or Cd^{2+} were ineffective and confirmed that Zn^{2+} is required for DNA binding activity (Maeo et al., 2001). Few researches have described substitutions of the conserved cysteine and histidine residues to alanine in the C_2H_2-type zinc finger-like motif in the WRKY domain. This replacement abolished the DNA-binding activity because the domain is stabilized by Zn^{2+} cation chelated by two cysteine residues appearing respectively at the end of strand 2 and at the beginning of strand 3 as well as the two conserved histidine residues occurring at the end of strand 4, which indicates that this structural motif is crucial for DNA binding (Maeo et al., 2001). Similarly, mutations within the consensus/invariable WRKYGQK sequence at the N-terminal side of the zinc finger-like motif also significantly reduced the DNA-binding activity. The mutation experiments have shown that the replacement of each of the conserved residues: Trp, Arg, two Lys, Tyr, and Gly to Ala significantly decreased or almost completely abolished the DNA-binding activity. These amino acid residues play an important role in the stabilization of the correct structure of DNA-protein complex and are critical for maintaining DNA-protein interactions (Maeo et al., 2001; Duan et al., 2007). Those experiments have been confirmed by the solved structure of AtWRKY4 domain in a complex with DNA (PDB:2LEX), suggesting that each of these residues together with Zn^{2+} cations is required for proper folding of the DNA-binding zinc finger and its binding activity.

Biological function

It is common for a single WRKY transcription factor to regulate transcriptional reprogramming associated with various biological processes. Studies carried out on different plants indicate that WRKY proteins are involved in the regulation of biotic or abiotic stress responses (Rushton et al., 2010) as well as plant development. The first experimentally confirmed function was that WRKY proteins play an essential role in the regulation of plant responses to pathogens as transcription factors. Many WRKY proteins are involved in the defense mechanism against an attack of pathogenic bacteria (Chen and Chen, 2002; Chen et al., 2002; Dellagi et al., 2000; Deslandes et al., 2002; Dong et al., 2003; Du and Chen, 2000; Gao et al., 2013), fungi (Chen et al., 2002; Chujo et al., 2013; Inoue et al., 2013; Schon et al., 2013), viruses (Chen et al., 2013; Chen et al., 2002; Wang et al., 1998; Yang et al., 1999) and oomycetes (Chen et al., 2002; Kalde et al., 2003; Mishra et al., 2013). Furthermore, WRKY proteins are upregulated upon the abiotic stress of wounding (Cheong et al., 2002; Hara et al., 2000; Mishra et al., 2013), salinity (Babitha et al., 2013; Bera et al., 2013; Brotman et al., 2013; Hu et al., 2013), drought

Fig. 2. Comparison of WRKY domain sequences from representatives of different groups of AtWRKY Transcription Factors. Gaps shown as dots have been inserted for optimal alignment. Residues that are highly conserved are in red and residues that bind zinc are highlighted in red boxes

(Babitha et al., 2013; Pnueli et al., 2002; Rizhsky et al., 2002; Wang et al., 2013), heat (Dang et al., 2013; Rizhsky et al., 2002), cold (Inoue et al., 2013; Pnueli et al., 2002), H_2O_2 effect (Vandenabeele et al., 2003) and UV radiation (Izaguirre et al., 2003). Some members of the family are implicated in other processes that are unique to plants, such as morphogenesis of trichomes and seeds (Johnson et al., 2002), senescence (Chen et al., 2002; Hinderhofer and Zentgraf, 2001; Robatzek and Somssich, 2001, 2002), dormancy (Pnueli et al., 2002), growth (C. Chen and Chen, 2002), starch (Sun et al., 2003), lignin (Guillaumie et al., 2010) and antocyanin biosynthesis (Johnson et al., 2002) and also metabolic pathways (Johnson et al., 2002; Rushton et al., 1995; Shim et al., 2013; Sun et al., 2003; Willmott et al., 1998). Moreover, a single WRKY transcription factor might be involved in regulating several apparently disparate plant processes. A single *WRKY* gene often responds to several factors evenly as a negative or positive regulator however, WRKY TF might also regulate the expression themselves. They have been isolated from different plants, but still, the role of an individual representative in regulating transcriptional reprogramming is not well characterized. This is due to the cross-talk and a very complex relationship between particular representatives.

The plant immune system

Plants are exposed to two types of stress: biotic and abiotic. Biotic stress is caused by parasitic microorganisms (viruses, bacteria, fungi), by other plants through overcrowding, allelopathy, or by trampling and gnawing animals. Plants become infected by pathogens of different lifestyles. Biotrophic pathogens are specialized to feed on living plant tissues and they have a narrow host range. Additionally, various strains of these pathogens have often adapted to a specific line of a given plant species. Many biotrophs live in the intercellular space between leaf mesophyll cells, and some produce haustoria. Necrotrophic pathogens are less specialized and they grow on plant tissues that are wounded, weakened or senescent. They frequently produce toxins that enable them to kill the host tissue prior to colonization. Abiotic stress factors are naturally occurring, often intangible, factors that may cause harm to the plants. The most basic stressors include: drought, wounding, salinity, extreme temperatures, H_2O_2 effect and UV radiation, as well as more extreme such as natural disasters: flood, tornadoes and wildfires. Abiotic stress is essentially unavoidable. Stress factors induce changes in the plant hormone homeostasis, which can cause programmed cell death. The genetic basis of this mechanism is still poorly understood. Therefore studies of the molecular basis of plant resistance to stress can contribute to the development of more resistant plants.

Plants have developed a highly complex immune system that enables them to respond to pathogen infection or environmental stress. Plants, unlike mammals, lack mobile defender cells. Without the adaptive immune

system, they rely on the innate immunity of each cell and on systemic signals originating from infection sites to defend against most potential pathogens.

Based mainly on studies with the model plant *Arabidopsis thaliana*, two branches of plant's innate immune system are currently distinguished: pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), depending on the manner by which pathogens are recognized (Dodds and Rathjen, 2010).

PTI is a type of plant innate immunity that is triggered upon the identification/recognition of a microbe associated molecular patterns (MAMPs) through the corresponding pattern recognition receptors (PRRs) located mainly in the plasma membrane. MAMPs are common molecules characteristic of microbes that are not found in host cells. Both non-pathogenic and pathogenic microbes produce effective MAMPs to activate immune responses. Specific receptors with extracellular leucine-rich repeats (LRRs) subsequently transduce signal through MAP-kinase cascades, which ultimately leads to the primary defense response. *A. thaliana* recognizes a variety of MAMPs including the most described flagellin (flg22), lipopolisaccharide (lps) and elongation factor Tu (elf18) originating from bacteria or fungal chitin and β-glucan (Tsuda et al., 2009; Wan et al., 2008; Zipfel et al., 2006; Zipfel et al., 2004). Plants also respond to other factors such as small molecules (ATP) and cell walls or cuticular fragments. The first identified and best studied PRR is the flagellin receptor FLS2. It consists of the N-terminal signal peptide, 28 LRRs, a transmembrane domain, and a cytoplasmic kinase domain. In Arabidopsis, it perceives a minimal motif of 22 amino acid residues of the flagellin protein of bacterial flagella (flg22). Binding of flg22 to the corresponding receptor FLS2 results in the endocytosis of the complex. The internalization of endosome is kinase dependent and relies on the PEST motif that is related to ubiquitinylation. Upon MAMPs recognition, the first line of defense is achieved and leads to a range of defense responses and reprogramming of the whole metabolism including activation, suppression, and modulation of various signaling pathways in plant cells, which prevents further pathogen expansion. After that, cell wall modification, callose deposition and accumulation of defense-related proteins are initiated. Such processes negatively affect the colonization of pathogens. PTI is an ancient conserved first layer of a plant innate immune response. To successfully grow and proliferate on their host, virulent pathogens have to override the first line of defense. Plants do not have an adaptive immune system to eliminate pathogens that have entered their intercellular spaces and vascular systems. PTI is therefore effective against a broad spectrum of invading microorganisms but has is a relatively weak immune response. Moreover, plant pathogens are able to break or suppress the basal defense activated in the primary innate immune system. Pathogens, by producing effectors, successfully proliferate on host plants and cause diseases.

The second type of immunity involves recognition of pathogen virulence molecules, called effectors, by intracellular receptors. This induces an effector-triggered immunity (ETI). ETI is the result of co-evolution between pathogens and plants. Viral, bacterial, fungal and oomycete pathogens, during evolution developed production of effector molecules which are secreted thus targeting key PTI elements to interfere with plant defence. Some plants have evolved R proteins to directly or indirectly detect these effectors named avirulence or Avr proteins. ETI is a faster and stronger version of PTI that often culminates in a hypersensitive response (HR) being a form of a programmed cell death. The hypersensitive response is a mechanism that prevents the spreading of infection to other parts of the plant. The HR causes a rapid death of cells in the local region surrounding an infection. HR cell death may, typically, retard or stop pathogen growth in some interactions, particularly those involving haustorial parasites. The resulting necrotic lesions are one of the first visible manifestations of defense responses and are thought to aid the confinement of the pathogen to the dead cells. HR is not always observed, nor required for ETI. Particularly the mechanism of HR is initiated by the activation of *R* genes, which in effect trigger ion flux and accumulation of reactive oxygen species (ROS): superoxide anions, hydrogen peroxide, hydroxyl radicals and nitrous oxide further inducing lipid peroxidation and membrane damage. HR causes disease resistance by depriving the incoming pathogen of nutrients or by releasing compounds from dying cells which are destructive to microbes. For a subset of effectors, the mechanism of suppression has been elucidated. The *Pseudomonas syringae* effector AvrPto promotes infection in susceptible plants and abolishes responses elicited by MAMPs. AvrPto binds receptor kinases, including Arabidopsis FLS2 and EFR, to block plant immune responses in the plant cell. The ability to

target receptor kinases is required for the virulence function of AvrPto in plants. This model illustrates the dynamic coevolution between plants and pathogens (Chisholm et al., 2006). Apart from suppressing the hypersensitive response, some plant pathogens produce small molecule effectors that mimic plant hormones. Pathogenic bacteria *P. syringae* AvrPtoB also induces the production of coronatine, a jasmonic acid (JA) analogue that suppresses salicylic acid-induced defense responses to biotrophic pathogens. It induces stomatal opening, helping pathogenic bacteria to gain access to the apoplast. Fungal pathogen of rice *Gibberella fujikuroi* produces a plant hormone that causes hypertrophy, etiolation and chlorosis. Affected plants are infertile with empty panicles, producing no edible grains ("foolish seedling disease"). Cytokinins produced by many pathogens can promote pathogen success through the retardation of senescence in an infected leaf tissue. ETI effectiveness is qualitatively stronger and faster than PTI and often involves a localized cell death called the hypersensitive response (HR) (Dangl et al., 1996). PTI is generally effective against non-adapted pathogens in a phenomenon called non-host resistance, whereas ETI is active against adapted pathogens. However, these relationships are not exclusive and depend on the elicitor molecules produced by each infectious pathogen. Extreme diversification of ETI receptors and pathogen effectors within and between species is common.

Besides local immune responses, PTI and ETI activate long-distance defense reactions such as systemic acquired resistance (SAR) which predispose plants to become more resistant to subsequent pathogen attacks (Mishina and Zeier, 2007). In *A. thaliana* and other higher plants, local and systemic defense responses are controlled by the balanced action of distinct, but partially interconnected pathways involving several phytohormones, including salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). In general SA signaling pathway is essential for the resistance toward biotrophic and hemibiotrophic pathogens whereas the JA and ET sectors are important for immunity toward necrotrophs.

Systemic acquired resistance (SAR) is a mechanism of induced defense that confers long-lasting protection against a broad spectrum of microorganisms. SAR requires the signal molecule (salicylic acid) and is associated with accumulation of pathogenesis-related proteins (PR proteins), which are thought to contribute to resistance. Up to date, PR proteins have been classified into 17 families (van Loon et al., 2006) based on their biological role and/or physicochemical properties (sequence similarity, molecular mass, isoelectric point). The biological functions of most classes of the defense proteins have been recognized, including chitinases, β-glucanases, peroxidases and protein inhibitors (Van Loon et al., 1994). Some of them are involved in antimicrobial metabolites production with a crucial role in induced plant disease resistance. The role of some PR proteins, including PR-10, in defense response still remains to be elucidated. In response to SA, the positive regulator protein NPR1 moves to the nucleus where it interacts with TGA transcription factors and induces a defense gene expression, thus activating SAR.

The role of WRKY transcription factors in plant defense

Extensive studies have demonstrated that plant WRKY transcription factors play important roles in the two branches of the plant innate immune system, PTI and ETI.

Studies using WRKY knockout or knockdown mutants or plant lines overexpressing *WRKY* genes have shown that WRKY TF can positively or negatively regulate various aspects of plant PTI and ETI. It was also well established that those regulators rarely act alone. Functional redundancy causes difficulties in linking specific WRKY with a definite process. For example, the AtWRKY70 protein acts as an integrator of a cross-talk between SA and JA in plant defense responses (Shim and Choi, 2013). It functions as an activator of SA-dependent defense genes and a repressor of JA-regulated genes. Moreover, AtWRKY70 is required for both, the basal defense and the full R-gene mediated disease resistance against the oomycete *Hyaloperonospora parasitica* (Knoth et al., 2007), bacteria *Erwinia carotovora* and *Pseudomonas syringae* (Dong et al., 2003) as well as the fungi *Erysiphe cichoracearum* (Li et al., 2006). Recent publications have provided a conclusive genetic proof that Arabidopsis WRKY proteins are crucial regulators of the defense responses against both biotrophic and necrotrophic pathogens. For example, a disruption of *AtWRKY33* enhances susceptibility to necrotrophic fungal pathogens *Botrytis cinerea* and *Alternaria brassicicola* (Zheng et al., 2006). Further investigations showed that AtWRKY33 physically interacts with genes in-

volved in the redox homeostasis, SA signaling, ethylene-JA mediated cross-communication, camalexin biosynthesis and thus is a key transcriptional regulator of hormonal and metabolic responses towards *Botrytis cinerea* infection (Birkenbihl et al., 2012). A functional analysis based on T-DNA insertion mutants and transgenic overexpression lines indicates that AtWRKY3 and AtWRKY4 also function as positive regulators in plant resistance against *B. cinerea* (Lai et al., 2008), similarly to AtWRKY8 (Chen et al., 2010). Several WRKY factors act as negative regulators of resistance. For instance, basal plant resistance triggered by avirulent *P. syringae* strain was enhanced in *Atwrky*7 and *Atwrky*11/*Atwrky*17 insertional mutants (Journot-Catalino et al., 2006). Likewise, the disruption of *AtWRKY38* or *AtWRKY62* enhances plant basal defense against *P. syringae*. Induction of *AtWRKY38* or *AtWRKY62* reduces disease resistance and *PR1* expression, thus they function additively as negative regulators of plant basal defense (Kim et al., 2008).

A recent study suggests that AtWRKY51 may have function as a positive regulator of basal defense against *P. syringae* (Gao et al., 2011). In addition, AtWRKY25 and AtWRKY72 were also shown as regulators in response to biotrophs *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis* (Bhattarai et al., 2010; Zheng et al., 2007), whereas three representatives of a small subgroup IIa of WRKY genes, comprising *AtWRKY18*, *AtWRKY40*, and *AtWRKY60*, play important functions in regulating plant disease resistance toward *P. syringae, B. cinerea* and *Golovinomyces orontii* infection. A functional analysis of single, double, and triple combinations of *wrky18*, *wrky40* and *wrky60* mutants for response to microbial pathogens indicated that AtWRKY18, AtWRKY40, and AtWRKY60 proteins have partially redundant roles in activating defense to the fungal necrotroph *Botrytis cinerea* and repressing basal resistance to a virulent strain of the bacterial biotroph *Pseudomonas syringae* (Xu et al., 2006). These three WRKY transcription factors play complex and antagonistic roles in plant disease resistance. Synthesis of Arabidopsis WRKY22 and WRKY29 is induced by a MAPK pathway that confers resistance to both bacterial and fungal pathogens and expression of *AtWRKY29 gene* in transiently transformed leaves led to reduced disease symptoms (Asai et al., 2002). Two additional WRKY transcription factors, AtWRKY53 and AtWRK58 were identified as modulators of SAR and they act as positive and negative regulators respectively (Wang et al., 2006). Furthermore, the AtWRKY52 representative of group III that possess an atypical structural feature – zinc finger motif, was shown to confer resistance toward the bacterial pathogen *Ralstonia solanacearum.* (Deslandes et al., 2002). It combines a typical for R proteins nucleotide binding leucine-rich repeat (NB-LRR) and Toll/interleukin-1 receptor (TIR) domain with WRKY domain.

These results indicate that the WRKY proteins interact functionally in a complex pattern of overlapping, antagonistic, and distinct roles in plant responses to different types of microbial pathogens. The above, are but a few examples of varied AtWRKY functions in plant immunity to indicate the complexity of this subject. More detailed information can be found in Table 1.

The role of WRKY transcription factors in abiotic stress

Plants are unable to move and therefore they are simultaneously subjected to different stress factors. Adaptation of plants to unfavorable environmental changes involves a series of complex physiological and biochemical mechanisms. Moreover, plant responses to abiotic stress conditions are very diverse among species. Also single representatives of the same species, even from a plant living in the same area, respond uniquely. There is no universal defense response although some common mechanisms can be elucidated. Compared to the research on biotic stress, little is known about the involvement of these TFs in abiotic stress responses. A single WRKY protein is often involved in several stress responses, and some of them are even involved in both abiotic and biotic stresses. A cross-talk between signaling networks involved in the responses to biotic and abiotic stress is very complex. Furthermore, it is difficult to distinguish unambiguously which WRKY is associated with a particular stress response.

Microarray profiling/analyses of the *A. thaliana* root transcriptome revealed induction of 18 *WRKY* genes and repression of 8 *WRKY* genes in response to the salinity stress. In other microarray experiments *Atwrky6* and *Atwrky75* were among the 27 transcripts elevated at least five-fold in data sets related to oxidative stress response (Gadjev et al., 2006). Similarly, Arabidopsis WRKY18, WRKY40 and WRKY60 proteins were reported to respond in a complex pattern not only to pathogens but also to salt and osmotic stress (Chen et al., 2010).

Table 1. List of WRKY transcription factors and its fuction

Gene	Induction factor	Function	Ref.
AtWRKY1	SA	defense response, SAR	(Duan et al., 2007)
AtWRKY2	NaCl, mannitol	negative regulator in ABA signaling, regulation of seed germination and post germination growth	(Jiang and Yu, 2009a, 2009b)
AtWRKY3	*B. cinerea*, SA, JA, ACC	positive role in plant resistance to necrotrophic pathogens	(Lai et al., 2008)
AtWRKY4	*P. syringae*, SA, JA, sucrose, senescence, cold, salinity	negative effect on plant resistance to biotrophic pathogens, carbohydrate metabolism	(Hammargren et al., 2008; Lai et al., 2008)
AtWRKY6	H_2O_2, methyl viologen, Pi and B starvation	negative regulator in low Pi stress and positive regulator in low B stress	(Chen et al., 2009; Kasajima et al., 2010)
AtWRKY7	SA, *P. syringae*	negative regulator of plant defense against *P. syringae*	(Kim et al., 2006)
AtWRKY8	NaCl, wounding, *P. syringae*	salinity stress tolerance, repressor of plant PTI signaling, defense response against TMV-cg	(Chen et al., 2013; X. Gao et al., 2013; Hu et al., 2013)
AtWRKY10		seed developement	(Luo et al., 2005)
AtWRKY11	*P. syringae*	negative regulator of basal resistance toward *P. syringae,* regulation of JA-dependent responses	(Journot-Catalino et al., 2006)
AtWRKY17	*P. syringae,* NaCl	negative regulator of basal resistance toward *P. syringae,* regulation of JA-dependent responses, NaCl tolerance	(Journot-Catalino et al., 2006) (Jiang and Deyholos, 2006)
AtWRKY18	ABA, SA, *P. syringae, B. cinerea*	ABA signaling, NaCl and mannitol tolerance, regulation of defense response to bacteria and fungi, resistance to *P. syringae*	(Chen and Chen, 2002; Chen et al., 2010; Schon et al., 2013; Shang et al., 2010)
AtWRKY22	H_2O_2, dark, chitin, flagellin	regulation of dark-induced senescence, resistance to pathogens	(Asai et al., 2002; Wan et al., 2008; Zhou et al., 2011)
AtWRKY23	*H. schachtii*, auxin	resistance to nematode, stem cell specification	(Grunewald et al., 2013; Grunewald et al., 2008)
AtWRKY25	*P. syringae,* ABA, ethylene, NO, NaCl, mannitol, cold, heat	tolerance to heat and NaCl, increased sensitivity to oxidative stress and ABA, negative regulator of defense response to *P. syringae*	(Jiang and Deyholos, 2009; Li et al., 2009; Li et al., 2011)
AtWRKY26	heat	heat tolerance, dehydration stress	(Li et al., 2011)
AtWRKY28	NaCl, mannitol, H_2O_2	dehydration, salt and oxidative stress	(Babitha et al., 2013)
AtWRKY29	chitin, flagellin, *P. syringae*	defense response	(Asai et al., 2002; Wan et al., 2008)
AtWRKY30	H_2O_2, ozone, SA	abiotic stress tolerance, regulation of senescence	(Besseau et al., 2012; Scarpeci et al., 2013)
AtWRKY33	NaCl, mannitol, cold, heat, H_2O_2, ozone, UV, chitin, *B. cinerea, P. syringae, A. brassiciola*	heat and NaCl tolerance, redox homeostasis, resistance to *B. cinerea and P. syringae*, SA signaling, ethylene-JA-mediated cross-communication, camalexin biosynthesis	(Birkenbihl et al., 2012; Y. Jiang and Deyholos, 2009; Li et al., 2011; Wan et al., 2008; Zheng et al., 2006)
AtWRKY34	cold, sucrose	cold tolerance, carbohydrate metabolism, pollen developement	(Hammargren et al., 2008; Zou et al., 2010)
AtWRKY38	chitin, SA, *P. syringae,*	negative regulator of plant basal defense, regulation of HR	(Hammargren et al., 2008; Kim et al., 2008)
AtWRKY39	heat, drought	tolerance to heat, dehydration stress	(Ding et al., 2014; Zou et al., 2010)

Gene	Induction factor	Function	Ref.
AtWRKY40	ABA, SA, chitin, wounding, *P. syringae, B. cinerea*	ABA signaling, defense response, thermotolerance	(Chen et al., 2010; Li et al., 2009; Liu et al., 2012; Shang et al., 2010; Shen et al., 2007)
AtWRKY41	*P. syringae, E. carotovora*	resistance to *P. syringae*, susceptibility to *E. carotovora*, regulator in the cross talk of salicylic acid and jasmonic acid pathways	(Higashi et al., 2008)
AtWRKY42	Pi starvation	Pi deficiency stress	(Chen et al., 2009)
AtWRKY44		proanthocyanidin synthesis, seed mucilate deposition, seed coat development,	(Johnson et al., 2002)
AtWRKY45	Pi starvation	Pi deficiency stress	(Wang et al., 2014)
AtWRKY46	heat, NaCl, K starvation, *P. syringae*	thermotolerance, osmotic stress, K deficiency stress, basal pathogen resistance	(Ding et al., 2014; Hu et al., 2012; Li et al., 2009; Meier et al., 2008)
AtWRKY48	*P. syringae*	repressors of plant PTI signaling	(Gao et al., 2013)
AtWRKY50	*B. cinerea*	SA- and low 18:1-dependent repression of JA signaling.	(Gao et al., 2011)
AtWRKY51	*B. cinerea*	SA- and low 18:1-dependent repression of JA signaling.	(Gao et al., 2011)
AtWRKY52	SA, *R. solanacearum*	resistance to Ralstonia solanacearum	(Deslandes et al., 2002)
AtWRKY53	Chitin, flagellin, *P. syringae*, SA, H2O2, wounding	tolerance to oxidative stress, regulator of SAR and basal pathogen response, leaf development, senescence	(Ding et al., 2014; Hu et al., 2012; Wan et al., 2008; Xie et al., 2014)
AtWRKY54	H_2O_2	oxidative stress, negative regulator of leaf senescence	(Besseau et al., 2012)
AtWRKY58		regulator of SAR	(Wang et al., 2006)
AtWRKY60	NaCl, SA, *P. syringae, B. cinerea*	salt and osmotic stress, ABA signaling, defense response	(Chen et al., 2010; Liu et al., 2012; Shang et al., 2010; Shen et al., 2007)
AtWRKY62	*P. syringae*	negative regulator of plant basal defense	(Kim et al., 2008)
AtWRKY63	water deficiency, ABA	positive regulator in drought tolerance, negative regulator in ABA signaling	(Ren et al., 2010)
AtWRKY65	Fe starvation	iron deficiency stress	(http://www.arabidopsis.org/browse/genefamily/WRKY.jsp)
AtWRKY70	H_2O_2, *H. parasitica, E. cichoracearum, P. syringae, E. carotovora*	response to reactive oxygen species, activator of SA-dependent defense genes and a repressor of JA-regulated genes, basal and full R-gene mediated pathogen resistance, negative regulators of leaf senescence	(Besseau et al., 2012; ong et al., 2003; Knoth et al., 2007; Li et al., 2006; Shim et al., 2013)
AtWRKY72	oomycete *H. arabidopsidis*	basal defense response	(Bhattarai et al., 2010)
AtWRKY75	Pi starvation	positive regulator in Pi starvation	(Devaiah et al., 2007)

In recent years, numerous groups have demonstrated that manipulation of WRKY TF levels in knockout or overexpressor plants affects specific stress responses. Two closely related AtWRKY25 and AtWRKY33 are involved in response to heat, drought and osmotic stress (Jiang and Deyholos, 2009). The *wrky25* mutants exhibited deficient thermotolerance at different stages of growth, while *AtWRKY25* overexpressing plants displayed enhanced thermotolerance compared to the wild-type plants (Li et al., 2009). Furthermore, an earlier study showed the induction of *AtWRKY25* during the oxidative stress (Rizhsky et al., 2004). Thus AtWRKY25 is involved in various stress responses. In other work, the *AtbHLH17* and *AtWRKY28* genes which are known to be upregulated

under drought and oxidative stress in Arabidopsis were expressed. The transgenic lines showed an enhanced tolerance to NaCl, mannitol, and oxidative stress. Under mannitol stress conditions, also a higher root growth was observed (Babitha et al., 2013). These examples demonstrate that the WRKYs might be a powerful tool for the generation of drought resistance plants.

WRKY might enhance cold as well as heat tolerance. The WRKY34 transcription factor negatively mediated cold sensitivity of mature Arabidopsis pollen. Otherwise, functional analysis indicated that the WRKY34 transcription factor was also involved in pollen development. Mature pollen is very sensitive to cold stress in chilling-sensitive plants. *AtWRKY34* gene might be involved in pollen viability, although the mechanism is unclear. Cold treatment increased *AtWRKY34* gene expression in wild-type plants, and promoter-GUS analysis revealed that *AtWRKY34* gene expression is pollen-specific (Zou et al., 2010).

Arabidopsis WRKY39 provides an evident example for a TF that is involved in heat acclimation of plants. Heat-treated seeds and *wrky39* knockdown mutants had increased susceptibility to heat stress, showing reduced germination, decreased survival and elevated electrolyte leakage compared to wild-type plants. Additionally, *AtWRKY39* gene overexpressing plants exhibited enhanced thermotolerance compared to wild-type plants (Li et al., 2010). WRKY also participate in tolerance to micro and macro nutrients deficiency. AtWRKY6 and AtWRKY42 are involved in Arabidopsis responses to low phosphate stress through regulation of *PHOSPHATE1* (*AtPHO1*) gene expression (Chen et al., 2009). Moreover, a transcriptome analysis around the root tip identified AtWRKY6 as essential for normal root growth under low boron conditions (Kasajima et al., 2010).

WRKYs also participate in responses to wounding. Two wounding-responsive *WRKY3* and *WRKY6* genes were identified in tobacco *Nicotiana attenuata*. Moreover, NaWRKY3 is required for NaWRKY6 elicitation by fatty acid-amino acid conjugates from the larval oral secretions that are released into the wounds during feeding. Silencing either *WRKY3* or *WRKY6*, or both, by stable transformation, makes plants highly vulnerable to herbivores and is associated with impaired accumulation of jasmonates. These observations indicate an important role of WRKY3 and WRKY6 in sustaining active JA levels during a continuous insect attack (Skibbe et al., 2008).

The role of WRKY transcription factors in other processes

In the past few years, there has been an increasing evidence that WRKY proteins actively participate in certain plant developmental and physiological processes such as trichome developement (Johnson et al., 2002), seed germination, senescence (Hinderhofer and Zentgraf, 2001; Robatzek and Somssich, 2002), fruit maturation and carbohydrate metabolism (Sun et al., 2003). Biosynthesis of anthocyanin (Johnson et al., 2002), starch (Sun et al., 2003), and sesquiterpene (Xu et al., 2004) are also dependent on WRKY proteins.

The expression of root genes in *A. thaliana* was mapped and the obtained gene expression pattern indicated a possible specialized role for 12 members of WRKY TF family in the root cell maturation (Birnbaum et al., 2003). AtWRKY44 is the first described member of the WRKY family involved in the morphogenesis of trichomes. AtWRKY44 is presumed to have a role in non-hair epidermis development, due to its preferential gene expression in differentiating non-hair cells (Johnson et al., 2002).

Several *WRKY* genes from different plant species are expressed during different stages of seed development. The *WRKY* gene *DGE1* of orchard grass (*Dactylis glomerata*) is expressed during a somatic embryogenesis (Alexandrova and Conger, 2002). Similarly, *ScWRKY1* gene, is strongly and transiently expressed in fertilized ovules at the late torpedo stage in wild potato and has a specific role during embryogenesis (Lagace and Matton, 2004). In barley, *SUSIBA2* is expressed in the endosperm and regulates starch production (Sun et al., 2003). Likewise, Arabidopsis *WRKY10* gene, also known as *MINISEED3*, is expressed in pollen, a globular embryo as well as in the developing endosperm from the 2-nuclei stage through the cellularization stage. Furthermore, *WRKY* genes may control seed germination and postgermination in rice. *OsWRKY71* encodes a transcriptional repressor of GA signal transduction in aleurone cells (Zhang et al., 2004).

Additionally AtWRKY44 plays role in the synthesis of mucilage and tannin in the seed coat and is synthesized in seed integument or endosperm. Experiments with w*rky44* mutants showed that they were defective in the synthesis of proanthocyanidin and seed mucilate deposition, thus the seeds were yellow colored and their size was reduced when the mutant allele was transmitted through the female parent (Johnson et al., 2002). AtWRKY18 and AtWRKY60 have a positive effect on plant ABA sensitivity,

for the inhibition of seed germination and root growth. On the other hand, AtWRKY40 antagonizes the AtWRKY18 and AtWRKY60 effect (Chen et al., 2010).

WRKY participates in the carbohydrate metabolism. AtWRKY45 and AtWRKY65 are involved in regulating genes which respond to carbon starvation (Contento et al., 2004). Three rice *WRKY* genes are also upregulated in sucrose-starved rice suspension cultures (Wang et al., 2007). Furthermore, sugar regulates the expression of the Arabidopsis *NUCLEOSIDE DIPHSOPHATE KINASE 3a* (*NDPK3a*) gene. NDPK3a is located in mitochondria because sugar metabolism is intricately connected with this organelle through the conversion of sugars to ATP, and through the production of carbon skeletons that can be used in anabolic processes. Regarding the *NDPK3a* gene, glucose-mediated induction of NDPK3ais decreased in the *wrky34* mutant, while sucrose-mediated induction of NDPK3a is increased in the *wrky4* mutant. AtWRKY4 and AtWRKY34 are involved in sugar regulation of the *NDPK3a* gene exerting opposite effects (Hammargren et al., 2008).

In cotton plant *Gossypium arboreum*, sesquiterpene phytoalexins are secondary metabolites induced by fungal and bacterial infection or other environmental stimuli. They accumulate in epidermal and subepidermal cells of roots. GaWRKY1 is a transcriptional activator of the *CAD1* gene participating in the biosynthesis of cotton sesquiterpene. (Xu et al., 2004)

The WRKY interactions

Transcriptional gene expression regulation is very complex. The gene expression programs that maintain specific cell states are controlled by thousands of transcription factors, cofactors, and chromatin regulators. Transcriptional regulation tends to involve combinatorial interactions between several transcription factors, which allow sophisticated response to multiple conditions in the environment. This is associated with the harmonious modulation of a large number of different proteins that directly interact with DNA but also require participation of other regulatory elements indirectly influencing gene expression. WRKYs similarly to other regulatory proteins rarely work alone and interact transiently or permanently with proteins that play role in transcription and chromatin remodeling, signaling and other cellular processes. WRKYs were classified into 3 large groups and 5 subgroups. Slight variations within DNA-binding domains and other sequence motifs conserved within each group participate in protein-protein interactions and mediate complex functional interactions between WRKYs and other factors that possess a regulatory and modulatory effect. Among partners interacting with WRKY TF the following proteins have been identified: MAP kinases, MAP kinase kinases, 14-3-3 proteins, calmodulin, histone deacetylases, resistance proteins and other WRKY transcription factors (Chi et al., 2013).

WRKY-WRKY interactions

The WRKY promoters are statistically enriched with W-box elements and this observation suggests a functional linkage of many WRKY genes by auto- and cross-regulatory mechanisms. Thus WRKY proteins provide a dynamic regulation of target genes by cooperation or antagonism. The extensive protein-protein interactions were found within members of the same subclass, but also between members of different subclasses. In *A. thaliana* three members of group IIa (WRKY18, WRKY 40, WRKY60) interact through the leucine zipper motifs present at the N-terminal end. Interestingly, *in vitro* assays have shown that heterocomplexes AtWRKY18/AtWRKY40 may have enhanced regulatory activities comparing to homodimers composed of one of the WRKYs. Furthermore AtWRKY60 alone has little DNA-binding activity for W-box sequences but could enhance the binding of AtWRKY18 to DNA in opposite to the reduction of AtWRKY40 DNA-binding activity. This phenomenon may have a role in the controlling intensity of the basal defense response (Xu et al., 2006). Moreover, AtWRKY40 and AtWRKY60 interact with AtWRKY36 (group IId) and AtWRKY38 (group III) as revealed by a yeast two-hybrid assay (Consortium, 2011). Within group IIb, AtWRKY6 and AtWRKY42 interact with each other (Chen et al., 2009). Similar examples are interactions of AtWRKY30 with 3 others members of group III (AtWRKY53, AtWRKY54 and AtWRKY70) (Besseau et al., 2012). An analysis of the WRKY sequence drew attention to multiple leucine/isoleucine/valine residues at seven residue intervals. This is not the canonical leucine zipper but it seems to be responsible for dimer formation through hydrophobic interactions. There are two more possible mechanisms of WRKY-WRKY interactions considering DNA organization. W-boxes that are recognized by WRKY proteins very often are clustered

and separated by short spaces. An interacting WRKY may bind the closely-spaced W-boxes and regulate the target gene cooperatively and antagonistically. If the W-boxes are separated by a substantial number of nucleotides, then the same WRKY complex may interact through the DNA loop formation. Furthermore, this mechanism may affect the binding of other TF.

WRKY-VQ interactions

WRKY transcription factors interact with proteins containing a conserved FxxxVQxLTG motif with two residues: valine (V) and glutamine (Q). There are 34 genes encoding proteins which possess VQ motif in *A. thaliana*. They are relatively small, 100-200 amino-acid in length. The sequence beyond the short conserved motif with VQ residues is very diverse but as shown by a yeast two-hybrid assay, all of these 34 VQ proteins are capable of interacting with WRKY proteins (Cheng et al., 2012). *A. thaliana* WRKY protein, members of group I and group IIc are able to interact with the VQ motif. Having analyzed the amino acid sequences of the C-terminal WRKY domain of group I and the single WRKY domain of group IIc, the conclusion is that these two groups share similar structural features that are part of an interface for interaction with a short VQ motif. The two aspartate residues preceding the WRKYGQK motif and four residues interfering with two cysteines engaged in a zinc finger are essential for the interaction with a VQ motif. What is interesting, the interaction is not restricted and a single WRKY protein may interact with several VQ proteins. For example, AtWRKY25 and AtWRKY33 may interact with a majority of VQ proteins with varying degrees while AtWRKY51 interact with about 50% of all tested VQ proteins (Cheng et al., 2012). Within VQ proteins are: MKS1 (MAP kinase substrate1) interacting with AtWRKY33 (Andreasson et al., 2005; Qiu et al., 2008), HAIKU1, responsible for endosperm growth and seed size, that interact with AtWRKY10 (Luo et al., 2005) and SIB1 (SIGMA factor interacting protein1) that enhance plant defense against necrotrophs (Lai et al., 2011).

WRKY-MAP-kinase interactions

MAPKs (Mitogen-Activated Protein Kinases) play a crucial role in plant responses to pathogens and environmental stress conditions. The majority of WRKY transcription factors are also engaged in response to various stresses. Functional analyses indicate that among substrates for identified as stress responsive MAPKs are WRKY TFs from group I. These WRKYs possess two WRKY domains and contain clustered proline-directed serines (SP clusters) that are postulated to be potential phosphorylation sites for MAPKs (Ishihama and Yoshioka, 2012). MAPK may phosphorylate also WRKYs from other groups, which suggests recognition of other phosphorylation sites. Some members of group I proteins contain MAPK-docking site named the D-domain with a cluster of basic residues upstream of LxL motif (Ishihama and Yoshioka, 2012). The diversity of MAPK interacting sites may force selectivity of their interactions with WRKYs (Ishihama and Yoshioka, 2012). For example AtWRKY 33 interacts with MKS1, a substrate for MPK4. More detailed analyses showed that in the absence of pathogens, MPK4 is presented in a nucleus in complex with AtWRKY33 and AtWRKY is released when infection occurs (Qiu et al., 2008). AtWRKY33 is also up-regulated by the MPK3/MPK6 cascade and therefore plays role in the regulation of a pathogen-induced biosynthesis of camalexin (Mao et al., 2011).

WRKY interactions with other proteins

There are evidences for the existence of other binding partners for WRKY transcription factors. They belong to various protein families.

Yeast two-hybrid screens identified Arabidopsis HDA19 (Histone Deacetylase 19) as an interacting partner of both AtWRKY38 and AtWRKY62 (Kim et al., 2008). The interaction occurs in the nucleus and is highly specific. Histone deacetylase removes acetyl groups from histones. Deacetylated histones have the ability to wrap the DNA more tightly. Deacetylation of histones leads to the repression of genes transcription. Overexpression of *HDA19* results in repression of the AtWRKY38 and AtWRKY62 activity as transcriptional activators.

Another binding partner for WRKY is calmodulin (CaM – Calcium Modulated Protein). CaM is a multifunctional intermediate messenger protein that transduces calcium signals by binding calcium ions and then modifying its interactions with various target proteins. Ten Arabidopsis WRKY proteins from group IId were recognized as CaM binding. The binding was verified by a gel mobility shift assay, a split-ubiquitin yeast two-hybrid assay and a competition assay with Ca^{2+}/CaM-dependant

phosphodiesterase (Park et al., 2005). WRKYs from IId group contain a short region called C-motif responsible for calmodulin binding. This domain has conserved amino acid sequence DxxVxKFKxVISLLxxxR. The functionally characterized WRKYs from IId roup: AtWRKY7, AtWRKY11 and AtWRKY17 act as regulatory repressors of the plant basal defense (Journot-Catalino et al., 2006; Kim et al., 2006). Moreover, if the C-motif is located close to the WRKY domain, then the binding of CaM will prevent RKY-WRKY interactions. This might be a possible mechanism for the regulation of WRKY-WRKY interaction by cellular Ca^{2+} levels.

Seven WRKYs from *A. thaliana* (AtWRKY6, AtWRKY16, AtWRKY18, AtWRKY19, AtWRKY27, AtWRKY32 and AtWRKY40) were identified as complexes with 14-3-3 proteins (Chang et al., 2009). These interactions are promoted by protein phosphorylation by pathogen-responsive kinase cascades. 14-3-3 proteins dimerize and might bind two target proteins. Among the targets are also other than WRKYs, phosphorylated proteins. 14-3-3 proteins often function as adaptor proteins that bind a multitude of regulatory and signaling proteins, thus they could have an important function in a complex WRKY interaction network (Schoonheim et al., 2007).

Structural studies of WRKY proteins

Structural studies of WRKY proteins are crucial to understand the mechanism of their interactions with both DNA and other potential binding partners. Each WRKY possesses, apart from the invariable DNA-binding WRKY domain, other motifs responsible for the interactions with different protein partners. Thus, determination of the global structure is essential to help us understand the complex mechanisms of signaling and transcriptional reprogramming of cell functioning controlled by WRKY proteins. Unfortunately, the solution of three dimensional (3D) structure is available only for highly conserved DNA-binding domain but not for full-length WRKY protein and there are no topological data regarding subgroup-specific motifs. The structural data on a full length WRKY protein will help us to understand how do they act as transcription regulators and to localize potential DNA and interacting partners binding sites. So far there have been only 3 structures of AtWRKY DNA-binding domains deposited in PDB (Fig. 3). Up to date one of them is solved using X-ray crystallography and the structure of the remaining two, using NMR spectroscopy. This known crystal structure represents the C-terminal domain of AtWRKY1 (PDB code: 2ayd) (Duan et al., 2007) and the two NMR structures mentioned above referred to the corresponding domain from AtWRKY4. One of them was solved in complex with DNA (PDB code: 1wj2) (Yamasaki et al., 2005) and the other without ligand (PDB code: 2lex) (Yamasaki et al., 2012). Both, crystal and NMR structures possess very similar globular architecture, composed of β-sheets. The crystal structure of the C-terminal part of AtWRKY1 (PDB: 2ayd) (Duan et al., 2007) determined at 1.6 Å resolution revealed that this domain is composed of a globular structure with 5 β-strands, forming an antiparallel β-sheet. Aditionally, a zinc binding site was found at one end of the β-sheet, between strands β4 and β5. DNA-binding residues of WRKY1 are located at β2 and β3 strands (Ciolkowski et al., 2008). 2-5 β-sheets correspond to 1-4 β-sheets from NMR structure of AtWRKY4 domain (PDB: 1wj2) (Yamasaki et al., 2005).

The major differences between the known structures were noticed in the region considered as C- and N-termini of the domain. The secondary structure elements of the above mentioned WRKY domains are β-strands forming an antiparallel β-sheet. Conserved Cys/His residues located at C-terminus of the β-sheet formed zinc binding pocket. WRKYGQK residues are present at the N-terminus of β-sheet. The structure of the C-terminal AtWRKY4 domain in complex with the DNA fragment (W-box) solved by NMR, allowed to deduce its DNA-binding mechanism (Yamasaki et al., 2012). A four stranded β-sheet enters the major groove of DNA in an atypical mode termed β-wedge, where the sheet is nearly perpendicular to the DNA helical axis. Residues in the conserved WRKYGQK motif (except tryptophane, W) contact DNA bases mainly through extensive apolar contacts and hydrogen-bonding interactions with thymine methyl groups (Yamasaki et al., 2012). The structure of the protein in complex with DNA, consists of four-stranded β-sheet which is similar to that without DNA, with a backbone root mean square deviation of 1.9 Å. The 16 bp DNA is in the B-form with a slight bent toward the protein. The major molecular interface is created by the β1-strand that contains an invariant WRKYGQK sequence (Fig. 3C). The formation of the complex has significantly altered the position of this strand to the others. The kink at Gly enabled a close contact of β1-strand with DNA bases (Yamasaki et al., 2012).

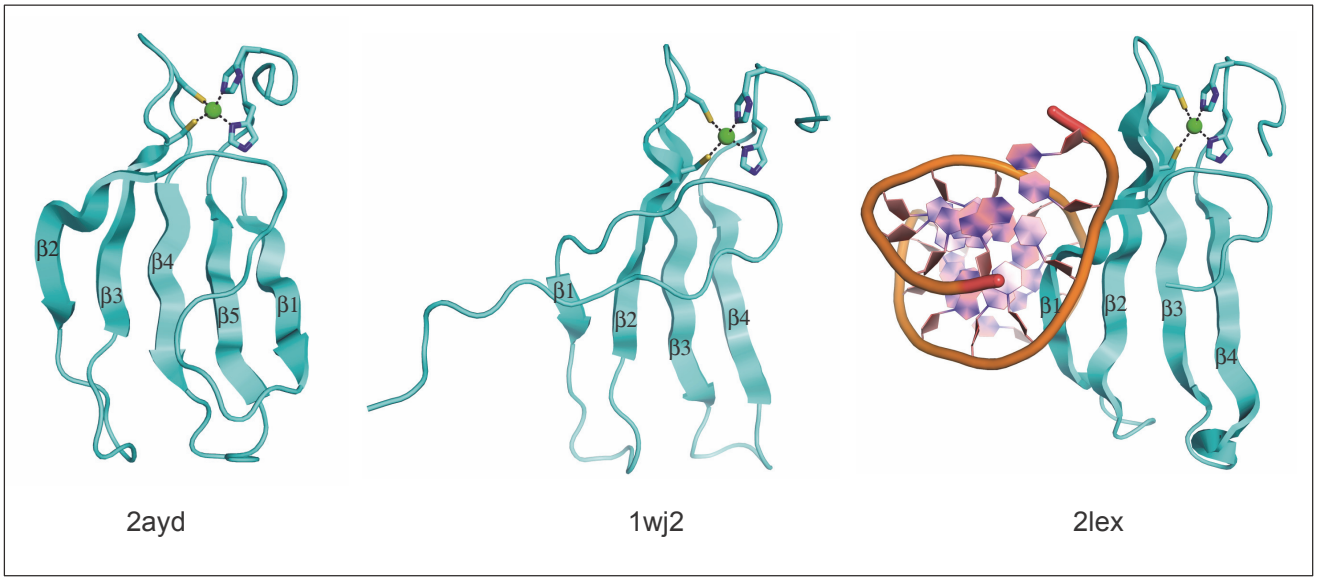

Fig. 3. Known structures of *Arabidopsis thaliana* WRKY domains solved by X-ray crystallography (2ayd) and NMR spectroscopy (1wj2 and 2lex). Detailed description in the text

Conclusions

WRKY proteins have been identified as a family of transcription factors restricted to the plant kingdom. The biggest progress in functional studies on the WRKY transcription factors has been achieved during the past 20 years. The use of diverse technologies and approaches, including plant physiology, genetic engineering, molecular biology techniques and bioinformatics helped to understand the complex mechanisms underlying the field of plant signal transduction and regulation of gene transcription. Current information suggests that WRKY factors play a key role in regulating the pathogen-induced defense program as well as in various aspects of plant responses to abiotic stresses and plant development, thus they appear to participate in controlling the expression of a plethora genes. Considering the number of genes in this gene family and complex crosstalk between WRKY pathways, the identification of the role of individual WRKY proteins appears very complicated. Further analyses covering structural studies of full length WRKY proteins and identification of their interacting partners will give prospects to a better understanding of their function.

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References

Alexandrova K.S., Conger B.V. (2002) *Isolation of two somatic embryogenesis-related genes from orchardgrass (Dactylis glomerata).* Plant Sci. 162: 301-307.

Andreasson E., Jenkins T., Brodersen P., Thorgrimsen S., Petersen N.H.T., Zhu S., Qiu J.-L., Micheelsen P., Rocher A., Petersen M., Newman M.-A., Nielsen H.B., Hirt H., Somssich I., Mattsson O., Mundy J. (2005) *The MAP kinase substrate MKS1 is a regulator of plant defense responses.* EMBO J. 24(14): 2579-2589.

Asai T., Tena G., Plotnikova J., Willmann M.R., Chiu W.-L., Gomez-Gomez L., Boller T., Ausubel F.M., Sheen J. (2002) *MAP kinase signalling cascade in Arabidopsis innate immunity.* Nature 415(6875): 977-983.

Babitha K.C., Ramu S.V., Pruthvi V., Mahesh P., Nataraja K.N., Udayakumar M. (2013) *Co-expression of AtbHLH17 and AtWRKY28 confers resistance to abiotic stress in Arabidopsis.* Transgenic Res. 22(2): 327-341.

Bera S.K., Ajay B.C., Singh A.L. (2013) *WRKY and Na+/H+ antiporter genes conferring tolerance to salinity in interspecific derivatives of peanut (Arachis hypogaea L.).* Austral. J. Crop Sci. 7(8): 1173-1180.

Besseau S., Li J., Palva E.T. (2012) *WRKY54 and WRKY70 co-operate as negative regulators of leaf senescence in Arabidopsis thaliana.* J. Exp. Bot. 63(7): 2667-2679.

Bhattarai K.K., Atamian H.S., Kaloshian I., 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(2): 229-240.

Birkenbihl R.P., Diezel C., Somssich I.E. (2012) *Arabidopsis WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward Botrytis cinerea infection*. Plant Physiol. 159(1): 266-285.

Birnbaum K., Shasha D.E., Wang J.Y., Jung J.W., Lambert G.M., Galbraith D.W., Benfey P.N. (2003) *A gene expression map of the Arabidopsis root*. Science 302(5652): 1956-1960.

Brotman Y., Landau U., Cuadros-Inostroza A., Tohge T., Fernie A.R., Chet I., Viterbo A., Willmitzer L. (2013) *Trichoderma-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance*. PLoS Pathog. 9(3): e1003221.

Chang I.F., Curran A., Woolsey R., Quilici D., Cushman J. C., Mittler R., Harmon A., Harper J.F. (2009) *Proteomic profiling of tandem affinity purified 14-3-3 protein complexes in Arabidopsis thaliana*. Proteomics 9(11): 2967-2985.

Chen C., Chen Z. (2002) *Potentiation of developmentally regulated plant defense response by AtWRKY18, a pathogen-induced Arabidopsis transcription factor*. Plant Physiol. 129(2): 706-716.

Chen H., Lai Z., Shi J., Xiao Y., Chen Z., Xu X. (2010) *Roles of arabidopsis WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress*. BMC Plant Biol. 10: 281.

Chen L., Zhang L., Li D., Wang F., Yu D. (2013) *WRKY8 transcription factor functions in the TMV-cg defense response by mediating both abscisic acid and ethylene signaling in Arabidopsis*. Proc. Natl Acad. Sci. USA 110(21): E1963-1971.

Chen L., Zhang L., Yu D. (2010) *Wounding-induced WRKY8 is involved in basal defense in Arabidopsis*. Mol. Plant Microbe. Interact. 23(5): 558-565.

Chen W., Provart N.J., Glazebrook J., Katagiri F., Chang H.S., Eulgem T., Mauch F., Luan S., Zou G., Whitham S.A. et al. (2002) *Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses*. Plant Cell 14(3): 559-574.

Chen Y.F., Li L.Q., Xu Q., Kong Y.H., Wang H., Wu W.H. (2009) *The WRKY6 transcription factor modulates PHOSPHATE1 expression in response to low Pi stress in Arabidopsis*. Plant Cell 21(11): 3554-3566.

Cheng Y., Zhou Y., Yang Y., Chi Y.J., Zhou J., Chen J.Y., Wang F., Fan B., Shi K., Zhou Y.-H. et al. (2012) *Structural and functional analysis of VQ motif-containing proteins in Arabidopsis as interacting proteins of WRKY transcription factors*. Plant Physiol. 159(2): 810-825.

Cheong Y.H., Chang H.S., Gupta R., Wang X., Zhu T., Luan S. (2002) *Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis*. Plant Physiol. 129(2): 661-677.

Chi Y., Yang Y., Zhou Y., Zhou J., Fan B., Yu J.Q., Chen Z. (2013) *Protein-protein interactions in the regulation of WRKY transcription factors*. Mol. Plant. 6(2): 287-300.

Chisholm S.T., Coaker G., Day B., Staskawicz B.J. (2006) *Host-microbe interactions: shaping the evolution of the plant immune response*. Cell 124(4): 803-814.

Chujo T., Miyamoto K., Shimogawa T., Shimizu T., Otake Y., Yokotani N., Nishizawa Y., Shibuya N., Nojiri H., Yamane H. (2013) *OsWRKY28, a PAMP-responsive transrepressor, negatively regulates innate immune responses in rice against rice blast fungus*. Plant Mol. Biol. 82(1-2): 23-37.

Ciolkowski I., Wanke D., Birkenbihl R.P., Somssich I.E. (2008) *Studies on DNA-binding selectivity of WRKY transcription factors lend structural clues into WRKY-domain function*. Plant Mol. Biol. 68(1-2): 81-92.

Consortium A.I.M. (2011) *Evidence for network evolution in an Arabidopsis interactome map*. Science 333: 601-607.

Contento A.L., Kim S.J., Bassham D.C. (2004) *Transcriptome profiling of the response of Arabidopsis suspension culture cells to Suc starvation*. Plant Physiol. 135(4): 2330-2347.

Dang F.-F., Wang Y.-N., Yu L., Eulgem T., Lai Y., Liu Z.-Q., Wang X., Qiu A.-L., Zhang T.-X., Lin J. et al. (2013) *CaWRKY40, a WRKY protein of pepper, plays an important role in the regulation of tolerance to heat stress and resistance to Ralstonia solanacearum infection*. Plant Cell Environ. 36(4): 757-774.

Dangl J.L., Dietrich R.A., Richberg M.H. (1996) *Death Don't Have No Mercy: Cell Death Programs in Plant-Microbe Interactions*. Plant Cell 8(10): 1793-1807.

de Pater S., Greco V., Pham K., Memelink J., Kijne J. (1996) *Characterization of a zinc-dependent transcriptional activator from Arabidopsis*. Nucl. Acids Res. 24(23): 4624-4631.

Dellagi A., Helibronn J., Avrova A.O., Montesano M., Palva E.T., Stewart H.E., Toth I.K., Cooke D.E.L., Lyon G.D., Birch P.R.J. (2000) *A potato gene encoding a WRKY-like transcription factor is induced in interactions with Erwinia carotovora subsp. atroseptica and Phytophthora infestans and is coregulated with class I endochitinase expression*. Mol. Plant Microbe. Interact. 13(10): 1092-1101.

Deslandes L., Olivier J., Theulieres F., Hirsch J., Feng D.X., Bittner-Eddy P., Beynon J., Marco Y. (2002) *Resistance to Ralstonia solanacearum in Arabidopsis thaliana is conferred by the recessive RRS1-R gene, a member of a novel family of resistance genes*. Proc. Natl Acad. Sci. USA 99(4): 2404-2409.

Dodds P.N., Rathjen J.P. (2010) *Plant immunity: towards an integrated view of plant-pathogen interactions*. Nat. Rev. Genet. 11(8): 539-548.

Dong J., Chen C., Chen Z. (2003) *Expression profiles of the Arabidopsis WRKY gene superfamily during plant defense response*. Plant Mol. Biol. 51(1): 21-37.

Du L., Chen Z. (2000) *Identification of genes encoding receptor-like protein kinases as possible targets of pathogen- and salicylic acid-induced WRKY DNA-binding proteins in Arabidopsis.* Plant J. 24(6): 837-847.

Duan M.R., Nan J., Liang Y.H., Mao P., Lu L., Li L., Wei C., Lai L., Li Y., Su X.D. (2007) *DNA binding mechanism re-*

vealed by high resolution crystal structure of Arabidopsis thaliana WRKY1 protein. Nucl. Acids Res. 35(4): 1145-1154.

Eulgem T., Rushton P.J., Robatzek S., Somssich I.E. (2000) *The WRKY superfamily of plant transcription factors*. Trends Plant Sci. 5(5): 199-206.

Eulgem T., Rushton P.J., Schmelzer E., Hahlbrock K., Somssich I.E. (1999) *Early nuclear events in plant defence signalling: rapid gene activation by WRKY transcription factors*. EMBO J. 18(17): 4689-4699.

Gadjev I., Vanderauwera S., Gechev T.S., Laloi C., Minkov I.N., Shulaev V., Apel K., Inzé D., Mittler R., Van Breusegem F. et al. (2006) *Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis*. Plant Physiol., 141(2): 436-445.

Gao Q.M., Venugopal S., Navarre D., Kachroo A. (2011) *Low oleic acid-derived repression of jasmonic acid-inducible defense responses requires the WRKY50 and WRKY51 proteins*. Plant Physiol. 155(1): 464-476.

Gao X., Chen X., Lin W., Chen S., Lu D., Niu Y., Li L., Cheng C., McCormack M., Sheen J. et al. (2013) *Bifurcation of Arabidopsis NLR immune signaling via Ca(2)(+)-dependent protein kinases*. PLoS Pathog. 9(1): e1003127.

Guillaumie S., Mzid R., Mechin V., Leon C., Hichri I., Destrac-Irvine A., Trossat Magnin C., Delrot S. Lauvergeat V. (2010) *The grapevine transcription factor WRKY2 influences the lignin pathway and xylem development in tobacco*. Plant Mol. Biol. 72(1-2): 215-234.

Hammargren J., Rosenquist S., Jansson C., Knorpp C. (2008) *A novel connection between nucleotide and carbohydrate metabolism in mitochondria: sugar regulation of the Arabidopsis nucleoside diphosphate kinase 3a gene*. Plant Cell Rep. 27(3): 529-534.

Hara K., Yagi M., Kusano T., Sano H. (2000) *Rapid systemic accumulation of transcripts encoding a tobacco WRKY transcription factor upon wounding.* Mol Gen Genet. 263(1): 30-37.

Hinderhofer K., Zentgraf U. (2001) *Identification of a transcription factor specifically expressed at the onset of leaf senescence.* Planta 213(3): 469-473.

Hu Y., Chen L., Wang H., Zhang L., Wang F., Yu D. (2013) *Arabidopsis transcription factor WRKY8 functions antagonistically with its interacting partner VQ9 to modulate salinity stress tolerance*. Plant J. 74(5): 730-745.

Inoue H., Hayashi N., Matsushita A., Xinqiong L., Nakayama A., Sugano S. et al. (2013) *Blast resistance of CC-NB-LRR protein Pb1 is mediated by WRKY45 through protein-protein interaction*. Proc. Natl Acad. Sci. USA 110(23): 9577-9582.

Ishiguro S., 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 beta-amylase from sweet potato*. Mol. Gen. Genet. 244(6): 563-571.

Ishihama N., Yoshioka H. (2012) *Post-translational regulation of WRKY transcription factors in plant immunity*. Curr. Opin. Plant Biol. 15(4): 431-437.

Izaguirre M.M., Scopel A.L., Baldwin I.T., Ballare C.L. (2003) *Convergent responses to stress. Solar ultraviolet-B radiation and Manduca sexta herbivory elicit overlapping transcriptional responses in field-grown plants of Nicotiana longiflora*. Plant Physiol. 132(4): 1755-1767.

Jiang Y., Deyholos M.K. (2009) *Functional characterization of Arabidopsis NaCl-inducible WRKY25 and WRKY33 transcription factors in abiotic stresses*. Plant Mol. Biol. 69(1-2): 91-105.

Jin J., Zhang H., Kong L., Gao G., Luo J. (2014) *PlantTFDB 3.0: a portal for the functional and evolutionary study of plant transcription factors*. Nucl. Acids Res. 42: D1182-1187.

Johnson C.S., Kolevski B., Smyth D.R. (2002) *TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor*. Plant Cell 14(6): 1359-1375.

Journot-Catalino N., Somssich I.E., Roby D., Kroj T. (2006) *The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in Arabidopsis thaliana*. Plant Cell 18(11): 3289-3302.

Kalde M., Barth M., Somssich I.E., Lippok B. (2003) *Members of the Arabidopsis WRKY group III transcription factors are part of different plant defense signaling pathways.* Mol. Plant Microb. Interact. 16(4): 295-305.

Kasajima I., Ide Y., Yokota Hirai M., Fujiwara T. (2010) *WRKY6 is involved in the response to boron deficiency in Arabidopsis thaliana*. Physiol. Plant, 139(1): 80-92.

Kim K.C., Fan B., Chen Z. (2006) *Pathogen-induced Arabidopsis WRKY7 is a transcriptional repressor and enhances plant susceptibility to Pseudomonas syringae*. Plant Physiol. 142(3): 1180-1192.

Kim K.C., Lai Z., Fan B., Chen Z. (2008) *Arabidopsis WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense*. Plant Cell 20(9): 2357-2371.

Knoth C., Ringler J., Dangl J.L., Eulgem T. (2007) *Arabidopsis WRKY70 is required for full RPP4-mediated disease resistance and basal defense against Hyaloperonospora parasitica*. Mol. Plant Microb. Interact. 20(2): 120-128.

Lagace M., Matton D.P. (2004) *Characterization of a WRKY transcription factor expressed in late torpedo-stage embryos of Solanum chacoense*. Planta 219(1): 185-189.

Lai Z., Li Y., Wang F., Cheng Y., Fan B., Yu J.Q., Chen Z. (2011) *Arabidopsis sigma factor binding proteins are activators of the WRKY33 transcription factor in plant defense*. Plant Cell 23(10): 3824-3841.

Lai Z., Vinod K., Zheng Z., Fan B., Chen Z. (2008) *Roles of Arabidopsis WRKY3 and WRKY4 transcription factors in plant responses to pathogens*. BMC Plant Biol. 8: 68.

Li J., Brader G., Kariola T., Palva E.T. (2006) *WRKY70 modulates the selection of signaling pathways in plant defense*. Plant J. 46(3): 477-491.

Li S., Fu Q., Huang W., Yu D. (2009) *Functional analysis of an Arabidopsis transcription factor WRKY25 in heat stress*. Plant Cell Rep. 28(4): 683-693.

Li S., Zhou X., Chen L., Huang W., Yu D. (2010) *Functional characterization of Arabidopsis thaliana WRKY39 in heat stress*. Mol. Cell 29(5): 475-483.

Liu J.J., Ekramoddoullah A.K. (2009) *Identification and characterization of the WRKY transcription factor family in Pinus monticola*. Genome 52(1): 77-88.

Luo M., Dennis E.S., Berger F., Peacock W.J., 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.

Maeo K., Hayashi S., Kojima-Suzuki H., Morikami A., Nakamura K. (2001) *Role of conserved residues of the WRKY domain in the DNA-binding of tobacco WRKY family proteins*. Biosci. Biotechnol. Biochem. 65(11): 2428-2436.

Mao G., Meng X., Liu Y., Zheng Z., Chen Z., Zhang S. (2011) *Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in Arabidopsis*. Plant Cell 23(4): 1639-1653.

Mishina T.E., Zeier J. (2007) *Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in Arabidopsis*. Plant J. 50(3): 500-513.

Mishra S., Triptahi V., Singh S., Phukan U.J., Gupta M.M., Shanker K., Shukla R.K. (2013) *Wound induced tanscriptional regulation of benzylisoquinoline pathway and characterization of wound inducible PsWRKY transcription factor from Papaver somniferum*. PLoS One 8(1): e52784.

Park C.Y., Lee J.H., Yoo J.H., Moon B.C., Choi M.S., Kang Y.H. et al. (2005) *WRKY group IId transcription factors interact with calmodulin*. FEBS Lett. 579(6): 1545-1550.

Pnueli L., Hallak-Herr E., Rozenberg M., Cohen M., Goloubinoff P., Kaplan A., Mittler R. (2002) *Molecular and biochemical mechanisms associated with dormancy and drought tolerance in the desert legume Retama raetam*. Plant J. 31(3): 319-330.

Qiu J.L., Fiil B.K., Petersen K., Nielsen H.B., Botanga C.J., Thorgrimsen S. et al. (2008) *Arabidopsis MAP kinase 4 regulates gene expression through transcription factor release in the nucleus*. EMBO J. 27(16): 2214-2221.

Rizhsky L., Davletova S., Liang H., Mittler R. (2004) *The zinc finger protein Zat12 is required for cytosolic ascorbate peroxidase 1 expression during oxidative stress in Arabidopsis*. J. Biol. Chem. 279(12): 11736-11743.

Rizhsky L., Liang H., Mittler R. (2002) *The combined effect of drought stress and heat shock on gene expression in tobacco*. Plant Physiol. 130(3): 1143-1151.

Robatzek S., Somssich I.E. (2001) *A new member of the Arabidopsis WRKY transcription factor family, AtWRKY6, is associated with both senescence- and defence-related processes*. Plant J. 28(2): 123-133.

Robatzek S., Somssich I. E. (2002) *Targets of AtWRKY6 regulation during plant senescence and pathogen defense*. Genes Dev. 16(9): 1139-1149.

Rushton P.J., Macdonald H., Huttly A.K., Lazarus C.M., Hooley R. (1995) *Members of a new family of DNA-binding proteins bind to a conserved cis-element in the promoters of alpha-Amy2 genes*. Plant Mol. Biol. 29(4): 691-702.

Rushton P.J., Somssich I.E., Ringler P., Shen Q.J. (2010) *WRKY transcription factors*. Trends Plant Sci. 15(5): 247-258.

Rushton P.J., Torres J.T., Parniske M., Wernert P., Hahlbrock K., Somssich I.E. (1996) *Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes*. EMBO J. 15(20): 5690-5700.

Schon M., Toller A., Diezel C., Roth C., Westphal L., Wiermer M., 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 Microb. Interact. 26(7): 758-767.

Schoonheim P.J., Sinnige M.P., Casaretto J.A., Veiga H., Bunney T.D., Quatrano R.S., de Boer A.H. (2007) *14-3-3 adaptor proteins are intermediates in ABA signal transduction during barley seed germination*. Plant J. 49(2): 289-301.

Shim J.S., Choi Y.D. (2013) *Direct regulation of WRKY70 by AtMYB44 in plant defense responses*. Plant Signal Behav. 8(6): e20783.

Shim J.S., Jung C., Lee S., Min K., Lee Y.W., Choi Y. et al. (2013) *AtMYB44 regulates WRKY70 expression and modulates antagonistic interaction between salicylic acid and jasmonic acid signaling*. Plant J. 73(3): 483-495.

Skibbe M., Qu N., Galis I., Baldwin I.T. (2008) *Induced plant defenses in the natural environment: Nicotiana attenuata WRKY3 and WRKY6 coordinate responses to herbivory*. Plant Cell 20(7): 1984-2000.

Sun C., Palmqvist S., Olsson H., Boren M., Ahlandsberg S., 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(9): 2076-2092.

Tsuda K., Sato M., Stoddard T., Glazebrook J., Katagiri F. (2009) *Network properties of robust immunity in plants.* PLoS Genet. 5(12): e1000772.

van Loon L.C., Rep M., Pieterse C.M. (2006) *Significance of inducible defense-related proteins in infected plants*. Ann. Rev. Phytopathol. 44: 135-162.

Van Loon L.C., Pierpoint W.S., Boller T., Conejero V. (1994) *Recommendations for naming plant pathogenesis-related proteins*. Plant Mol. Biol. Rep. (12): 245-264.

Vandenabeele S., Van Der Kelen K., Dat J., Gadjev I., Boonefaes T., Morsa S. et al. (2003) *A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco.* Proc. Natl Acad. Sci. USA 100(26): 16113-16118.

Wan J., Zhang X.C., Neece D., Ramonell K.M., Clough S., Kim S.Y. et al. (2008) *A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis*. Plant Cell 20(2): 471-481.

Wang D., Amornsiripanitch N., Dong X. (2006) *A genomic approach to identify regulatory nodes in the transcriptio-*

nal network of systemic acquired resistance in plants. PLoS Pathog. 2(11): e123.

Wang H.J., Wan A.R., Hsu C.M., Lee K.W., Yu S.M., Jauh G.Y. (2007) *Transcriptomic adaptations in rice suspension cells under sucrose starvation*. Plant Mol. Biol. 63(4): 441-463.

Wang X., Du B., Liu M., Sun N., Qi X. (2013) *Arabidopsis transcription factor WRKY33 is involved in drought by directly regulating the expression of CesA8*. Amer. J. Plant Sci. 4(6A): 21-27.

Wang Z., Yang P., Fan B., Chen Z. (1998) *An oligo selection procedure for identification of sequence-specific DNA-binding activities associated with the plant defence response*. Plant J. 16(4): 515-522.

Willmott R.L., Rushton P.J., Hooley R., Lazarus C.M. (1998) *DNase1 footprints suggest the involvement of at least three types of transcription factors in the regulation of alpha-Amy2/A by gibberellin*. Plant Mol. Biol. 38(5): 817-825.

Wu K.L., Guo Z.J., Wang H.H., Li J. (2005) *The WRKY family of transcription factors in rice and Arabidopsis and their origins*. DNA Res. 12(1): 9-26.

Xie Z., Zhang Z.L., Zou X., Huang J., Ruas P., Thompson D., Shen Q.J. (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(1): 176-189.

Xu X., Chen C., Fan B., Chen Z. (2006) *Physical and functional interactions between pathogen-induced Arabidopsis WRKY18, WRKY40, and WRKY60 transcription factors*. Plant Cell 18(5): 1310-1326.

Xu Y.H., Wang J.W., Wang S., Wang J.Y., Chen X.Y. (2004) *Characterization of GaWRKY1, a cotton transcription factor that regulates the sesquiterpene synthase gene (+)-delta-cadinene synthase-A*. Plant Physiol. 135(1): 507-515.

Yamasaki K., Kigawa T., Inoue M., Tateno M., Yamasaki T., Yabuki T. et al. (2005) *Solution structure of an Arabidopsis WRKY DNA binding domain*. Plant Cell 17(3): 944-956.

Yamasaki K., Kigawa T., Watanabe S., Inoue M., Yamasaki T., Seki M. et al. (2012) *Structural basis for sequence-specific DNA recognition by an Arabidopsis WRKY transcription factor*. J. Biol. Chem. 287(10): 7683-7691.

Yang P.Z., Chen C.H., Wang Z.P., Fan B.F., Chen Z.X. (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(2): 141-149.

Zhang H., Jin J., Tang L., Zhao Y., Gu X., Gao G., Luo J. (2011) *PlantTFDB 2.0: update and improvement of the comprehensive plant transcription factor database*. Nucl. Acids Res. 39: D1114-1117.

Zhang Z.L., Xie Z., Zou X., Casaretto J., Ho T.H., Shen Q.J. (2004) *A rice WRKY gene encodes a transcriptional repressor of the gibberellin signaling pathway in aleurone cells*. Plant Physiol. 134(4): 1500-1513.

Zheng Z., Mosher S.L., Fan B., Klessig D.F., Chen Z. (2007) *Functional analysis of Arabidopsis WRKY25 transcription factor in plant defense against Pseudomonas syringae*. BMC Plant Biol. 7: 2.

Zheng Z., Qamar S.A., Chen Z., Mengiste T. (2006) *Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens*. Plant J. 48(4): 592-605.

Zipfel C., Kunze G., Chinchilla D., Caniard A., Jones J.D., Boller T., Felix G. (2006) *Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation*. Cell 125(4): 749-760.

Zipfel C., Robatzek S., Navarro L., Oakeley E.J., Jones J.D., Felix G., Boller T. (2004) *Bacterial disease resistance in Arabidopsis through flagellin perception*. Nature 428 (6984): 764-767.

Zou C., Jiang W., Yu D. (2010) *Male gametophyte-specific WRKY34 transcription factor mediates cold sensitivity of mature pollen in Arabidopsis*. J. Exp. Bot. 61(14): 3901-3914.