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WRKY Transcription Factor Family in Plant Defense Mechanism

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Different transcription factor families in plants have been identified and played important roles in development and coping with either biotic or abiotic stress. Plants require sophisticated regulation mechanisms to control their complex biological processes. Several families of the transcription factors in plants have been extensively studied. For example, MADS-box transcription factors, as homeotic selectors, control the transition from vegetative to generative growth and determine inflorescence meristem identity in plants (Ng and Yanofsky, 2001). In addition, bZIP transcription factors have been implicated in much more diverse biological processes such as pathogen defense, light and stress signaling, seed maturation, and flower development (Jakoby et al., 2002).

Among the processes by which plants react to external stimuli, such as pathogen attack, wound, and various abiotic stresses, resistance (R) gene-mediated defense responses are the most prevalent and well-characterized mechanisms in plants (Dangl and Jones, 2001). Recently, the information provided by the *Arabidopsis* genome initiative has accelerated the study of the signaling pathways triggered by the interaction between avirulence product of an invading pathogen and corresponding resistant gene of the host plant (Austin et al., 2002; Azevedo et al., 2002). In addition, increasing evidence indicates that the regulatory activity of a family of plant transcription factors is indispensable in controlling the expression defense response genes (Eulgem et al., 2000; Heise et al., 2002).

Common Features of WRKY Transcription Factors

Plants have evolved a wide variety of mechanisms to regulate the transcription of defense-related genes that are induced upon pathogen attack. The comparative analysis of the whole genome of *Arabidopsis* has revealed several families of the transcription factors directly involved in plant defense. Excellent review and research articles on this are available (Eulgem et al., 2000; Heise et al., 2002; Jakoby et al., 2002).

Among these transcription activators, WRKY transcription factors have been shown to regulate the transcription of a wide range of defense-related genes. WRKY proteins seem to be ubiquitous in the plant kingdom and characteristically contain highly conserved amino acid sequence motifs containing the sequences, WRKYGQK, followed by a Cys_2-His_2 or Cys_2-His-Cys zinc-binding motif. Plant WRKY transcription factors were first identified on the basis of the DNA binding properties in sweet potato (Ishiguro and Nakamura, 1994). WRKY proteins bind to regions upstream of regulated genes that contain the nucleotide sequence motif (T)(T)TGAC(C/T), which is called a W-box (de Pater et al., 1996; Rushton et al., 1996).

After searching the *Arabidopsis* genome for genes containing the WRKYGQK amino acid sequence motifs, Eulgem et. al (2000) divided *Arabidopsis* WRKY proteins into three groups by the number and type of WRKY domains and the type of zinc finger motif they contain. Group I contains two WRKY domains, designated either as N-terminal or C-terminal WRKY domain (Fig. 1). Based on comparisons of the predicted amino acid sequences near WRKY domains among three groups and previously characterized WRKY proteins, the C-terminal WRKY domain is likely to mediate sequence-specific binding to the target sequences (Eulgem et al., 1999). Both group I and II proteins contain the same type of zinc-finger motifs, Cys_2-His_2, but group II has a single WRKY domain. Group III proteins like group II have a single WRKY domain, but contain a different type of zinc-binding motif, Cys_2-His-Cys. Most previously characterized WRKY proteins contain basic nuclear localization signals (Eulgem et al., 2000).

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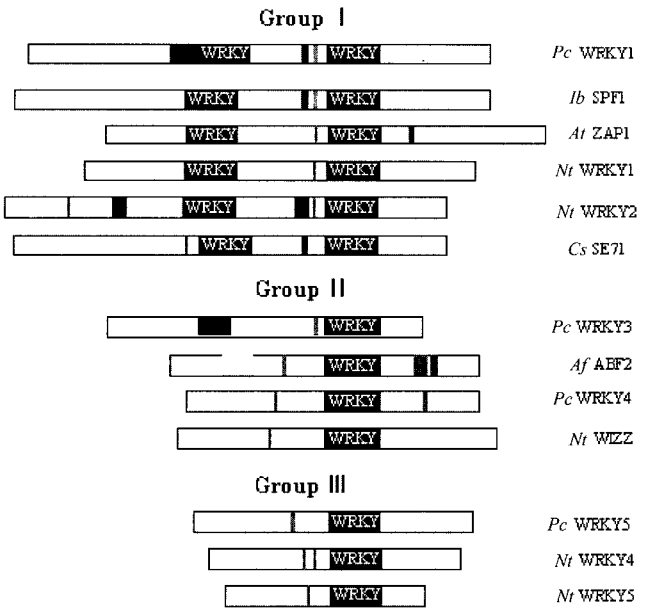

Fig. 1. Schematic representation of published full-length WRKY proteins from parsley (*Pc*), sweet potato (*Ib*), *Arabidopsis* (*At*), tobacco (*Nt*), cucumber (*Cs*), and wild oat (*Af*). They have been divided into three groups based on the number and type of the WRKY domains they contain. WRKY domains are black, putative basic nuclear localization signals are blue, and leucine zippers are pink. Serine-threonine-rich regions are yellow, glutamine-rich regions are purple, proline-rich regions are green, and acidic regions are red (Eulgem et al., 2000).

Biological Roles of WRKY Factors

In a recent analysis of 72 *Arabidopsis* WRKY genes, Dong et al. (2003) showed that WRKY genes are differentially regulated by salicylic acid (SA) treatment and infection by *Pseudomonas syringae*. In addition, the expression of some of the WRKY genes was tissue specific and regulated developmentally. Yoda et al. (2002) identified a tobacco WRKY gene, *TIZZ*, whose transcription is rapidly and transiently activated in response to *Tobacco mosaic virus* (TMV) infection in a SA-independent manner. The protein was shown to localize to the nucleus and bind W-box sequences *in vitro*. Even though WRKY genes are involved in early responses to biological and abiotic insults, some WRKY gene affects morphogenesis in *Arabidopsis*. The *Arabidopsis* plants with mutants *TTG2* have abnormal trichome and seed coat development. *Arabidopsis WRKY6* is expressed at high levels during leaf senescence and floral organ abscission (Robatzek and Somssich, 2002). Hence, like many resistance genes, the *WRKY6* expression is associated with programmed cell death. WRKY6 is both a positive and negative regulator of transcription-interestingly it negatively regulates its own transcription. Similarly, *Arabidopsis AtWRKY18*, a WRKY transcription factor that is rapidly induced in response to SA treatment, negatively regulates its own transcription (Chen and Chen, 2002). Even though ectopic expression of *AtWRKY18* provided enhanced resistance to *Pseudomonas syringae*, the constitutive, high-level expression of *AtWRKY18* produced stunted plants.

A Disease Resistance Gene Containing WRKY Domain

The products of resistance genes are believed to participate in the recognition of the specific pathogen-encoded avirulence factors in gene-for-gene interactions that activate defense response signal pathways. More than 30 disease resistance genes from either model or crop plants have been cloned so far (Dangl and Jones, 2001). The analysis of the *Arabidopsis* genome identified approximately 100 R gene loci (Sanderfoot and Raikhel, 2000). This is a relatively small number of genes compared with the number of pathogens that potentially can infect *Arabidopsis*. However, *Arabidopsis* and other plants compensate for its relatively small number of R genes by possessing multiple disease defense pathways and expressing R-proteins that recognize multiple avirulence factors. For example, the *Arabidopsis* resistance gene *RPM1* confers resistance to the bacterial pathogen *Pseudomonas syringae* that expresses either *avrRpm1* or *avrB* avirulence genes (Bisgrove et al., 1994; Grant et al., 1995). In addition, the tomato *Mi* and *Arabidopsis HRT/RPP8* confer resistance to unrelated pathogen species. These genes confer resistance to both nematode (*Meloidogyne incognita*) and insect (*Macrosiphum euphorbiae*), and viral (*Turnip crinkle virus*) and oomycete (*Peronospora parasitic*) pathogens, respectively (Rossi et al., 1998; Cooley et al., 2000). The *Arabidopsis RPW8* gene has little sequence homology to previously cloned R genes and confers broad-spectrum, SA-dependent resistance to powdery mildew and as such represents a new class of R genes (Xiao et al., 2001). Plants also produce more general responses to combat pathogen infection such as the induction of systemic acquired resistance (SAR) (Maleck et al., 2001) and the sequence specific degradation of viral RNAs by post-transcriptional gene silencing pathways (PTGS) (Marathe et al., 2000).

As described above, plants have evolved different defense strategies, such as disease resistance proteins recognizing multiple avirulence factors, SAR, and PTGS. Recent findings also indicate that recombination events have produced new combinations of functional domains that compensate for the relatively small number of R genes in plants. The *RRS1-R* gene, which confers resistance to several strains of *Ralstonia solanacearum*, is a recessive allele in *Arabidopsis* (Deslandes et al., 1998). The predicted amino acid sequences of *RRS1-R* unexpectedly reveal that it has TIR-NBS-LRR plus a single WRKY domain on the C-termini, which had been designated as *AtWRKY52* earlier (Deslandes et al., 2002;

Eulgem et al., 2000). Based on the predicted structures of the proteins they encode, R genes have been divided into five different classes (Dangl and Jones, 2001). The products of all previously isolated R genes are predicted to reside in either cytoplasm or plasma membrane including extracellular membrane where they perceive ligands. Although *RRS1-R* has not been experimentally localized to a subcellular region, its predicted amino acid sequence contains basic nuclear localization signals, suggesting that it is targeted to the nucleus unlike any other R genes (Lahaye, 2002).

In addition to the linkage, TIR-NBS-LRR and WRKY transcription factor domains, *RRS1-R* behaved in an unusual genetic manner in complementation experiments. *RRS1-R* acted as a dominant trait when delivered into *RRS1-S* genotype by *Agrobacterium*-mediated transformation (Deslandes et al., 2002). This contradicted the results from genetic study in which *RRS1-R* functioned as a recessive Mendelian trait (Deslandes et al., 1998). This phenomenon remains unexplained, although the authors proposed that *RRS1-S* is a suppressor of *RRS1-R* in F1 and F2 heterozygous plants (Deslandes et al., 2002).

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