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Plant-specific VQ-domain proteins as interaction partners of WRKY transcription factors

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Abstract

VQ-domain proteins are known to interact with WRKY transcription factors and have been reported to be involved in plant defence responses to environmental stresses in *Arabidopsis*. Thus, elucidation of the defence mechanisms during the interaction of VQ-domain proteins and WRKY transcription factors could provide useful insights into the regulation of VQ-domain protein-mediated WRKY transcription factors. As the focus of this review, we summarize the genomic analysis of the VQ-domain proteins as one of the WRKY-interacting proteins and their biological effects during plant stress conditions in *Arabidopsis* and rice.

Keywords: regulators; VQ-domain proteins; W-box elements; WRKY transcription factors

Introduction

Plants have developed defence mechanisms to survive in various stressful environments. Plants encounter abiotic and biotic stresses such as drought, salinity, freezing, nutrient deficiency and pathogen infection. The ability to survive in the face of stress determines, in part, the geographical distribution of species as well as their growth habits and life cycles. Therefore, plants have developed complicated mechanisms involving morphological, physiological and biochemical processes to cope with these stresses.

The WRKY transcription factors in plants, characterized by the WRKYGQK amino-acid sequence at the N-terminal domain and by a zinc-finger-like motif, are important regulators of stress-related genes in plants. This group is one of the largest families of transcriptional regulators in *Arabidopsis* (>70 genes) and in rice (>100 genes) (Chi *et al.*, 2013). WRKY transcription factors have been classified into three groups: groups I–III, depending on the number and structure of the WRKY zinc-finger motifs (Eulgem *et al.*, 2000). WRKY transcription factors physically interact with other proteins, such as 14-3-3-proteins, calmodulin, histone deacetylases, mitogen-activated protein (MAP) kinase, resistance proteins, VQ-domain proteins and other WRKY transcription factors (Park *et al.*, 2005; Kim *et al.*, 2008; Chang *et al.*, 2009; Popescu *et al.*, 2009; Rushton *et al.*, 2010; Cheng *et al.*, 2012; Chi *et al.*, 2013). They influence a range of biological activities including development and defence signalling in both monocotyledonous and dicotyledonous plants, including rice and *Arabidopsis* (Eulgem and Somssich, 2007).

VQ-domain proteins in *Arabidopsis*

VQ-domain proteins have a region consisting of 57 amino acids with the highly conserved 'FXXXVQX(L/V/F)TG' motif and physically interact with WRKY transcription factors (Cheng *et al.*, 2012). In *Arabidopsis*, 34 *Arabidopsis thaliana* VQ (*AtVQ*) genes have been identified, each with the conserved VQ motif. The smallest, AtVQ1,

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Table 1. Genes of VQ-domain proteins involved in stress responses

Gene names	Gene locus	Description	References
In Arabidopsis			
AtVQ9	At1G78310	Interaction with AtWRKY8 as a repressor	Hu *et al.* (2013)
		Enhanced tolerance to salt stress in *AtVQ9* mutants	
AtVQ14 (*IKU1*)	At2G35230	Interaction with AtWRKY10	Wang *et al.* (2010)
		Regulation of endosperm and seed growth	
AtVQ15 (*CAMBP25*)	At2G41010	Unknown to AtWRKY-interacting partner	Perruc *et al.* (2004)
		Response to osmotic stress	
AtVQ16 (*SIB2*)	At2G41180	Interaction with AtWRKY33	Lai *et al.* (2011)
		Defence response against necrotrophic pathogens	
AtVQ21 (*MKS1*)	At3G18690	Interaction with AtWRKY 25 and AtWRKY33	Andreasson *et al.* (2005)
		Involved in MPK4-regulated defence activation as a substrate	Qiu *et al.* (2008)
AtVQ23 (*SIB1*)	At3G56710	Interaction with AtWRKY33	
		Chloroplast-localized protein	Lai *et al.* (2011)
		Induction by the *Pst* infection	Xie *et al.* (2010)
		Defence response against necrotrophic pathogens	
In rice			
OsVQ2	LOC_Os01g46440	Induction of *Xoo* (compatible and incompatible bacteria) inoculation	Kim *et al.* (2013)
OsVQ7	LOC_Os02g15290	Tissue-specific expression	Wang *et al.* (2010)
		Induction of ABA treatment	Kim *et al.* (2013)
OsVQ12	LOC_Os03g26990	Down-regulation during panicle development	Wang *et al.* (2010)
OsVQ18	LOC_Os05g12090	Variety-specific expression pattern	Wang *et al.* (2010)
		Induction of drought treatment	Kim *et al.* (2013)
OsVQ22	LOC_Os06g33970	Up-regulation against rice blast fungus	Kawahara *et al.* (2012)
OsVQ27	LOC_Os07g06760	Induction of *Xoo* (incompatible bacteria) inoculation	Kim *et al.* (2013)
OsVQ31	LOC_Os07g48800	Tissue-specific expression	Wang *et al.* (2010)
		Induction of *Xoo* (compatible bacteria) inoculation	Kim *et al.* (2013)
OsVQ36	LOC_Os10g01240	Variety-specific expression pattern	Wang *et al.* (2010)

AtVQ; *Arabidopsis thaliana VQ*; *IKU1*, HAIKU1; *CAMBP25*, calmodulin-binding protein 25; *SIB1* and *SIB2*, sigma factor-binding proteins 1 and 2; *MKS1*, mitogen-activated protein kinase 4 substrate 1; *Pst*, *Pseudomonas syringae* pv. *tomato*; *OsVQ*, *Oryza sativa VQ*; *Xoo*, *Xanthomonas oryzae*.

protein contains 430 amino acids. There is little sequence homology among the AtVQ proteins except for the short VQ motif (Cheng *et al.*, 2012). To date, six AtVQ proteins have been functionally analysed and five of them, AtVQ9, AtVQ14, AtVQ16, AtVQ21 and AtVQ23, have been found to interact with WRKY transcription factors (Table 1). AtVQ15/AtCAMBP25, known as the calmodulin-binding protein, was the first to be reported as an AtVQ protein, although it was not called a VQ-domain protein at the time (Perruc *et al.*, 2004). The results of a functional study indicate that AtVQ15 is a negative regulator of osmotic stress responses. In a study, transgenic plants that overexpressed *AtVQ15* were found to be hypersensitive to osmotic stress during seed development (Perruc *et al.*, 2004). The second reported AtVQ protein, AtVQ21, is MAP kinase 4 substrate 1, which interacts with MAP kinase 4. The AtVQ21 protein has been reported to form complexes with AtWRKY25 and AtWRKY33, which were members of the Group I WRKY family (Andreasson *et al.*, 2005; Qiu *et al.*, 2008). AtVQ14, also called HAIKU1, regulates endosperm growth and seed development through its interaction with AtWRKY10, called MINI3 (Wang *et al.*, 2010). Recently, AtVQ23 and AtVQ16, also called sigma factor-binding proteins 1 and 2 (SIB1 and SIB2), have been identified and shown to interact with AtWRKY33, an important WRKY transcription factor involved in plant disease resistance to necrotrophic pathogens (Xie *et al.*, 2010; Lai *et al.*, 2011). These proteins complex with AtWRKY33 through the recognition of the C-terminal WRKY domain and increase the DNA-binding activity of AtWRKY33. Support for the role of these proteins as dual activators of AtWRKY33 comes from a study in which resistance to *Botrytis cinerea*, a necrotrophic pathogen, was found to be compromised in *AtVQ23* and *AtVQ16* mutants, but enhanced in transgenic plants overexpressing *AtVQ23* (Lai *et al.*, 2011). However, Xie *et al.* (2010) reported that AtVQ23-overexpressing plants exhibit resistance to only *Pseudomonas syringae* and not to *B. cinerea*. More recently, AtWRKY8 has been reported to interact with AtVQ9, which is localized in the nucleus, and this interaction has been found to decrease DNA binding to W-box repeats in target genes. Even though the *AtVQ9* gene has been found to be highly expressed during salt treatment, *AtVQ9* mutant plants exhibit enhanced tolerance to salt stress, and AtVQ9 may be a negative regulator of the AtWRKY8-mediated signalling response (Hu *et al.*, 2013).

VQ-domain proteins in rice

Accumulating evidence suggests that *Oryza sativa* WRKY (*OsWRKY*) genes (*OsWRKY6*, *OsWRKY12*, *OsWRKY13*, *OsWRKY30*, *OsWRKY45*, *OsWRKY53*, *OsWRKY71* and *OsWRKY89*) are also major regulators of the transcriptional activation of defence-related genes in rice (Liu et al., 2005, 2007; Qiu *et al.*, 2007; Shimono *et al.*, 2007; Wang *et al.*, 2007; Hwang *et al.*, 2011; Lee *et al.*, 2013). However, the VQ-domain proteins of rice have not been studied to the same extent as those of *Arabidopsis*. Thirty-nine *Oryza sativa* VQ (*OsVQ*) genes have been identified in the rice genome and found to have the conserved VQ motif, except two VQ-domain proteins (OsVQ37 and OsVQ39) (Kim *et al.*, 2013). The putative OsVQ proteins contain 77 (OsVQ2) to 439 (OsVQ35) amino-acid residues. Although expression patterns have been examined for all these *OsVQ* genes, their functions have not been assessed (Table 1).

Recently, using the rice Affymetrix GeneChip Arrays with 39 tissues of two rice indica varieties (Minghui 63 and Zhenshan 97), the expression profiles of *OsVQ* genes have been examined during development (Wang *et al.*, 2010). Notably, two genes, *OsVQ7* and *OsVQ31*, displayed tissue-specific expression at the late stage of leaf development, while variety-specific expression patterns were observed for the *OsVQ18* and *OsVQ36* genes. The expression of the *OsVQ12* gene was gradually down-regulated during panicle development (Wang *et al.*, 2010). In addition, according to the RNA-Seq analysis of *Xanthomonas oryzae* (*Xoo*)-infected rice, the *OsVQ22* gene was highly expressed following infection with the rice blast fungus, *Magnaporthe oryzae* (Kawahara *et al.*, 2012).

Additionally, we studied 39 OsVQ-domain proteins in rice and analysed the phylogenetic relationship between the VQ domains of *Arabidopsis* and those of rice. We also examined the expression profiles of *OsVQ* genes during biotic stress, specifically upon inoculation with the bacterial pathogen *Xoo*, and exposure to the abiotic stresses abscisic acid and drought (Kim *et al.*, 2013). From these initial studies, we hope to be able to elucidate the biological functions of the VQ-domain proteins in rice.

Concluding remarks

Over the past decade, a number of proteins that interact with WRKY transcription factors have been identified, using various approaches such as yeast two-hybrid screening and co-immunoprecipitation assays. Some of the proteins, including VQ-domain proteins, may activate or suppress the expression of target genes through their compatible interaction with WRKY transcription factors. The study of the chemical and/or physical interactions between WRKY transcription factors and VQ-domain proteins may permit the identification of the specific mechanisms that regulate DNA-binding activity and

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transcription efficiency during environmental stresses. Eventually, understanding the interaction between VQ-domain proteins and WRKY transcription factors may help obtain information on the molecular mechanisms of WRKY transcription factors in the regulation of stress-induced signal transduction.

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