Supporting Information

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SI Text

SI Experimental Procedures

Plasmids and Cloning. Cloning of constructs VIP1-myc_pRT100 and PR1::GUS VIP1 has been described in ref. 1. VIP1 Lysine 212 derivatives K212R and K212T as well as Trxh8_m266::GUS were generated by site-directed mutagenesis (Stratagene) using primers listed in Table S1 and verified by sequencing. Promoter::GUS constructs were prepared using the previously described vector pGreenII 0029-GUS (2) as backbone. Promoter regions of *MYB44* and *Trxh8* were PCR-amplified from genomic *Arabidopsis* Col-O DNA with primers that introduced restriction sites (Table S1) and ligated into the corresponding sites of the vector backbone. For the synthetic promoter constructs, a DNA fragment was amplified from 35S_pGreen-GUS (1) using primers VRE-35Smin or mVRE-35Smin and a reverse primer located in the GUS-coding region. PCR products were cloned as 5′ blunt-3′ *Nco*I fragments into *Sma*I-*Nco*I-opened pGUS.

Co-Immunoprecipitation. Protein extracts of protoplasts transformed with VIP1-Myc and VIP1-HA overexpression constructs were immunoprecipitated with polyclonal anti-HA antibody (rabbit) and immunoblotted with monoclonal anti-Myc antibody (mouse) as described in ref. 1.

Generation of Transgenic Plants. Transgenic *Arabidopsis* lines were generated using the floral dipping method (3) with Col-O wild-type plants. VIP1 was cloned into the binary expression vector pGreenII 0029 (4) under the control of the 35S promoter and transformed as HA epitope-tagged version. Transformed plants were selected by growth on kanamycin-containing medium. Expression of the transgene was analyzed by immunoblotting, and selected homozygous lines of the second generation after transformation used for the experiments.

Plant RNA Extraction and RT-PCR Analysis. RNA of 14-day-old seedlings was extracted using the TRIzol reagent (Sigma) optimized for plant RNA extraction. First-strand cDNA was synthesized from 2 μg total RNA using reverse transcriptase (Fermentas) with oligo(dT) and diluted 1:5 in RNase-free water. For RT-PCR analysis, aliquots of the same cDNA sample were used for all primer sets (Table S1).

Expression and Purification of VIP1 Recombinant Protein. *Escherichia coli* strain BL-21 codon plus (Stratagene) was transformed with pTX3b (New England Biolabs) carrying the VIP1 coding sequence. Growth of bacteria and isolation of VIP1 protein were performed according to the instructions of the manufacturer. Extraction, column-binding, washing, and elution were performed in buffer 1 (20 mM Tris, pH 8.0, 0.5 M NaCl, 0.1 mM EDTA, 0.1% Tween 20). After washing, chitin bead-bound VIP1 was either processed for RDSA or eluted by incubating the beads overnight in buffer 1 supplemented with 40 mM DTT. Purity of eluted protein was checked by running an aliquot on a 10% SDS gel, followed by Coomassie blue staining.

Random DNA Binding Selection Assay. Oligonucleotides RDSA_1 or RDSA_2 carrying a 17- or 18-nt degenerate region, respectively (Table S1), were rendered double-stranded by primed synthesis using primer RDSA_1re or RDSA_2re (Table S1) and Klenow fragment (Fermentas) and purified via a PCR-purification column (Jetstar). To exclude the possibility, that false positive DNA elements might be isolated because of the possible presence of VIP1-targeted motifs within the nonrandom flanking sites, the RDSA was performed in duplicates with two independent sets of input random DNA fragments (RDSA_1 and RDSA_2) flanked by two independent sets of primer annealing sites.

Chitin beads with bound recombinant VIP1 protein were washed three times in RDSA buffer (5 mM Tris, pH 8.0, 75 mM NaCl, 2.5 mM $MgCl_2$, 0.5 mM EDTA, 5% glycerol, 1% Tween, 1 mM DTT), resuspended in RDSA buffer, divided into 2× five aliquots and kept on ice. The first aliquots were incubated in 30 μL RDSA buffer with 0.1 nmol double-stranded RDSA_1 or RDSA_2 at room temperature for 40 min. Following three 1-mL washes of the beads in RDSA buffer, the DNA was released by boiling the beads in 50 μL water and purified via a PCR purification column (Jetstar). The recovered DNA was amplified in a 20-μL PCR (nine cycles) containing primers RDSA_1fo/re or RDSA_2fo/re, respectively. PCR products were column-purified (Jetstar) and subjected to another cycle of RDSA by incubating them with the second aliquot of chitin bead-bound recombinant VIP1 protein. Five such cycles were performed, with increasing stringency by raising the amounts of the competitor poly(dIdC) (0, 10, 20, 50, and 100 ng) and lowering the amounts of protein in the binding reaction. PCR products recovered after the fifth RDSA cycle were cloned into pGemTeasy (Promega) and sequenced.

Motif Abundance Calculation. The Patmatch tool (http://www.arabidopsis.org/cgi-bin/patmatch/nph-patmatch.pl) was used to identify all *Arabidopsis* genes with ACNGCT motifs in the 500-bp upstream regulatory region. A gene ID list of all genes whose promoters harbor at least two such motifs was generated ("list VRE"). IDs of treatment-responsive genes were extracted from published microarray data ("list array 1, 2,… etc.). Of each microarray study, the set of stress-responsive genes with at least two VRE within the 500-bp promoter region was determined through pairwise comparison of "list arrays 1, 2,… etc." with "list VRE" using the AttedII minitool (http://atted.jp/tool.html). The proportion of multiple-ACNGCT-motif-harboring promoters within a given dataset (number of genes overlapping in "list array" and "list VRE" divided by the number of all treatment-responsive genes in the respective microarray) was calculated and compared with the relative abundance of such promotors among all *Arabidopsis* genes. Statistical analysis (Chi^2 test) was performed using the GraphPad software.

1. Djamei A, Pitzschke A, Nakagami H, Rajh I, Hirt H (2007) Trojan horse strategy in *Agrobacterium* transformation: Abusing MAPK defense signaling. *Science* 318:453–456.
2. Doczi R, et al. (2007) The *Arabidopsis* mitogen-activated protein kinase kinase MKK3 is upstream of group C mitogen-activated protein kinases and participates in pathogen signaling. *Plant Cell* 19:3266–3279.
3. Clough SJ, Bent AF (1998) Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16:735–743.
4. Hellens RP, Edwards EA, Leyland NR, Bean S, Mullineaux PM (2000) pGreen: A versatile and flexible binary Ti vector for *Agrobacterium*-mediated plant transformation. *Plant Mol Biol* 42:819–832.

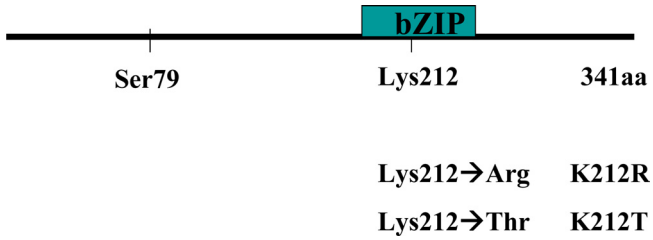

Fig. S1. VIP1 protein structure. MPK3 phosphorylation site (Ser-79), bZIP domain, and DNA binding specificity-determining residue Lys-212 are indicated. In mutant variants K212R and K212T, Lys-212 had been replaced by a similar (Arg) or nonsimilar (Thr) residue.

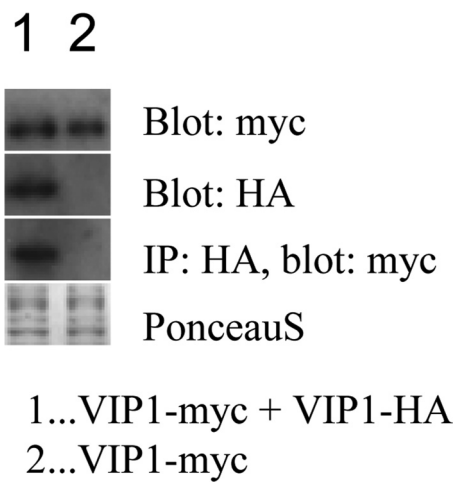

Fig. S2. VIP1 forms homodimers. Coimmunoprecipitation of VIP1. Protein extracts from *Arabidopsis* protoplasts overexpressing VIP1-Myc and VIP1-HA (lane 1) or VIP1-Myc only (lane 2) were subjected to immunoblotting before and after immunoprecipitation with anti-HA antibody. Equal protein loading was visualized by PonceauS staining.

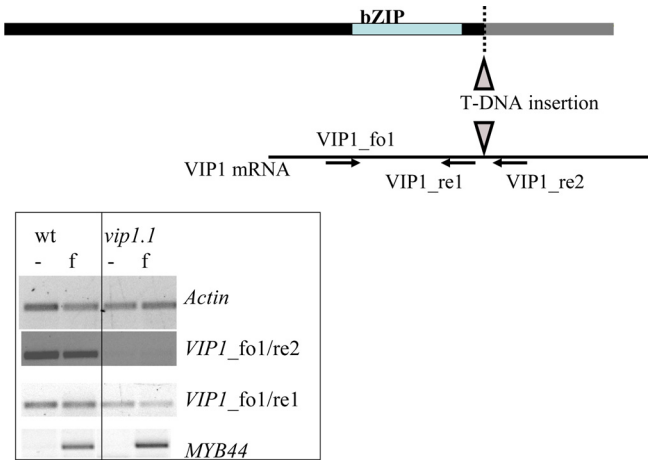

Fig. S3. *vip1-1* mutants form a truncated but functional bZIP protein and are not affected in stress gene expression. *Upper*: schematic presentation of the VIP1 coding sequence. The bZIP domain and T-DNA insertion site are indicated. Primer annealing sites are indicated by arrows. *Lower*: semiquantitative RT-PCR analysis of 14-day-old wild-type and *vip1-1* seedlings after 20-min mock (-) or flg22 (f) treatment.

Table S1. List of oligonucleotides

Primer name	Gene ID	Sequence	Restriction site
		Primers for cloning of promoters	
Trx8Prom_fo	At1g69880	GTTAGATGGAcTGCAgTTGGAAC	*PstI*
Trx8Prom_re	At1g69880	ACGTTAGCACCCATgGAGTGTG	*NcoI*
MYB44Prom_re	At5g67300	TATCAGCCATgGATTTTGGAATG	*NcoI*
MYB44Prom_fo	At5g67300	GTCTGCAGACAGCTGTACCGCAAACTTTGA	*PstI*
MYB44Prom_fo_m1	At5g67300	GTCTGCAGAaAaCTGAACCGCAAACTTTGA	*PstI*
MYB44Prom_fo_m2	At5g67300	GTCTGCAGAaAaCTGAACCGCAAACTTTGAAAAAtCGCT	*PstI*
VRE1–35Smin		TACAGCTGTCTACAGCTGTCATGGCAAGACCCTTCCTC	
mVRE1–35Smin		TACAAAAGTCTACAAAAGTCATGGCAAGACCCTTCCTC	
GUS_re		CCACACTTTGCCGTAATGAGTG	
		Primers for site-directed mutagenesis	
VIP1_K212R	At1g43700	GCGAGGTCGAgAGAGAGGAAGATTAGGTATACTGG and TATACCTAATCTTCCTCTCTcTCGACCTCGCCGCA	
VIP1_K212T	At1g43700	GCGAGGTCGAcAGAGAGGAAGATTAGGTATACTGG and TATACCTAATCTTCCTCTCTgTCGACCTCGCCGCA	
Trxh8_m266	At1g69880	CATGCGATAGATATAGCGTAAGACAataTTTAGAATTCC and GGAATTCTAAAtatTGTCTTACGCTATATCTATCGCATG	
		Primers for RDSA	
RDSA_1		TAGTTGAGTCTCACAAACGAACAC(N17)CATTCCAAAATCCATGGCTGATA	
RDSA_1fo		TAGTTGAGTCTCACAAACGAACAC	
RDSA_1re		CATTCCAAAATCCATGGCTGATA	
RDSA_2		AATGGATCCAAGCTTAAGC(N18)CGTTGAATTCCCATGGACA	
RDSA_2fo		AATGGATCCAAGCTTAAGC	
RDSA_2re		TGTCCATGGGAATTCAACG	
		Primers for EMSA	
as-1_fo		CTCTACGTCACTATTTTACTTACGTCATAGATG	
as-1_re		CATCTATGACGTAAGTAAAATAGTGACGTAGAG	
E1_fo		TAGTTGAGTCTCACAAACGAACACGACAGCTCAGTGAAGGCCATTCCAAAA TCCATGGCTGATA	
E1_re		TATCAGCCATGGATTTTGGAATGGCCTTCACTGAGCTGTCGTGTTCGTTTGTG AGACTCAACTA	
E1m_fo		TAGTTGAGTCTCACAAACGAACACGACAGaaCAGTGAAGGCCATTCCAAAA TCCATGGCTGATA	
E1m_re		TATCAGCCATGGATTTTGGAATGGCCTTCACTGttCTGTCGTGTTCGTTTGTGA GACTCAACTA	
		Primers for RT-PCR	
Actin_fo	At2g37620	ATGGTTAAGGCTGGTTTTGC	
Actin_re	At2g37620	AGCACAATACCGGTAGTACG	
VIP1_fo1	At1g43700	ATCGAACGGTGTTGTTCCTC	
VIP1_re1	At1g43700	GGATCAAGCAAAGCAAGCTC	
VIP1_re2	At1g43700	AGCTCTGATGTTCCTCTCTG	
Trxh8_fo1	At1g69880	GTGTGGACCATGTAAAACCCTTG	
Trxh8_re1	At1g69880	GTGTGGACCATGTAAAACCCTTG	
Primers for ChIP			
180repeat_fo		ACCATCAAAGCCTTGAGAAGCA	
180repeat_re		CCGTATGAGTCTTTGTCTTTGTATCTTCT	
WRKY18 ChIP_fo	At4g31800	CCTAACATATCCCAAAACCTATC	
WRKY18			
ChIP_re	At4g31800	AACCGTCCATAAAAGAAACCTTTATC	
MYB44 ChIP_fo	At5g67300	AAACCGCTTGCGTGGAAGAAGC	
MYB44 ChIP_re	At5g67300	TATCAGCCATGGATTTTGGAATG	

Restriction sites introduced by primers used for cloning are underlined. Mismatches to wild-type sequences are shown in lower case.