BIOLOGIA PLANTARUM 59 (1): 18-28, 2015 DOI: 10.1007/s10535-014-0478-6

In silico characterization and expression analysis of selected *Arabidopsis* receptor-like kinase genes responsive to different MAMP inducers

S-A. NEW, L.A. PIATER, and I.A. DUBERY*

Department of Biochemistry, University of Johannesburg, P.O. Box 524, Auckland Park, 2006, South Africa

Abstract

Candidate *Arabidopsis thaliana* S-domain receptor-like kinase (*SD-RLK)* (*At1g11330*, *At1g61430*, and *At1g61610*) and leucine-rich repeat receptor-like kinase *(LRR-RLK)* (*At1g51850*, *At2g19190*, and *At5g45840*) genes were characterized utilizing *PlantPAN*, *AGRIS*, and *AthaMap* databases. Following determination of the main conserved domains, both classes of RLKs were found to be structurally similar with extracellular, transmembrane, and intracellular domains including a serine/threonine kinase domain, which might suggest a functional role in intracellular signal transduction. This established that the *RLK* genes had a superficially similar structure but distinct ligand binding domains. The expressions of these genes in response to a treatment with microbe-associated molecular pattern molecules (MAMPs), namely lipopolysaccharides, flg22 peptide from flagellin, peptidoglycan, chitosan, and ergosterol were compared. The candidate *RLKs*, potentially involved in surveillance, were found to be responsive to the elicitation treatments. Furthermore, differential regulation that was observed at the transcriptional level as well as the intensity of responses could possibly be correlated to the promoter architecture. With the use of *in silico* analyses, the architectures of 1 000 bp promoter regions upstream from the transcription start sites were determined. The analyses also revealed putative defense-related *cis*-regulatory elements that included W-boxes, MYB factor, AP2/ERF-, GT1- and ATHB-5 binding sites. The frequency at which these *cis*-elements occurred in each promoter differed, and the number or clusters within the core-regulatory region of individual promoters might be indicative of the responsiveness of each gene to the MAMP elicitation.

Additional key words: *cis*-elements, elicitation, defense, lipopolysaccharides, transcription factors.

Introduction

Plant cells respond to perception of bacterial lipopolysaccharides (LPS) through the up-regulated expression of receptor-like kinase (*RLK*) and resistance (*R*) genes (Sanabria and Dubery 2006, Antolín-Llovera *et al.* 2012). This phenomenon is not limited to LPS, but also observed in response to other microbe-associated molecular pattern (MAMP) molecules (Navarro *et al.* 2004, Sanabria *et al.* 2008). A positive feedback regulation operates in the MAMP-triggered immunity (MTI) branch of plant innate immunity, with a transcriptional activation of the components involved in perception and signaling (Navarro *et al.* 2004, Sanabria *et al.* 2012). However, it is not known if this represents a generic response (Kemmerling *et al.* 2011) or whether some stimulus-specificity (Antolín-Llovera *et al.* 2012) is involved.

RLKs constitute one of the largest classes of plant defense genes in *Arabidopsis thaliana,* and it contains the well-known leucine-rich repeat (LRR) and lysin-motif (LysM) RLKs as potential pattern-recognition receptors (PRRs) (Antolín-Llovera *et al.* 2012, Wu and Zhou 2013). In addition, members of the S-domain RLKs (SD-RLKs) may also be involved in pathogen perception (Sanabria *et al.* 2008, 2012). Some members of these RLKs are able to detect MAMPs, which are conserved microbe-derived molecules, and trigger a battery of basal defense responses. The growing list of studied MAMPs include LPS, peptidoglycan (PGN), and flagellin (FLG) from bacteria, and chitin (CHIT) and ergosterol (ERG) from fungi (Gerber *et al.* 2004, Gust *et al.* 2007, Aslam *et al.* 2009, Zipfel and Robatzek 2010, Erbs and Newman 2012, Vidhyasekaran 2014).

———

Submitted 13 March 2014, *last revision* 7 June 2014, *accepted* 17 June 2014.

Abbreviations: CHIT - chitosan, CRE - cis-regulatory element, ERG - ergosterol, Flg22 - flagellin-derived peptide, LPS - lipopolysaccharide, MAMP - microbe associated molecular pattern, MTI - MAMP-triggered immunity, PGN - peptidoglycan, RLK - receptor-like kinase, TF - transcription factor.

Acknowledgements: This study was supported by the South African National Research Foundation and the University of Johannesburg, South Africa. We thank Robert Gerrard for technical assistance.

* Corresponding author, fax: (+27) 0115592605, e-mail: idubery@uj.ac.za

The up-regulated expression of *RLK* genes in response to MAMPs presumably leads to an enhanced sensitivity of the plant to further RLK-associated stimuli, sensing the presence of invading micro-organisms, *i.e.* a primed or sensitized state (Newman *et al*. 2007, Sanabria *et al*. 2010). This might enable the plant to have an increased ability to recognize pathogens and to respond more rapidly as well as more intensely to subsequent pathogen challenge (Navarro *et al*. 2004, Sanabria *et al*. 2008, 2010). Roles for SD-RLKs and LRR-RLKs in perception and signal transduction events might be through recognition and binding ligands, or in an indirect manner involving ligand-induced conformational changes, dimerization, or recruitment of a co-receptor to initiate both auto- and trans-phosphorylation of itself and other proteins. Moreover, PRRs may interact with additional transmembrane proteins that act as signaling adapters or amplifiers to achieve full functionality (Zipfel and Robatzek, 2010).

Signal integration and weighing diverse input signals within signaling networks occurs at different sites including the activation of transcription factors (TFs) that result in changes in gene expression (Riechmann 2002). Multiple signaling pathways regulate defense responses, and these often overlap (Hu *et al*. 2010). The timing of and the levels to which stress-related genes are induced depend on *de novo* synthesis of proteins or signaling molecules, such as salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA). In response to pathogen attack, plants are able to rapidly generate new transcripts, resulting in a stress-related coping mechanism which is activated to counter the potential risk of infection. A large fraction of the *Arabidopsis* genome encodes TFs, *i.e.*, over 1 500 TFs that usually form large plant-specific families (Singh *et al.* 2002, Priest *et al.* 2009). TFs play vital roles in activation of plant defense genes that are pivotal in defense mechanisms (Rushton and Somssich 1998, Chen *et al*. 2002).

Much remains to be discovered about the *cis*-regulatory elements (CREs) and TFs, and deciphering the molecular mechanisms behind the events pertaining to plant defense responses is a challenging field of research (Van Veck *et al.* 2011). Due to the intricate nature of plant signaling networks, using wet bench molecular tools like homo- and heterologous expressions, deletion analysis, and reporter genes to identify and characterize all defense-related genes would be unfeasible. To assist in new developments, *in silico* analyses or bioinformatic web-based tools can be used for the prediction or analysis of domain architecture and gene products. Such analyses allow for a starting point in understanding the key TFs involved in plant defenses as well as the signature motif regions found in defense genes. Various databases are available to gather information in this regard (Shameer *et al.* 2009), and by using bioinformatic tools, promoters may be analyzed for over-represented *cis*-elements (Priest *et al.* 2009). Locating TF binding sites (TFBS) in complete genome sequences allows identification of distinct TFs and thereby making it possible to generate models to demonstrate gene regulation (Bülow *et al.* 2006). This type of data allow a defense-related gene promoter to be charted/annotated with possible defense-related CREs, and can further be probed to ascertain which combinations of putative TFBS and TFs exist in such promoter sequences.

In this report, candidate *SD-RLK* and *LRR-RLK* genes that were found to be up-regulated/co-expressed in response to a LPS treatment (*AtGenExpress*, expression set: 1008080727; Livaja *et al.* 2008), were chosen to investigate whether converging or specific responses exist for individual MAMPs. These included the flg22 peptide of flagellin, lipopolysaccharide (a lipoglycan with lipid A, core oligosaccharide and O-polysaccharide moieties), peptidoglycan (an alternating copolymer of β-(1→4)-N-acetylglucosamine and N-acetyl-muramic acid), chitosan (a polycation, polyamine of β-(1→4) glucosamine) and ergosterol (an ergosta-5,7,22-trien-3β-ol sterol). The selected *SD-RLK* (*At1g11330*, *At1g61430*, and *At1g61610*) and *LRR-RLK* (*At1g51850*, *At2g19190*, and *At5g45840*) genes and their promoters were characterized at an *in silico* level utilizing bioinformatic tools. Subsequent expression analyses in response to LPS, PGN, Flg22, ERG, and CHIT were also performed to evaluate the responsiveness of the genes towards elicitation by different MAMPs.

Materials and methods

Selection of target genes: Both the *Genevestigator* (plant biology) (https://www.genevestigator.com) and *TAIR* (The *Arabidopsis* Information Resource, www.arabidopsis.org) *AtGenExpress* microarray data expression set 1008080727 were used to identify *SD-RLK* and *LRR-RLK* genes that showed up-regulation upon a LPS or flg22 treatment. *At2g19190*, which is also known as *flg22-induced receptor-like kinase 1* (*FRK1*), was included as known marker gene associated with MAMP-triggered immunity (MTI). Table 1 lists the fold induction of the selected genes at a 4 h time point when induced with LPS or flg22.

Bioinformatic tools for sequence retrieval: Full genomic sequences of the selected *SD-RLK* (*At1g11330*, *At1g61430*, and At1g61610) and *LRR-RLK* (*At1g51850*, *At2g19190*, and *At5g45840*) genes were retrieved from *NCBI Gene* (www.ncbi.nlm.nih.gov) using *GenBank*, and the architecture of each gene determined. In conjunction with the sequence data, *NCBI Gene* indicates genomic regions, transcripts, and products. Genomic annotations of the relative positions of the conserved domains within each gene were gathered and compared using *NCBI* and *SMART* (Simple Modular Architecture Research Tool, www.smart.embl-heidelberg.de). Most of the data on

NCBI is curated from various sources, main being *TAIR*, and these usually only differ in output format. *NCBI* additionally provides direct links to supporting databases. Following analyses, the collected data allowed the genes to be graphically represented, demonstrating conserved domains, exons, introns, and start/stop codons.

Elicitor preparation and elicitation of *Arabidopsis thaliana* cells: Lipopolysaccharides (LPS) from *Burkholderia cepacia* (Coventry and Dubery 2001), peptidoglycan (PGN) from *Streptomyces* sp. (*Sigma-Aldrich,* St Louis, MO, USA), and chitosan (CHIT) purified from chitin (*Sigma-Aldrich*) according to Louw and Dubery (2000) were prepared as stock solutions of 1 mg cm^{-3} and added to *Arabidopsis thaliana* cells at final concentrations of 100 μg cm^{-3}. Flagellin peptide (flg22, FLG, *GL Biochem Laboratories*, Shanghai, China) was prepared as a 1.0 μM stock in water, and ergosterol (ERG, *Sigma-Aldrich*) as a 1 mM stock in 0.2 % (v/v) ethanol. Final concentrations of FLG and ERG were 100 nM and 100 μM, respectively, in a half-strength Murashige and Skoog (1962; MS) medium.

Arabidopsis thaliana L. (ecotype Columbia) callus cells were used for all treatments and prepared as previously described (Huang *et al.* 2012). A friable *A. thaliana* callus (100 mg) was placed together with a half-strength MS medium (pH 5.8) into separate RNase-free tubes for each treatment with the respective elicitors. The expression of target genes was followed 0 and 4 h after the treatment and compared with a control. The tubes were placed horizontally on an orbital shaker (*Labcon*, Johannesburg, South Africa) at 120 rpm and 25 °C for 1 h. Then, the cells were treated with 0.2 cm^3 of each elicitor to the final concentrations as specified. Control cells were treated only with the MS medium. All the tubes were placed back on the shaker and kept for the specified time intervals.

Expression analyses of selected *RLK* genes in response to lipopolysaccharides, peptidoglycan, chitosan, flagellin peptide-22, and ergosterol: Following induction, the MS medium was removed from the treated cells and RNA isolated using the *Trizol* reagent (Huang *et al.* 2012). A DNase treatment and RNA precipitation with 8 M lithium chloride was included according to Sambrook and Russell (2000). Isolated RNA was quantified, a RNase inhibitor (recombinant *RNasin*®, *Promega*, Madison, WI, USA) was added and the quality and integrity of RNA was analyzed by gel electrophoresis (Huang *et al.* 2012). RNA free of DNA contamination was divided into aliquots and stored at -20 °C.

To examine the expression profiles of target genes, semi-quantitative RT-PCR was employed using total RNA isolated from an *A. thaliana* callus elicited with MAMPs. All gene-specific primers for *A. thaliana SD-RLK* and *LRR-RLK* target genes were designed utilizing *Beacon Designer* (http://www.premierbiosoft.com/molecular_beacons/index.html) and *QuantPrime* (http://www.quantprime.de/). An *18S rRNA* gene (*18S*) was used as internal control reference gene (Dean *et al.* 2002). Gene-specific amplicons were sequenced to confirm their identity.

The RNA (250 ng) was used as template for the amplification of each gene using gene-specific primers (Table 1*A* Suppl.) and a *Robust II* RT-PCR kit (*Finnzyme*, *Thermo Scientific,* Waltham, MA, USA) according to the manufacturer's instructions. RT-PCR cycling conditions were programmed into a *Mastercycler*® thermal cycler (*Eppendorf*, Hamburg, Germany) as follows: a reverse transcription using an oligo-dT primer at 42 °C for 60 min and an enzyme inactivation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, annealing primers (Table 1*A* Suppl.) for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR products were separated and visualized on a 1 % agarose gel stained with ethidium bromide. Densitometry was performed and band intensities quantified using image analysis and the *Quantity One*™ software (*Bio-Rad Laboratories*, Hercules, CA, USA). The mean results were combined and fold-induction calculated.

Identification of *cis*-regulatory elements: In order to identify defense-related *cis*-elements in the promoters of the selected *RLKs*, *PlantPAN* was used as primary database (http://PlantPan.mbc.nctu.edu.tw, Chang *et al.* 2008), as well as *AGRIS* (http://arabidopsis.med.ohio-state.edu, Davuluri *et al.* 2003), *PLACE* (http://www.dna.affrc.go.jp/PLACE, Higo *et al.* 1999), and *AthaMap* (http://www.athamap.de, Bülow *et al.* 2006). A 1 kb (-1000 bp) promoter sequence upstream of the predicted start codon (ATG) was chosen since most *A. thaliana* promoters display *cis*-elements within this region (Maleck *et al.* 2000). From these databases, a list of *cis*-elements, corresponding TFs, and *cis*-element sequences and positions within the promoters was generated.

Databases for gene and promoter analysis included *NCBI*, *SMART*, Genevestigator, (https://www.genevestigator.com), and *TAIR*, and supplied data, such as the complete genome sequence, domain architecture, gene products, and gene expression profiles of target genes. Promoter analysis using a database, such as *PlantPAN*, was used for identifying and collecting putative *cis*-elements found in promoters. *PlantPAN* consists of TFs profiles curated from databases, such as *PLACE* and *AGRIS*. Probing other databases for promoter analysis studies ensured that the data collected from *PlantPAN* were validated for each target gene. As such, *AGRIS*, *AthaMap*, and *PLACE* provided information about the TFs and *cis*-elements found in *A. thaliana*, which can be cross-referenced with data collected from other sources.

The *Arabidopsis Stress Responsive Transcription Factor Data Base* (*STIFDB*) (http://caps.ncbs. res.in/stifdb) was used to collect data on putative stress-related TFs and the frequency with which TFBS may occur in a promoter. Like other databases, such as *NCBI* or *PlantPAN*, this site allows direct links to alternative search engines including *TAIR* (Shameer *et al.* 2009). Plant TF databases

(http://plntfdb.bio.uni-potsdam.de/v3.0 and http://planttfdb.cbi.pku.edu.cn) added additional information regarding plant TF families.

Results

The genomic sequence of each target gene was retrieved from *NCBI*, and the gene architecture located within the sequences analyzed (Table 1*A-F* Suppl., http://goo.gl/olAUgi). The *SD-RLK* genes demonstrated similar gene architecture. Subsequently, the genomic sequence for each gene was used to obtain a graphic representation of the conserved domains found in the selected genes utilizing *SMART*. The similarity between the main conserved domains can clearly be observed (Fig. 1).

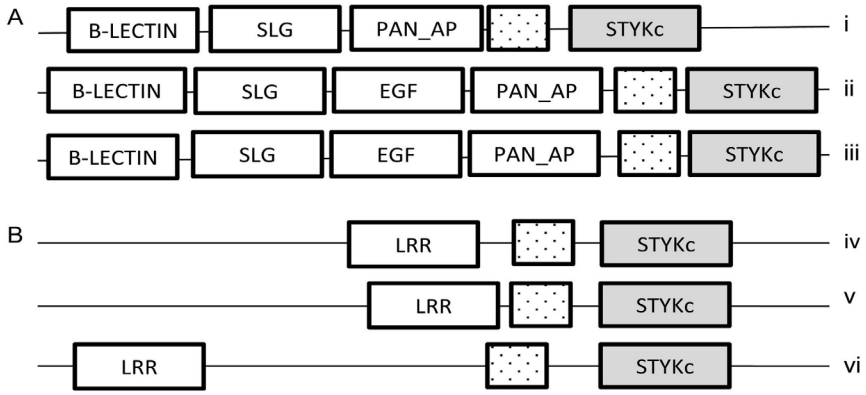

Fig. 1. *A* - The main conserved domains within *SD-RLK* genes: i - *At1g11330*, ii - *At1g61430*, and iii - *At1g61610*. The domains are as follows: B-lectin, S-locus glycoprotein (SLG), epidermal growth factor (EGF), plant PAN/APPLE-like domain (PAN_AP), transmembrane domain, and the serine/threonine (STYKc) domain *in grey*. *B* - The main conserved domains within *LRR-RLK* genes: iv - *At1g51850*, v - *At2g19190*, and vi - *At1g45840*. The domains are as follows: leucine rich-repeat (LRR), transmembrane domain, and the serine/threonine (STYKc) domain *in grey*.

At1g11330 and *At1g61610* were annotated as encoding S-locus lectin protein kinase family proteins, whereas *At1g61430* encoded a putative protein from the same family. These genes contained a characteristic serine/ threonine protein kinase domain, S-glycoprotein domain, and B-lectin domain-bulb-type mannose-specific lectin (Fig. 1*A*). The *SD-RLKs* also contained a plant PAN/APPLE-like domain which was found in plant S-receptor protein kinases and secreted glycoproteins. Lastly, an epidermal growth factor (EGF)-like domain was found in both *At1g61430* and *At1g61610*. Therefore, the *SD-RLKs* consisted of different characteristic domains which are usually found in this family and involved in ligand binding (Sanabria *et al.* 2012, Xing *et al.* 2013).

The *LRR-RLK* genes demonstrated a similar gene architecture and conserved domains found in receptor-like kinases (Fig. 1). *At1g51850* was annotated as encoding a putative RLK protein, whereas *At2g19190*, which is also known as the Flg22-induced receptor-like kinase 1 (*FRK1*), encoded a protein kinase, and *At5g45840* was a serine/threonine protein kinase-encoding gene. All three genes contained a LRR-domain which is a repetitive motif responsible for ligand binding, and a serine/threonine protein kinase domain containing an ATP binding site. The genes were annotated as functioning in amino acid phosphorylation.

To examine the expression of the target genes in *Arabidopsis*, experimental conditions were determined by semi-quantitative (sq) RT-PCR analysis using FLG22, LPS, PGN, CHIT, and ERG as MAMPs to induce a response over a time course of 1 and 4 h. These time intervals were chosen to coincide with the time frame during which the genes are reported to be induced. (Laquitaine *et al.* 2006, Sanabria *et al*. 2012)

The sqRT-PCR results illustrate that the reference gene, *18S rRNA,* exhibited a stable expression over these time points and was not responsive to any of the MAMPs. Clear profiles for each target transcript displayed either up- or down-regulation, establishing comparative expression over the time-course of MAMP treatment (Fig. 2*A*,*B*). *At2g19190*, the FLG/flg22-induced RLK (*FRK1*), a known marker gene associated with MTI, was induced as expected. Different basal expressions as well as different responses to the various MAMPs were seen. In general, the effect of the MAMPs on the *LRR-RLK* expression could be discerned at 4 h post-elicitation, *e.g.*, for the *SD-RLK At1g11330* in response to LPS and FLG and for *At1g61610* towards CHIT. As reported below, these observations generally correlated to the promoter architecture of the corresponding genes with regard to the number of defense-related CREs within the core/proximal regulatory region and/or clusters of *cis*-elements within these regions (approximately -250 bp upstream from the start codon). Minimal or no increase in

the expression was associated with promoter architecture which consisted of fewer *cis*-elements found in the core regulatory regions.

The intergenic regions upstream of the genes ranged from approximately 900 to 3 200 bp. The -1000 bp regions upstream of the ATG start site of each candidate gene were analyzed using *PlantPAN*, and a list of all putative defense-related *cis*-elements with regard to the position and strand was generated. This information was used to chart the promoter sequence and demonstrate the occurrences of putative CREs that are most likely recognized by defense-related TFs, for example W-boxes and their corresponding WRKY TFs. This initial step aided in identifying the promoter regions of selected genes that are most likely involved in defense responses and generated a number of defense-related regulatory elements. The main TFs that play a role in defense are WRKY, MYB, AP2/ERF, and bZIP (Riechmann 2002, Singh *et al.* 2002), all of which were identified in the current study (Table 2) and correlated well with the *cis*-elements related to defense signaling. The W-boxes, MYB factors, and AP2/ERF *cis*-elements were chosen for further investigation.

The -1000 bp promoter sequences of each *SD-RLK* and *LRR-RLK* gene that had been annotated with putative CREs are presented in detail in Fig. 1*A-F* Suppl. The

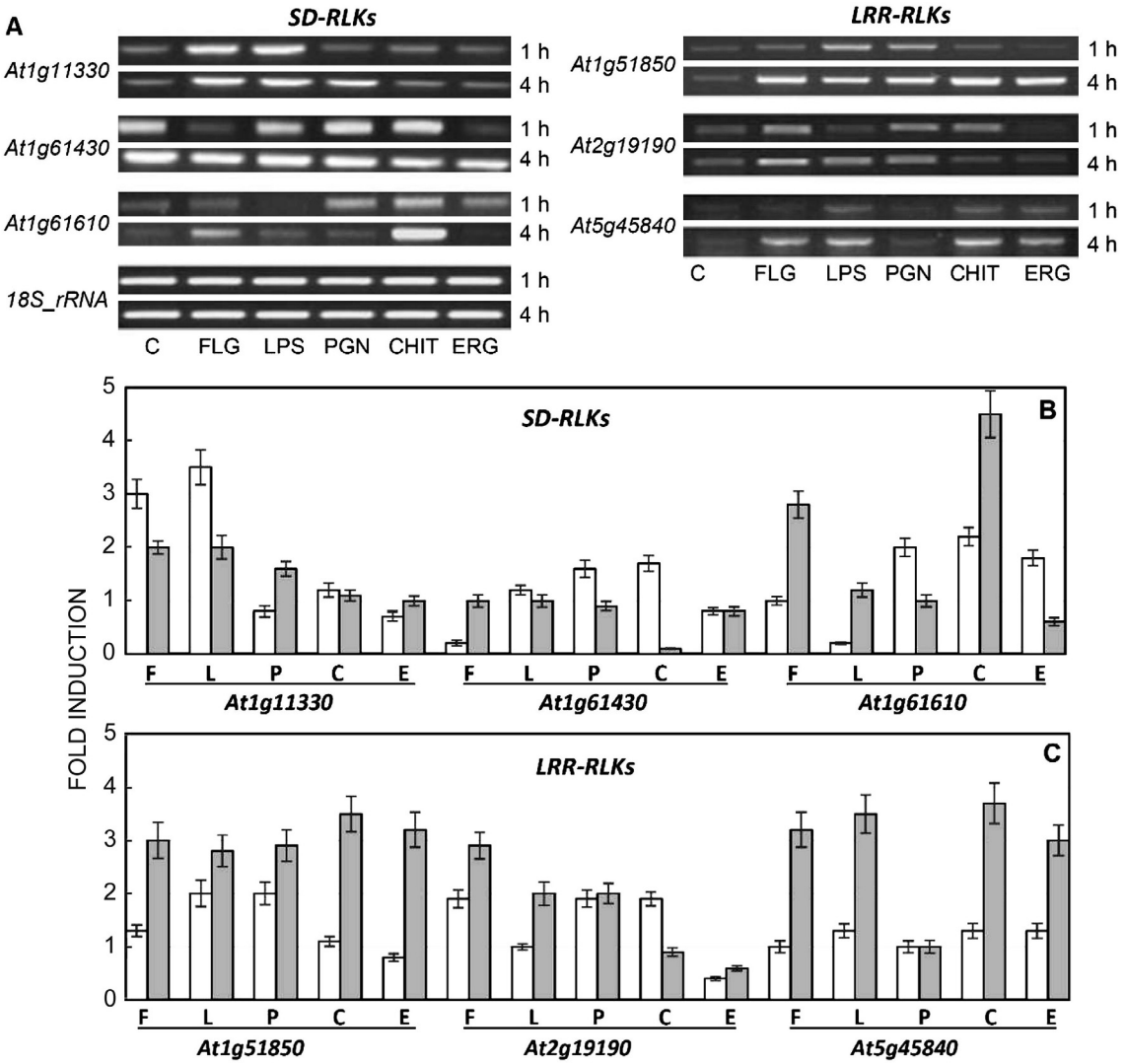

Fig. 2. *A* - Semi-quantitative RT-PCR results of the expressions of *SD-RLKs* - *At1g11330* (342 bp), *At1g61430* (312 bp), *At1g61610* (308 bp), *LRR-RLKs* - *At1g51850* (685 bp), *At2g19190* (253 bp), *At5g45840* (488 bp), and *18S* (200 bp) amplicons after 35 cycles. The intensity of the amplicons indicates the responses after 1 and 4 h elicitation using FLG (F), LPS (L), PGN (P), CHIT (C), ERG (E), and control (0 h, no MAMP) treatments. sqRT-PCR of constitutively expressed *18S rRNA* as invariant reference gene allowed for the comparison of the relative expressions of target/reference genes (*B* and *C*): A graphical presentation of fold-induction with standard deviations of *SD-RLK* and *LRR-RLK* genes relative to the 0 h control after the elicitations for 1 h (the *white bars*) and 4 h (the *grey bars*). The results recorded for each target gene are representations of four independent biological repeats.

Table 1. Description of *SD-RLK* and *LRR-RLK* genes selected according to microarray data from a *TAIR* expression set: 1008080727 *AtGenExpress*. Microarray data of the effect of different pathogen-derived elicitors 1 and 4 h after infiltration into leaves from 5-week-old *Arabidopsis* seedlings. Transcripts from each gene showed up-regulation at 4 h in response to LPS or flg22.

Gene	Number of exons	Description	Elicitor	Log_2 signal value	Notes
At1g11330	7	S-locus lectin protein kinase family protein	LPS flg22	11.75 11.65	homologous to *Nt-SD-RLK*
At1g61430	7	S-locus protein kinase family protein	LPS flg22	7.41 7.36	
At1g61610	7	S-locus lectin protein kinase family protein	LPS flg22	8.75 8.67	At1g11330 syntenic pair
At1g51850	14	leucine-rich repeat protein kinase protein	LPS flg22	10.87 14.97	
At2g19190	13	leucine-rich repeat protein kinase protein	LPS flg22	13.00 16.26	flg22-induced RLK 1 *(FRK1)*
At5g45840	13	leucine-rich repeat protein kinase protein	LPS flg22	8.10 7.98	

Table 2. Summary of putative *cis*-elements and transcription factors related to resistance against pathogens found within the promoters of the selected *SD-RLK* and *LRR-RLK* genes (regulatory elements in *bold* were focussed on in this study).

Transcription factor	TF family	Response	Reference
ACGTATERD1, ABRERATCAL ACGT ABRE-MOTIF, A2OSEM	ABRE	biotic and abiotic responses, ABA	Lenka *et al.* 2008
ASF1MOTIFCAMV	bZIP	pathogen and SA response, as-1 element	Redman *et al.* 2002
ARFAT	ARF	found in the promoters of primary/early auxin response genes.	*PlantPAN*
ATHB-5	HD-ZIP	ABA responsiveness	Johannesson *et al.* 2003
ATHB-9	HD-Zip	environmental stresses	Chen *et al.* 2002
AtSR1-6	CAMTA/AtSR	stress signalling (ET and ABA signalling), calmodulin	Yang and Poovaiah 2002
DPBFCOREDCDC3 / DPBF1&2	bZIP	ABA-responsive	Finkelstein and Lynch 2000
GBF5	bZIP	stress signalling	Fusco *et al.* 2005
GT1	Trihelix	abiotic and biotic stress	Redman *et al.* 2002,
GT-3b		pathogen response	Park *et al.* 2004
HAHB4	HD-ZIP	biotic stress, JA and ethylene signaling, wounding	Manavella *et al.* 2008
MYB (1, 2, 3, 4)	R2/R3-MYB	wounding, anaerobic stress and pathogen attack	Stracke *et al.* 2001
MYB1LEPR	MYB	tomato Pti4 (ERF) regulates defence genes by using a GCC box and non-GCC box *cis* element	*PlantPAN*
AtMYC2	bHLH	AtMYC2 is involved in the JA signalling pathway, protein regulator involved with wounding, and a negative regulator of pathogen related genes	Jalali *et al.* 2006
RAV1	AP2/ERF	low temperature, pathogen attack, wounding, drought, and salt stresses	Kagaya *et al.* 1998
TGA (1, 2, 3, 4, 5)	bZip	PR-gene regulation, disease resistance	Kesarwani *et al.* 2007
WBOXATNPR1	WRKY	defense-related environmental stress	Chen *et al.* 2002
W-BOX	WRKY	abiotic and biotic stresses interacting physically and functionally in a complex pattern of overlapping, antagonistic distinct roles in plant responses to different types of pathogens	Pandey and Somssich 2009

figure includes all putative defense *cis*-elements (over and above those significant to this study). The number/frequency of occurrence of defense-related elements that were found in all three *SD-RLK* and *LRR-RLK* promoters, respectively, are in Table 3. Of the *LRR-RLK* genes, *At2g19190* (*FRK1*) contained more of the selected CREs, whereas no clear pattern emerged for the *SD-RLK* genes.

Table 3. Summary of the *in silico* analysis of the occurrence of selected, predicted *cis*-elements in the -1000 bp upstream promoter region of target *SD-RLK* and *LRR-RLK* genes associated with plant defense (refer to Fig. 1*A-F* Suppl. for promoter sequences).

Gene family	Gene	W-box	MYB	AP2/ERF/RAV1	ATHB-5	GT-1
SD-RLK	*At1g11330*	3	1	3	7	7
	At1g61610	2	2	3	14	12
	At1g61430	3	2	3	3	7
LRR-RLK	*At2g19190 (FRK1)*	10	7	2	15	7
	At1g51850	9	5	6	6	8
	At5g45840	1	5	3	7	11

Discussion

RLK genes are important components in the defense arsenal of plants (Antolín-Llovera *et al.* 2012, Wu and Zhou 2013). Proteins encoded by defense-associated *RLK* genes are able to perceive the presence of pathogens and initiate defense responses. The inducibility of RLKs has been proposed to be a requirement to prepare the cell to survive future stresses (Sanabria *et al.* 2012). Furthermore, RLKs that are not known to act as PRRs may contribute to the diversity of RLKs and be part of a broad-spectrum resistance against multiple pathogens.

The analysis of the architecture of the *SD-RLK* genes indicates that they consisted of a number of subdomains, and the characteristic main domains were found in all three *SD-RLKs* (*At1g11330*, *At1g61430*, and *At1g61610*), namely the B-lectin, S-locus glycoprotein (SLG), PAN_AP, and serine/threonine kinase domains (Fig. 1). These RLKs are plausible receptor candidates (Shiu *et al.* 2004, Sanabria *et al.* 2012). The B-lectin domain, consisting of a consensus sequence motif QXDXNXVXY, specifically recognizes diverse sugars involved in cell-cell and host-pathogen interactions, glycoprotein turnover, and innate immune responses (Sanabria *et al.* 2012). The SLG domain is involved in self-incompatibility (SI) and found in SD-RLKs (Nasrallah 1997). Similarly, the PAN domain is known to be present in plant SD-RLKs and secreted glycoproteins involved in SI. In addition, an EGF domain, which contains six conserved cysteines and is highly similar to the conserved sequence of S-locus glycoprotein domains, was found in *At1g61430* and *At1g61610*. The catalytic domains of the SD-RLKs, consisting of the serine/threonine kinase domain and including the tyrosine protein kinase domain (STYKc), were found in the intracellular region. These catalytic domains are known to regulate an array of biological processes, including innate immune responses through phosphorylation of target proteins.

Similar to the *SD-RLKs*, the selected *LRR-RLKs* (*At1g51850*, *At2g19190*, and *At5g45840*) consist of characteristic domains which allow the classification of such RLKs (Afzal *et al.* 2008). These RLKs possess the characteristic LRR, a structural motif of approximately 24 amino acids, with a repetitive sequence pattern rich in leucine (Shiu and Bleecker 2003). This characteristic feature is found in the extracellular region of a LRR-RLK and might represent a potential binding site for extracellular ligand(s). LRRs may play a role in ligand binding, but are also involved in protein-protein interactions (Zipfel and Robatzek 2010, Antolín-Llovera *et al.* 2012). The intracellular region usually contains a protein kinase (PKc) domain with serine/threonine recognition specificity involved in protein phosphorylation.

Even though the structures of the SD-RLKs and LRR-RLKs are broadly similar, there are functional differences in the two RLK classes. The extracellular S-glycoprotein and repetitive LRR domains allow the LRR-RLKs a possible binding specificity to a variety of MAMPs and/or microbes. Moreover, the similar intracellular protein kinase activity supplies the molecular architecture to transduce extracellular signals intracellularly. This might be indicative that the same type of signaling mechanisms are carried out *via* SD-RLKs and LRR-RLKs in response to pathogens to trigger similar transcriptional regulation which can lead to a potent generically-regulated defense response that forms part of the innate MTI.

The rapid initiation of MAMP-triggered signaling is potentially important in eliciting spatial and temporal enhancement of plant immunity. A combination of microarray data, bioinformatic analyses, and gene expression studies allows for a better understanding of the target *RLKs* which demonstrate various expressions when induced with MAMPs. All MAMPs used in this study elicited some typical plant immunity-associated responses, such as ion fluxes across the plasma membrane, the increase in calcium content, the production of reactive oxygen species, and the activation of mitogen-activated protein kinase (MAPK) (Gerber *et al.* 2004, Piater *et al.* 2004, Gust *et al.* 2007, Antolín-Llovera *et al.* 2012, Erbs and Newman 2012, Vidhyasekaran 2014), and one would expect that all of the genes will be similarly up-regulated if solely responsive to the same second messenger. However, this was not the case, as differential expression patterns for each gene was observed with regard to the time of elicitation as well as the MAMP. Factors like the bio-availablity of the MAMPs (especially for the LPS, PGN,

and CHIT), the specificity of the perception, and the effectiveness of signal transduction could be determinants of the responses.

Where the induced expression of the selected *RLK* genes was observed, it was relatively low in comparison to studies where the same genes were reported in microarray experiments with whole *A. thaliana* plants and live phytopathogens. This might be due to different experimental models, conditions, and induction kinetics. Furthermore, a combination of MAMPs might be required, as would be found in the case of live pathogens, to trigger an effective defense response, and a single MAMP perception event might not trigger sufficient signaling molecules to cross a threshold to be able to activate a strong enough response that would result in increased gene expression. The expression of genes is influenced by the tissue/organ type and developmental stage of the plant (*Arabidopsis ePF browser*, http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi), and the genes may also be regulated by tissue-specific TFs not active in cultured cells (Gurr and Rushton 2005).

Cooperative interactions of diverse TFs contribute to gene expression in response to external stimuli and precise combinations of many TFs are essential for differential gene expression. Although the *SD-RLK* and *LRR-RLK* genes exhibited co-expression in the microarray analysis in response to LPS, the RT-PCR expression analysis indicated differential responses to LPS and the other MAMPs, raising the question about the TFs involved in their activation.

The *in silico* analysis of the promoters of the target *SD-RLK* and *LRR-RLK* genes show an array of probable defense-related CREs and TFs that were found in a -1000 bp region upstream of the gene transcriptional start site. The number of stress-related regulatory elements points towards a link with defense-related responses found to be involved in biotic stresses in *A. thaliana.* The segments of the promoter form (a) cluster(s) of TFBSs (Fig. 1 Suppl). These clusters may also include over-lapping CREs which allows the responses to TFs to converge and generate a stronger response to pathogen attack (Singh *et al.* 2002, Shameer *et al.* 2009). The promoters furthermore contain multiple binding sites for the same TF, which may suggest evolutionary adaptions in changeable *cis*-elements to create multiple sites of *cis* control (Shameer *et al.* 2009).

As seen in the promoters of the target genes, the TFBSs are short functional DNA motifs that contain conserved core sequences. It has been shown across multiple pathogen-plant interaction studies that *cis*-elements are keys in orchestrating and targeting transcriptional processes (Vandepoele *et al.* 2006). Identification of *cis*-element core(s) provides the basis of generating models that can be utilized in developing databases for bioinformatic analysis and, consequently, theoretically identifying TFBS/TF combinations in promoters as done in this study. The combinations and clusters of TFBS/TF form *cis*-regulatory modules (CRMs) which are important in the design of synthetic promoters engineered with *cis*-elements that may improve crop resistance (Rushton *et al.* 2002, Gurr and Rushton 2005).

The frequent occurrence of W-box, MYB, and AP2/ERF (including RAV1AAT) *cis*-elements in the *SD-RLK* and *LRR-RLK* promoter regions was observed. This is expected in defense-related genes as shown in active pathogen- or elicitor-responsive promoters (Maleck *et al.* 2000, Vandepoele *et al.* 2006, Beets *et al.* 2012). Moreover, within some of the short sequences which form the core CREs, putative binding sites for more than one defense-related TF were found, forming a potential TF cluster site. This suggests that the target genes were involved in plant defense and, furthermore, that differential regulation at a transcription level was effected by different TFs.

WRKY TFs usually bind to the corresponding W-box (C/TTGACT/C) *cis*-elements that are found in promoters of defense-related genes and genes involved in SA signaling and show up-regulation following pathogen perception (Maleck *et al.* 2000, Singh *et al.* 2002, Qu and Zhu 2006, Pandey and Somssich 2009, Cho *et al*. 2012). The plant-specific R2/R3-MYB TFs are known to be involved in environmental stresses and in responses to wounding and microbes (Dubos *et al.* 2010, Prouse and Campbell 2012). MYB4 binding sites (CC(TA)AACC) were found in all six of the promoters of the *SD-RLK* and *LRR-RLK* genes investigated here.

The AP2/ERF family contains plant-specific TFs, such as AP2, RAV, ERF, and a dehydration-responsive element-binding protein (DREB). The AP2 subfamily contains two AP2/ERF motifs, whereas RAV members have an AP2/ERF motif and a B3 DNA binding domain recognising CAACA and CACCTG sequences, respectively (Mizoi *et al.* 2012). These ERF proteins bind to two *cis*-elements, the GCC box, found in a number of PR (pathogenesis-related) gene promoters, and to the C-repeat (CRT)/dehydration responsive element (DRE) motif [A/G]CCGAC (Singh *et al.* 2002, Mizoi *et al.* 2012). The AP2/ERF family, including RAV1 found in the promoter regions of the investigated genes, form part of the superfamily involved mainly in response to patho-gen perception and bacterial infection (Qu and Zhu 2006).

The GT1 consensus *cis*-element occurs in many light-regulated genes and also plays a role in SA- and pathogen-induced gene expression (Zhou 1999). The frequent occurrence of the ATHB-5 *cis*-elements in the promoters is also of interest. ATHB-5 is a homeodomain-leucine zipper (HD-ZIP) TF in *A. thaliana* and a positive regulator of ABA responsiveness (Johannesson *et al.* 2003).

The *in silico* analyses of the promoters signify that CREs most likely play important roles in the activation of the *RLK* gene expression. In general, the total number of defense-related *cis*-elements as well as their relative positions within the promoters appear to be influenced in determining the timing and intensity of the responses, thereby suggesting a complex transcriptional network regulating MAMP-triggered responses.

Conclusion

Developments in genomic data, *in silico* analyses, and molecular techniques have formed the basis of investigating defense mechanisms at a molecular level (Gururani *et al.* 2012). Characterization of the LPS-responsive *SD-RLK* and *LRR-RLK* genes investigated in this study demonstrated the expected features found in plant defense-related genes and the molecular architecture associated with extracellular sensors and signals transducers. The semi-quantitative analysis demonstrated that the genes differed in their responsiveness towards LPS and the other MAMPs investigated, have different expression profiles when induced, and that the intensity of response could be correlated to the unique features of the individual MAMP inducers and the respective promoter architectures. The number and type of *cis*-regulatory elements found suggest that the genes are linked to a role in defense. All the promoters were found to contain numerous defense-related CREs, and interestingly, mostly the same types of *cis*-elements in all cases. Each promoter contained more than one of the W-box, MYB-, AP2/ERF-, GT1- and ATHB-5 binding sites found near to the transcription start site indicating that they form part of the core regulatory region which is involved in transcriptional control. The clusters of *cis*-elements found in these proximal regions of each promoter correlated with the responsiveness of the genes to the MAMP elicitation. In addition, the frequency with which these *cis*-elements occur may be a determinant of the degree to which the genes are expressed.

References

Antolín-Llovera, M., Ried, M.K., Binder, A., Parniske, M.: Receptor kinase signaling pathways in plant-microbe interactions. - Annu. Rev. Phytopathol. **50**: 451-473, 2012.

Afzal, A.J., Wood, A.J., Lightfoot, D.A.: Plant receptor-like serine threonine kinases: roles in signalling and plant defense. - Mol. Plant Microbe Interact. **21**: 507-517, 2008.

Aslam, S.N., Erbs, G., Morrissey, K.L., Newman, M.-A., Chinchilla, D., Boller, T., Molinaro, A., Jackson, R.W., Cooper, R.M.: Microbe-associated molecular pattern (MAMP) signatures, synergy, size and charge: influences on perception or mobility and host defence responses. - Mol. Plant Pathol. **10**: 375-387, 2009.

Beets, C., Huang, J-C., Madala, N.E., Dubery, I.A.: Bio-synthesis of camalexin in *Arabidopsis thaliana* in response to lipopolysaccharide elicitation: a gene-to-metabolite study. - Planta **236**: 261-272, 2012.

Bülow, L., Steffens, N.O., Galuschka, C., Schindler, M., Hehl, R.: *AthaMap*: from *in silico* data to real transcription factor binding sites. - *In Silico* Biol. **6**: 23, 2006.

Chang, W.C., Lee, T.Y., Huang, H.D., Huang, H.Y., Pan, R.L.: *PlantPAN*: plant promoter analysis navigator for identifying combinatorial *cis*-regulatory elements with distance constraint in plant gene group. - BMC Genom. **9**: 561, 2008.

Chen, W., Provart, N.J., Glazebrook, J., Katagiri, F., Chang, H-S., Eulgem, T., Mauch, F., Luan, S., Zou, G., Whitham, S.A., Budworth, P.R., Tao, Y., Xie, Z., Chen, X., Lam, S., Kreps, J.A., Harper, J.F., Si-Ammour, A., Mauch-Mani, B., Heinlein, M., Kobayashi, K., Hohn, T., Dangl, J.L., Wang, X., Zhu, T.: Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. - Plant Cell **14**: 559-574, 2002.

Cho, S.M., Kang, E.Y., Min, K.H., Lee, A.K., Kim, Y.C., Yang, K.Y., Kim, K.S., Choi, Y.S., Cho, B.H.: A positive regulatory role of the watermelon *ClWRKY70* gene for disease resistance in transgenic *Arabidopsis thaliana*. - Biol. Plant. **56**: 560-565, 2012.

Coventry, H.S., Dubery, I.A.: Lipopolysaccharides from *Burkholderia cepacia* contribute to an enhanced defense capacity and the induction of pathogenesis-related proteins in *Nicotiana tabacum*. - Physiol. mol. Plant Pathol. **58**: 149-158, 2001.

Davuluri, R.V., Sun, H., Palaniswamy, S.K., Matthews, N., Molina, C., Kurtz, M., Grotewold, E.: *AGRIS*: Arabidopsis Gene Regulatory Information Server, an information resource of *Arabidopsis cis*-regulatory elements and transcription factors. - BMC Bioinform. **4**: 25, 2003.

Dean, J.D., Goodwin, P.H., Hsiang, T.: Comparison of relative RT-PCR and northern blot analyses to measure expression of β-1,3-glucanase in *Nicotiana benthamiana* infected with *Colletotrichum destructivu*. - Plant mol. Biol. Rep. **20**: 347-356, 2002.

Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., Lepiniec, L.: MYB transcription factors in *Arabidopsis*. - Trends Plant Sci. **15**: 573-581, 2010.

Erbs, G., Newman, M.-A.: The role of lipopolysaccharide and peptidoglycan, two glycosylated bacterial microbe-associated molecular patterns (MAMPs), in plant innate immunity. - Mol. Plant Pathol. **13**: 95-104, 2012.

Finkelstein, R.R., Lynch, T.J.: The Arabidopsis abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. - Plant Cell **12**: 599-610, 2000.

Fusco, N., Micheletto, L., Dal Corso, G., Borgato, L., Furini, A.: Identification of cadmium-regulated genes by cDNA-AFLP in the heavy metal accumulator *Brassica juncea* L. - J. exp. Bot. **56**: 3017-3027, 2005.

Gerber, I.B., Zeidler, D., Durner, J., Dubery, I.A.: Early perception responses of *Nicotiana tabacum* cells in response to lipopolysaccharides from *Burkholderia cepacia*. - Planta **218**: 647-657, 2004.

Gerber, I.B., Dubery, I.A.: Protein phosphorylation in *Nicotiana tabacum* cells in response to perception of lipopoly-saccharides from *Burkholderia cepacia*. - Phytochemistry **65**: 2957-2966, 2004.

Gurr, S.J., Rushton, P.J.: Engineering plants with increased disease resistance: how are we going to express it? - Trends Biotechnol. **23**: 283-290, 2005.

Gururani, M.A., Venkatesh, J., Upadhyaya, C.P., Nookaraju, A., Pandey, S.K., Park, S.W.: Plant disease resistance genes: current status and future directions. - Physiol. mol. Plant Pathol. **78**: 51-65, 2012.

Gust, A.A., Biswas, R., Lenz, H.D., Rauhut, T., Ranf, S., Kemmerling, B., Götz, F., Glawischnig, E., Lee, J., Felix, G., Nürnberger, T.: Bacteria-derived peptidoglycans

constitute pathogen-associated molecular patterns triggering innate immunity in *Arabidopsis*. - J. biol. Chem. **262**: 32338-32348, 2007.

Higo, K., Ugawa, Y., Iwamoto, M., Korenaga, T.: Plant *cis*-acting regulatory DNA elements (*PLACE*) database. - Nucl. Acids Res. **27**: 297-300, 1999.

Hu, X.J., Zhang, Z.B., Xu, P., Fu, Z.F., Hu, S.B., Song, W.Y.: Multifunctional genes: the cross-talk among the regulation networks of abiotic stress responses. - Biol. Plant. **54**: 213-223, 2010.

Huang, J.-C., Piater, L.A., Dubery, I.A.: Identification and characterization of a differentially expressed NAC transcription factor gene in MAMP-treated *Arabidopsis thaliana*. - Physiol. mol. Plant Pathol. **80**: 19-27, 2012.

Jalali, B.L., Bhargava, S., Kamble, A.: Signal transduction and transcriptional regulation of plant defence responses. - J. Phytopathol. **154**: 65-74, 2006.

Johannesson, H., Wang, Y., Hanson, J., Engström, P.: The *Arabidopsis thaliana* homeobox gene ATHB5 is a potential regulator of abscisic acid responsiveness in developing seedlings. - Plant mol. Biol. **51**: 719-729, 2003.

Kagaya, Y., Ohmiya, K., Hattori, T.: RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants. - Nucl. Acids Res **27**: 470-478, 1998.

Kemmerling, B., Halter, T., Mazzotta, S., Mosher, S., Nürnberger, T.: A genome-wide survey for *Arabidopsis* leucine-rich repeat receptor kinases implicated in plant immunity. - Front. Plant Sci. **2**: 88, 2011.

Kesarwani, M., Yoo, J., Dong, X.: Genetic interactions of TGA transcription factors in the regulation of pathogenesis-related genes and disease resistance in *Arabidopsis*. - Plant Physiol. **144**: 336-346, 2007.

Laquitaine, L., Gomès, E., François, J., Marchive, C., Pascal, S., Hamdi, S., Atanassova, R., Delrot, S., Coutos-Thévenot, P.: Molecular basis of ergosterol-induced protection of grape against *Botrytis cinerea*: induction of type I LTP promoter activity, WRKY, and stilbene synthase gene expression. - Mol. Plant Microbe Interact. **19**: 1103-1112, 2006.

Lenka, S.K., Lohia, B., Kumar, A., Chinnusamy, V., Bansal, K.C.: Genome-wide targeted prediction of ABA responsive genes in rice based on over-presented *cis*-motif in co-expressed genes. - Plant mol. Biol. **69**: 261-271, 2009.

Livaja, M., Zeidler, D., Von Rad, U., Durner, J.: Transcriptional responses of *Arabidopsis thaliana* to the bacteria-derived PAMPs harpin and lipopolysaccharide. - Immunobiology **213**: 161-171, 2008.

Louw, A.E., Dubery, I.A.: Plant defence responses in isonicotinamide-treated tobacco cells. Evidence supporting a role for nicotinamide related metabolites as stress mediators in plant defense metabolism. - J. Plant Physiol. **156**: 26-32, 2000.

Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K.A., Dangl, J.L., Dietrich, R.A.: The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. - Nat. Genet. **26**: 403-410, 2000.

Manavella, P.A., Dezar, C.A., Bonaventure, G., Baldwin, I.T., Chan, R.L.: HAHB4, a sunflower HD-Zip protein, integrates signals from the jasmonic acid and ethylene pathways during wounding and biotic stress responses. - Plant J. **56**: 376-388, 2008.

Mizoi, J., Shinozaki, K., Yamaguchi-Shinozaki, K.: AP2/ERF family transcription factors in plant abiotic stress responses. - Biochim. biophys. Acta **1819**: 86-96, 2012.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-479, 1962.

Nasrallah, J.B.: Evolution of the *Brassica* self-incompatibility locus: A look into S-locus gene polymorphisms. - Proc. nat. Acad. Sci. USA **94**: 9516-9519, 1997.

Navarro, L., Zipfel, C., Rowland, O., Keller, I., Robatzek, S., Boller, T., Jones, J.D.G.: The transcriptional innate immune response to flg22. Interplay and overlap with *Avr* gene-dependent defense responses and bacterial pathogenesis. - Plant Physiol. **135**: 1113-1128, 2004.

Newman, M., Dow, J.M., Molinaro, A., Parrilli, M.: Priming, induction and modulation of plant defense responses by bacterial lipopolysaccharides. - J. Endotoxin Res. **13**: 69-84, 2007.

Pandey, S.P., Somssich, I.E.: The Role of WRKY transcription factors in plant immunity. - Plant Physiol. **150**: 1648-1655, 2009.

Park, H.C., Kim, M.L., Yun, H.K., Jeon, J.M., Moon, B.C., Lee, J.H., Yoon, H.W., Lee, S.H., Chung, W.S., Lim, C.O., Lee, S.Y., Hong, J.C., Cho, M.J.: Pathogen- and NaCl-induced expression of the SCaM-4 promoter is mediated in part by a GT-1 box that interacts with a GT-1-like transcription factor. - Plant Physiol. **135**: 2150-2161, 2004.

Piater, L.A., Nürnberger, T., Dubery, I.A.: Identification of a lipopolysaccharide responsive erk-like MAP kinase in tobacco leaf tissue. - Mol. Plant Pathol. **5**: 331-341, 2004.

Priest, H.D., Filichkin, S.A., Mockler, T.C.: *cis*-Regulatory elements in plant cell signalling. - Curr. Opin. Plant Biol. **12**: 643-649, 2009.

Prouse, M.B., Campbell, M.M.: The interaction between MYB proteins and their target DNA binding sites. - Biochim. biophys. Acta **1819**: 67-77, 2012.

Qu, L.-J., Zhu, Y.-X.: Transcription factor families in *Arabidopsis*: major progress and outstanding issues for future research. - Curr. Opin. Plant Biol. **9**: 544-549, 2006.

Redman, J., Whitcraft, J., Johnson, C., Arias, J.: Abiotic and biotic stress differentially stimulate as-1 element activity in *Arabidopsis*. - Plant Cell Rep. **21**: 180-185, 2002.

Riechmann, J.L.: Transcriptional regulation: a genomic overview. - In: Somerville, C.R., Meyerowitz, E.M. (ed.): The *Arabidopsis* Book. Pp. 1-46. American Society of Plant Biologists, Rockville 2002.

Rushton, P.J., Reinstädler, A., Lipka, V., Lippok, B., Somssich, I.E.: Synthetic plant promoters containing defined regulatory elements provide novel insights into pathogen- and wound-induced signalling. - Plant Cell **14**: 749-762, 2002.

Rushton, P.J., Somssich, I.E.: Transcriptional control of plant genes responsive to pathogens. - Curr. Opin. Plant Biol. **1**: 311-315, 1998.

Sambrook, J., Russell, D.W.: Extraction, purification and analysis of mRNA from eukaryotic cells. - In: Sambrook, J., Russell, D.W. (ed.): Molecular Cloning. Vol. 1. Pp. 7.4-7.8. Cold Spring Harbour Laboratory Press, New York 2000.

Sanabria, N.M., Dubery, I.A.: Differential display profiling of the *Nicotiana* response to LPS reveals elements of plant basal resistance. - Biochem. biophys. Res. Commun. **344**: 1001-1007, 2006.

Sanabria, N.M., Goring, D., Nürnberger, T., Dubery, I.A.: Self/nonself perception and recognition mechanisms in plants: a comparison of self-incompatibility and innate immunity. - New Phytol. **178**: 503-514, 2008.

Sanabria, N.M., Huang, J.-C., Dubery, I.A.: Self/non-self perception in plants in innate immunity and defense. - Self/Non-Self Immun. Recog. Signal. **1**: 40-45, 2010.

Sanabria, N.M., Van Heerden, H., Dubery, I.A.: Molecular characterization and regulation of a *Nicotiana tabacum* S-domain receptor-like kinase gene induced during an early rapid response to lipopolysaccharides. - Gene **501**: 39-48, 2012.

Shameer, K., Ambika, S., Varghese, S.M., Karaba, N., Udayakumar, M., Sowdhamini, R.: STIFDB-*Arabidopsis* stress responsive transcription factor database. - Int. J. Plant Genom. **2009**: 583429, 2009.

Shiu, S-H., Bleecker, A.B.: Expansion of the receptor-like kinase/pelle gene family and receptor-like proteins in *Arabidopsis*. - Plant Physiol. **132**: 530-543, 2003.

Shiu, S-H., Karlowski, W.M., Pan, R., Tzeng, Y-H., Mayer, K.F.X.: Comparative analysis of receptor-like kinase family in *Arabidopsis* and rice. - Plant Cell **16**: 1220-1234, 2004.

Singh, K.B., Foley, R.C., Oñate-Sánchez, L.: Transcriptional factors in plant defense and stress responses. - Curr. Opin. Plant Biol. **5**: 430-436, 2002.

Stracke, R., Werber, M., Weisshaar, B.: The R2R3-MYB gene family in *Arabidopsis thaliana*. - Curr. Opin. Plant Biol. **4**: 447-456, 2001.

Vidhyasekaran, P. (ed.): PAMP Signals in Plant Innate Immunity. - Springer, Dordrecht 2014.

Vandepoele, K., Casneuf, T., Van de Peer, Y.: Identification of novel regulatory modules in dicotyledonous plants using expression data and comparative genomics. - Genome Biol. **7**: R103.1-R103.14, 2006.

Van Veck, M.C., Bol, J.F., Lindhorst, J.M.: Prospecting for genes involved in transcriptional regulation of plant defenses, a bioinformatics approach. - BMC Plant Biol. **11**: 88, 2011.

Wu, Y., Zhou, J.-M.: Receptor-like kinases in plant innate immunity. - J. Integr. Plant. Biol. **55**: 1271-1286, 2013.

Xing, S., Li, M., Liu, P.: Evolution of S-domain receptor-like kinases in land plants and origination of S-locus receptor kinases in Brassicaceae. - BMC Evol. Biol. **13**: 69, 2013.

Yang, T., Poovaiah, B.W.: CGCG box is recognized by AtSR1-6 (CAMTA3) Ca^{2+} and calmodulin binds to AtSRs. - J. biol. Chem. **47**: 45049-45058, 2002.

Zhou, D.X.: Regulatory mechanism of plant gene transcription by GT-elements and GT-factors. - Trends Plant Sci. **4**: 210-214, 1999.

Zipfel, C., Robatzek, S.: Pathogen-associated molecular pattern-triggered immunity: Veni, Vidi…? - Plant Physiol. **154**: 551-554, 2010.