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Transcriptional responses of *Arabidopsis thaliana* to the bacteria-derived PAMPs harpin and lipopolysaccharide

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Abstract

Many plant–pathogen interactions are controlled by specific interactions between pathogen avirulence (*avr*) gene loci and the corresponding plant resistance *R* locus (gene-for-gene-hypothesis). Very often, this type of interaction culminates in a hypersensitive reaction (HR). However, recently pathogen-associated molecular patterns (PAMPs) such as flagellin or lipopolysaccharides (LPS) that are common to all bacteria have been shown to act as general elicitors of basal or innate immune responses in several plant species. Here, we summarize the genetic programs in *Arabidopsis thaliana* behind the LPS-induced basal response and the HR induced by harpin, respectively. Using Agilent Arabidopsis cDNA microarrays consisting of ~15,000 oligomers, changes in transcript accumulation of treated cells were monitored over a period of 24 h after elicitor treatment. Analysis of the array data revealed significant responses to LPS (309 genes), harpin (951 genes) or both (313 genes). Concentrating our analysis on the genes encoding transcription factors, defence genes, cell wall biogenesis-related genes and signal transduction components we monitored interesting parallels, but also remarkably different expression patterns. Harpin and LPS induced an overlapping set of genes involved in cell wall biogenesis, cellular communication and signalling. The pattern of induced genes associated with cell rescue and general stress responses such as small heat-shock proteins was highly similar. In contrast, there is a striking difference regarding some of the most prominent, central components of plant defence such as WRKY transcription factors and oxidative burst-associated genes like NADPH oxidases, whose expression became apparent only after treatment with harpin. While both harpin and LPS can stimulate plant immunity in *Arabidopsis*, the PAMP LPS induces much more subtle host reactions at the transcriptome scale. The defence machinery induced by harpin resembles the known HR-type host responses leading to cell death after treatment with this elicitor. LPS is a weak inducer of basal resistance and induces a different pattern of genes. Strikingly the biggest overlap (40) of responding genes was found between the early harpin response (30 min) and the late LPS response (24 h).

Keywords: Defence response; Harpin; Innate immunity; Lipopolysaccharides (LPS); Microarrays; PAMPs

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Abbreviations: HR, hypersensitive reaction; LPS, lipopolysaccharide; NO, nitric oxide; PAMP, pathogen-associated molecular pattern; ROS, reactive oxygen species; SA, salicylic acid; SAR, systemic acquired resistance; sHSP, small heat-shock protein.

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Introduction

Plants possess an innate immune system enabling them to defend themselves against attacking pathogens. Using a large set of receptors, the immune system developed different strategies to realize disease resistance. Cultivar-specific resistance conforms to the gene-for-gene-hypothesis (see Fig. 1), and is genetically determined by complementary pairs of pathogen-encoded avirulence (*AVR*) genes and plant resistance (*R*) genes (Dangl and Jones, 2001). In other cases receptors have broad range specificity and recognize many related molecular structures called pathogen-associated molecular patterns (PAMPs). Among these general elicitors are essential polysaccharides and polynucleotides, which are not found in the host plant and differ only slightly from pathogen to pathogen. The most important PAMPs are conserved cell-surface structures like flagellin, lipopeptides (LP), peptidoglycanes (PG) and lipopolysaccharides (LPS) which are unique to bacteria. In addition, PAMPs might be group-specific such as type III secretion peptides (Nurnberger et al., 2004).

LPS are major parts of Gram-negative bacteria cell surfaces, composed of a hydrophobic lipid A, a covalently linked non-repetitive core oligosaccharide, divided into inner and outer core, and the O-antigen of oligosaccharide-repeating units (Meyer et al., 2001). LPS from various sources could trigger defence-related responses in several plant species without triggering an oxidative burst (Newman et al., 2002). On the other hand, LPS from the phytopathogen *Xanthomonas campestris* pv. *campestris* could induce an oxidative burst reaction with accumulation of hydrogen peroxide (H_2O_2) in tobacco cell cultures (Meyer et al., 2001), and LPS isolated from *Burkholderia cepacia* was found to trigger a rapid influx of Ca^{2+} into the cytoplasm of cells (Gerber et al., 2004). Furthermore, LPS induces a strong release of nitric oxide (NO) accompanied by an up-regulation of a set of local and systemic defence genes (Zeidler et al., 2004). It should be noted, however, that LPS is also a communication signal for progression of legume symbiosis (Mathis et al., 2005). Recently it has been shown that LPS infiltrated into *Arabidopsis* leaves was able to trigger accumulation of free and glucosidic salicylic acid (SA) in local and systemic leaves(Mishina and Zeier, 2007). This induction was significant but not as strong as the induction by living *Pseudomonas* cells.

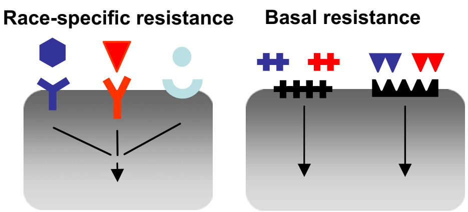

Fig. 1. Race-specific and basal resistance responses. Bacterial plant pathogens often are recognized in a gene-for-gene manner. The recognition of specific avirulence factors (Avr) results in race-specific resistance. In addition, most plants exhibit a basal defence mechanism, which is regulated through perception of pathogen-associated molecular patterns (PAMPs) such as flagellin or lipopolysaccharides.

Harpin is an acidic, heat-stable, glycine- and leucine-rich, water-soluble protein, secreted by bacteria with a type III secretion system such as *Pseudomonas syringae* and *Erwinia amylovora* (Dong et al., 1999). Harpin is not a general feature of bacteria. However, secretion of harpin is common to many pathogenic bacteria, and harpin induces strong responses in both host as well as non-host plants (He et al., 1994). Its release into plants elicits cell death, probably initiated by an apoptotic inactivation of mitochondria (He et al., 1993; Krause and Durner, 2004). The harpin-mediated systemic acquired resistance (SAR) in plants is characterized by an increase of reactive oxygen species (ROS) such as H_2O_2 and NO (Baker et al., 1993; Strobel et al., 1996; Desikan et al., 1998; Krause and Durner, 2004) as well as by an enhanced accumulation of SA which is modulated by a strong pH shift (Clarke et al., 2005). It stimulates calcium influx across the plasma membrane of cells, essential for initiation of defence mechanisms (Blume et al., 2000). A very recent study supports that harpin alters chloroplast function through modifications of the thylakoid membrane structure leading to minor photosynthetic activity (Boccara et al., 2007). Thus, harpin induces plant responses, which are frequently observed during hypersensitive reaction (HR)-type responses.

Pathogenic bacteria possess several sets of pathogenic components like the Gram-negative *P. syringae*, which contain LPS and additional PAMPs, and produce harpins. Especially in case of avirulent strains, it is difficult to distinguish defence response after recognition of non-host and race-specific elicitors, respectively. In pepper, basal defences induced by LPS, were suppressed by infection with *X. campestris* pv. *Vesicatoria* (Keshavarzi et al., 2004). A recent study analysed the immune response of *Arabidopsis* cell cultures to bacterial flagellin flg22 at the transcriptional level (Navarro et al., 2004). Flagellin is recognized as a PAMP, its perception requires the FLS2 leucine-rich repeat receptor kinase (Zipfel et al., 2004). A substantial overlap between non-host (flagellin) and race-specific (to the elicitor Avr9) immune response became apparent (Navarro et al., 2004). However, in contrast to race-specific defence responses the basal resistance is still poorly defined (Tao et al., 2003; Glazebrook, 2004). Here, we compare the genetic programs behind the response to LPS and the harpin resistance response in *Arabidopsis thaliana*. Concentrating our detailed analysis on defence-associated genes, we will highlight interesting parallels, but also striking different expression patterns for the examined elicitors.

Initial responses to PAMPs

There are several comparing studies on *Arabidopsis* plant gene expression during pathogenic stresses such as *P. syringae* pv. *tomato* carrying the AvrRpt2 gene (Scheideler et al., 2002), treatment with *Alternaria brassicicola* (Schenk et al., 2000) or infection by rhizobacteria *Pseudomonas thivervalensis* (Cartieaux et al., 2003). Further reported are transcriptome analyses of *Arabidopsis* plants stressed with defence-related signaling molecules like SA, methyl jasmonate (MJ), ethylene (Schenk et al., 2000; Zhong and Burns, 2003), and experiments with SAR-inducing or SAR-repressing treatments (Maleck et al., 2000). However, only few studies addressed basal defence or gene induction by PAMPs (Navarro et al., 2004; Sanabria and Dubery, 2006).

In our studies we used high-density cDNA microarrays (Agilent) consisting of about 15,000 oligomers to study the transcriptomes of *A. thaliana* to the elicitor harpin and the PAMP LPS, respectively. Both effectors induce NO and ROS (Gerber et al., 2004; Krause and Durner, 2004), and for both effectors immunization effects were demonstrated (Grisham, 2000; Newman et al., 2002; Mishina and Zeier, 2007) (Table 1). Strikingly, while harpin treatment causes cell death in every plant species analysed so far, LPS does not (Newman et al., 2002; Zeidler et al., 2004). Due to their homogeneity and repeatability, we preferred *Arabidopsis* cell suspension cultures for our transcription profiling.

Global transcriptional changes in *Arabidopsis*

Expression profiling was performed on cells, treated with 100 μg/ml LPS from *B. cepacia* or 50 μg/ml harpin from *P. syringae*, respectively. Changes in transcript accumulation of treated cells and non-treated cells were monitored at six time points within 24 h after elicitor treatment. Analysis of the array data revealed 1573 (see Supplement 1) genes whose expression showed significant changes in transcript abundance in response to LPS (309 genes), harpin (951 genes) or both (313 genes) elicitors. Based on the TIGR and the MIPS *A. thaliana* databases, the elicitor-specific changes in transcript abundance were categorized by function (Fig. 2). Harpin induced most transcripts very rapidly within 2 h, while the number of repressed genes remained on a comparatively low level. The great efforts of harpin-induced cells to withstand the pathogenic attack seemed to decline between 2 and 8 h after treatment, which is reflected in the simultaneous onset of cell death of harpin-treated *tobacco* and *Arabidopsis* cells (Xie and Chen, 2000; Krause and Durner, 2004). In contrast, LPS-induced transcript levels much slower and weaker. However, 24 h after treatment we observed a high number of LPS-induced genes. The number of down-regulated genes seemed to be nearly constant over 1 day. Analysed at the level of functional categories, a strong similarity between the 24-h LPS pattern and the 30-min pattern of harpin-elicited cells is striking. At the level of individual genes, we observed a very small overlap consisting of 25 genes after 30 min and 14 genes after 24 h, respectively. Interestingly, the biggest overlap (40) of genes was found between the early harpin response (30 min) and the late LPS response (24 h).

Here, we concentrate our analysis on genes encoding transcription factors, defence proteins, cell wall biogenesis-related proteins and signal transduction components.

Activation of transcription factor genes

Based on the Arabidopsis Gene Regulatory Information Server (AGRIS) database of *Arabidopsis* transcription factors (Davuluri et al., 2003) we analysed the time-dependent regulation of genes encoding transcription factors in *Arabidopsis* suspension cells after LPS and harpin exposure. Based on sequence similarity the genes that are involved in transcriptional regulation were classified by families. Currently there are 35 families listed consisting of 1466 genes. The Agilent array used

Table 1. Comparison of already known LPS and harpin caused events in *Arabidopsis thaliana* suspension cells

Examples for effects in plants	LPS	Harpin	Citation
Increase of cytosolic Ca^{2+}-levels	+	+	Blume et al. (2000), Meyer et al. (2001), Gerber et al. (2004)
H_2O_2 generation (oxidative burst)	+	+	Desikan et al. (1998), Meyer et al. (2001), Gerber et al. (2004)
Synthesis of SA	+	+	Samuel et al. (2005), Mishina and Zeier (2007)
Media alkalinization	+	+	Wei (1992), Baker et al. (1993), Gerber et al. (2004)
NO generation	+	+	Zeidler et al. (2004), Krause and Durner (2004)
Cell death	–	+	Xie and Chen (2000), Krause and Durner (2004)
Induction of resistance	+	+	Newman et al. (2002), Dong et al. (2004)

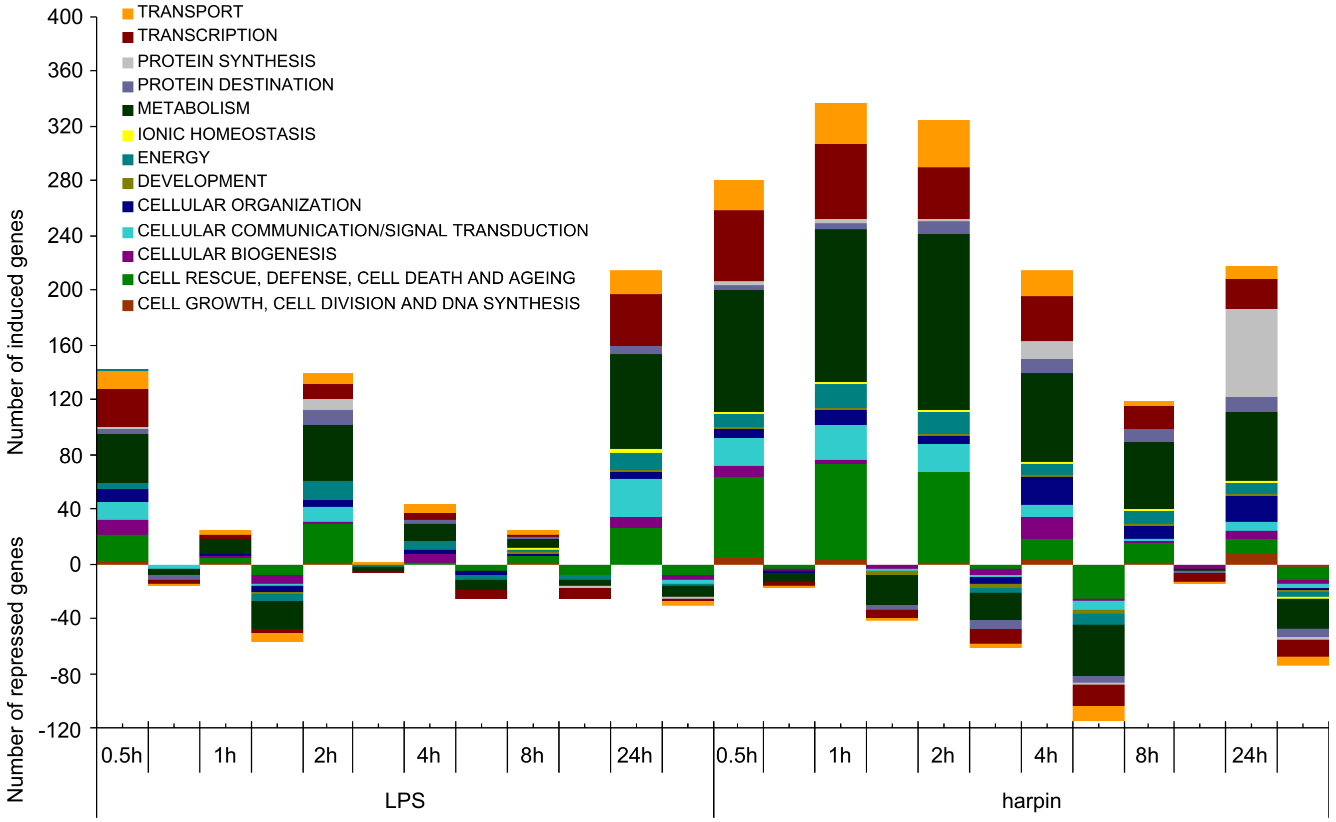

Fig. 2. Gene expression in functional categories after LPS and harpin treatment, respectively. Shown are the gene expression dynamics for the first 24 h after LPS and harpin treatment. Differentially transcribed genes were grouped into 13 functional categories on basis of the TIGR and the MIPS *Arabidopsis thaliana* databases. Note that some genes can have more than one annotated function.

contained 1138 transcription factor genes, of which 1011 could be detected (Table 2).

In sum, the WRKY-family, AP2-EREBP-family, HB-family, MYB-family, NAC-family and the C2H2-family were identified as significantly participating in transcriptional regulation after treatment. Again, in the case of harpin most genes encoding transcription factors responded quickly while LPS induced a much slower response (Fig. 3; see also Supplement 2).

In higher plants, the MYB protein family is extraordinarily diverse. They are known to be involved in a variety of cellular processes such as the regulation of biosynthetic pathways like phenylpropanoid or tryptophan biosynthesis, control of cell fate determination and regulation of the cell cycle (Zimmermann et al., 2004). AP2 APETALA2 and ethylene-responsive element binding proteins (EREBPs) are prototypic members of a family of transcription factors unique to plants. AP2/EREBP genes form a multigene family, and they play a variety of roles throughout the plant life cycle, from being key regulators of several developmental processes, like floral organ identity determination or control of leaf epidermal cell identity, to forming part of the mechanisms used by plants to respond to various types of biotic and environmental stress (Riechmann and Meyerowitz, 1998). MYB-type and AP2/EREBP-type transcription factors are reported as significantly induced by wounding stress (Cheong et al., 2002).

The WRKY family of *Arabidopsis* contains 74 members, which are identified as W box (C/T)TGAC (T/C) binding proteins, a DNA sequence found in promoters of several defence-related genes (Ulker and Somssich, 2004). WRKY transcription factors are reported as differentially regulated in *Arabidopsis* treated with an avirulent *P. syringae* strain and/or SA. Different sets of AtWRKY genes were found as significantly induced or repressed by wounding stress (Cheong et al., 2002) in *Arabidopsis* plants (Jiao et al., 2003) as well as during flagellin treatment of *Arabidopsis* suspension cells (Navarro et al., 2004).

Our comparing data analysis of the main transcription factor families for LPS and harpin time course studies is shown in Fig. 3. Remarkable is the strong induction of up to 13 AtWRKY genes after harpin exposure, together with an almost complete absence of any AtWRKY gene regulation after LPS treatment. A comparison of the 14 LPS and harpin-regulated

Table 2. Summary of LPS- and harpin-induced transcription factor genes

Family name	Total	On array	Detected	Percent	LPS regulated	Harpin regulated	Total regulated	Percent
WRKY	74	55	49	89.1	4	13	14	28.6
AP2-EREBP	120	112	100	89.3	15	15	24	24.0
C2C2-Gata	28	18	13	72.2	2	2	2	15.4
G2-like	40	23	21	91.3	2	3	4	19.0
HB	66	60	56	93.3	7	5	10	17.9
MYB	137	124	108	87.1	9	14	18	16.7
bZIP	70	58	46	79.3	3	4	7	15.2
NAC	90	77	68	88.3	3	8	10	14.7
ABI3VP1	18	17	14	82.4	0	2	2	14.3
C2H2	98	85	77	90.6	5	10	11	14.3
C2C2-Dof	36	33	29	87.9	2	4	4	13.8
C3H	164	97	88	90.7	5	7	9	10.2
ARR-B	15	11	11	100.0	0	1	1	9.1
CCAAT-HAP5	13	12	11	91.7	2	1	1	9.1
C2C2-CO-like	30	26	24	92.3	1	1	2	8.3
GRAS	25	24	24	100.0	1	1	2	8.3
SBP	16	12	12	100.0	1	0	1	8.3
ARF	22	16	15	93.8	0	1	1	6.7
HSF	21	19	18	94.7	1	1	1	5.6
bHLH	146	73	66	90.4	1	2	3	4.5
Trihelix	29	24	22	91.7	0	1	1	4.5
MADS	100	79	63	79.7	0	2	2	3.2
Alfin	7	7	7	100.0	0	0	0	0.0
C2C2-YABBY	5	2	2	100.0	0	0	0	0.0
CCAAT-DR1	2	2	2	100.0	0	0	0	0.0
CCAAT-HAP2	10	10	9	90.0	0	0	0	0.0
CCAAT-HAP3	10	9	9	100.0	0	0	0	0.0
CPP	8	4	3	75.0	0	0	0	0.0
E2F-DP	8	5	5	100.0	0	0	0	0.0
EIL	6	6	5	83.3	0	0	0	0.0
GRF	9	1	1	100.0	0	0	0	0.0
MYB-related	9	8	8	100.0	0	0	0	0.0
Orphan	3	1	1	100.0	0	0	0	0.0
TCP	26	18	14	77.8	0	0	0	0.0
TUB	10	10	10	100.0	0	0	0	0.0

The numbers reflect classified, detected and regulated family members. The classification is based on Arabidopsis Gene Regulatory Information Server (AGRIS) database of *Arabidopsis* transcription factors (http://arabidopsis.med.ohio-state.edu; Davuluri et al., 2003).

AtWRKY genes with reported AtWRKY genes from pathogen, SA, wound and flagellin stress treatments in *Arabidopsis* is summarized in Table 3. While some of the WRKY genes showed only regulation by harpin (AtWRKY10, AtWRKY17 and AtWRKY75) or harpin and *P. syringae*, respectively (AtWRKY8 and AtWRKY31), two genes are induced by all stressors except LPS (AtWRKY22 and AtWRKY33). No surprise is the activation of the AtWRKY22 and AtWRKY29 genes by harpin. Both transcription factors are associated with the defence-induced mitogen-activated protein kinase (MAPK) signalling pathway which leads to resistance against bacteria and fungi (Asai et al., 2002). In sum, harpin but not LPS did induce a typical pattern of defence-related WRKYs.

Genes involved in cell wall biogenesis

Modification of cell wall architecture is an essential part of plant response to invading pathogens, a reason for us to focus on this area. Our comparative transcriptional analysis of genes which are involved especially in cell wall biogenesis and organization revealed mostly similar effects in gene expression of *Arabidopsis* cells after LPS and harpin treatment. Primary cell walls from higher plant cells are composed predominantly of polysaccharides whose main parts are cellulose, hemicellulose and pectin, whereas the major component of secondary walls is lignin (Micheli, 2001). We found 55 regulated genes (see Supplement 3) which are involved in cell wall biogenesis. About 20% of them were induced in

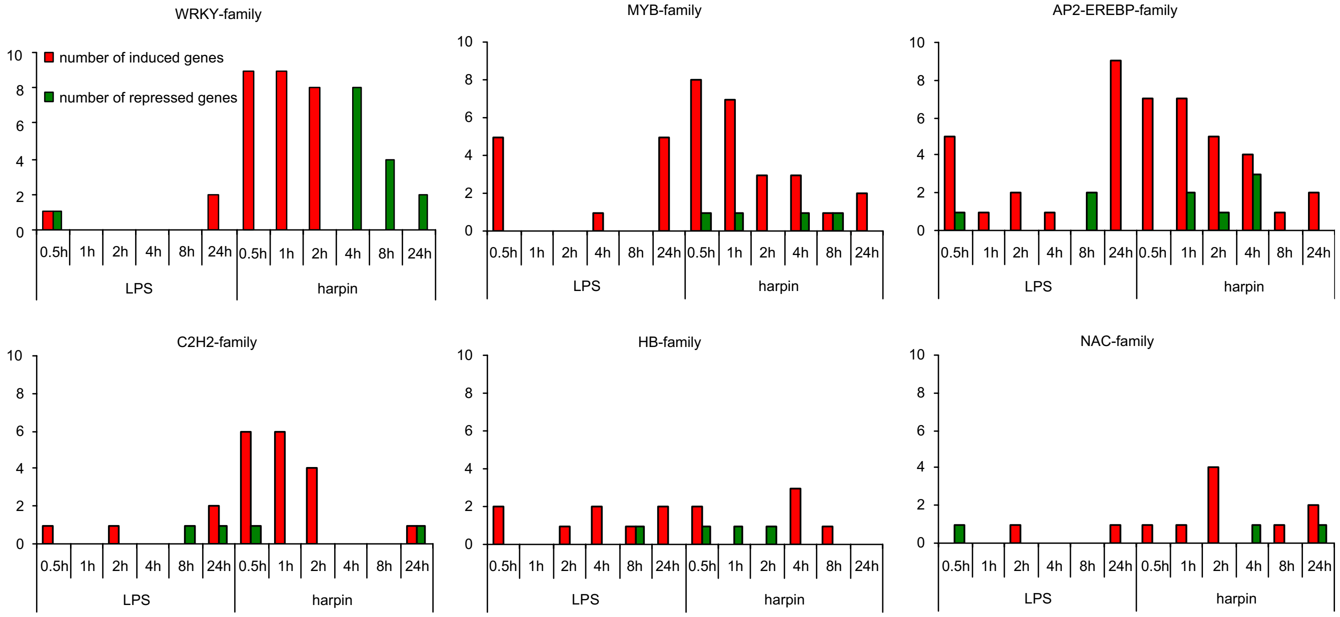

Fig. 3. Expression patterns of six important transcription factor families regulated by LPS and harpin. Genes with mRNA abundance induced or repressed above two-fold are grouped into families on the basis of the Arabidopsis Gene Regulatory Information Server (AGRIS) database of *Arabidopsis* transcription factors [http://www.arabidopsis.med.ohio-state.edu], (Davuluri et al., 2003). Overrepresented families were selected according to (Hennig et al., 2004) after following criteria: Families with less than two regulated genes were neglected. In case (i) two to nine members were regulated and (ii) at least 30% of the family was represented, the family was chosen. The same holds true if 10 or more regulated genes were counted and at least 5% of the family size was represented.

Table 3. Comparison of AtWRKY genes differentially regulated during different stress treatments in *Arabidopsis*

WRKY type	ID	LPS	Harpin	Wounding[a]	Flagellin (flg22)[b]	Pathogen[c,d]	Salicylic acid[c]
AtWRKY8	At5g46350	–	+	–	–	+	–
AtWRKY9	At1g68150	+	+	–	–	–	–
AtWRKY10	At1g55600	–	+	–	–	–	–
AtWRKY17	At2g24570	–	+	–	–	–	–
AtWRKY22	At4g01250	–	+	+	+	+	+
AtWRKY25	At2g30250	–	+	–	–	+	+
AtWRKY29	At4g23550	–	+	–	+	+	–
AtWRKY31	At4g22070	–	+	–	–	+	–
AtWRKY33	At2g38470	–	+	+	+	+	+
AtWRKY40	At1g80840	–	+	+	–	+	+
AtWRKY48	At5g49520	+	–	–	–	+	–
AtWRKY53	At4g23810	–	+	+	+	–	+
AtWRKY55	At2g40740	–	+	–	–	–	+
AtWRKY75	At5g13080	–	+	–	–	–	–

The classification is based on Arabidopsis Gene Regulatory Information Server (AGRIS) database of *Arabidopsis* transcription factors (http://arabidopsis.med.ohio-state.edu; Davuluri et al., 2003).

[a] Cheong et al. (2002).

[b] Navarro et al. (2004).

[c] Dong et al. (2003).

[d] Lippok et al. (2007).

both LPS- and harpin-treated cells, respectively. A few genes (cinnamoyl-CoA reductase (At5g14700 and At1g80820 cinnamyl-alcohol dehydrogenase At1g09510 and At1g09500 caffeoyl-CoA 3-*O*-methyltransferase At1g67980) involved in lignin synthesis were found transiently induced early (0.5–2 h) after harpin treatment. No genes involved in callose synthesis were found induced by either harpin or LPS. Considering the function of encoded proteins the majority is responsible for cell wall modification, alteration and degradation. We found six pectin esterases whose action can result in loosening or disassembly of cell walls (Jiang et al., 2001), and two pectate lyases which degrade the middle lamella of plant cell walls. Furthermore, we detected one xyloglucan endotransglycosylase which catalyses the depolymerization or solubilization of the hemicelluloses formating xyloglucan (Micheli, 2001). However, the very same gene has a reported role in restructuring primary walls at the time when secondary wall layers are deposited (Bourquin et al., 2002). The four pectin methylesterases induced by LPS and harpin catalyse the demethylesterification of homogalacturonic acid units of pectin and are known to be involved in stiffening and loosening of cell walls (Micheli, 2001; Al-Qsous et al., 2004). They are also reported to play a role in pathogen–plant interactions (Giovane et al., 2004). Harpin induced several putative cellulose synthases, which may be involved in cell wall assembly. After LPS treatment, the cells activated one gene coding for the glycoprotein extensin whose synthesis appears to structurally reinforce the wall, thereby enabling the plant to withstand environmental stress. Further, a significant induction of two expansins was observed. In plants, expansins cause loosening and extension of cell walls (Rose and Bennett, 1999; Kalamaki et al., 2003), and might play a role in LPS-dependent progression of endosymbiosis (Mathis et al., 2005).

Different effects on cell rescue and defence

Cell rescue and defence-related genes (see Supplement 4) represent more than 10% of the LPS- and harpin-regulated genes (165). Thereby 81 of them are regulated by both stresses but at different time points or for different periods. A comparison of the expression changes across the defence genes for the two treatments is visualized by the clustergram (Fig. 4), wherein genes are ordered by related regulation patterns and expression amplitudes. LPS and harpin caused very different transcriptional answers. Harpin induced a very strong and transient response. After LPS treatment most of the defence-related genes showed a relative low level of transcripts, most of them are repressed or even not expressed, and the reduction of early induced genes is not as dramatic as in the case of harpin elicited cells. The strongest answer to LPS regarding induction or repression of defence genes was measured 24 h post treatment.

Because LPS as well as harpin are known to cause oxidative stress in *Arabidopsis* cells (Desikan et al., 1998; Gerber et al., 2004) we asked for the expression pattern of ROS-associated genes (Supplement 5). Remarkable is the strong and primarily synchronous induction of superoxide forming respiratory burst oxidases and superoxide preventing alternative oxidases (Mittler et al., 2004) within 30 min after harpin treatment. After 4 h these genes are again down-regulated, and the superoxide scavenging ferritin one precursor (op den Camp et al., 2003; Mittler et al., 2004) is activated.

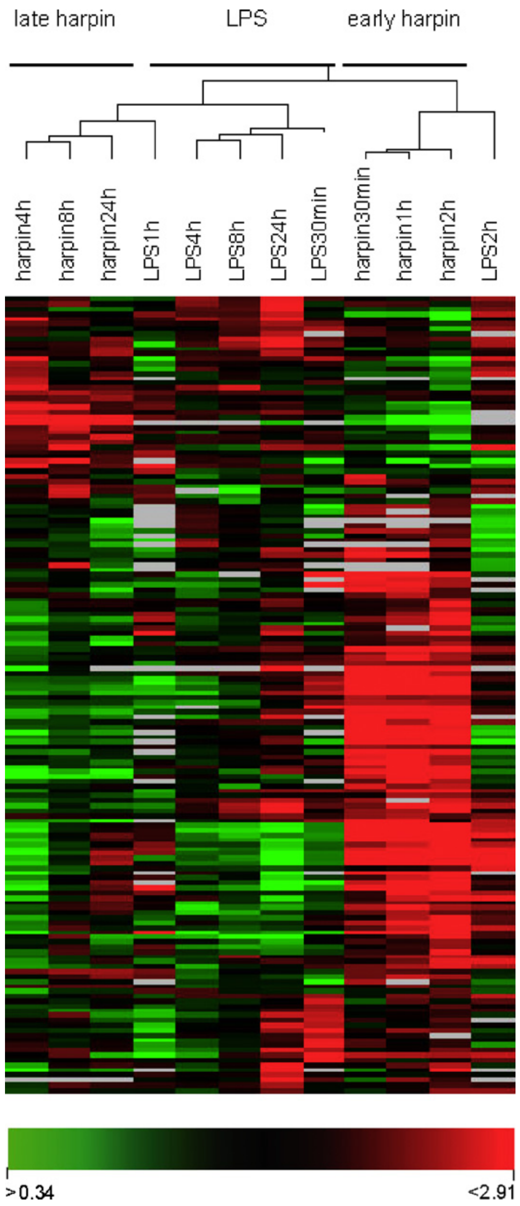

Fig. 4. Clustering of *Arabidopsis* gene expression profiles after treatment with LPS and harpin. Transcript levels of 167 cellular rescue- and defence-related genes were analyzed 0.5, 1, 2, 4, 8 and 24 h after elicitor treatment. Each horizontal line displays the expression data for one gene after normalization at time points as indicated. The cluster tree at the left side displays the nodes of co-regulated gene expression over all 24 h and both treatments. The relative abundance of any transcript in treated suspension cells was compared to untreated control cells. The color scale at the bottom shows the normalized expression level.

In case of LPS, the superoxide producing and scavenging enzymes (i.e. their genes) (Mittler, 2002) are regulated at a very low level. However, after 8 h a superoxide dismutase (SOD) and a ferritin one precursor gene became very strongly induced. In sum, only in harpin-elicited cells we observed a massive induction of ROS associated genes or genes involved in redox control. This transcriptional pattern may reflect the HR-type answer, which harpin can induce in most plants (He et al., 1994).

Next we focussed our analysis on inducible pathogenesis-related proteins (PR-genes), and proteins associated with activation of pathogen defence (Kinkema et al., 2000; Schenk et al., 2000) such as phytoalexin production, oxidative stress protection, tissue repair and lignification. Most pathogen-related proteins have a damaging action on the cellular structures of a parasite, PR-1 and PR-5 interact with the plasma membrane, whereas β-1,3-glucanases (PR-2) and chitinase (PR-3, PR-4, PR-8 and PR-11) attack β-1,3-glucans and chitin of cell walls in most higher fungi. PR-5 proteins are thought to create transmembrane pores and therefore they have been named permatins. Chitinases can also display lysosyme activity and hydrolyze bacterial peptidoglycan (Odjakova and Hadjiivanova, 2001). Surprisingly, both harpin and LPS induced only very few PR-proteins (PR-3-type chitinase and PR-4 hevein). This finding contrasts with previous findings of induction of PR genes in *Arabidopsis* plants treated with harpin or LPS (Zeidler et al., 2004; Mishina and Zeier, 2007). From this data we conclude that the cell culture system responds in some ways different to bacterial elicitors than a whole plant.

In contrast, a remarkable induction was found for small heat-shock protein genes (sHSPs). HSPs have been demonstrated to prevent cytochrome *c* release and they disrupt the apoptosome by binding to cytochrome *c* (Hoeberichts and Woltering, 2003). Recently it was shown that LPS causes a dephosphorylation of HSPs in tobacco. It is still unclear if this action entails activation or deactivation of these proteins (Gerber et al., 2006). In *Arabidopsis,* there are 13 sHSPs, divided into six classes on the basis of their intracellular localization (Scharf et al., 2001). Although the strength of induction differed after LPS and harpin treatment we found high similar expression patterns (Supplement 5). Both elicitors caused a strong, transient induction of all known sHSP genes with rapid down-regulation (Supplement 6). In addition to mitochondrial and cytosolic sHSPs the chloroplast-localized sHSP which is suggested to protect the photosystem II against oxidative stress and photoinhibition (Heckathorn et al., 1998) was strongly induced (Supplement 6) by harpin. In sum, while harpin did induce at least subgroups of typical HR- and/or SAR associated genes (Schenk et al., 2000), LPS did elicit a more general, albeit weakly pronounced stress response.

Cellular communication and signal transduction

Among the 101 LPS and harpin-regulated genes assigned to signal transduction components, we found 54 protein kinases. Most of them are receptor-like kinases, whose induction was observed throughout the whole period of treatment. Almost 50% belong to leucine-rich repeat receptor-like kinases (LRR-RLK). Harpin caused an induction of 31 receptor-like kinases, whereas 16 are up-regulated by LPS during 24 h (Supplement 7). Interestingly, LPS induced a LRR VI receptor-like kinase by nearly 17-fold after 30 min. A comparison of our expression data for harpin- and LPS-activated RLK genes with those published for *Arabidopsis* challenged with the PAMP flg22, a peptide corresponding to the most conserved domain of flagellin, revealed that six of the 25 significantly by flg22 regulated RLK genes show nearly the same expression pattern 1 h post-treatment (Navarro et al., 2004).

The majority of the genes involved in cellular communication and signal transduction could be assigned to phytohormone- or calcium/calmodulin-related groups, respectively (Supplement 8). Harpin induced the gene encoding the abscisic acid-responsive protein and LPS the gibberellic acid-regulated GASA4 transcript, respectively (Aubert et al., 1998). Auxin-, ethylene- and calcium/calmodulin-related signalling component transcripts were induced in response to both treatments. However, as mentioned above, responses to harpin were most prominent in the early phase after treatment, while transcriptional changes after LPS-treatment became apparent at the end of the 24 h period.

Regarding MAPK and their regulating upstream kinases, we found a very strong and immediate induction of AtMPK11 by harpin. Surprisingly, we did not observe an activation of any stress and/or pathogen-associated MAPKs such as AtMPK4 and AtMPK6 at the transcript level. LPS did not influence any of the currently annotated *Arabidopsis* MAPKs genes. While harpin and in some instances LPS-induced genes encoding the upstream components MAPKKK Raf27, MPKKK5, MPKKK15, MPKKK16 and MPKKK19 (with the exception of the Raf-protein all belonging to the MEKK subfamily, Mizoguchi et al., 2000), the overall impact of these elicitors on genes of MAPK signalling networks was weak. On the other hand, transcriptional activation of plant MAPK signalling components by stress, pathogens or elicitors seems to be only one aspect of stress-induced MAPK activation which very often is controlled by post-translational mechanisms (Zhang and Klessig, 2001).

Conclusions

After identification of about 600 LPS- and 1300 harpin-regulated genes in *Arabidopsis*, we concentrated our data analysis on genes associated with defence and/or adaptation to stress. While both harpin and LPS induced an overlapping array of defence genes, we observed a striking difference regarding some of the prominent defence components such as WRKY transcription factors or receptor kinases. The defence machinery induced by harpin reflects the much more pronounced host response including cell death induction after treatment with this elicitor. It is an exciting goal for the future to investigate whether genes induced specifically by PAMPs are components of innate immunity and whether they contribute to the phenomenon of basal resistance.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.imbio.2007.10.004.

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