Transcriptome Analysis of Induced Systemic Drought Tolerance Elicited by *Pseudomonas chlororaphis* O6 in *Arabidopsis thaliana*

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(Received on July 15, 2012; Revised on September 21, 2012; Accepted on September 30, 2012)

Root colonization by *Pseudomonas chlororaphis* O6 induces systemic drought tolerance in *Arabidopsis thaliana*. Microarray analysis was performed using the 22,800-gene Affymetrix GeneChips to identify differentially-expressed genes from plants colonized with or without *P. chlororaphis* O6 under drought stressed conditions or normal growth conditions. Root colonization in plants grown under regular irrigation condition increased transcript accumulation from genes associated with defense, response to reactive oxygen species, and auxin- and jasmonic acid-responsive genes, but decreased transcription factors associated with ethylene and abscisic acid signaling. The cluster of genes involved in plant disease resistance were up-regulated, but the set of drought signaling response genes were down-regulated in the *P. chlororaphis* O6-colonized under drought stress plants compared to those of the drought stressed plants without bacterial treatment. Transcripts of the jasmonic acid-marker genes, *VSP1* and *pdf-1.2*, the salicylic acid regulated gene, *PR-1*, and the ethylene-response gene, *HEL*, also were up-regulated in plants colonized by *P. chlororaphis* O6, but differed in their responsiveness to drought stress. These data show how gene expression in plants lacking adequate water can be remarkably influenced by microbial colonization leading to plant protection, and the activation of the plant defense signal pathway induced by root colonization of *P. chlororaphis* O6 might be a key element for induced systemic tolerance by microbes.

Keywords : induced systemic tolerance, plant disease resistance, plant growth-promoting rhizobacteria, transcript analysis

Root colonization by certain microbes increases plant fitness (Van Loon and Glick, 2004). Mechanisms of plant growth promotion by rhizobacteria include elimination of deleterious pathogens, increases availability of nutrients, production of phytohormones such as auxins, and cytokinins (Defago and Haas, 1990; Glick et al., 1998; Kim et al., 2011; Kloepper et al., 1980; Timmusk and Wagner, 1999) or reduction of the plant growth regulator ethylene production (Glick, 1995). Induced systemic resistance (ISR) by root colonization with nonpathogenic rhizobacteria is effective in various plants against different types of pathogens (Kloepper et al., 2004; Ramamoorthy et al., 2001; Van Loon et al., 1998).

Drought and salt tolerance in plants could be induced by root colonization of certain rhizobacteria. Induced drought and salt tolerance was observed in plants with root colonization by the endophytic fungus *Piriformospora indica* and *Trichoderma* species (Scharidl et al., 2004; Waller et al., 2005). Root colonization by the gram-positive bacterium, *Paenibacillus polymyxa* also increases survival *Arabidopsis thaliana* under drought stress (Timmusk and Wagner, 1999). Drought tolerance induced by *Achromobacter piechaudii* has been correlated with the lowered ethylene levels due to the bacterial cells producing 1-aminocyclopentane-1-carboxylate (ACC)-deaminase (Mayak et al., 2004). *Pseudomonas chlororaphis* O6 is one of these beneficial root colonizers. *P. chlororaphis* O6 root colonization reduced symptom development caused by the leaf pathogens, *Erwinia carotovora* and *Pseudomonas syringae* pv. *tabaci* in tobacco (Han et al., 2006; Spencer et al., 2003) and in cucumber against *Corynespora* leaf spot disease (Kim et al., 2004). In addition, root colonization by *P. chlororaphis* O6 increases leaf surface area in tobacco (Kang et al., 2006; Ryu et al., 2007) and induces tolerance to drought and salinity in *Arabidopsis* (Cho et al., 2008; 2011; 2012).

Previous works indicated that certain plant genes were altered upon root colonization by certain beneficial microbes.

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Increases in transcripts of genes involved in cell division and proliferation, but down-regulation of genes related to stress were determined in plants with colonization by an ACC-deaminase-producing strain *Enterobacter cloacae* UW4 (Hontzeas et al., 2004). Up-regulation of genes involved in auxin responses and down-regulation of genes involved in ethylene responses were identified in plants colonized by another plant growth-promoting root colonizer, *P. fluorescens* FPT9601-T5 (Wang et al., 2005). Increases in defense and abiotic stress-related transcripts, and repression of photosynthesis-related transcript occurred with colonization by *P. thivervalensis* MLG45 (Cartieaux et al., 2003). Our previous work showed that the potential priming genes in *A. thaliana* upon root colonization by *P. chlororaphis* O6 were identified and characterized (Cho et al., 2011). Expression of the identified priming genes as well as jasmonic acid- and ethylene-regulated defense genes were predicted as important roles in the *P. chlororaphis* O6-mediated systemic tolerance against abiotic and biotic stresses (Cho et al., 2011).

For better understanding of the plant genes involved in stress tolerance induced by *P. chlororaphis* O6, in this study, we identified *A. thaliana* transcript changes using microarray approach in the leaves of plants treated with drought stress on the *P. chlororaphis* O6 root-colonized and non-colonized plants. We extended studies to examine the transcription of other plant defense genes that are associated with signaling pathways involving jasmonic acid, ethylene, and salicylic acid pathway under drought stress conditions.

Materials and Methods

Plant materials and growth conditions. Surface sterilized seeds of *Arabidopsis thaliana* ecotype Columbia (Col-0), were planted into sterile soil-less medium (peat moss: vermiculite: perlite, 7:3:3 v/v) using 500 cm³ of the medium in a 10.5 × 10.5 × 9 cm pot, and 15–30 seeds per pot. *Arabidopsis* plants were watered with 20 ml of sterile water per pot in every two days. Seedlings were grown with a 16-h-light/8-h-dark cycle under 40-W fluorescent lights (2,000 lux, 80 μmol photons m⁻²s⁻¹). The temperature was maintained at 22 ± 1 °C with a relative humidity of 50–60%.

Bacterial strains and cultural condition. *P. chlororaphis* O6 was stored at –70 °C in 15% glycerol and prepared for experimental use as described previously (Cho et al., 2011). Briefly, frozen cells were streaked onto King's medium B plates (KB) agar plates without antibiotics and incubated at 28 °C for 2 days. These cells were inoculated into KB broth without antibiotics and grown overnight. The cells were obtained as a pellet by centrifugation at 10,000 × g for 10 min, washed once with sterile water, and re-suspended to 1 × 10⁸ colony forming unit (cfu)/ml to use as an inoculum.

Induced drought tolerance bioassay. *Arabidopsis* plants were inoculated with bacterial suspension after two weeks of growth. Plants in pots were treated with 35 ml of the suspended *P. chlororaphis* O6 or water as control. Seven days after bacterial root treatment, one group of plants was stopped watering, whereas a second group was continued watering at two day intervals. At the defined days, the number of the survived seedling under drought stress was determined by determination of the percentages of recovery of the plants showing no wilt symptoms after rehydration. Three independent experiments were performed with at least 30 plants per treatment. Data were analyzed by one way ANOVA using SPSS Statistics v.19 (IBM Corporation, Somers, NY). The significance of treatments was determined by Duncan's multiple range test ($P < 0.05$).

RNA isolation and gene chip microarray experiment.

Arabidopsis plants were inoculated after two weeks of growth with *P. chlororaphis* O6 or water and incubated under drought or normal growth conditions as described above. At 15 day of drought, aerial parts of the plants were harvested, and frozen in liquid nitrogen for isolation of total RNA for gene chip analysis. Total RNA was prepared using RNeasy Plant Mini kits (Qiagen Inc., Valencia, CA) for microarray analysis. The quality of RNA was assessed by appearance of the bands after agarose gel electrophoresis and UV-absorbance spectrophotometry. For microarray analysis, total RNA was processed for use on Affymetrix *Arabidopsis* genome GeneChip arrays according to the manufacturer's protocol (Santa Clara, CA). In brief, 10 μg of total RNA from each treatment used in a reverse transcription reaction to generate first-strand cDNA, using the SuperScript Choice System (Invitrogen, Carlsbad, CA) with oligo (dT)₂₄ primer fused to T7 RNA polymerase promoter. After second strand synthesis, biotin-labeled target complementary RNA (cRNA) was prepared using the BioArray high-yield RNA transcript labeling kit (Enzo Biochem., New York, NY) in the presence of biotinylated UTP and CTP. After purification and fragmentation, 15 μg of cRNA was used in a 300 μl hybridization mixture containing added hybridization controls. A total of 200 μl of the mixture was hybridized on arrays for 16 h at 45 °C. Standard post-hybridization wash and double-stain protocols were used on an Affymetrix GeneChip fluidics station 450. Arrays were scanned on an Affymetrix GeneChip scanner 2500. The microarray study was duplicated with two independent RNA samples.

Affymetrix Gene Chip data analysis. The data were first analyzed with Microarray Suite 5.0 software (Affymetrix, Santa Clara, CA). For each microarray, overall intensity normalization for the entire probe sets was performed using

Affymetrix Microarray Suite 5.0. Using the GeneChip Suite 5.0 default parameters, the detection of P-value ($P < 0.05$) and the signal value were calculated for each probe set from

each independent sample hybridization. For the microarray analysis, overall intensity normalization for the entire probe set was performed as described (Irizarry et al., 2003), and

Table 1. Genes with up-regulated transcripts in plants colonized with *P. chlororaphis* O6 grown under normal watering condition compared with genes in control plants grown under normal watering condition

Functional categories ^a	Ratio (log ₂) ^b	Probe set number ^c	AGI number ^d
Biogenesis of Cellular component			
germin-like protein (GLP2a)	1.20	249474_s_at	At5g39190
expansion (At-EXP1)	1.50	256299_at	At1g69530
Cell and protein fate			
senescence-associated protein (SAG29)	1.05	245982_at	At5g13170
E2, ubiquitin-conjugating enzyme 17 (UBC17)	1.27	246195_at	At4g36410
Cell rescue, defense and virulence			
putative zinc finger protein salt-tolerance protein	1.01	252924_at	At4g39070
glutaredoxin family protein	1.01	261958_at	At1g64500
putative disease resistance protein	1.02	267411_at	At2g34930
putative disease resistance response protein	1.04	254906_at	At4g11180
EXOSTOSIN-1	1.14	246682_at	At5g33290
catalase	1.20	253174_at	At4g35090
band 7 family protein	1.22	260401_at	At1g69840
glutathione S-transferase	1.30	250083_at	At5g17220
germin - like protein germin-like protein GLP2a	1.30	249491_at	At5g39130
zinc finger (C2H2 type) protein 3 (AZF3)	1.31	249139_at	At5g43170
broad-spectrum mildew resistance RPW8 family protein	1.42	252170_at	At3g50480
peroxidase	1.45	250798_at	At5g05340
allene oxide cyclase, putative / early-responsive to dehydration protein	1.50	257644_at	At3g25780
cytochrome P450	1.69	266246_at	At2g27690
proline oxidase	1.75	257315_at	At3g30775
beta-1,3-glucanase 2 (BG2) (PR-2)	2.01	251625_at	At3g57260
several small proteins induced by heat, auxin, ethylene and wounding	2.24	255479_at	At4g02380
band 7 family protein	3.32	259272_at	At3g01290
heat shock protein	4.31	254384_at	At4g21870
Cellular communication/signal transduction			
serine/threonine kinase - like protein	1.02	254255_at	At4g23220
putative receptor-like protein kinase	1.06	264107_s_at	At2g13790
ethylene responsive element binding factor	1.13	247540_at	At5g61590
MAP kinase ATMPK9	1.23	260979_at	At1g53510
calcium-binding protein	1.25	254487_at	At4g20780
protein kinase-like protein Pto kinase interactor 1	1.53	251494_at	At3g59350
Cellular transport			
nonspecific lipid-transfer protein precursor	1.02	247717_at	At5g59320
putative ABC transporter	1.03	260496_at	At2g41700
sodium-dicarboxylate cotransporter-like	1.11	248756_at	At5g47560
C4-dicarboxylate transporter	1.21	249765_at	At5g24030
sucrose transport protein	1.38	260143_at	At1g71880
putative sulphate transporter protein	1.71	264901_at	At1g23090
putative protein cation transport protein chaC	1.72	246884_at	At5g26220
CLC-b chloride channel protein	1.75	256751_at	At3g27170
P-glycoprotein-like protein	3.39	251248_at	At3g62150

Table 1. (Continued)

Functional categories ^a	Ratio (log ₂) ^b	Probe set number ^c	AGI number ^d
Development			
embryo-specific protein 3 (ATS3)	1.37	250620_at	At5g07190
Energy			
pyruvate, orthophosphate dikinase	1.11	245528_at	At4g15530
Interaction with the environmental stimuli			
oxidoreductase	1.09	262616_at	At1g06620
Metabolism			
cytochrome P450	1.02	257636_at	At3g26200
putative protein SRG1 protein	1.07	252265_at	At3g49620
S-adenosyl-L-methionine	1.09	259286_at	At3g11480
flavanone 3-hydroxylase (FH3)	1.09	252123_at	At3g51240
laccase-like protein	1.14	251124_s_at	At5g01040
cellulose synthase like protein	1.20	245465_at	At4g16590
nitrate reductase	1.22	261979_at	At1g37130
AMP-binding protein	1.23	262698_at	At1g75960
flavonoid 3-hydroxylase-like protein	1.28	250558_at	At5g07990
xyloglucan endo-1,4-beta-D-glucanase-like protein	1.31	253628_at	At4g30280
6-phosphogluconolactonase-like protein	1.34	249732_at	At5g24420
reticuline oxidase-like protein	1.52	254432_at	At4g20830
11-beta-hydroxysteroid dehydrogenase-like	1.55	248520_at	At5g50600
UDP-glucose glucosyltransferase	1.89	260799_at	At1g78270
tyrosine transaminase like protein	2.12	254232_at	At4g23600
oleosin	2.22	249353_at	At5g40420
leucoanthocyanidin dioxygenase-like protein	2.32	250793_at	At5g05600
oleosin, 18.5K	2.39	254095_at	At4g25140
putative tyrosine aminotransferase	2.67	263539_at	At2g24850
Storage protein			
NWMU1-2S albumin 1 precursor	2.55	253904_at	At4g27140
putative cruciferin 12S seed storage protein	2.57	265095_at	At1g03880
Bet v I allergen family protein	2.61	263836_at	At2g40330
12S cruciferin seed storage protein	2.61	253767_at	At4g28520
NWMU2-2S albumin 2 precursor	2.99	253894_at	At4g27150
legumin-like protein	3.09	249082_at	At5g44120
Transcription			
myb family transcription factor	1.15	247768_at	At5g58900
zinc finger protein CONSTANS-LIKE 1 (COL1)	1.29	246523_at	At5g15850
CONSTANS	1.87	246525_at	At5g15840
ethylene-responsive element-binding protein (EREBP)	2.62	260451_at	At1g72360
WRKY family transcription factor	3.03	253485_at	At4g31800
Others			
auxin-responsive family protein	1.07	262092_at	At1g56150
auxin-responsive family protein	1.11	262703_at	At1g16510
auxin efflux carrier family protein	1.16	263073_at	At2g17500
class I non-symbiotic hemoglobin (AHB1)	1.07	263096_at	At2g16060
nodulin-like protein	1.13	266993_at	At2g39210

^a Average fold change value which measure in expression for a transcription from two replicated experiments.^b Described name of probe set on Affymetrix chip.^c AGI locus numbers.

then the signal value, which assigns a relative measure of abundance of the transcript, and the detection of P-value, which indicates whether a transcripts is reliably detected, were calculated for each probe set. The normalized data were examined by comparison of the ratio expression under the four conditions, control (non-colonized plants grown with normal watering), *P. chlororaphis* O6 root-colonized plants grown with normal watering, non-colonized plants grown with drought treatment and *P. chlororaphis* O6 root-colonized plants grown with drought treatment.

The change algorithm assigned a call of “increase” or “decrease”, when the signal $\log_2$ ratio was changed. The used signal log ratio cut-offs were corresponded to approximately a 2.0-fold change. In addition, the P-value, measuring the probability that the expression levels of each probe set are different between two arrays, was calculated for all comparison analyses that we performed. A probe set was designated as being up regulated, if the detection algorithm assigned a call of ‘present’ in the *P. chlororaphis* O6 treated sample and the signal log ratio was greater than or equal to 1.0 in both duplicated samples. A probe set was designated as being down regulated, if the detection algorithm assigned a call of ‘present’ in the *P. chlororaphis* O6 treated sample and the signal log ratio was less than or equal to -1.0 in both duplicated samples.

We compared each array of each treatment and then normalized all of each comparison array for preprocessing applied by Robust Multiarray Average (RMA) methods (Irizarry et al., 2003). Genes preferentially expressed in the plants inoculated with *P. chlororaphis* O6 were identified using the same criterion, i) fold change cutoff > 2 and ii) significance level (T-test, 0.05) and iii) volcano plotting, showed both i and ii commonly significant probe sets, and iv) up-regulated and down-regulated gene probe sets were detected the algorithm assigned a call of ‘present’ by GCOS filtering.

Reverse transcription polymerase chain reaction (RT-PCR) analysis. Total RNA, isolated as described above, was used for RT-PCR to determine changes in transcript abundance for discrete genes. The plants for these studies were from different experiments than those used for the microarray analysis. RT-PCR with specific primer pairs (Supplemental Table 1) was performed using Reverse Transcription PCR kit (Qiagen Inc., Valentia, CA). Primers for the actin gene of *A. thaliana*, forward (5'-CATCAGGAAGGACTTGTTACG-3') and reverse (5'-GATGGACCTGACTCGTC ATAC-3'), were used as an internal standard. The 25 μ l reaction mixtures were incubated at 50 °C for 30 min for reverse transcription, followed by PCR for 35 cycles with denaturation at 94 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. A thermal cycler

machine was used. Controls consisted of omission of one of the primers and analysis of the RNA without reverse transcription to determine that DNA was not present in the plant samples. The amplified PCR products were separated in 2% agarose gel stained with ethidium bromide. The results provided are typical of three independent studies.

Results

***P. chlororaphis* O6 induced drought tolerance phenotypes in *Arabidopsis* plant.** *Arabidopsis* plants, in which water was withheld, showed reduction in survival after 14 days of drought. Survival rate rapidly decreased in control plants at 15 and 16 days of drought treatment. Plants inoculated with *P. chlororaphis* O6 did not show such a loss in viability upon water withholding (Fig. 1). Average populations of *P. chlororaphis* O6 was recovered from the roots of inoculated plants at about 3.5×10^6 cfu/g of root, but no bacteria was recovered from roots of non-colonized control plants.

Analysis of gene expression by microchip array detection. A set of 5 representative control genes were identified that did not showed a significant change in expression under any of the treatments (Supplemental Table 2). The expression of five genes that are often regarded as having

Fig. 1. Drought tolerance in *Arabidopsis thaliana* is affected by root colonization with *P. chlororaphis* O6. Survival of *A. thaliana* cultivar Col-0 (n=30) was determined with plants grown in soil-less medium for 2 weeks and inoculated with 10^8 cfu/ml of *P. chlororaphis* O6 (O6). After one week of growth under normal watering conditions (non-inoculated [C+W] and inoculated [O6+W]) plants were subjected to water withholding for a further 16 days (non-inoculated, [C+D] and inoculated [O6+D]). The survival rates of control plants grown under normal watering conditions were overlapped with those of O6 treated plants grown under normal watering conditions. Each data point represents the mean of three replicate experiments. Photographic images of plant appearance after drought stress or normal growth conditions for 16 days. Each data point represents the mean of three replicate experiments with 30 plants/treatment.

constitutive expression was examined for changes in expression induced by colonization with and without water withholding (Supplemental Table 2). Genes encoded for actin, glyceraldehyde phosphate dehydrogenase and ubiquitin did not show fold changes above 2 under the designated growth conditions. However, the 25S rRNA gene transcript was

decreased in non-colonized and drought stressed plants compared to the control plants. There was increased transcript accumulation of 5S rRNA transcripts in *P. chlororaphis* O6 root-colonized plants grown with water withholding compared with the control plants (Supplemental Table 2).

Table 2. Genes with down-regulated transcripts in plants colonized with *P. chlororaphis* O6 grown under normal watering condition compared with genes in control plants grown under normal watering condition

Functional categories ^a	Ratio (log ₂) ^b	Probe set number ^c	AGI number ^d
Abiotic stress response			
salt-tolerance protein identical to salt-tolerance protein	-1.53	260956_at	At1g06040
cold-regulated protein cor15b precursor	-1.52	263495_at	At2g42530
cold-regulated protein cor15a precursor	-1.43	263497_at	At2g42540
Cellular communication/signal transduction			
serine threonine protein kinase	-1.37	248910_at	At5g45820
putative protein	-1.26	250942_at	At5g03350
Cell rescue, defense and virulence			
disease resistance RPP5 like protein (fragment)	-1.39	245450_at	At4g16880
thionin Thi2.2	-1.15	249645_at	At5g36910
Development			
putative protein ABI3-interacting protein	-2.26	250971_at	At5g02810
Energy			
lysophospholipase homolog	-1.04	245734_at	At1g73480
Metabolism			
xyloglucan endo-transglycosylase	-1.37	252607_at	At3g44990
putative alpha-amylase similar to alpha-amylase	-1.19	260412_at	At1g69830
cytochrome P450 homolog	-1.00	256598_at	At3g30180
Photosynthesis			
putative chlorophyll A-B binding protein	-1.10	258239_at	At3g27690
putative chlorophyll a/b binding protein	-1.10	263345_s_at	At2g05070
Lhcb3 chlorophyll a/b binding protein	-1.03	248151_at	At5g54270
Transcription			
H-protein promoter binding factor-2a	-1.85	252429_at	At3g47500
CONSTANS-like B-box zinc finger protein-like	-1.81	247921_at	At5g57660
putative transcription factor	-1.57	259751_at	At1g71030
Transcription			
myc-like protein	-1.56	264107_s_at	At5g04150
DNA-binding protein-like DNA-binding protein	-1.46	251705_at	At3g56400
Expressed protein	-1.39	253423_at	At4g32280
myb-related protein	-1.21	246253_at	At4g37260
ethylene-responsive transcriptional coactivator	-1.20	258133_at	At3g24500
SigA binding protein	-1.13	246293_at	At3g56710
unknown protein contains similarity to phytochrome interacting factor	-1.12	259417_at	At1g02340
DNA-binding homeotic protein Athb-2	-1.06	245276_at	At4g16780
putative protein putative DNA-binding protein	-1.05	249383_at	At5g39860

^a Functional categories from MIPS database.

^b Signal log value which measure the change in expression for a transcription between arrays. This change is expressed as the log₂ ratio a log₂ ratio of 1 is the same as a fold change of 2. Average fold change value which measure in expression for a transcription from two replicated experiments.

^c Described names of probe set on Affymetrix chip.

^d AGI locus numbers.

Identification of genes preferentially expressed in plants colonized with *P. chlororaphis* O6.

Our criteria to designate genes that were differentially expressed in leaves due to systemic effects induced by root colonization with *P. chlororaphis* O6 in plants grown under normal watering conditions were determined as described in method. The majority of *P. chlororaphis* O6-regulated genes were in the unknown function category, 53% for up-regulated genes and 34% for down regulated genes (Supplemented Fig. 1). Large differences between the up- and down-regulated were observed for genes involved in transcription and cellular transport. The up-regulated genes by root colonization of *P. chlororaphis* O6 were involved in plant growth promotion such as the auxin-induced genes (glucanase At4g30280, At1g56150, auxin responsive-family protein and the auxin efflux carrier protein (At2g17500) (Table 1). Genes involved in response to reactive oxygen species showed increased transcripts: glutaredoxin (At1g64500), glutathione S-transferase (At5g17220), peroxidase (At5g05340) and catalase (At4g35090). Genes encoding other stress-related proteins with increased transcripts included those induced by drought (At4g39070, At3g25780, and At5g43170), and a small protein (At4g02380), were also increased. Other increased transcripts were related to disease resistance included the

PR protein (At5g57260), the broad spectrum mildew resistance gene RPW (At3g50480) and disease resistance proteins (At2g34930, At4g11180) (Table 1). Among the most expressed transcript was that for WRKY 18 involved in transcription of defense genes.

Down-regulated genes upon *P. chlororaphis* O6 root-colonization were ABA- and ethylene-related: the ethylene transcription factors, ERF (At3g24500), Myb (At4g37260), Myc-like (At5g0415), and a negative regulator of ABA signaling, AB13 interacting protein At5g02810 (Table 2). Additionally, the transcript levels for three genes involved in salt and cold tolerance At1g06040, At2g42530, and At2g42540 were decreased.

Genes preferentially expressed in *P. chlororaphis* O6-colonized and drought stressed plants. Up-regulated drought responsive genes in non-colonized drought stress treatment compared to normal watered control *Arabidopsis* plant were listed in Table 3. Transcripts of late embryogenesis abundant protein (At3g17520), low-temperature-responsive 65 kDa protein (At3g15670), Em-like protein GEA1 (At3g51810), putative dehydrin (At3g50980), and putative protein phosphatase 2C (At1g07430) were showed over 100 fold changes (Table 3).

Table 3. Representative up-regulated drought responsive genes in the control plant treated with stress compared with genes in control plants without drought treatment

Annotation	Average fold change ^a	Probe set number ^b	AGI number ^c
late embryogenesis abundant protein (LEA)	2402.55	258347_at	AT3g17520
low-temperature-responsive 65 kD protein (LTI65)	1380.44	258224_at	AT3g15670
Em-like protein GEA1 (EM1)	564.06	246299_at	AT3g51810
dehydrin, putative	327.68	252137_at	AT3g50980
protein phosphatase 2C, putative	100.35	261077_at	AT1g07430
DRE2B	78.36	267026_at	AT2g38340
DRE-binding protein (DREB2A)	72.08	250781_at	AT5g05410
homeobox-leucine zipper protein 7 (HB-7)	59.95	266327_at	AT2g46680
myb family transcription factor	57.48	259618_at	AT1g48000
no apical meristem (NAM) (RD26)	56.11	253872_at	AT4g27410
dehydrin (RAB18)	50.35	247095_at	AT5g66400
ABA-responsive protein (HVA22b)	47.89	247437_at	AT5g62490
zinc finger (AN1-like) family protein	42.66	256576_at	AT3g28210
L-ascorbate peroxidase 1b (APX1b)	42.31	258695_at	AT3g09640
zinc finger (C2H2 type) family protein (ZAT10)	38.90	261648_at	AT1g27730
AP2 domain-containing protein RAP2.1	38.76	245807_at	AT1g46768
drought-induced protein (Di21)	34.09	245523_at	AT4g15910
9-cis-epoxycarotenoid dioxygenase, putative	30.21	257280_at	AT3g14440
abscisic acid-insensitive 2 (ABI2)	27.90	247957_at	AT5g57050
low-temperature-responsive protein 78 (LTI78)	23.91	248337_at	AT5g52310

^a Average fold change value which measure in expression for a transcription from two replicated experiments.

^b Described name of probe set on Affymetrix chip.

^c AGI locus numbers.

Fig. 2. Hierarchical cluster and expression pattern profiles of genes expressed differentially. Hierarchical cluster analysis of the 1919 differentially expressed genes. Up-regulated genes (red) cluster in sections I (211 genes) and II (797 genes) and down-regulated genes (green) in clusters III (299 genes) and IV (612 genes). The letters indicate comparison of expression profiles for each gene (t1c1 is commonly compare with O6 and control; t2c1 is commonly compare with O6 and drought treated plants versus control plants grown normal watering conditions; t2c2 is commonly compare with O6 and drought treated plants versus control plants treated with drought).

Hierarchical clustering based on expression patterns in the plants colonized with *P. chlororaphis* O6 and treated drought stress showed that expression clusters I and II were up-regulated transcripts and cluster III and IV down-regulated genes (Fig. 2). Genes in cluster I or III were up- or down-regulated in the plants challenged with *P. chlororaphis* O6 compared to non-colonized control plants, whereas genes in cluster II or IV were up- or down-regulated in the *P. chlororaphis* O6 colonized and drought stressed plants

compared to the drought stressed non-colonized plants. We focused on characterization of cluster II and cluster IV, because the genes were more changes in drought stress of *P. chlororaphis* O6-colonized plants than drought stress of non-inoculated plants.

Clusters II and IV contained many genes that have no known function. However, genes with known function in cluster II and cluster IV are summarized in Supplemental Tables 3, 4, 5 and 6. Up-regulated genes in the cluster II were plant growth promotion auxin-induced genes (At1g78060, At3g13560, and At3g23730); genes related to elevated disease resistance include PR protein (At5g42100), anthranilate N-hydroxycinnamoyl/benzoyltransferase (At3g50270), genes related to carbon utilization (At1g70410, At5g14740) and stress response protein such glutathione-transferase like protein (At3g43800), salt stress induced proteins (At1g65860) (Tables 5 and 6); genes related to other metabolic pathway include glycolysis (At4g26530, At1g70820 and At1g56190), cell wall degrading genes (At1g60590, At4g22010, and At3g53190), sugar signaling (At5g66530), and gene of biosynthesis flavonoid (At1g65060) (Supplemental Table 3).

Some of the up-regulated genes in the cluster II involved in cellular communication; the calmodulin and calcium-binding protein (At3g52290, At2g41100, At3g49260, At5g07240, and At2g41090), receptor-like protein kinase (At5g67280, At5g61480, At5g16000, At4g34220, At5g16590, At5g01890, At3g49670, At2g36570, At3g56370, At3g14840 and At5g54380) protein phosphatase-2C (At4g38520), and serine-threonine-specific protein kinase (At5g59670) (Supplemental Table 4).

Down-regulated genes in the cluster IV were included; in metabolic pathway of plant growth related 3-ketoacyl-CoA thiolase (At2g33510); genes related to regulate carbon flux include acyl-CoA oxidase like protein (At4g167760), acyl-CoA oxidase 2 (At5g65110), and triacylglycerol lipase like protein (At5g18630); salt and drought stress induced proteins (At1g62570, At2g32090, and At5g11110), abiotic, biotic and senescence induced protein (At3g57520), sugar utilization related protein (At1g60140, At2g21590); genes related to other metabolic pathway include nitrogen metabolism (At5g11520), mitochondrial respiration related protein (At2g07687), phosphate starvation (At3g52780) (Supplemental Table 5).

The down-regulated genes in the cluster IV involve in cellular transcription; Myb transcription factors MYB94 (At3g47600), MYB43 (At5g16600), MYB102 (At4g21440), MYB74 (At4g05100), cpm10 (At1g48000), and Myb related transcription factor (At5g04760), homeodomain transcription factor (At2g46680), ABA responsive AtHB-7 (At2g46680), AP2 domain transcription factor (At3g61630, At5g13330), CCAAT-box binding transcription factor

Fig. 3. RT-PCR analyses of selected genes from cluster II and cluster IV from the microchip array analyses. RT-PCR was performed using total RNA isolated 15 days from plants that were non-inoculated and watered (C+W), water withheld for 15 days (C+D), inoculated with *P. chlororaphis* O6 and watered (O6+W), inoculated with *P. chlororaphis* O6 and water withheld for 15 days (O6+D). The genes examined were: thionin [At1g66100], PR5, myrosinase-associated protein [At3g14210], PP2C [At4g38520], CaBP-22 [At2g41090], and dehydration response protein ERD [At1g04430], ABF3, ABI1, LEA3 [At4g02380], dehydrin [At3g50980], and HNL4 [At5g10300]. These results are the end points from 25 RT-PCR cycles and are typical of three independent studies.

(At1g54830, At1g72830), bZIP transcription factor (At2g46270, At3g62420, At3g49760), and Zinc finger ethylene-response transcription factor (At4g00940, At4g29190, At5g04340) (Supplemental Table 6).

RT-PCR confirmation of hybridization data from the microarray. To confirm the reliability of results from gene chip expression profile analyses, RT-PCR experiments were performed using cDNAs synthesized from total RNA from plants grown four different conditions. Expression of the representative genes in the cluster II: Thionin (At1g66100), PR5, myrosinase associated protein (At3g14210), and Ca²⁺ binding protein (At2g41090), was the highest levels in the O6 colonized and drought stressed plants, whereas expression of the five representative genes in the cluster IV, ABF3, ABI1, LEA3 (At4g02380), dehydrin (At3g50980), and alpha hydroxynitrile lyase (HNL4) (At5g10300), showed the highest expression in the non-colonized and drought-stressed plants (Fig. 3).

Expression patterns of defense related genes. We extend-

Fig. 4. Expression of plant disease resistance marker genes in *P. chlororaphis* O6-inoculated *Arabidopsis thaliana* Col-0. Transcript levels of designated genes in aerial parts of plants without roots were determined by RT-PCR. Expression levels in plants at 13 and 15 days of drought stress (D) with (O6) and without *P. chlororaphis* O6-inoculation (C) are shown. These results are the end points from 25 RT-PCR cycles and are typical of three independent studies.

ed our studies to examine the effects of the *P. chlororaphis* O6 root colonization and drought stress for defense-related genes associated with different signaling pathways: *PR1* for the salicylic acid pathway, *pdf1.2* and *VSP1* for the jasmonate pathway and *HEL* for the ethylene pathway. Extension of the drought stress period for 13 and 15 days showed increasing transcript levels of *VSP1* in the drought stressed plants, whereas the *pdf1.2* transcript level was decreased in the plants under drought condition. However, the enhanced expression of the *pdf1.2* and for *HEL* genes was detected in the O6-colonized plants grown under normal growth condition with normal watering. In drought stressed plants, the transcripts of the *PR1*, *pdf1.2* and *VSP1* were increased in the O6-treated plants under drought stress condition (Fig. 4).

Discussion

Plant is able to response and to survive drought stress depending on plant mechanism that integrates cellular responses (Kim et al., 2012). Plant drought tolerance can be achieved key genetical, biochemical and physiological changes. One of key hormones involved in the response of drought stress is the abscisic acid (ABA). The ABA regulates down-stream signal pathways involving in drought tolerance in plants (Shinozaki and Yamaguchi-Shinozaki, 1997). Our findings indicated that *P. chlororaphis* O6 root colonization induced abiotic stress (Cho et al., 2008; 2012) and induced resistance to pathogens (Kim et al., 2004; Ryu et al., 2007; Spencer et al., 2003). Priming effect by rhizobacteria has been associated with several types of induced resistance.

The molecular mechanism of priming is still unclear. It is hypothesized that induction of the primed state results in an increase in the amount or activity of cellular components with important roles in defense signaling (Conrath et al., 2002). We believe the identified genes in this study will open opportunities for identification of novel plant genes or signal transduction pathways that are involved in induced drought tolerance by rhizobacteria.

We are intrigued by these studies to deduce the cell signaling pathways involved in the induced systemic tolerance by microbes. Our previous study with genes identified by differential expression approach in plants that were colonized for three days by *P. chlororaphis* O6, but were grown without water stress, confirmed a rapid induction of the auxin-related gene *NIT1* (At3g44310) encoding nitrilase 1 (Cho et al., 2011). In this study, at least five auxin-related genes were increased in *P. chlororaphis* O6 colonized plants. In the *P. fluorescens* FPT9601-T5 system, certain ethylene-pathway genes, the ethylene transcription factors, ERF (At3g24500), Myb (At4g37260) and Myc-like (At5g0415), were down-regulated. However, we observed up-regulation of drought- and salt-stress-related genes (At4g39070, At5g39130, At5g43170, and At3g25780).

In this study, we confirmed that a concept in induced drought tolerance or induced systemic disease resistance in prior colonization of the plant by the rhizobacteria speed up the rate of transcript accumulation and increase transcript levels for the defense genes upon pathogen challenge or abiotic stresses. Normal grown plants under watering conditions for 13 days after inoculation with *P. chlororaphis* O6 showed increased transcript levels of the jasmonic acid-related genes, *pdf1.2*, and the ethylene responsive gene, hevein-like protein *HEL* supporting the data with other defense genes studied by Spencer et al. (2003). Other studies indicated that common defense and stress-response genes were beta-1,3-glucanase (*PR-2*), band 7 family protein (At3g01290), and UDP-glucose transferase (At1g78270). In *Arabidopsis* plants, induced systemic resistance by root colonization with *P. fluorescens* W417r was correlated with induction of plant defense related genes (Verhagen et al., 2004). Several defense responsive genes are induced response to abiotic stress such as salt and drought stress (Mayak et al., 2004; Timmusk and Wagner, 1999). Pepper endochitinase gene over-expressing transgenic plants show bacterial infection disease resistance as well as exhibit increase tolerance to NaCl during germination and seedling growth (Hong and Hwang, 2006). Other defense responsive gene, pepper basic *PR1* gene promoter activated not only defense response material such as bacterial pathogen, ethylene, salicylic acid but also high salt, drought, and low temperature (Hong et al., 2005). These results indicated that up-regulation of the identified genes related to plant

defense related genes might be correlated in the induced systemic tolerance or resistance against plant diseases and abiotic stresses by root colonization of *P. chlororaphis* O6.

Our RT-PCR results confirmed the finding that *P. chlororaphis* O6 induces systemic activation of plant defenses under drought stress condition. The genes include thionin, *PR5* and myrosinase associated protein were belonged in the cluster II. Thionins, 5 kDa, basic, cysteine-rich anti-microbial peptides, have been identified in a large number of plant species, and are either expressed constitutively or induced by pathogen attack or elicitors (Epple et al., 1997, 1998; Fernandez de Caley et al., 1972). *PR5* proteins are also called thaumatin-like proteins because of their striking sequence similarity with thaumatin, a sweet-tasting protein from *Thaumatococcus daniellii* (Cornelissen et al., 1986; Edens et al., 1982; Pierpoint et al., 1990). Although the biological function of thaumatin-like proteins has not yet been established, members of this group have been shown to have antifungal activity against a broad spectrum of fungal pathogens (Hajgaard et al., 1991; Vigers et al., 1992). Myrosinases may be involved in plant defense in the damaged plant tissues by insects or microorganisms, because they involve in releasing toxic glucosinolates (Wittstock et al., 2002).

Five genes exhibiting decrease expression in cluster IV tested are including ABF3, ABI1, LEA3, dehydrin and HNL4. This cluster IV is very interesting, because ABF3, ABI1 and dehydrin are known to representative drought signaling response gene. Constitutive over-expression of the binding factors ABF3, which is regulated through the ABA-responsive elements (ABRE), induced drought tolerance in *Arabidopsis* (Choi et al., 2000). We believe that the root colonization by *P. chlororaphis* O6 reduces the drought stress phenotypes, thus alleviating the need to express drought signaling response genes in the protected plants. Our findings using *Arabidopsis* mutants altered in cell signaling pathways also suggested that ABA is not the major player in drought tolerance induced by *P. chlororaphis* O6, rather jasmonic acid might be involved (Cho et al., 2011; 2012).

In summary, we observed that root colonization by *P. chlororaphis* O6 stimulated genes responsive to drought as well those genes with defense roles. It is interesting that resilience to both abiotic stress and to biotic challenge is simultaneously activated by interaction with certain rhizosphere microbial colonizers. We speculate that induced drought resistance by O6 could be resulted of priming effect by unknown microbial determinants produced by this bacterium and resulted of activation of complex signal network mediating by plant hormones, and plant defense resistance might be played an important role in induced systemic tolerance against abiotic stresses.

Acknowledgments

This work was supported by a grant from BioGreen 21 program (code# 2007041034005), Rural Development Administration and by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0011555).

References

- Carteaux, F., Thibaud, M. C., Zimmerli, L., Lessard, P., Sarrobert, C., David, P., Gerbaud, A., Robaglia, C., Somerville, S. and Nussaume, L. 2003. Transcriptome analysis of *Arabidopsis* colonized by a plant-growth promoting rhizobacterium reveals a general effect on disease resistance. *Plant J.* 36:177–188.
- Cho, S. M., Kang, B. R., Han, S. H., Anderson, A. J., Park, J.-Y., Lee, Y.-H., Cho, B. H., Yang, K.-Y., Ryu, C.-M. and Kim, Y. C. 2008. 2R,3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* 21:1067–1075.
- Cho, S. M., Kang, B. R., Kim, J. J. and Kim, Y. C. 2012. Induced systemic drought and salt tolerance by *Pseudomonas chlororaphis* O6 root colonization is mediated by ABA-independent stomatal closure. *Plant Pathol. J.* 28:202–206.
- Cho, S. M., Park, J. Y., Han, S. H., Anderson, A. J., Yang, K. Y., McSpadden Gardener, B. and Kim, Y. C. 2011. Identification and transcriptional analysis of priming genes in *Arabidopsis thaliana* induced by root colonization with *Pseudomonas chlororaphis* O6. *Plant Pathol. J.* 27:272–279.
- Choi, H. I., Hong, J. H., Ha, J. O., Kang, J. Y. and Kim, S. Y. 2000. ABFs, a family of ABA-responsive element binding factors. *J. Biol. Chem.* 275:1723–1730.
- Cornelissen, B. J. C., Hooft van Huijsduijnen, R. A. and Bol, J. F. 1986. A tobacco mosaic virus-induced protein is homologous to the sweet-tasting protein thaumatin. *Nature* 321:531–532.
- Conrath, U., Pieterse, C. M. and Mauch-Mani, B. 2002. Priming in plant-pathogen interactions. *Trends Plant Sci.* 7:210–216.
- Defago, G. and Hass, D. 1990. Pseudomonads as antagonists of soilborne pathogens: modes of action and genetic analysis. In *Soil Biochemistry*, Vol. 6, ed. Bollag, J. M., and Stotzky, G., pp. 249–291. New York: Marcel Dekker.
- Edens, L., Hesling, L., Klock, R., Ledeboer, A. M., Maat, J., Toonen, M. Y., Visser, C. and Verrips, C. T. 1982. Cloning of cDNA encoding the sweet-tasting plant protein thaumatin and its expression in *Escherichia coli*. *Gene* 18:1–12.
- Epple, P., Apel, K. and Bohlmann, H. 1997. Overexpression of an endogenous thionin enhances resistance of *Arabidopsis* against *Fusarium oxysporum*. *Plant Cell* 9:509–520.
- Epple, P., Vignutelli, A., Apel, K. and Bohlmann, H. 1998. Differential induction of the *Arabidopsis thaliana* Thi2.1 gene by *Fusarium oxysporum* f. sp. *matthiolae*. *Mol. Plant-Microbe Interact.* 11:523–239.
- Fernandez de Caley, R., Gonzalez-Pascual, B., Garcia-Olmedo, F. and Carbonero, P. 1972. Susceptibility of phytopathogenic bacteria to wheat purothionins *in vitro*. *Appl. Microbiol.* 23:998–1000.
- Glick, B. R. 1995. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* 41:109–117.
- Glick, B. R., Penrose, D. M. and Li, J. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *J. Theor. Biol.* 190:63–68.
- Hajgaard, J., Jacobsen, S. and Svendsen, I. 1991. Two antifungal thaumatinlike proteins from barley grain. *FEBS Lett.* 291:127–131.
- Han, S. H., Lee, S. J., Moon, J. H., Park, K. H., Yang, K. Y., Cho, B. H., Kim, K. Y., Kim, Y. W., Lee, M. C., Anderson, A. J. and Kim, Y. C. 2006. GacS-dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *tabaci* in tobacco. *Mol. Plant-Microbe Interact.* 19:924–930.
- Hong, J. K. and Hwang, B. K. 2006. Promoter activation of pepper class II basic chitinase gene, *CACi2*, and enhanced bacterial disease resistance and osmotic stress tolerance in the *CACi2*-overexpressing *Arabidopsis*. *Planta* 22:433–448.
- Hong, J. K., Lee, S. C. and Hwang, B. K. 2005. Activation of pepper basic PR-1 gene promoter during defense signaling to pathogen, abiotic and environmental stresses. *Gene* 356:169–180.
- Hontzeas, N., Saleh, S. S. and Glick, B. R. 2004. Changes in gene expression in canola roots induced by ACC-deaminase-containing plant-growth-promoting bacteria. *Mol. Plant-Microbe Interact.* 17:865–871.
- Irizarry, R. A. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4:249–264.
- Kang, B. R., Yang, K. Y., Cho, B. H., Han, T. H., Kim, I. S., Lee, M. C., Anderson, A. J. and Kim, Y. C. 2006. Production of indole-3-acetic acid in the plant beneficial strain *Pseudomonas chlororaphis* O6 is negatively regulated by the global sensor kinase GacS. *Curr. Microbiol.* 52:473–476.
- Kim, M. S., Kim, Y. C. and Cho, B. H. 2004. Gene expression analysis in cucumber leaves primed by root colonization with *Pseudomonas chlororaphis* O6 upon challenge-inoculation with *Corynespora cassiicola*. *Plant Biol.* 6:105–108.
- Kim, Y. C., Glick, B. R., Bashan, Y. and Ryu, C. M. 2012. Enhancement of plant drought tolerance by microbes. In: *Plant responses to drought stress: From Morphological to molecular feature*. Aroca, R. (ed), Springer-Verlag, Berlin Heidelberg, in press.
- Kim, Y. C., Leveau, J., McSpadden Gardener, B. B., Pierson, E. A., Pierson III, L. S. and Ryu, C. M. 2011. The factorial basis for plant health promotion by plant-associated bacteria. *Appl. Environ. Microbiol.* 77:1548–1555.
- Kloepper, J. W., Leong, J., Teintze, M. and Schroth, M. N. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* 286:885–886.
- Kloepper, J. W., Ryu, C. M. and Zhang, S. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus*

- spp. *Phytopathology* 94:1259–1266.
- Mayak, S., Tirosh, T. and Glick, B. R. 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.* 42:565–572.
- Pierpoint, W. S., Jackson, P. J. and Evans, R. M. 1990. The presence of a thaumatin-like protein, a chitinase and a glucanase among the pathogenesis-related proteins of potato (*Solanum tuberosum*). *Physiol. Mol. Plant Pathol.* 36:325–338.
- Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V. and Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Prot.* 20:1–11.
- Ryu, C. M., Kang, B. R., Han, S. H., Cho, S. M., Kloepper, J. W., Anderson, A. J. and Kim, Y. C. 2007. Tobacco cultivars vary in induction of systemic resistance against *Cucumber mosaic virus* and growth promotion by *Pseudomonas chlororaphis* O6 and its *gacS* mutant. *Eur. J. Plant Pathol.* 119:383–390.
- Schardl, C. L., Leuchtmann, A. and Spiering, M. J. 2004. Symbioses of grasses with seedborne fungal endophytes. *Annu. Rev. Plant Biol.* 55:315–340.
- Shinozaki, K. and Yamaguchi-Schinozaki, K. 1997. Gene expression and signal transduction in water-stress response. *Plant Physiol.* 115:327–334.
- Spencer, M., Ryu, C. M., Yang, K. Y., Kim, Y. C., Kloepper, J. W. and Anderson, A. J. 2003. Induced defence in tobacco by *Pseudomonas chlororaphis* strain O6 involves at least the ethylene pathway. *Physiol. Mol. Plant Pathol.* 63:27–34.
- Timmusk, S. and Wagner, E. G. H. 1999. The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. *Mol. Plant-Microbe Interact.* 12:951–959.
- Van Loon, L. C., Bakker, P. A. H. M. and Pieterse, C. M. J. 1998. Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol.* 36:453–483.
- Van Loon, L. C. and Glick, B. R. 2004. Increased plant fitness by rhizobacteria. In: *Molecular ecotoxicology of plants*, Sander-mann, H. (ed), Springer-Verlag, Berlin Heidelberg, pp 177–205.
- Verhagen, B. W., Glazebrook, J., Zhu, T., Chang, H. S., van Loon, L. C. and Pieterse, C. M. J. 2004. The transcriptome of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Mol. Plant-Microbe Interact.* 17:895–908.
- Vigers, J. A., Roberts, W. K. and Selitrennikoff, C. P. 1992. A new family of plant antifungal proteins. *Mol. Plant-Microbe Interact.* 4:315–323.
- Waller, F., Archatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Huckelhover, R., Neumann, C., von Wettstein, D., Franken, P. and Kogel, K.-H. 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc. Natl. Acad. Sci. USA* 102:13386–13391.
- Wang, Y., Ohara, Y., Nakayashiki, H., Tosa, Y. and Mayama, S. 2005. Microarray analysis of the gene expression profile induced by the endophytic plant growth-promoting rhizobacteria, *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. *Mol. Plant-Microbe Interact.* 18:385–396.
- Wittstock, U. and Halkier, B. A. 2002. Glucosinolate research in the *Arabidopsis* era. *Trends Plant Sci.* 7:263–270.