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Transcript profiling of oilseed rape (*Brassica napus*) primed for biocontrol differentiate genes involved in microbial interactions with beneficial *Bacillus amyloliquefaciens* from pathogenic *Botrytis cinerea*

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Abstract Many microorganisms interact with plants but information is insufficient concerning requirements for plant colonization and if interactions become beneficial or detrimental. Pretreatment of oilseed rape (*Brassica napus*) with Bacillus results in disease suppression upon challenge with pathogens. We have studied transcriptome effects on oilseed rape primed with the *Bacillus amyloliquefaciens* 5113 biocontrol strain and compared that with effects of the fungal pathogen *Botrytis cinerea*. Using the cDNA-AFLP technique 21,700 transcript fragments were obtained of which 120 were differentially expressed and verified by northern blot analysis for selected transcripts. Priming with Bacillus caused greater effect on leaf than root transcripts where sequencing and BLAST analysis suggested many of the transcripts to be involved in metabolism and bioenergy. Bacillus and Botrytis treatment also changed metabolic gene expression in addition to signaling and transcription control genes as well as a potential disease resistance (TIR-NBS-LRR) gene. The pathogen provoked non-primed plant profile was less dominated by metabolism than Bacillus and Bacillus–Botrytis treated plants. Several transcripts were homologues to unknown genes in the different treatments. Altogether Bacillus treatment of roots cause a systemic gene expression in leaves suggested to result in a metabolic reprogramming as a major event during priming.

Keywords Plant priming · *Bacillus amyloliquefaciens* · *Brassica napus* · Plant growth promotion

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Abbreviations

hpi	Hours post inoculation
ISR	Induced systemic resistance
JA	Jasmonic acid
LPS	Lipopolysaccharides
PGPR	Plant Growth Promoting Rhizobacteria
SA	Salicylic acid
SAR	Systemic acquired resistance
TDF	Transcript derived fragment

Introduction

Soil is a complex habitat containing a multitude of microorganisms that can interact with plants. Symbiotic interactions include microorganisms spanning from pathogens to mutualists that colonize the plants as endophytes or epiphytes. Rhizobacteria colonizing the root system of plants is an example of intricate and important interactions in the rhizosphere (Denison and Kiers 2004). Colonization of plants by microorganisms is complex and seems to be species specific. The microcosm present in the rhizosphere not only differs because of soil conditions but also depends on earlier and present plants (Garbeva et al. 2004). Certain bacteria of the *Bacillus*, *Pseudomonas* and *Serratia* families (Lucy et al. 2004) can improve plant growth. The Plant Growth Promoting Rhizobacteria (PGPR) effect can be due to e.g. production of plant hormones or by increasing the amount of minerals and nitrogen available for the plant (Bloemberg and Lugtenberg 2001).

Microorganisms can also confer disease suppression to plants by different mechanisms. Bacteria living in vascular tissue of plants, in the rhizosphere or phyllosphere are in an appropriate position to protect the plant from deleterious organisms. Competition for growth space and nutrients by

the beneficial bacteria can indirectly protect the plant from harmful microorganisms (Idriss et al. 2002). Bacteria can produce antibiotics towards different fungi and bacteria (Raaijmakers et al. 2002). Alteration of the plant cell wall by certain bacteria causes an increased protection to pathogens (Benhamou et al. 1996; Walker et al. 2004). Bacteria can make the plant more tolerant to pathogens by stimulating defense as systemic acquired resistance (SAR) (Bostock 2005). *Streptomyces*, *Pseudomonas* and *Bacillus* have been shown to induce another plant defense system called induced systemic resistance (ISR). ISR is effective against many pathogens and has been shown to be operational many weeks after induction. In contrast to SAR, this defense system depends on jasmonic acid (JA) and ethylene (van Loon et al. 1998). SAR is dependent on salicylic acid (SA) and involves the production of pathogenesis related (PR) proteins not found in ISR. These systems both converge at the downstream control gene *NPR1* (Bostock 2005) although alternative pathways exist (Ryu et al. 2004b). Rhizobacteria seem to prepare the response to pathogens by priming the plant to respond more rapidly to a pathogen rather than activating a constitutive defense. Genes important for resistance are induced faster and/or stronger than in unprimed control plants when attacked by a pathogen. A recent study of plant resource allocation has shown that priming has a lower fitness cost than a constitutive active defense even when under pathogen pressure (van Hulten et al. 2006).

The genus *Bacillus* is characterized by rod shaped, facultative aerobe, endospore forming bacteria that live in soil and often colonize the plant rhizosphere. Several *Bacillus* spp. produce antibiotics and some *Bacillus* strains, mainly *B. subtilis*, *B. cereus* and *B. amyloliquefaciens*, are known to mediate protection against pathogens on plants (Kloepper et al. 2004) and several commercial preparations are available (Schisler et al. 2004). *B. amyloliquefaciens* UCMB-5113 is a red pigmented strain originally isolated from soil, which is able to colonize oilseed rape (*Brassica napus*) (Reva et al. 2004) and provide protection against the fungal phytopathogens *Alternaria brassicae*, *Botrytis cinerea*, *Leptosphaeria maculans* and *Verticillium longisporum* (Danielsson et al. 2007).

The aim of this study was to evaluate the effects of treatment of oilseed rape with Bacillus UCMB-5113 on the Brassica transcriptome to improve our understanding of how a biocontrol bacterium mediates disease suppression in *B. napus* to fungal pathogens such as Botrytis. This information will be used as a basis to address how plants allow co-existence with certain microorganisms without deploying a defense program as for pathogens and also the relationship between belowground and aboveground responses in defense priming. For transcript fingerprinting we used the cDNA-AFLP technique, which is sensitive, robust and has high resolution. We have earlier established this technique for *B. napus* to analyze effects of wounding (Sarosh and Meijer 2007).

Materials and methods

Plant material

Oilseed rape (*B. napus*, cv. Westar) seeds were surface sterilized for 20 min in 20% sodium hypochlorite followed by a brief rinse with 50% methanol before planting in sterile soil into 11 × 11 × 5 cm pots. The pots were grown in controlled environment using a 16/8 h photoperiod with light of 200 µmol m^{-2} s^{-2} at 22/18°C. The pots received a specific amount of water to exclude differences between treatments.

Bacteria

Bacillus UCMB-5113 was grown in LB media at 28°C with agitation for 3 days to allow for production of spores. The bacterial cultures were heat treated at 75°C for 10 min to select for *Bacillus* spores and to kill possible contaminants. After centrifugation, spores were washed in sterile water and the concentration was determined by viable count analysis on LB plates and the stock solution was kept refrigerated until use.

Pathogen

B. cinerea (strain 30158) was grown on PDA plates (16/8 h photoperiod at 21/16°C), for a month or until spores had been produced. The spores were harvested and filtered through miracloth. The concentration of spores was measured with a Bürkner chamber and adjusted to 10^7 spores ml^{-1}. The spore solution was stored at 4°C until use.

Biocontrol bacterial treatment

Plants were left to dry for 2 days before application of *Bacillus* spore solution (10^7 spores ml^{-1}) by adding 100 ml to each pot 7 days before infection so that only soil but no aboveground parts received any bacterial spores.

Pathogen inoculation and sampling

Plants that had a similar size (five true leaves) were chosen from each treatment. Ten µl of *B. cinerea* solution was drop inoculated on the first leaf of each plant. The inoculated leaf was monitored for local effects while the non-inoculated second leaf was used to analyse systemic effects. Plants were kept in mini greenhouses 12 h before

and 12 h after inoculation to maintain high humidity and facilitate infection. Plants were scored at 72 h as (1) uninfected; (2) <1\2 of the leaves infected; (3) >1\2 of the leaves infected; or (4) dead plants. Roots or leaves (local and systemic harvested separately where relevant) from three plants were pooled together and collected at 0, 12, 24 h and 3 d after infection. Water treated non-primed plants with or without pathogen inoculation were collected at the same intervals.

RNA isolation and cDNA-AFLP analysis

Total RNA was isolated from frozen leaf tissue (Chomczynski and Sacchi 1987) and amount and purity assessed by absorbance at 260 nm or by the A_{260}/A_{280} ratio using a Nanodrop spectrophotometer. cDNA-AFLP analysis was carried out on two biological replicates. cDNA was synthesized using the mRNA Capture Kit (Roche Applied Science, Germany). The subsequent cDNA-AFLP analysis was performed as described by Breyne et al. (2002). mRNA was converted to cDNA from 5 µg of total RNA using a biotinylated oligo-dT primer in streptavidin-coated PCR tubes. The purified cDNA templates were first digested with *Bst*YI restriction enzyme (New England Biolabs, Beverly, MA, USA). Subsequently, the 3′-ends of the cDNAs were captured with streptavidin-coated PCR tubes. Digestion with the second enzyme *Mse*I (New England Biolabs), released the transcript tags. Pre-amplification was performed by a *Mse*I primer without a selective nucleotide combined with a *Bst*YI primer containing either T or C at the 3′ end. The pre-amplification reaction was carried out using 20 cycles (94°C for 30 s; 56°C for 1 min; 72°C for 1 min). The amplified reaction was diluted 600-fold and 5 µl was used for final selective amplification using a touchdown amplification programme (Vos et al. (1995). *Bst*T and *Mse*I primers and *Bst*C and *Mse*I primers with one selective nucleotide, respectively, were used for the cDNA-AFLP analysis and all 16 primer combinations were performed. Selective [^{33}P]-ATP labeled amplification products were separated on a 6% polyacrylamide gel run at constant power (100 W) until 4,300 Vh was reached. Gels were dried before scanning with a Phosphor Imager (Bio-Rad) and exposure to Kodak Biomax film for 3 d.

Isolation, quantification and sequencing of transcript derived fragments

Gel profiles were quantified using Quantity One software (Bio-Rad). Lane-based background subtraction was carried out and the bands were then normalized to compensate for differences in any loading effects in the different lanes. The intensity of each band was quantified using volume units (intensity units × mm^2).

Bands were excised from the gel and boiled in water for 5 min. The DNA was precipitated and reamplified using the *Bst*YI(T)-0, *Bst*YI(C)-0 and *Mse*I-0 primers. For PCR, the same reaction conditions as in the preamplification were used.

The reamplified products were cloned into pbluescript SK+ vector and sequenced using the M13 F/R primers. Sequencing of the transcript derived fragments (TDFs) was carried out at Macrogen Inc., Korea. Database searches were performed using the BLASTN and BLASTX programs (Altschul et al. 1997) at NCBI, EMBL and TAIR. Only the best hit is presented in Tables 1, 2, 3, 4. Sequence data from this article can be found in the GenBank/EMBL data bases under accession numbers GH70926263778115–GH70930963778162.

Use of the Genevestigator software revealed the response profiles of genes to different stimuli or genes that respond to selected factors (Zimmermann et al. 2005).

Northern blot analysis

Northern analysis was carried out on the same RNA preparation used in the cDNA-AFLP analysis and repeated with two biological replicates. Ten µg of total RNA was fractionated on a 1.2% denaturing formaldehyde agarose gel and transferred onto Hybond N+ membranes (Amersham, UK) as described (Sambrook et al. 1989). cDNA clones were isolated after agarose gel electrophoresis of restriction digested plasmids. The *PR-1a* and *PDF1.2* genes were used as probes (Uknes et al. 1992; Penninckx et al. 1996). The probes were labeled with [α-^{32}P]-dATP using Rediprime II Random Prime Labelling System (Amersham Biosciences, Sweden). Prehybridization and hybridization were performed in 50% formamide at 42°C (Sambrook et al. 1989). After hybridization, the membranes were washed at 42°C in 0.5 × SSC, 0.1% SDS. A Phosphor Imager (Bio-Rad) was used for imaging and quantification.

Results

Partial transcriptome analysis of *Brassica napus* primed with *Bacillus* sp.

Application of *B. amyloliquefaciens* strain UCMB-5113 to oilseed rape plants indeed conferred protection to Botrytis (Fig. 1). Phenotypic evidence for disease suppression provided the basis for molecular studies of the underlying processes. Plants were collected and scored after 3 days and the disease suppression was easily discerned. Use of UCMB 5113, as well as several other closely related strains (Danielsson et al. 2007), result in approximately 40% decrease of disease symptoms 1 week after inoculation with Botrytis.

Table 1 TDF homologues expressed in *B. napus* roots 24 h after Bacillus treatment

No.	cDNA-AFLP fragment	Size (bp)	Homology[a]	GenBank homolog[b]	Blast score *E*-value[c]	Genevestigator[d]
1.	BnBacR15A (+)[e]	188	Expressed protein	AT4G04330	3e-09	Hormone: BL/H_3BO_3, ABA
2.	BnBacR22A (+)	237	GLN1;4 glutamine synthetase	AT5G16570	3e-09	BL/H_3BO_3, SA, *P. syringae*
3.	BnBacR15B (+)	128	Expressed protein	AT1G32290	3e-09	Norflurazon, SA
4.	BnBacR08 (+)	184	Thiol methyltransferase	AT2G43940	2e-06	*P. infestans*, ethylene
5.	BnBacR13B (+)	120	Meprin and TRAF homology domain-containing protein	AT2G42470	1e-05	–
6.	BnBacR18A (+)	106	Expressed protein	AT1G16630	6e-12	–
7.	BnBacR20B (+)	173	Glycine-rich protein	AT5G47020	1e-04	Syringolin, MJ
8.	BnBacR28B (+)	159	Expressed protein	AT1G74950	4e-16	MJ, Wounding, *Pseudomonas syringae* pv. tomato DC3000
9.	BnBacR13A (−)	120	Expressed protein, contains Pfam profile	AT5G25590	6e-05	H_2O_2, BL H_3BO_3, ethylene, MJ
10.	BnBacR6B (+)	160	Protein kinase	AT3G22750	1e-06	Ethylene, *P. syringae*, wounding
11.	BnBacR5B (−)	173	Histidinol dehydrogenase, putative HDH	AT5G63890	1e-05	Norflurazon
12.	BnBacR1C (+)	156	NAC transcription factor	AT3G15500	2e-10	*P. syringae*, PCD
13.	BnBacR2C (+)	210	GTP binding protein	AT5G03520	4e-12	BL
14.	BnBacR3C (+)	189	MYB transcription factor	AT5G02320	1e-10	
15.	BnBacR4C (+)	140	ATMYB 30	AT3G28910	5e-10	Nematode, H_2O_2
16.	BnBacR6C (+)	155	bZIP Transcription factor	AT5G06840	4e-06	
17.	BnBacR7C (−)	110	Glycosyl transferase	AT2G43820	2e-10	Nematode, ethylene
18.	BnBacR8C (+)	188	GTPase family	AT5G20010	3e-12	*P. syringae*, glucose
19.	BnBacR9C (−)	80	Chlorophyll A-B protein	AT5G01530	5e-10	Sucrose, *P. syringae*
20.	BnBacR10C (−)	133	Amino acid permease	AT5G49630	6e-12	BL/H_3BO_3
21.	BnBacR11C (+)	192	AP2 transcription factor	AT5G25810	8e-10	Light, BL

[a] Homolog of best hit from BLAST search using the TDF is provided

[b] The gene identifier for the best hit is provided based on the BLAST search

[c] The *E*-score from the best hit of the BLAST analysis is provided

[d] Factors affecting expression of the Arabidopsis homologue is indicated based on Genevestigator

[e] The effect on the TDF relative expression level is stated as + (up-regulated) or − (down-regulated)

This reduction was observed even during optimal conditions for the fungus, i.e., enclosure in mini-greenhouses providing high humidity as well as a very high inoculation dose. A detailed transcript profiling of *B. napus* roots in response to priming by *B. amyloliquefaciens* strain UCMB-5113 was carried out using the cDNA-AFLP technique. Roots and leaves from three plants were collected at 0, 12, 24 h and 3 d after priming with UCMB-5113. Water treated control plants were collected at the same intervals. RNA was extracted from three biological replicates and cDNA-AFLP profiles were obtained using 16 primer combinations. To identify genes involved during priming, TDFs resulting for the four time intervals using 16 primer combinations were analyzed. Bands were scored based on presence/absence and intensity of bands and quantified. A representative cDNA-AFLP autoradiogram developed due to priming in roots is shown in Fig. 2. Approximately 3,000 fragments were quantified with band sizes ranging from approximately 50–550 bp. Based on the quantification data, 27 TDFs in roots were selected that showed a two to three-fold difference compared to the control. Of these, 22 were up-regulated after priming and the remaining five fragments were found to be down-regulated. In total, 21 TDFs from roots were successfully cloned and sequenced.

BLAST analysis of the sequenced TDFs resulted in identification of 16 homologues to known genes in the database and five TDFs corresponding to expressed proteins without any assigned function in *Arabidopsis thaliana* (Table 1). Of the 21 TDFs, seven were annotated to be involved in metabolism, two in signal transduction, two likely to be involved in energy generation and four transcription factors (Fig. 3A). A significant up-regulation of

Table 2 TDF homologues expressed in *B. napus* leaves 24 h after Bacillus treatment

No.	cDNA-AFLP fragment	Size (bp)	Homology[a]	GenBank homolog[b]	Blast score *E*-value[c]	Genevestigator[d]
1.	BnBacL16A (+)[e]	142	PSBO 1, Encodes a protein which is an extrinsic subunit of photosystem II	AT5G66570	9e-05	Hormone: BL/H_3BO_3
2.	BnBacL18B (−)	132	GDSL-motif lipase/hydrolase family protein	AT4G28780	2e-04	Ethylene
3.	BnBacL19A (+)	215	Glycine-rich protein	AT5G46730	2e-07	*P. syringae*, drought
4.	BnBacL01(+)	247	Similar to protein kinase family protein	AT2G05060	9e-39	–
5.	BnBacL30A (+)	113	Glycosyl hydrolase family 3 protein, beta-D-glucan exohydrolase	AT3G47050	1e-04	SA, Nitrate low
6.	BnBacL5A (+)	89	Expressed protein	AT2G27230	3e-04	MJ, SA
7.	BnBacL6A (+)	74	Protein kinase, putative, similar to protein kinase AKIN betagamma-2	AT1G09020	8e-04	Hormone: BL/H_3BO_3
8.	BnBacL6B (+)	75	Expressed protein	AT5G48120	2e-04	Syringolin, ethylene
9.	BnBacL7B(−)	90	Chloroplast ribosomal protein L2	ATCG01310	8e-26	6-Benzyladenine, hydrogen peroxide
10.	BnBacL8B (+)	145	Endo-1,4-beta-glucanase	AT1G64390	4e-04	Glucose, sucrose
11.	BnBacL 21A (−)	228	Chlorophyll A-B binding protein	AT3G54890	2e-13	Light, 6-benzyl adenine
12.	BnBacL05 (+)	164	Multidrug resistance P-glycoprotein	AT1G74220	6e-05	SA, MJ, wounding
13.	BnBacL04 (+)	150	Malate dehydrogenase	AT5G58330	8e-14	Light, BL H_3BO_3
14.	BnBacL03 (−)	224	Methyltransferase family protein	AT1G69523	3e-10	Syringolin, *B. cinerea*, *P. syringae*, wounding, SA
15.	BnBacL23A (+)	127	PSBO2 encodes an extrinsic subunit of photosystem II	AT3G50820	0.13	BL/H_3BO_3
16.	BnBacL24A (−)	119	Expressed protein	AT4G29590	7e-04	Light, nematode
17.	BnBacL24B (+)	120	Phosphoethanolamine *N*-methyltransferase 3, (NMT3)	AT1G73600	1e-10	Glucose, SA
18.	BnBacL27A (+)	191	bZIP Transcription factor family protein	AT2G18160	1e-18	Brassinolide H_3BO_3
19.	BnBacL30A (+)	113	Root hair defective 3 GTP-binding (RHD3)	AT5G45160	5e-10	Syringolin
20.	BnBacL5B (+)	173	Histidinol dehydrogenase, putative/HDH	AT5G63890	1e-05	Norflurazon
21.	BnBacL5D (+)	205	Protein kinase, putative (MRK1)	AT3G63260	2e-06	Syringolin, H_2O_2, *P. syringae*
22.	BnBacL6C (+)	171	Protein kinase	AT3G22750	1e-09	Ethylene, *P. syringae*, wounding
23.	BnBacL20A (+)	149	mRNA-binding protein	AT3G63140	3e-17	Light, BL/H_3BO_3
24.	BnBacL13A (+)	72	Photosystem I reaction center subunit II, chloroplast, putative	AT4G02770	4e-10	Light, BL/H_3BO_3
25.	BnBacL13B (+)	61	Homeobox-leucine zipper transcription factor (HB-9), identical to HD-Zip protein	AT1G30490	2e-09	BL/H_3BO_3, SA
26.	BnBacL1D (+)	177	AAP1 (Amino acid permease 1)	AT1G58360	1e-07	ABA, *B. cinerea*
27.	BnBacL2D (−)	203	Kelch repeat containing protein	AT1g08420	4e-10	SA, nematode
28.	BnBacL3D (+)	124	Serine carboxypeptidase	AT3G10410	3e-10	ABA, osmotic
29.	BnBacL4D (−)	125	Isocitrate dehydrogenase	AT2G17130	1e-05	BL, ABA
30.	BnBacL5D (−)	100	Unknown protein	AT5G12050	5e-09	IAA, BL
31.	BnBacL8D (+)	125	ATCUL3A	AT1G26830	3e-09	Syringolin, *P. syringae*
32.	BnBacL9D (+)	248	Peroxidase	AT4G11290	4e-10	*P. infestans*, BL, sucrose
33.	BnBacL10D (+)	180	ASK19	AT2G03160	1e-09	BL, MJ
34.	BnBacL11D (+)	210	MYC2 bHLH protein	AT1G32640	4e-12	MJ
35.	BnBacL12D (+)	189	MYB transcription factor	AT5G02320	1e-07	–

[a] Homolog of best hit from BLAST search using the TDF is provided

[b] The gene identifier for the best hit is provided based on the BLAST search

[c] The *E*-score from the best hit of the BLAST analysis is provided

[d] Factors affecting expression of the Arabidopsis homologue is indicated based on Genevestigator

[e] The effect on the TDF relative expression level is stated as + (up-regulated) or − (down-regulated)

Table 3 TDF homologues expressed in Bacillus treated *B. napus* leaves at 24 h after challenge inoculation with *B. cinerea*

No.	cDNA-AFLP fragment	Size (bp)	Homology[a]	GenBank homolog[b]	Blast Score *E*-value[c]	Genevestigator[d]
1.	BnBacBc21B (+)[e]	465	Serine/threonine protein kinase	AT2G05060	3e-38	–
2.	BnBacBc03A (−)	236	Methyltransferase family protein	AT1G69523	4e-10	Syringolin, *B. cinerea*, *P. syringae*, wounding, SA
3.	BnBacBc04 (+)	155	Malate dehydrogenase	AT5G58330	9e-14	Light, BL H_3BO_3
4.	BnBacBc06 (+)	180	Ulp1 protease family protein	AT1G35770		Zearalenone, SA
5.	BnBacBc09 (+)	129	Expressed protein tropomyosin-related	AT5G48160	2e-04	
6.	BnBacBc10 (+)	130	F-box family protein (FBL3), contains similarity to leucine-rich repeats	AT5G01720	2e-05	Cycloheximide
7.	BnBacBc12 (+)	190	WRKY family transcription factor	AT1G80840	7e-14	Cycloheximide, H_2O_2, MJ
8.	BnBacBc13 (−)	194	LHCA1 chlorophyll A-B binding protein/LHCI type I (CAB)	AT3G54890	2e-06	Light, 6-benzyl adenine
9.	BnBacBc14 (+)	206	Clathrin adaptor complex	AT5G46630	2e-09	Syringolin, SA
10.	BnBacBc15 (+)	103	Disease resistance protein (TIR-NBS-LRR class)	AT1G64070	3e-04	Brassinolide, MJ
11.	BnBacBc17B (+)	138	PSBO 1, encodes an extrinsic subunit of photosystem II	AT5G66570	6e-08	Hormone: BL/H_3BO_3
12.	BnBacBc21C (−)	465	Chlorophyll A-B binding protein LHCB2:4, nearly identical to Lhcb2 protein	AT3G27690	5e-41	Hormone: BL/H_3BO_3, light
13.	BnBacBc19A (+)	210	Glycine-rich protein	AT5G46730	2e-05	Nematode, BL/H_3BO_3
14.	BnBacBc6A (+)	78	Protein kinase, putative, similar to protein kinase AKIN betagamma-2	AT1G09020	7e-05	BL/H_3BO_3, Syringolin
15.	BnBacBc8B (+)	145	Endo-1,4-beta-glucanase	AT1G64390	4e-04	Glucose, sucrose
16.	BnBacBc13A (−)	115	Expressed protein, contains Pfam profile	AT5G25590	5e-05	Aminoethoxyvinylglycine (ethylene inhibitor)
17.	BnBacBc25A (+)	166	Lactoylglutathione lyase family protein/glyoxalase I family protein	AT5G57040	6e-05	Abscisic acid
18.	BnBacBc25B (+)	229	Expressed protein	AT5G14370	4e-06	H_2O_2, MJ
19.	BnBacBc26A (−)	165	UbiE/COQ5 methyltransferase family protein	AT1G69526	6e-13	Ethylene
20.	BnBacBc29B (+)	138	Expressed protein	AT1G68380	3e-12	MJ, *B. cinerea*
21.	BnBacBc6C (+)	154	Protein kinase, putative (MRK1)	AT3G63260	1e-06	Syringolin, H_2O_2, *P. syringae*
22.	BnBacBc6D (+)	134	Expressed protein	AT5G05950	3e-09	6-Benzyl adenine, syringolin
23.	BnBacBc15A (+)	79	MYC2 basic helix-loop-helix (bHLH) protein (RAP-1)	AT1G32640	4e-12	MJ, *B. cinerea*, wounding
24.	BnBacBc18B (+)	151	Plastocyanin	AT1G20340	2e-20	Light, 6-benzyl adenine

[a] Homolog of best hit from BLAST search using the TDF is provided

[b] The gene identifier for the best hit is provided based on the BLAST search

[c] The *E*-score from the best hit of the BLAST analysis is provided

[d] Factors affecting expression of the Arabidopsis homologue is indicated based on Genevestigator

[e] The effect on the TDF relative expression level is stated as + (up-regulated) or − (down-regulated)

TDFs corresponding to metabolism and energy production was observed. Genevestigator program was used to correlate the role of Arabidopsis orthologs response during different stress challenges (Table 1). Genevestigator analysis of the BnBacR28B (expressed protein recently denoted as JAZ2), BnBacR20B (glycine-rich protein) and BnBacR13A (expressed protein) orthologs in Arabidopsis showed high induction by methyl jasmonate (MJ). Some TDFs were ethylene responsive like BnBacR08 (thiomethyltransferase), BnBacR6B (protein kinase) and BnBacR13A (expressed protein). Two TDFs, BnBacR15A (expressed protein) and BnBacR22A (glutamine synthetase) were induced by brassinosteroids.

Systemic gene expression in leaves after priming

Root treatment of *B. napus* with Bacillus strain UCMB-5113 resulted in a systemic gene expression in leaves. A representative cDNA-AFLP profile due to Bacillus

Table 4 TDF homologues expressed in *B. napus* leaves at 24 h after challenge inoculation with *Botrytis cinerea*

No.	cDNA-AFLP fragment	Size (bp)	Homology[a]	GenBank homolog[b]	Blast score *E*-value[c]	Genevestigator[d]
1.	BnBc28A (−)[e]	159	Leucine-rich repeat protein kinase, putative, similar to light repressible receptor protein kinase	AT1G51805	1e-18	Cycloheximide (protein synthesis inhibitor); light, SA
2.	BnBc29A (−)	137	Similar to geranylgeranyl transferase alpha subunit-related/RAB geranylgeranyl transferase alpha subunit-related	AT4G24490	3e-12	Naphthylphthalamic acid (auxin transport inhibitor);
3.	BnBc24B (+)	86	MATE efflux family protein	AT4G21900	2e-10	
4.	BnBc21C (+)	89	Expressed protein	AT5G66658	3e-11	
5.	BnBc23A (+)	128	Expressed protein	AT1G32290	3e-09	Norflurazon, SA
6.	BnBc23B (−)	81	Cinnamoyl-CoA reductase family	AT2G02400	2e-12	*B. cinerea*, MJ
7.	BnBc24A (+)	84	Chitinase, putative, similar to basic endochitinase CHB4 precursor SP:Q06209 from *Brassica napus*	AT2G43620	4e-10	
8.	BnBc07 (+)	161	Clathrin heavy chain, putative, similar	AT3G11130	5e-04	Naphthylphthalamic acid; *B. cinerea*
9.	BnBc15B (+)	190	Amino acid permease I (AAP1)	AT1G58360	1e-07	*B. cinerea*, ABA
10.	BnBc14A (−)	196	Acyl-CoA oxidase, putative, strong similarity to acyl-CoA oxidase	AT2G35690	1e-10	N-octyl-3-nitro-2,4,6-trihydroxybenzamide (photosystem II inhibitor); $AgNO_3$ (ethylene inhibitor)

[a] Homolog of best hit from BLAST search using the TDF is provided

[b] The gene identifier for the best hit is provided based on the BLAST search

[c] The *E*-score from the best hit of the BLAST analysis is provided

[d] Factors affecting expression of the Arabidopsis homologue is indicated based on Genevestigator

[e] The effect on the TDF relative expression level is stated as + (up-regulated) or − (down-regulated)

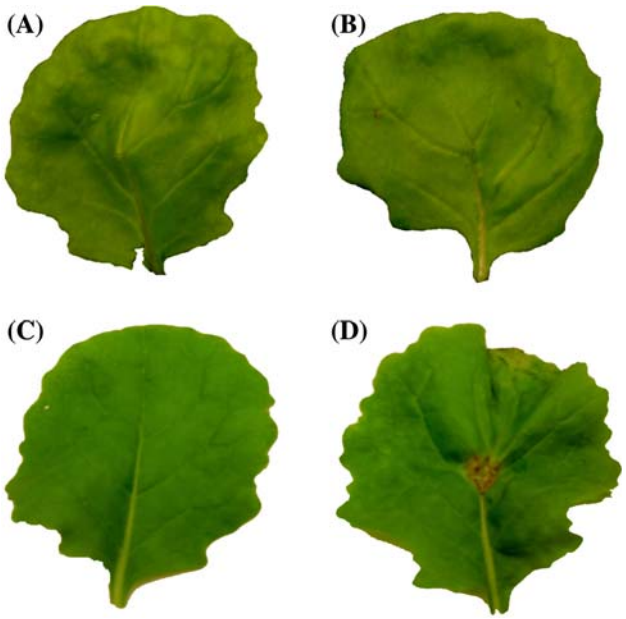

Fig. 1 Effects of Bacillus inoculation on *Botrytis* disease on *B. napus* leaves. *B. napus* leaves were detached from five-leaf stage plants primed with *B. amyloliquefaciens* (**A**), water treated (**B**), primed plants 3 days after inoculation with *B. cinerea* (**C**), and water treated control plants challenge inoculated with *B. cinerea* (**D**)

treatment is shown in Fig. 2. Approximately 6,200 fragments were obtained using 16 primer combinations. In leaves, 41 TDFs corresponded to up-regulated genes and nine corresponded to down-regulated genes. In total, 35 TDFs were cloned and sequenced.

BLAST analysis using the TAIR tBLASTx tool on the differentially expressed TDFs indicated 31 TDFs with high identity to other known genes while unknown genes four TDFs were assigned to genes of expressed proteins without any assigned function in Arabidopsis (Table 2). The TDFs were annotated as: 12 predicted to be involved in metabolism, five in signal transduction, four in intracellular traffic, five in energy generation and four as transcription factors (Fig. 3B). The majority of the TDFs corresponded to genes involved in metabolism indicating a role in enhanced plant nutrition, growth and disease resistance. Differential expression of photosynthetic/chlorophyll genes was observed indicating that resources were being diverted to other metabolic pathways leading to plant nutrition and growth.

Based on the response viewer profiles of the Genevestigator, BnBacL16A (photosystem II), BnBacL04 (malate dehydrogenase), BnBacL23A (photosystem II), BnBacL27A (bZIP transcription factor), BnBacL13A (photosystem I), and

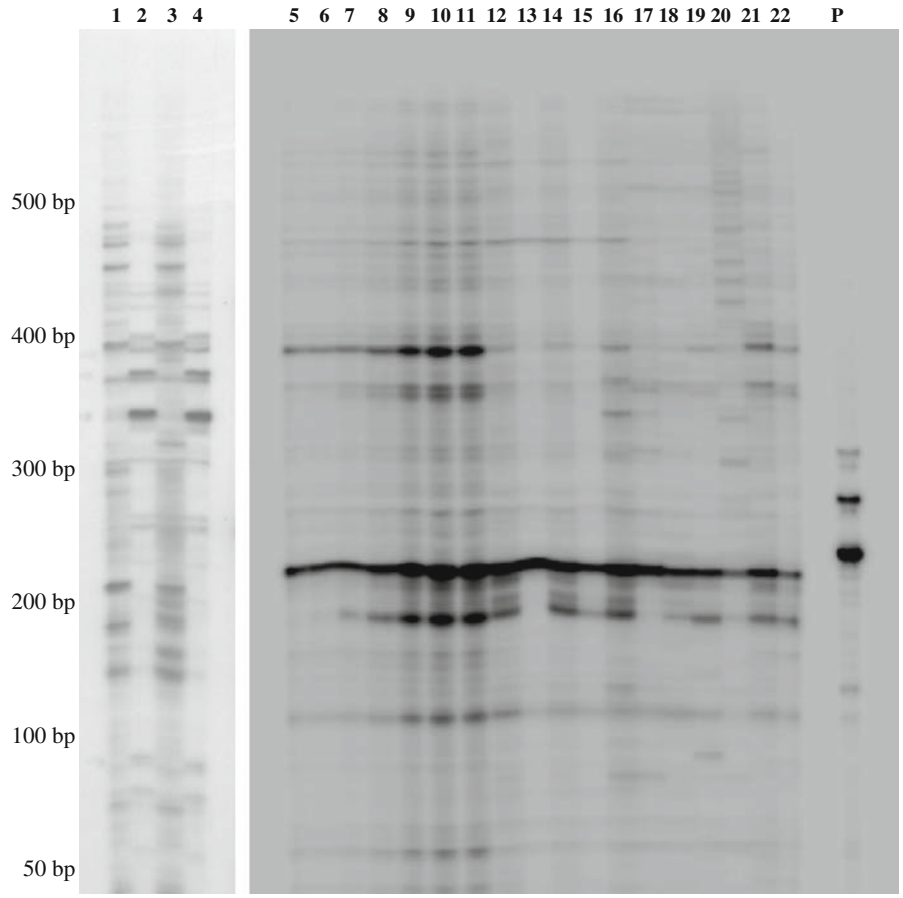

Fig. 2 cDNA-AFLP analysis of *B. napus* roots and leaves after Bacillus treatment and challenge inoculation with Botrytis. cDNA-AFLP fragment profiles displayed after Bacillus treatment of roots of 3-week-old plants and subsequent *B. cinerea* inoculation. The samples correspond to: Lanes 1–4, root samples prior to Botrytis inoculation. 1, 3, Bacillus treated; 2, 4, control (water treated); Lanes 5–10, leaf samples 0 h after treatment; 5, control; 6, Bacillus treated; 7, *B. cinerea* inoculated local leaves; 8, *B. cinerea* inoculated systemic leaves; 9, Bacillus + *B. cinerea* local leaves; 10, Bacillus + *B. cinerea* systemic leaves; Lanes 11–16, leaf samples 24 h after treatment; 11, control; 12, Bacillus treated; 13, *B. cinerea* inoculated local leaves; 14, *B. cinerea* inoculated systemic leaves; 15, Bacillus + *B. cinerea* local leaves; 16, Bacillus + *B. cinerea* systemic leaves; Lanes 17–22, leaf samples 72 h after treatment; 17, control; 18, Bacillus treated; 19, *B. cinerea* inoculated local leaves; 20, *B. cinerea* inoculated systemic leaves; 21, Bacillus + *B. cinerea* local leaves; 22, Bacillus + *B. cinerea* systemic leaves

BnBacL13B (leucine zipper transcription factor) orthologs were induced by brassinosteroids. Some TDFs were found to be elicited after MJ treatment like BnBacL5A (expressed protein) and BnBacL05 (multidrug resistant glycoprotein).

Differential gene expression in Bacillus treated and non-Bacillus primed plants challenged with the pathogen

B. amyloliquefaciens primed and non-primed plants were challenge inoculated with *B. cinerea* on the first leaves. Inoculated first leaves (local) and uninoculated second leaves (systemic) were harvested at 0, 12, 24 and 72 h post inoculation (hpi). RNA was extracted from the three replicates, each comprising three local and systemic leaves from three plants. Using 16 primer combinations the cDNA-AFLP analysis generated 12,500 fragments with an average of 50 fragments per lane (Fig. 2). The responses in local leaves were similar to those in systemic leaves. Quantification showed 24 differentially expressed TDFs in Bacillus treated-challenge inoculated plants. Of these, 19 genes were up-regulated and five genes down-regulated. Of the 10 TDFs identified in water treated-challenge inoculated plants, six were up-regulated and four down-regulated.

Sequence analysis of these TDFs revealed significant similarity to many known genes from GenBank (Table 3). A few unknown genes (expressed proteins) with unassigned function were also identified. Of the 19 TDFs with similarity to other known genes, eight were predicted to be involved in metabolism, four each in signal transduction and energy, one each in intracellular traffic and defense, and two likely to serve as transcription factors (Fig. 3C). Five TDFs (BnBacBc09, BnBacBc13A, BnBacBc25B,

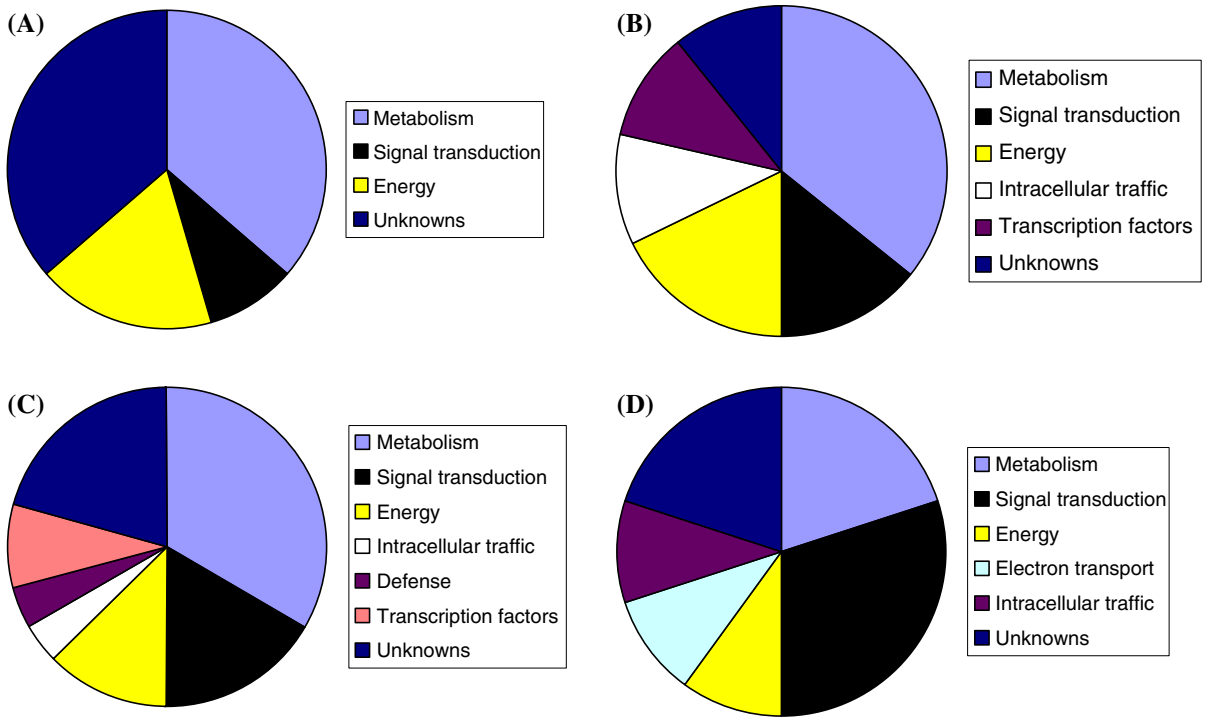

Fig. 3 Relationships among *B. napus* TDFs upon different microbial treatments. *B. napus* TDFs were analyzed from root (**A**) or leaf (**B**) tissues after treatment with Bacillus UCMB-5113; leaf (**C**) from Bacillus primed and Botrytis challenged plants; and leaf (**D**) from Botrytis challenged non-primed plants

BnBacBc29B, BnBacBc6D) were annotated as 'expressed proteins' without any assigned function in Arabidopsis. BnBacBc15 is an TIR-NBS-LRR disease resistance protein ortholog.

The Genevestigator response profile, showed several of the TDFs to be elicited by brassinosteroids. These TDFs included BnBacBc04 (malate dehydrogenase), BnBacBc15 (disease resistance protein), BnBacBc17B (photosystem II), BnBacBc21B (chlorophyll A-B binding protein), BnBacBc19A (glycine rich protein) and BnBacBc6A (putative regulator of SNF1 related protein kinase). A few other TDFs were induced by MJ treatment—BnBacBc12 (WRKY transcription factor), BnBacBc25B (expressed protein), BnBacBc29B (expressed protein) and BnBacBc15A (bHLH protein). BnBacBc06 (Ulp1 protease) and BnBacBc14 (clathrin adaptor) were affected by SA.

Table 4 lists the 10 fragments differentially expressed in the non-primed plants inoculated with *B. cinerea*. The TDFs were assigned into functional categories; metabolism, signal transduction, energy, electron transport and intracellular traffic (Fig. 3D). Half of the TDFs were found to be sharing the metabolic and signal transduction functions. This is unlike the earlier trend, where a majority of the TDFs seemed to play a role in metabolism as observed in Bacillus treated or Bacillus treated-pathogen inoculated plants. Two TDFs (BnBc23A, BnBc21c) of unknown function were also found.

Northern blot analysis of expressed TDFs

To validate the differential expression of TDFs observed from the cDNA-AFLP analysis, selected clones were analyzed by northern blot analysis (Fig. 4). For Bacillus treated roots (Fig. 4A), BnBacR18A (expressed protein) showed strong induction in Bacillus treated roots in comparison with the water treated control at all time intervals tested. Transcript levels of BnBacR28B (expressed protein) were found to be up-regulated after 24 and 72 h of bacterial colonization in the roots. A MYB transcription factor (BnBacR3C) was observed to be strongly expressed at 12 h after priming compared to the control. Expression of a TDF (BnBacR4C) coding for an unknown protein was found to be highly up-regulated at later time point of 24 h after priming and maintained its course up to 72 h. Three TDFs were selected to confirm their systemic expression in leaves after Bacillus treatment (Fig. 4B). BnBacL19A (glycine rich protein), increased at 12 hpi and reached its highest expression at 72 hpi. BnBacL05 (multidrug resistant P-glycoprotein) showed a two-fold induction at 24 hpi compared to control. BnBacL6C (protein kinase) showed a four-fold increase at 12 hpi, indicating a role as an early

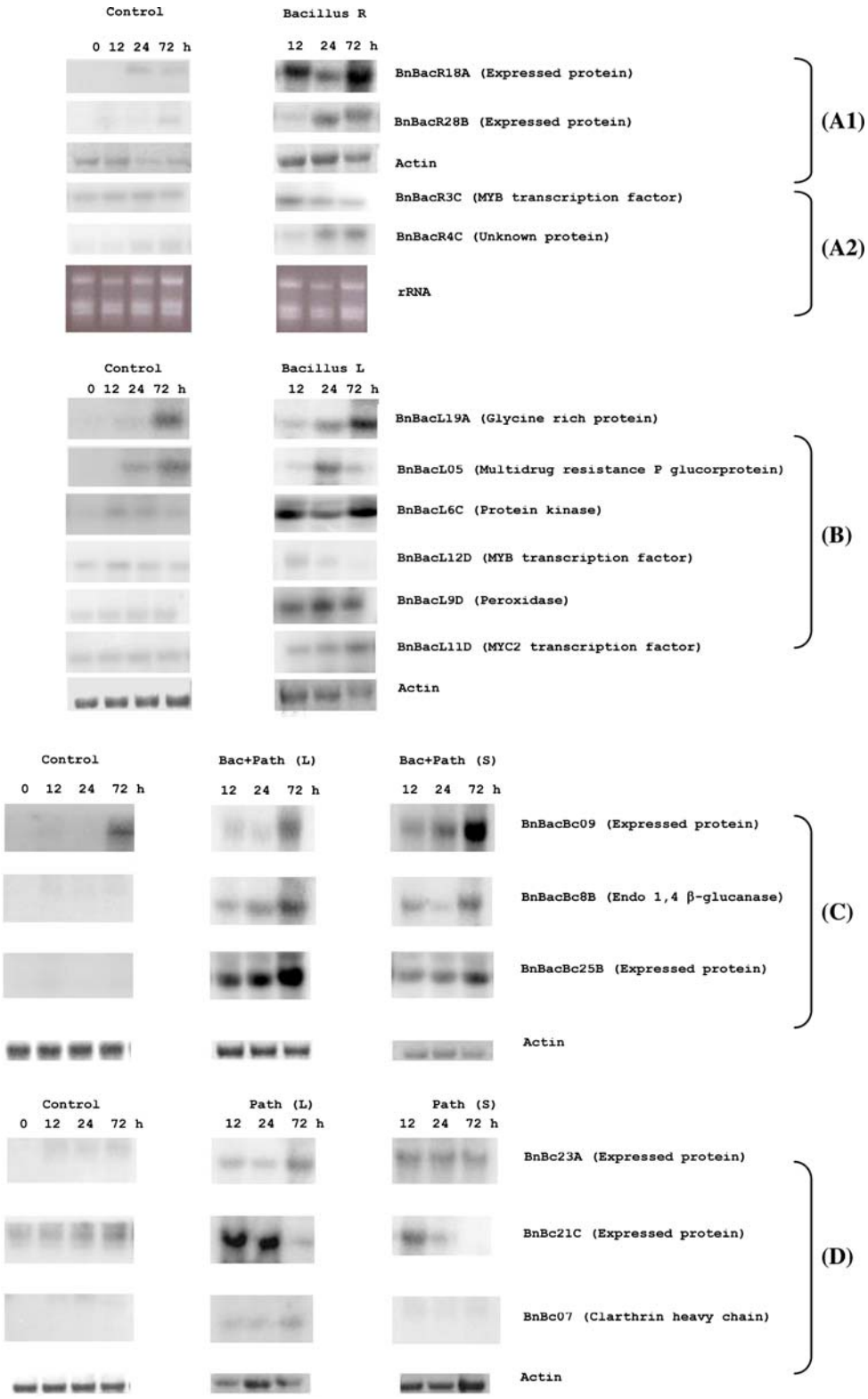

Fig. 4 Northern blot analysis of TDFs expressed in *B. napus* tissues. Total RNA (10 µg) was separated on 1.2% formaldehyde agarose gels and stained with ethidium bromide to verify equal loading and transferred onto Hybond N+ membranes. **A1** and **A2** Root RNA after Bacillus priming was probed with the following TDFs; BnBacR18A (expressed protein) and BnBacR28B (expressed protein). *Left panel* shows control leaf samples, *right panel* shows UCMB-5113 treated root samples. **B** The blots containing *B. napus* leaf RNA after Bacillus priming in roots were probed with TDFs corresponding to; BnBacL19A (glycine-rich protein); BnBacL05 (Multidrug resistance P-glycoprotein) and BnBacL6B (expressed protein). The samples were water treated control (*left*) or UCMB-5113 primed (*right*) leaf samples. **C** The blots containing *B. napus* leaf RNA after Bacillus priming and challenge with *B. cinerea* were probed with TDFs corresponding to; BnBacBc09 (expressed protein); BnBacBc8B (endo-1,4-beta-glucanase) and BnBacBc25B (expressed protein). The samples were control leaves (*left*), challenged local leaves (*middle*) and systemic leaves (*right*). **D** The blots containing *B. napus* leaf RNA after challenge inoculation with *B. cinerea* were probed with TDFs corresponding to; BnBc23A (expressed protein); BnBc21C (expressed protein) and BnBc07 (Clathrin heavy chain). The samples were control leaves (*left*), challenged local leaves (*middle*), and challenged systemic leaves (*right*).

player in priming. BnBacL9D (peroxidase) transcripts was found to be two-fold up-regulated in leaves at 12 h upon treatment and maintain upregulated to 72 hpi. TDF (BnBac5D) an unknown protein was observed to be down regulated at 24 h after priming compared to the control.

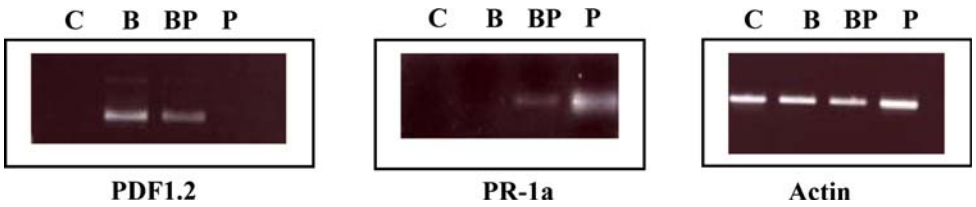

Fig. 5 Northern blot analysis using Arabidopsis probes on *B. napus* leaf RNA. Leaf samples were taken from *B. napus* plants pretreated with Bacillus and 2 days after challenge with Botrytis. The samples correspond to: C, control non-treated plants; B, Bacillus primed plants; BP, Bacillus primed and Botrytis challenged leaves; P, Botrytis challenged leaves. The blots were probed with Arabidopsis *PR1a* and *PDF1.2* cDNA while actin served as a RNA control

Northern blot analysis of TDFs from plants grown with or without Bacillus and challenged with Botrytis (Fig. 4C) showed increased levels in primed and challenged local and systemic leaves at 72 hpi for BnBacBc09 (expressed protein) and BnBacBc8B (glucanase). An augmented systemic expression was found with the BnBacBc09 indicating the systemic role of this expressed protein during priming and subsequent challenge inoculation. BnBacBc25B (expressed protein), was strongly up-regulated at 12–72 hpi in both local and systemic leaves but to a higher degree in local leaves.

Northern expression data (Fig. 4D) of the TDFs isolated after challenge inoculation with *B. cinerea* indicated that BnBc23A (expressed protein) was up-regulated in both local and systemic leaves but higher expressed in systemic leaves at 12–24 hpi. Expression analysis of the TDF BnBc21C (expressed protein) showed an augmented expression pattern in primed and challenged local leaves at 12–24 h after infection but a lower expression after 72 h compared with the control. A similar pattern of gene expression was observed in the systemic leaves but with a reduced amount compared to the local leaves. TDF BnBc07 (Clathrin), was more strongly expressed in local leaves after infection compared to both control and systemic leaves.

Bacillus priming seems to involve ISR

Gene expression of the *PR-1a* gene was investigated by northern analysis in leaves of Bacillus primed and Botrytis challenge inoculated plants (Fig. 5). The mRNA level of *PR-1a* was found to be very low or negligible in the UCMB-5113 primed plants but a low induction was observed in the UCMB-5113 primed and challenged plants. A high accumulation of *PR-1a* ortholog transcripts was observed in the *B. cinerea* challenged leaves, indicating that PR-1a is not playing a role during *B. amyloliquefaciens* mediated priming in oilseed rape. *PDF1.2* mRNA was found to be elicited 2-fold in the 24 h old Bacillus primed leaves compared with the Bacillus primed and pathogen inoculated plants but with no detectable expression in the pathogen inoculated plants (Fig. 5). The lack of SA effect but presence of JA effect as a result of Bacillus treatment on *B. napus* suggests that ISR to Botrytis is occurring as a result of Bacillus priming.

Discussion

Priming of defense has been considered to be an efficient strategy to induce resistance in plants against a variety of pathogens (Beckers and Conrath 2007). Immunization of Arabidopsis by certain bacteria has been demonstrated to enhance defense capacity against a broad range of pathogens (Pieterse et al. 2002; Ahn et al. 2007). Bacteria mediated ISR is not associated with induced expression of PR genes in contrast to pathogen induced SAR (Pieterse et al. 1996; Verhagen et al. 2004). Literature till date on the molecular and physiological mechanisms of ISR has been concentrated on the Pseudomonads. The main objective of this study was to identify plant transcripts affected by beneficial Bacillus bacteria and pathogens for future studies to elucidate their role in plant–microbe interactions. Hence, the present study provides impetus on the mechanistic role of Bacillus primed defense in Brassicas. In order to identify genes associated with colonization, potential priming of ISR and concomitant disease suppression, we applied the cDNA-AFLP technique to analyze the transcript profile of roots and leaves of *B. napus* plants undergoing Bacillus UCMB 5113 mediated defense priming as visualized by the healthy phenotype observed after Botrytis challenge. cDNA-AFLP analysis of the different samples generated approximately 21,700 fragments, of which about 120 corresponded to differentially expressed genes. Several of these fragments in different samples probably represent the same gene so the number of unique genes is likely to be lower. In total, 21 TDFs from roots and 35 TDFs from leaves of Bacillus treated plants were cloned. Intriguingly, fewer transcripts were observed in roots compared to leaves of Bacillus treated plants showing a strong systemic effect in priming. Localized signals may be fewer in whole root, not detected because the changes in gene expression occurred prior to the time of sampling or that many genes just are slightly upregulated. Hence these

transcripts were not amplified with the primers used or may rely more on post-translational modifications. We observed increased root biomass in the primed plants compared with the control indicating changed root morphology and physiology during Bacillus colonization. Root changes increase the capacity of the plants to absorb nutrients and enable compatible interactions in the rhizosphere (Lopez-Bucio et al. 2003). Rhizobacteria stimulate exudation of organic carbon by roots (Meharg and Killham 1995) suggesting enhanced photosynthetic rates to accommodate the sink in carbon source. Indeed changes in the expression of photosynthesis associated genes like photosystem I reaction center subunit II (BnBacL13A), PSBO2 photosystem II (BnBacL23A) and PSB01 photosystem II (BnBacL16A) were observed in the Bacillus primed plants.

ISR was originally described as the resistance conferred to Arabidopsis by the non-pathogenic root bacterium *Pseudomonas fluorescens* (Pieterse et al. 1996). In our experiments, the effectiveness of priming ISR like defense of oilseed rape by the Bacillus UCMB 5113 strain was tested towards *B. cinerea.* Under similar conditions Bacillus priming is also effective against *A. brassicae*, *L. maculans* and *V. longisporum* (Danielsson et al. 2007). Our studies indicated that *PR-1a*, dependent on SA, was not induced by Bacillus priming, while the elicitation of JA-dependent *PDF1.2* indicated that *B. amyloliquefaciens* indeed mediated ISR in oilseed rape. Both JA and ethylene are needed for Bacillus UCMB5113 primed ISR in Arabidopsis to *Pseudomonas syringae* as deduced from mutant studies (Danielsson and Meijer, unpublished). Medium potent antibiotic substances in Bacillus extracts retard Botrytis growth in vitro (Danielsson et al. 2007). While such a direct effect can contribute to disease suppression, it seems more likely that the major effect is mediated through the plant as ISR considering the lower Bacillus density and spatial separation from Botrytis in a more natural plant soil system. ISR has been observed in other species e.g. tomato (Yan et al. 2002) and tobacco (Zhang et al. 2002) suggesting this to be a common phenomenon in plants. Certain bacteria also can provide protection to different plants and pathogens. For example *Pseudomonas putida* LSW17S protects tomato to *Fusarium oxysporum* but also prime and induce cellular and molecular defense mechanisms in Arabidopsis against *P. syringae* DC3000 (Ahn et al. 2007).

To investigate if ISR is associated with transcriptional changes only apparent after pathogen attack, we analyzed the expression profile in local and systemic leaves of primed plants upon challenge inoculation with *B. cinerea* vs. the primed non-challenged leaves. Of the 12,500 TDFs expressed, 30 TDFs were found to show an augmented change in ISR expressing leaves and 24 TDFs were sequenced. Blast analysis showed that genes involved in protection against pathogens and oxidative stress were activated systemically in leaves of colonized plants. Among the primed and pathogen responsive genes, the majority of the genes were predicted to be influenced by JA or ethylene, indicating that both signals play an important role.

Based on the Genevestigator profiles, BnBacR28B, homologous to an expressed protein (At1g74950) in Arabidopsis, showed an upward trend by MJ and wounding. This expressed protein has recently been designated as JAZ2 belonging to a family of Jasmonate Zim-Domain (JAZ) genes based on the JIM domains (Chinni et al. 2007; Thines et al. 2007). JAZ proteins have been shown to play an essential role in plant defense against insect herbivores (Chung et al. 2008). Hence, our studies indicate that Bacillus priming of the plants by root treatment might trigger these JAZ genes and in turn prepare the plant by eliciting the primary defense genes suggesting increased protein turnover to be an important early event in priming. Genvestigator analysis showed several of the genes that had differential expression in Bacillus treated plants to be induced also by brassinosteroids and other hormones triggering plant growth. Accordingly Bacillus colonization may affect metabolism of hormones that promote growth e.g. of root tissue. Other factors reported to mediate bacterial primed biocontrol are volatile organic compounds (Ryu et al. 2004a; Han et al. 2006). However, experiments conducted to study effects of volatiles from the Bacillus 5113 strain on Arabidopsis showed no protection to *P. syringae* suggesting volatiles not to be an important factor at least in that interaction (Danielsson and Meijer, unpublished). The role of plant candidate genes to study for their role in priming of ISR includes induced TDFs coding for methyl transferase (BnBacBc03A), F-box family protein (BnBacBc10), WRKY transcription factor (BnBacBc12), disease resistance protein (BnBacBc15), glycine-rich protein (BnBacBc19A), endo-1,4-β-glucanase (BnBacBc8B), MYC2 transcription factor (BnBacL11D and BnBacBc15A) and putative regulators of SNF1-protein kinase (BnBacBc6A). Recently it has been reported that qPCR analysis of the MYC2 transcript levels were upregulated in WCS417r-ISR expressing Arabidopsis plants. Functional analysis of the MYC2 impaired mutants *jin1-1* and *jin1-2* failed to develop ISR against *P. syringae* DC3000 or *Hyaloperonospora parasitica* (Pozo et al. 2008). Plant SNF1-related kinases are known to regulate the activity of rate limiting metabolic enzymes as well as the transcription of glucose and stress-regulated genes (Bhalerao et al. 1999). Studies of this regulator in primed plants could unravel the role in regulation of metabolism for growth or defense after bacterial priming. Very little is known about signals leading to ISR but mitogen-activated protein kinase3 has been proposed as a candidate for priming mediated signaling in Arabidopsis (Beckers and

Conrath 2007). This study found several kinases to be affected by Bacillus treatment, which enable analysis of their specific role in priming. In our study we also observed an early up-regulated expression of a MYB transcription (BnBacR28B) in the roots upon colonization with the Bacillus. A recent study has shown that MYB72 is responsible for early induction of ISR in Arabidopsis roots upon treatment with *P. putida* WCS417r (van der Ent et al. 2008).

Another question is how Bacillus colonization is enabled without provoking a defense response by the innate immunity surveillance system operating in plants (Newman et al. 2007). If beneficial bacteria are recognised by pathogen- or microbe-associated microbial pattern receptors in the plant the bacteria must be able to suppress downstream signaling that otherwise would elicit negative defense factors. Recognition of lipopolysaccharides (LPS) and flagella proteins can trigger plant defense or elicit ISR when challenge inoculated with pathogens (Newman et al. 2007). LPS can suppress the hypersensitive response and programmed cell death associated with the defense responses induced by avirulent bacteria (Newman et al. 2007). In our study we identified a few genes as constituents in LPS mediated signaling during bacterial priming on Brassica. In addition, some of the genes of unknown function being up-regulated during priming may be involved in colonisation and suppression of defense. Further characterisation of these unknown genes would provide information regarding factors involved in priming. Bacterial components that could be involved in elicitation of ISR are e.g. lipopeptides (Ongena et al. 2007). The role and identity of bacterial factors in the specific interaction studied here remains to be elucidated.

This study showed that Bacillus colonization of oilseed rape roots cause a genetic reprogramming of plant cells both in local (root) and distal (leaf) tissues. Many of the genes affected seem to be involved in metabolism, energy generation and regulation. Other investigations also report that primed Arabidopsis plants underwent a transcriptional reprogramming that changed e.g. metabolic processes. Cartieaux et al. (2003) reported that Arabidopsis defense and carbon metabolism was affected with reduced carbon fixation after priming by *Pseudomonas thivervalensis*. They also observed small effects on root transcripts although overall morphological effects on roots were obvious. Another study (Verhagen et al. 2004) that analysed Arabidopsis primed by *P. fluroescens* WCS417r found a larger change in root transcripts but little effect on leaf transcripts. Many of these genes are controlled by JA or ethylene. Wang et al. (2005) primed Arabidopsis with *P. fluorescens* FPT9601-T5 and found shoot tissues to respond by differences mostly in genes connected with metabolism followed by transcription and communication. A recent investigation using Arabidopsis primed with Bradyrhizobium found effects on many genes in leaves regulated by JA or ethylene (Cartieaux et al. 2008). Most of the genes were down-regulated and belonged to several functional categories including metabolism and regulation to be common. It is clear that priming is not a universal response but seems to depend on the interaction studied. Obviously the resource allocation choice made by primed plants is a delicate balance to assure fitness (Bostock 2005; Heil 2001). In the Bacillus treated oilseed rape plants, genes related to metabolism were found to represent at least 40% of the genes isolated. The dramatic reduction in the proportion of the genes linked to metabolism and a subsequent increase of the disease resistance genes clearly illustrates a mechanism in which the plant recognizes the onset of pathogen attack and deviate the resources towards defense. These changes most likely alter metabolism and affect source–sink relationships and resource allocation in the plant and somehow prime defense as illustrated by disease suppression towards Botrytis. Metabolic re-programming during defense in Arabidopsis occurs following compatible and incompatible interactions (Scheidler et al. 2002). The precise mechanism of colonization and priming by beneficial bacteria is unclear but elucidating the role of the novel TDFs identified in this study may provide explanations for the molecular repertoire behind successful long term colonization and protection. This study also showed a clear difference in plant transcript profiles when exposed to beneficial vs. pathogenic microorganisms as a basis for studies of plant–microbe interactions and plant ability to differentiate among microorganisms and allow or counteract colonization. Future analysis of plant and bacterial factors can thus provide information about the role in colonization, priming and elicitation of ISR as well as the resource strategy of a primed plant.

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