Copyright © Physiologia Plantarum 2008, ISSN 0031-9317

Phytophthora infestans-triggered response of growth- and defense-related genes in potato cultivars with different levels of resistance under the influence of nitrogen availability

Barbara Ros[a,†,*], Volker Mohler[a], Gerhard Wenzel[a] and Fritz Thümmler[b]

[a]Department of Plant Sciences, Center for Life and Food Sciences Weihenstephan, Technische Universität München, Am Hochanger 2, 85350 Freising, Germany
[b]vertis Biotechnologie AG, Lise-Meitner-Str. 30, 85354 Freising, Germany

Correspondence
*Corresponding author,
e-mail: barbara.ros@fabi.up.ac.za

Received 4 September 2007; revised 7 December 2007

doi: 10.1111/j.1399-3054.2008.01048.x

The effects of high and low N concentrations on the *Solanum tuberosum–Phytophthora infestans* interaction were studied in the potato cultivars Bettina, New York 121, Indira and Arkula, which exhibited different levels of resistance. Aboveground biomass and Chl and N content were significantly higher in all cultivars grown in higher N environments, while C:N ratios were lower, confirming successful application of N. High availability of N significantly increased susceptibility of three of the four potato cultivars, and amounts of pathogen within the infected leaflets determined in a quantitative real-time reverse transcriptase–polymerase chain reaction reflected this. Differential gene expression of *P. infestans*-induced and -repressed genes derived from three subtracted cDNA libraries at 0, 24, 48 and 72 h post-inoculation was studied in parallel. *P. infestans* attack led to an induction of defense-related and at the same time repression of growth-related potato genes mainly encoding photosynthetic genes. High N supply led to higher transcript abundance of photosynthetic genes such as Chl *a*/*b*-binding protein and ribulose bisphosphate carboxylase. N-dependent suppression of defense-related compounds in absence of the pathogen was not observed. Better N nutrition appeared to allow the plants to invest more resources in defense reactions.

Introduction

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases in potato production worldwide (Fry et al. 1993). Global appearance of both *P. infestans* mating types has become a reality over the past 20 years (Goodwin and Drenth 1997). Therefore, it can be expected that sexual reproduction enhances development of more aggressive and virulent strains of the pathogen. Keeping this in mind, the most durable type of resistance against *P. infestans* at present is of race non-specific and polygenic nature. This so-called quantitative resistance is controlled by many interacting genes that do not prevent infection but slow down the development of the pathogen at individual infection sites on the plant (Turkensteen 1993). Successful potato breeding has become very difficult as a result of

Abbreviations – hpi, hours post-inoculation; qRT-PCR, quantitative real-time reverse transcriptase–polymerase chain reaction.

†Present address: Forestry & Agricultural Biotechnology Institute, Room 6-25, Agricultural Sciences Building, Lunnon Road, University of Pretoria, Pretoria 0002, South Africa

a demand not only for high levels of durable resistance combined with early maturity but also for complex quality traits and high tuber yield (Bradshaw and Birch 2006).

Improvement of tuber quality and crop yield can additionally be achieved by a suitable nitrogen (N) fertilization regimen. In conventional potato production, N dressings with up to 200 kg ha^{-1} are usually high (Vos 1996). However, many reports have shown that high N supply not only results in enhanced growth, reflecting stimulation of the primary metabolism, but is also simultaneously accompanied by lower levels of resistance to different pathogens because of a reduction of components of the secondary metabolism, which mirrors the defense reaction (Anglberger and Halmschlager 2003, Balestra and Varvaro 1997, Blodgett et al. 2005, Entry et al. 1991, Hakulinen et al. 1995, Lavola and Julkunen-Tiitto 1994, Leser and Treutter 2005). A few studies have confirmed that high N concentrations lead to an increase in susceptibility of potato cultivars to *P. infestans* (Carnegie and Colhoun 1983, Herlihy 1970, Mittelstraß et al. 2006). These observations follow the carbon–nutrient balance hypothesis proposed by Herms and Mattson (1992) on allocation strategies. An alternative hypothesis suggests that a better N nutrition in plant tissues provides favorable growth conditions for the invading pathogen (Snoeijers et al. 2000).

The recognition and defense of *P. infestans* by its host, and the ability of the pathogen to avoid or overcome the host's defensive shields, imply a complex, dynamic communication network between the interacting organisms. The onset of biochemical response pathways requires the up- or downregulation of genes. Simultaneous monitoring of expression of genes participating in these complex plant–pathogen interactions has become an easier task with the development of cDNA arrays. Therefore, subtractive cDNA libraries are the most used approach for investigating which genes are induced or repressed by a pathogen.

In this study, we address the question if plants grown under high and low N concentrations show different expression patterns of defense- and growth-related potato genes on *P. infestans* inoculation. Therefore, four potato cultivars with different levels of resistance were grown under two N conditions. Influence of N on growth and susceptibility was determined. We generated and screened two subtractive cDNA libraries enriched for *P. infestans*-induced genes during early [24 h post-inoculation (hpi)] and late (72 hpi) plant–pathogen interaction and one additional library that contained *P. infestans*-repressed genes at 72 hpi. We analyzed differential expression of these genes in the potato cultivars grown under high and low N concentrations at 0, 24, 48 and 72 hpi.

Materials and methods

Plant material and treatment

To compare gene induction after *P. infestans* infection under different N fertilization schemes, four potato cultivars (*Solanum tuberosum* ssp. *tuberosum* L. cv. Bettina, New York 121, Indira and Arkula) were grown from tubers of uniform size in a greenhouse at 18 ± 4°C under a 16 h day length. Each pot with a volume of 10 l was filled with 'Fruhstorfer Einheitserde Typ N' (Archut, Nürnberg, Germany) containing 200–400 mg N l^{-1}, 200–400 mg P l^{-1} and 400–600 mg K l^{-1}. After planting, no additional N was supplied to half of the tubers (N0) and 14.3 g ENTEC 26 (COMPO, Münster, Germany) containing 26% N was applied to the other half (N1). The total amounts of N were 0.6–0.8 g per pot or 27–36 kg N ha^{-1} for plants grown under N0 conditions and 3.9–4.1 g per pot or 175–185 kg N ha^{-1} for N1 plants. To provide the plants with all essential nutrients except N, the soil was fertilized with 13.4 g Hakaphos basis (COMPO) every 14 days.

Pooled third and fourth leaves from five different 4-week-old plants formed one sample. Thirty freshly collected leaflets were dipped into a suspension of 10^4 sporangia ml^{-1} for 5 s. The inoculum was a combination of the *P. infestans* mating type A1 strains 31 (race 1,3,4,5,7,8,10,11), 38 (race 1,3,4,7,8,10,11) and 57 (race 1,3,4,7,8,10,11) obtained from the Bayerische Landesanstalt für Landwirtschaft, Freising, Germany. Each isolate contributed equally to the mixture. Mock inoculations were performed by dipping 30 control leaflets for 5 s into sterile water. The detached leaflets were incubated in Petri dishes containing water agar in a growth chamber at 16°C under a 16 h light period (200 μmol m^{-2} s^{-1}, HQI; Osram, München, Germany). Samples were taken 0, 24, 48 and 72 hpi, frozen in liquid nitrogen and stored at −80°C.

Carbon, nitrogen and Chl content determination

Analysis of carbon (C) and N content in whole 4-week-old potato plants was carried out with an elemental analyzer coupled to a mass spectrometer at the Chair of Ecological Cultivation, Technische Universität München, Germany. In addition, Chl content of greenhouse-grown potato plants was measured once a week using a handheld Hydro N-Tester (YARA, Dülmen, Germany). The Chl meter readings are an average of 30 measurements and the values are without unit.

Total RNA isolation, purification and in vitro RNA synthesis

Total RNA from the leaves and the *P. infestans* strains grown on V8 medium was isolated three times using TRIzol® Reagent (Invitrogen, Karlsruhe, Germany) according to manufacturer's instructions. After adding one-third volume of 8 *M* LiCl, the RNA was pooled and precipitated overnight at 4°C and harvested by centrifugation at 12,000 *g* for 20 min.

Plasmid pMM14 was used to synthesize the RNA used as a non-potato control for the array hybridization experiments. The plasmid comprises the 3′ untranslated region of the luciferin-binding protein from the dinoflagellate *Gonyaulax polyedra* (Mittag et al. 1994), cloned in pTZ18R (Pharmacia, Karlsruhe, Germany) and was kindly provided by Dr M. Mittag (University of Jena, Germany). RNA was synthesized in vitro from the T7 promoter according to the instructions of the supplier of the T7 polymerase (MBI Fermentas, St. Leon-Rot, Germany).

The quality of all RNAs was verified by gel electrophoresis, and RNAs were quantified with a spectrophotometer at 260 nm.

Quantitative real-time one-step reverse transcriptase–polymerase chain reaction

Quantitative real-time reverse transcriptase–polymerase chain reaction (qRT-PCR) was performed as previously described using primers to a *P. infestans* elongation factor 1-beta-like gene (Ros et al. 2004). Each sample was run in triplicate in addition to non-template controls containing water instead of RNA. No signal above the level of the non-template control was observed. Polymerase chain reaction products were verified by gel electrophoresis to confirm that the signal was the result of product amplification and not because of primer dimers.

Generation and contents of the subtractive cDNA libraries

The construction of cDNA libraries highly enriched for *P. infestans*-induced genes by vertis Biotechnologie AG, Freising, Germany, was described previously (Ros et al. 2004). The library B24i consisted of genes induced 24 hpi in the 4-week-old cultivar Bettina. The tester was prepared from RNA isolated from leaflets inoculated with *P. infestans* for 24 h. The driver was prepared from a mixture of RNA isolated from mock-treated leaflets and *P. infestans* RNA to eliminate pathogen-derived genes. B72i consisted of *P. infestans*-induced Bettina genes collected 72 hpi. The tester was prepared from RNA isolated from leaflets inoculated with *P. infestans* for 72 h. The driver was prepared from a mixture of RNA isolated from mock-treated leaflets and *P. infestans* RNA to eliminate pathogen-derived genes. Opposite choice of tester and driver RNA in the library B72r led to an enrichment of Bettina genes that were repressed 72 hpi.

cDNA sequences reported in this article have been deposited in the GenBank and accession numbers are given in Fig. 3 and Table 3.

Expression profiling

Generation of cDNA arrays, first-strand cDNA synthesis, concurrent labeling with ^{32}P-modified nucleotides and hybridization were performed as described previously (Ros et al. 2005). Hybridization was conducted twice to obtain more than three data for each cultivar. Hybridization signals on the imaging plates were detected using STORM 860 (Molecular Dynamics, Sunnyvale, CA) with a resolution of 50 μm. The image data obtained were imported into the program package Array Vision (Imaging Research, St. Catharines, Canada) for spot detection and quantification of hybridization signals. The signals derived from hybridization of the cDNA transcribed from the non-potato RNA of pMM14 plasmid were defined for reference determination. For normalization, the individual spot was divided by the reference. Average gene induction or repression levels were calculated by dividing the sum of the induction or repression factors by the amount of genes that were up- or downregulated.

Statistical analysis

The statistical analyses were performed with the software SAS 9.1 (SAS Institute, München, Germany). One-tailed *t*-test was used to test for differences among treatments. Differences were considered significant at $P < 0.05$.

Results and discussion

Increasing N fertilization stimulates biomass production and decreases the C:N ratio

Monitoring Chl levels with a Hydro N-Tester reflects N content in the plant because Chl depends on N for its synthesis. The successful application of such a Chl meter in potato has been shown previously (Denuit et al. 2002). Throughout the cultivation period, all four cultivars responded equally to the different N supplies. Because of the fertilizing schemes, the N1 plants possessed a significantly higher Chl content than the N0 plants, reflecting the amount of N supplied (Fig. 1; $P < 0.05$). In 4-week-old plants, aboveground biomass and N contents were significantly higher when more N was available (Table 1;

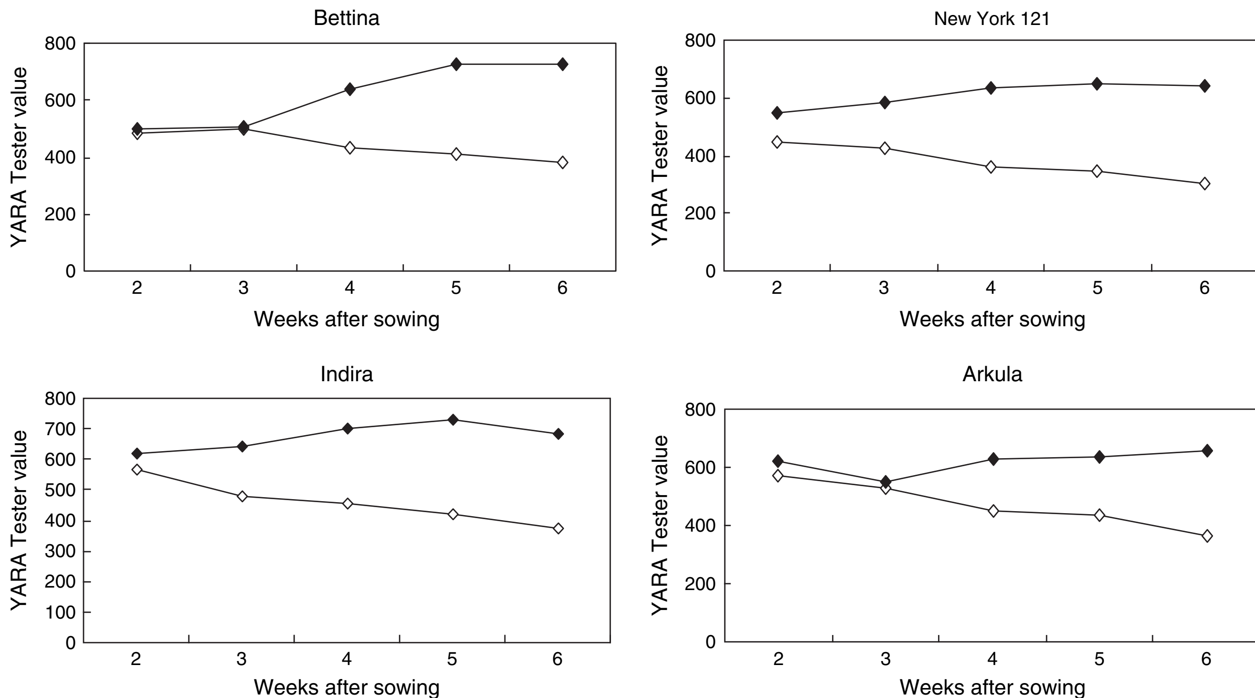

Fig. 1. Chl content of greenhouse-grown potato plants measured using a handheld Hydro N-Tester (YARA). Plants were grown under N0 (open symbols) or N1 (closed symbols) conditions. Values are means of 30 measurements.

$P < 0.05$). These findings verified the successful application of different N amounts in the greenhouse experiment, despite the preconception that potatoes grown from tubers might not respond to N in the same way as other plants grown from seeds with far less storage proteins. Because of constant C levels, an increase of N fertilization led to a drastic decrease of the C:N ratio (Table 1).

Increasing N fertilization increases susceptibility to *P. infestans*

Four-week-old potato plants grown under N0 and N1 conditions were inoculated with *P. infestans*. Susceptibility of the potato cultivars was investigated 24, 48 and 72 hpi. Macroscopically visible lesions were detected on the leaves 72 hpi. Bettina displayed very few lesions that did not differ between N0 and N1 conditions. New York 121 was more susceptible than Bettina but less susceptible than Indira. Arkula was the most susceptible potato cultivar. In New York 121, Indira and Arkula, the higher N supply led to more lesions after pathogen attack, supporting the hypothesis that an increase in N availability leads to an increase in susceptibility.

The amount of *P. infestans* within the leaflets was determined on a molecular level using qRT-PCR. At 72 hpi, more *P. infestans* was detected in New York 121, Indira and Arkula plants grown under N1 condition compared with those grown under N0 condition, reflecting the increased susceptibility of these plants ($P < 0.005$ and <0.001). Bettina, the most resistant cultivar in our experiment, did not show an altered level of resistance (Fig. 2). In this cultivar, only a few lesions containing low amounts of *P. infestans* were formed, which made molecular pathogen detection impossible as had been reported previously (Ros et al. 2005).

Table 1. Effect of N nutrition (N0, N1) on FW, N and C content (% dry weight) and C:N ratio of the potato plants. Data are means of five plants. According to *t*-test, significant differences at $P < 0.05$ are indicated with different letters.

Cultivar	N condition	FW (g)	N (%)	C (%)	C:N
Bettina	N0	110[a]	2.4[a]	39.3[a]	16[a]
	N1	617[b]	7.5[b]	42.9[a]	6[b]
New York 121	N0	109[a]	2.8[a]	40.8[a]	15[a]
	N1	504[b]	7.4[b]	43.1[a]	6[b]
Indira	N0	105[a]	3.5[a]	39.9[a]	12[a]
	N1	470[b]	7.5[b]	43.6[a]	6[b]
Arkula	N0	128[a]	2.2[a]	40.3[a]	19[a]
	N1	400[b]	7.6[b]	41.3[a]	5[b]

Contents of the subtractive cDNA libraries

To obtain a comprehensive set of genes for expression analyses, three subtractive libraries were generated from

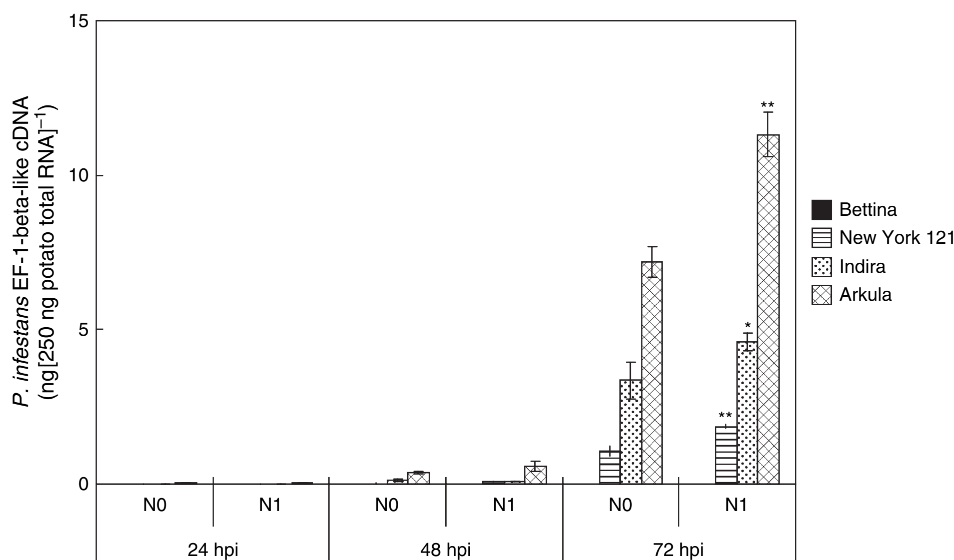

Fig. 2. Quantitative *P. infestans* detection in the potato cultivars in the course of infection. Plants were grown under N0 and N1 conditions. Thirty detached leaflets were dipped in *P. infestans* sporangium suspension. Leaflets were harvested 24, 48 and 72 hpi. qRT-PCR was performed using *P. infestans*-specific primers to an EF-1-beta-like gene. A standard curve over a 5-log range using RNA from the pathogen was used to calculate the amount of *P. infestans* RNA in the potato leaflets. Values are means ± SD of three replicates. Significant differences in the amount of *P. infestans* detected per time point in N0 and N1 plants of one cultivar are indicated by asterisks (according to *t*-test: $*P < 0.05$ and $**P < 0.01$). EF-1, elongation factor 1.

Bettina cDNA. Early- and late-induced defense responses were covered with the libraries B24i and B72i. The third library (B72r) contained genes repressed during late plant–pathogen interaction.

Two hundred and two cDNA clones from B24i, 169 from B72i and 270 from B72r were randomly picked and partially sequenced. BLASTn and BLASTx searches were performed for each cDNA clone (Altschul et al. 1997). E values greater than E−10 were considered non-significant (no similarities found). The cDNA libraries were designed in such a way that most defense-/stress-related genes were found in B24i and B72i, whereas most genes involved in primary metabolism were identified in B72r (Table 2). These findings suggest a possible induction of defense-related and repression of growth-related potato genes after *P. infestans* attack. No non-plant genes were detected in any library.

For expression analyses, 59 unigenes from B24i, 56 from B72i and 69 from B72r were selected, each library contributing about equal numbers of unigenes to the array. These 184 cDNAs could be classified into 10 functional categories according to partial sequence identities to known enzymes and proteins (Table 2) and covered a representative proportion of genes of the defense/stress related, primary metabolism and genes with unknown biological function.

Table 2. Contents of the chosen clones from the cDNA libraries and composition of the cDNA array.

Function	B24i: cDNA clones		B72i: cDNA clones		B72r: cDNA clones		cDNA array: cDNA clones	
Transcription	2	46% (rows Transcription–Phytohormone related)	3	50% (rows Transcription–Phytohormone related)	2	67% (rows Transcription–Phytohormone related)	7	55% (rows Transcription–Phytohormone related)
Photosynthesis/chloroplast	1		0		5		6	
Cellular metabolism	16		20		31		67	
Signaling	3		1		1		5	
Transporters	0		1		4		5	
Growth/development	3		1		1		5	
Phytohormone related	2		2		2		6	
Stress/defense	22	37%	21	38%	16	23%	59	32%
Unknown protein	8	14%	3	5%	4	6%	15	8%
No similarities found	2	3%	4	7%	3	4%	9	5%
Sum	59	100%	56	100%	69	100%	184	100%

N effect on differential gene expression

To determine the proportion of the 184 unigenes that was up- or downregulated during infection, the expression for each gene was studied 0, 24, 48 and 72 hpi with cDNA array hybridization. The printed cDNA arrays were hybridized with ^{32}P-labeled probes prepared from total RNA. The RNAs had been isolated from leaves of 4-week-old potato plants prior to any treatment, inoculated with *P. infestans* or from respective water controls. Gene induction or repression above two-fold was considered significant. A complete list of up- or downregulated transcripts is given in Table 3.

Increasing N fertilization increases constitutive expression of photosynthetic genes but does not affect constitutive expression of defense-/stress-related genes

Some transcripts encoding photosynthetic (*cab*, *psaH*, *psaN*, *rbcS*) and defense-/stress-related (*til*, *mta*, *pin*) genes possessed high expression levels in the pathogen-free plants at 0 h (Fig. 3). High expression levels of these genes could be found to a similar extent in the mock-treated leaves. This indicates that incubation of the detached leaves for up to 72 h in Petri dishes in itself did not lead to an activation of these genes. Neither function nor transcript abundance of these genes can explain why some potato cultivars were more resistant than others. However, all these constitutively expressed genes except *pin* showed significantly higher transcript levels when N supply was higher (Fig. 3; $P < 0.05$). Therefore, expression patterns of the photosynthetic genes directly reflect the enhanced Chl content on a molecular level (Figs 1 and 3, Table 1). Our findings are in agreement with an increase in *cab* and *rbcS* abundance in *Arabidopsis thaliana* at higher N concentrations (Martin et al. 2002).

According to Herms and Mattson (1992), high N availability stimulates the primary metabolism (growth) and suppresses the secondary metabolism (defense), which increases susceptibility to pathogens. However, this growth–differentiation balance hypothesis could only be applied to the expression of photosynthetic gene transcripts in unchallenged potato plants at 0 h (Fig. 3). No defense-related genes possessed maximum expression level in the N0 plants, although transcripts of various well-known enzymes involved in secondary metabolism such as chalcone isomerase (*chi*), cinnamic acid 4-hydroxylase and leucoanthocyanidin dioxygenase were on the cDNA array. Thus, in our study, no N-dependent suppression of defense-related compounds in absence of the pathogen was observed.

Impact of N concentration on P. infestans*-triggered induction of defense-/stress-related genes and repression of growth-related genes*

While gene induction in Bettina and New York 121 already took place 24 hpi in N0 plants, the defense reaction was only switched on in Indira and Arkula when more N was available (Fig. 4, Table 4). Higher N supply led to an induction of more defense-/stress-related genes at 24 hpi. The cultivars with a higher susceptibility induced fewer defense-/stress-related genes compared with the plants with a higher level of resistance (Table 4). These early-induced genes encode mainly well-known PR proteins (PR-1, PR-2, PR-3, PR-14, PR-17), a *chi*, a glutathione S-transferase, wound-induced genes *win1*

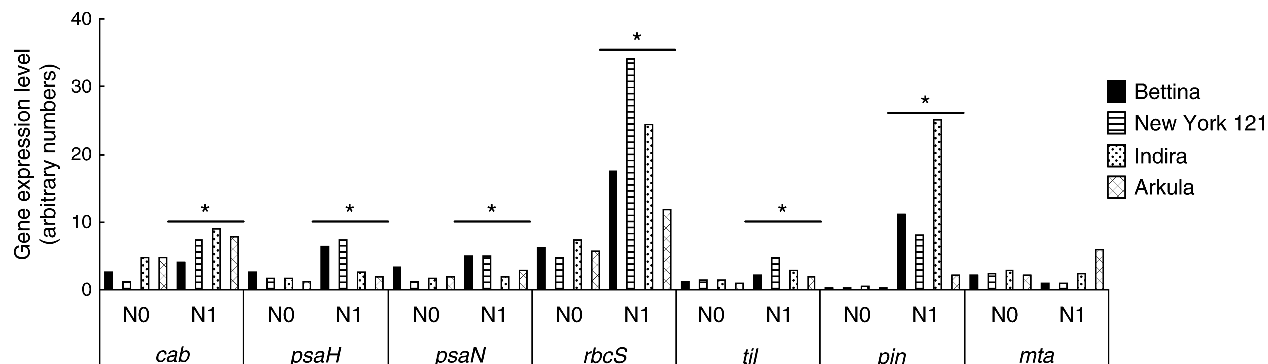

Fig. 3. Gene expression levels in the untreated potato cultivars at 0 h. Plants were grown under N0 or N1 conditions. Expression levels were deduced from cDNA array hybridization experiments. *cab*, Chl *a/b*-binding protein gene (GenBank accession no. EG009406); *psaH*, *psaH* gene for photosystem I (EG009401); *psaN*, photosystem I reaction center subunit *psaN* precursor (EG009403); *rbcS*, ribulose bisphosphate carboxylase mRNA (EG009421); *til*, temperature-induced lipocalin mRNA (EG009416); *pin*, auxin-induced proteinase inhibitor (EG009419); *mta*, metallothionein-like protein (EG009398). Significant differences in the gene expression level in N0 and N1 plants are indicated by an asterisk (according to *t*-test: $P < 0.05$).

Table 3. *P. infestans*-induced defense-/stress-related genes and *P. infestans*-repressed primary metabolism genes.

Clone	GenBank accession number	Homology	E value
		P. infestans-induced defense-/stress-related genes	
B72i 3C1	EG009390	mRNA for pathogenesis-related protein isoform b1 (*pr1b1* gene), *Solanum phureja*, AJ493450	4E−177
B72i 1E3	EG009373	mRNA for pathogenesis-related protein isoform b2 (*pr1b2* gene), *S. phureja*, AJ493451	2E−136
B72i 1D4	EG009371	Basic PR-1 protein precursor, *Capsicum annuum*, AY560589	3E−91
B24i 1A10	EG009370	Pathogenesis-related protein 1b (*pr1b*) precursor mRNA, *S. tuberosum*, AY050221	0
B72i 3A10	EG009388	TomQ'b mRNA for beta(1,3)glucanase, *Lycopersicon esculentum*, X74906	0
B72i 1A4	EG009364	PR-P2 mRNA for pathogenesis-related protein P2, *L. esculentum*, X58548	2E−120
B24i 1F1	EG009424	1,3-beta-glucan glucanohydrolase gene, *S. tuberosum*, AF067863	0
B72i 3D6	EG009396	Class II chitinase (ChtA2) mRNA, *S. tuberosum*, U49969	0
B72r 8D2	EG009417	Non-specific lipid transfer protein (*le16*) mRNA, *L. esculentum*, U81996	2E−141
B72i 3C8	EG009393	NtPRp27-like protein mRNA, *S. tuberosum*, AY185207	0
B24i 3A9	EG009359	Putative pathogenesis-related protein, *Oryza sativa*, NP_913675	2E−49
B72i 1A5	EG009365	Chalcone isomerase gene, *Hordeum vulgare*, AF474923	2E−166
B72i 3D1	EG009395	Cinnamic acid 4-hydroxylase mRNA, *Capsicum chinense*, AF088847	7E−157
B24i 1B10	EG009381	Cyt P450, *A. thaliana*, BAB02398	5E−52
B24i 2G1	EG009356	StGST type 3 mRNA for glutathion S-transferase, *S. tuberosum*, AB061250	0
B72i 2D11	EG009384	Glutathione S-transferase/peroxidase, *C. chinense*, CAI48072	2E−43
B24i 1D2	EG009413	Glutathione S-transferase (*gst1*) gene, *S. tuberosum*, J03679	2E−163
B72r 6H12	EG009411	TAS14 mRNA inducible by abscisic acid and environmental stress, *L. esculentum*, X51904	2E−49
B24i 2D8	EG009354	Universal stress protein family protein, *A. thaliana*, AAY78733	6E−18
B24i 2A1	EG009343	Biotic cell death-associated protein (CDM1) mRNA, *Nicotiana glutinosa*, AF208022	4E−69
B24i 3H4	EG009363	Wound-induced genes *win1* and *win2*, *S. tuberosum*, X13497	0
B72i 2B9	EG009383	Putative cold acclimation protein, *O. sativa*, XP_469914	E−40
B72r 6H1	EG009410	Zinc finger like, *O. sativa*, XP_468430	E−25
B72r 8A4	EG009414	Zinc finger homeodomain protein SZF-HD1, *Glycine max*, AAW22594	3E−38
B72i 1G7	EG009377	Putative RING-H2 zinc finger protein, *A. thaliana*, AAM65842	4E−31
B24i 3E11	EG009361	Putative short-chain-type alcohol dehydrogenase, *S. tuberosum*, AAK29646	2E−53
B24i 2D5	EG009352	mRNA for DC1.2 homologue, *Nicotiana tabacum*, AB009888	5E−53
B24i 3B10	EG009360	Pollen Ole e 1 allergen and extensin family protein like, *O. sativa*, XP_465260	3E−37
B24i 2D6	EG009353	Expansin-like protein B, *O. sativa*, AAO33384	4E−33
B72i 3C10	EG009394	Syntaxin of plants, *Medicago sativa*, AAZ32890	2E−42
B72r 8E9	EG009420	RHA2A; protein binding/ubiquitin-protein ligase/zinc ion binding, *A. thaliana*, NP_172962	8E−34
B24i 2D3	EG009351	Putative E2 ubiquitin-conjugating enzyme UBC7, *A. thaliana*, AAL86003	6E−49
B72i 1G8	EG009378	Immunophilin/FKBP-type peptidyl-prolyl *cis-trans* isomerase family protein, *A. thaliana*, NP_567750	E−28
		P. infestans-repressed primary metabolism genes	
B72r 65	EG009406	Chl *a/b*-binding protein (*Lhcb1-2*) gene, *S. tuberosum*, U20983	2E−32
B72r 1D5	EG009401	*psaH* gene for photosystem I, *Nicotiana sylvestris*, X61664	7E−117
B72r 1E6	EG009403	Photosystem I reaction center subunit *psaN* precursor, *A. thaliana*, AAM10156	8E−31
B72r 8G4	EG009421	Ribulose bisphosphate carboxylase mRNA, *S. tuberosum*, J03613	2E−129
B72r 8G5	EG009422	Glyceraldehyde-3-phosphate dehydrogenase, *C. annuum*, AJ246008	0
B72r 8H9	EG009423	*Cy-F1* mRNA for cytosolic fructose-1.6-biphosphatase, *S. tuberosum*, X76946	E−167
B72r 6F12	EG009409	Acyl carrier protein mRNA, *L. esculentum*, AY568716	0
B72r 8D5	EG009418	Granule-bound starch synthase, *S. tuberosum*, X58453	E−152
B72r 6E4	EG009408	mRNA for P-protein, *S. tuberosum*, Z99770	0

and *win2* and two stress-induced genes (Table 3). Some of these genes like PR-1, PR-2, PR-3 and PR-17 are associated with downstream plant defenses and systemic acquired resistance (Austin et al. 2002, Brederode et al. 1991, Ward et al. 1991, Okushima et al. 2000). The genes *win*1 and *win*2 encode cysteine-rich proteins, which might be involved in chitin binding (Stanford et al. 1989). Even though *P. infestans* does not contain chitin in its cell walls, chitinases (PR-3, PR-4, PR-8, PR-11) and glucanases (PR-2) are part of the defense response against this oomycete (Schröder et al. 1992). There is evidence that the co-expression of these two PR proteins results in an enhanced defense reaction (Boller 1993).

At 48 hpi, all cultivars showed induction of defense-/stress-related genes in N0 and N1 plants. However, in New York 121, Indira and Arkula, an increase in N fertilization

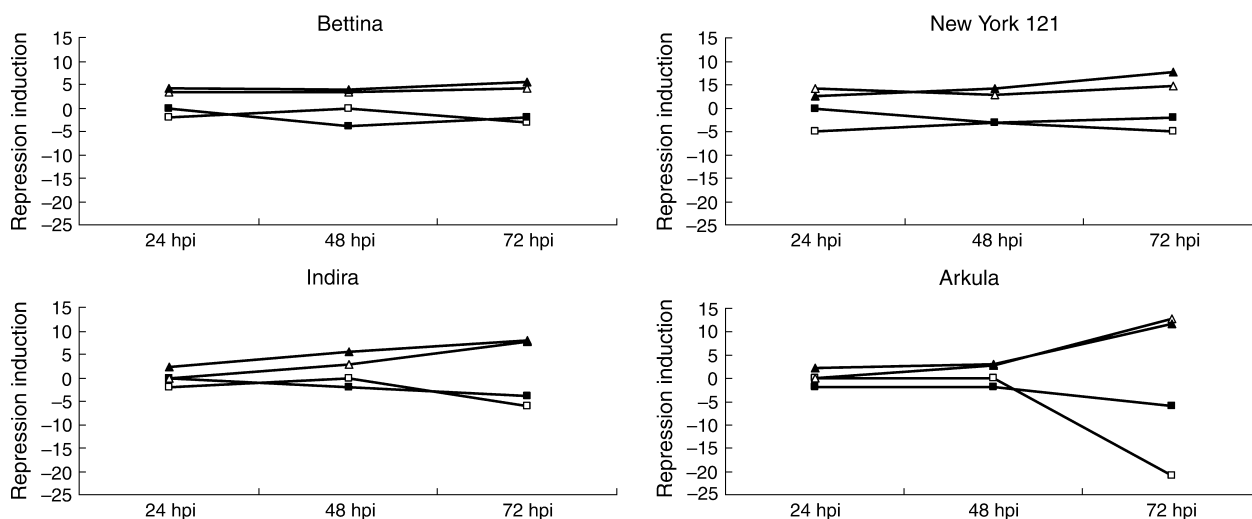

Fig. 4. *P. infestans*-triggered average gene expression patterns in the potato cultivars with time. Plants were grown under N0 (open symbols) or N1 (closed symbols) conditions. Thirty detached leaflets were dipped in either water or *P. infestans* sporangium suspension. Leaflets were harvested 24, 48 and 72 hpi. Expression levels were deduced from cDNA array hybridization experiments. Average inductions or repression levels were calculated by dividing the sum of the induction or repression factors by the amount of genes up- or downregulated at a respective time point. (▲) Defense-/stress-related genes; (■) genes involved in the carbohydrate-generating metabolism.

resulted in an increase of induced defense-/stress-related transcripts. N availability did not have much impact on average gene induction levels (Fig. 4, Table 4).

At 72 hpi, defense-/stress-related genes were induced in all N0 and N1 plants. A higher N availability led, only in Arkula and New York 121, to an increase in the number of induced defense-/stress-related genes at 72 hpi (Table 4). Maximum average induction level of defense-/stress-related genes was found in all plants at 72 hpi. Arkula, the most susceptible cultivar, showed the strongest average gene induction under N0 and N1 conditions (Fig. 4).

More defense-/stress-related genes were induced at 72 hpi compared with 24 hpi in all plants during the time course. N supply did not seem to affect average induction levels of the potato cultivars, with the exception of New York 121, which had a stronger average defense reaction 72 h after pathogen attack when more N was applied (Fig. 4).

The *P. infestans*-induced transcripts encoded mainly pathogenesis-related proteins such as PR-1, PR-2, PR-3, PR-14 and PR-17 (Table 3), all of which have previously been described in compatible and incompatible potato–*P. infestans* interactions (Restrepo et al. 2005, Ros et al.

Table 4. Total number of *P. infestans*-induced or -repressed genes in the potato cultivars. Overlap of genes between N0- and N1-treated plants and overlap between two neighboring time points within a cultivar are indicated in bold.

Cultivar	Bettina			New York 121			Indira			Arkula		
Treatment	N0		N1	N0		N1	N0		N1	N0		N1
					Induced defense-/stress-related genes							
24 hpi	9	**5**	11	9	**6**	12	0	**0**	2	0	**0**	5
	8		**7**	**7**		**8**	**0**		**2**	**0**		**4**
48 hpi	19	**11**	13	12	**12**	19	10	**8**	19	11	**5**	12
	12		**10**	**11**		**14**	**9**		**13**	**9**		**9**
72 hpi	20	**11**	12	16	**16**	20	23	**14**	14	18	**18**	25
					Repressed carbohydrate-generating genes							
24 hpi	5	**0**	0	5	**0**	0	1	**0**	0	0	**0**	1
	0		**0**	**3**		**0**	**0**		**0**	**0**		**0**
48 hpi	0	**0**	5	5	**1**	1	0	**0**	1	0	**0**	1
	0		**1**	**5**		**1**	**0**		**1**	**0**		**1**
72 hpi	2	**0**	3	9	**3**	3	3	**3**	8	9	**6**	6

2004, 2005, Tian et al. 2006, Wang et al. 2005). Overlap of defense-/stress-related genes between N0- and N1-treated plants of one cultivar at one time point was high (Table 4), indicating that upregulation of certain genes occurs independently of N concentration.

While *P. infestans* infection triggered induction of defense-/stress-related genes, it also caused downregulation of growth-related genes. At 24 hpi, higher N supply delayed gene repression in all cultivars, except Arkula. In contrast, repression of growth-related genes in N0 plants was observed in Bettina, New York 121 and Indira (Table 4).

In the N0 plants 48 hpi, repression of five growth-related genes was only found in New York 121, whereas in all N1 plants, carbohydrate-generating genes were suppressed to about the same extent (Fig. 4).

In all plants, growth-related genes were downregulated 72 hpi. In Arkula, Indira and New York, 121 more carbohydrate-generating genes were downregulated when more N was available, but average repression levels were lower. Arkula, the most susceptible cultivar, displayed an extreme difference in the average gene repression between the N0 and the N1 treatment (Fig. 4).

Decrease in transcript abundance after pathogen attack mainly affected C-generating genes (Table 3). All down-regulated cDNA clones were derived from the subtractive library B72r, which was enriched for *P. infestans*-repressed genes, confirming the concept and design of the subtractive cDNA libraries used in this study. Down-regulation of photosynthetic genes such as *cab* and *rbcS* has been shown in different compatible and incompatible plant–pathogen interactions (Berger et al. 2004, Chou et al. 2000, Scholes et al. 1994, Swarbrick et al. 2006). It has been suggested that changes in photosynthetic gene expression are caused by death of the invaded host cells and alterations in source–sink relations and C utilization (Swarbrick et al. 2006).

Concluding remarks

Slight differences in gene expression of the individual genotypes during pathogenesis could be because of the presence and nature of R genes, maturity type and starch content of the cultivars. Nevertheless, the following observations were obvious. N supply did have an impact on *P. infestans*-triggered gene expression patterns (Fig. 4, Table 4). Better N nutrition tended to allow the plants to induce more defense-/stress-related genes. Higher N supply also allowed Indira and Arkula an earlier induction of these genes. From other plant–pathogen interactions, it is known that defense reaction in a susceptible plant is often delayed (Ho and Yang 1999, Lamb et al. 1992, Tao et al. 2003, Tör et al. 2002). It is tempting to speculate that a better plant nutrition allowed the plants to invest more resources in defense reactions.

When more N was available, more growth-related genes were downregulated. But repression occurred later and was weaker. This again indicates that through a better nutrition, the plants were able to compensate for resources needed during defense reaction (Fig. 4, Table 4).

Despite all these findings, it remains to be shown why plants grown under higher N concentrations are more susceptible. Because we, as mentioned above, consider an effect of a trade-off between primary and secondary metabolites on transcript level unlikely, an alternative hypothesis has to be considered. Because pathogens invade host tissues to obtain nutrient resources, Snoeijers et al. (2000) speculated that a better plant nutrition of the host leads to a better growth environment for the pathogen, which then triggers genes involved in pathogenicity.

Information on the reaction of N metabolism and its regulation in *P. infestans* is currently very limited. A nitrate reductase gene from *P. infestans* has been cloned, but N-dependent expression during pathogenesis has not been studied (Pieterse et al. 1995). Recently, 8469 expressed sequence tags from *P. infestans* mycelium grown under limited N have been identified (Randall et al. 2005), and it is only a matter of time before other large-scale sequencing and expression profiling projects will contribute to a better understanding of *P. infestans* biology.

Acknowledgements – We thank the breeders Firlbeck (Germany), NORIKA (Germany) and Bavaria Saat (Germany) and the University of Cornell (USA) for the potato tubers; the Bayerische Landesanstalt für Landwirtschaft in Freising (Germany) for the *P. infestans* strains; COMPO (Germany) for the fertilizer used in the greenhouse experiments and Agathe Zach and Christina Hartmann for excellent technical assistance. The funding for this work as part A10 of 'Sonderforschungsbereich 607' was provided by the Deutsche Forschungsgemeinschaft.

References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402

Anglberger H, Halmschlager E (2003) The severity of *Sirococcus* shoot blight in mature Norway spruce stands with regard to tree nutrition, topography and stand age. Forest Ecol Manage 177: 221–230

Austin MJ, Muskett P, Kahn K, Feys BJ, Jones JD, Parker JE (2002) Regulatory role of SGT1 in early *R* gene-mediated plant defenses. Science 295: 2077–2080

Balestra GM, Varvaro L (1997) Influence of nitrogen fertilization on the colonization of olive phylloplane by

Pseudomonas syringae subsp. *savastanoi*. In: Rudolph K, Burr TJ, Mansfield JW, Stead D, Vivian A, von Kietzell J (eds) Pseudomonas syringae Pathovars and Related Pathogens. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 88–92

Berger S, Papadopoulos M, Schreiber U, Kaiser W, Roitsch T (2004) Complex regulation of gene expression, photosynthesis and sugar levels by pathogen infection in tomato. Physiol Plant 122: 419–428

Blodgett JT, Herms DA, Bonello P (2005) Effects of fertilization on red pine defence chemistry and resistance to *Sphaeropsis sapinea*. Forest Ecol Manage 208: 373–382

Boller T (1993) Antimicrobial functions of the plant hydrolases, chitinase and β-1,3-glucanase. In: Fritig B, Legrand M (eds) Mechanisms of Plant Defense Responses. Developments in Plant Pathology, Vol. 2. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 391–401

Bradshaw JE, Birch PJR (2006) Breeding potatoes in Scotland for resistance to late blight. In: Heilbronn TD (ed) Proceedings of the Crop Protection in Northern Britain, Dundee, 28 February to 1 March 2006. Association for Crop Protection in Northern Britain, Edinburgh, Great Britain, pp 249–254

Brederode FT, Linthorst HJM, Bol JF (1991) Differential induction of acquired resistance and PR gene expression in tobacco by virus infection, ethephon treatment, UV light and wounding. Plant Mol Biol 17: 1117–1125

Carnegie S, Colhoun S (1983) Effects of plant nutrient on susceptibility of potato leaves to *Phytophthora infestans*. Phytopathol Z 108: 242–250

Chou H-M, Bundock N, Rolfe SA, Scholes JD (2000) Infection of *Arabidopsis thaliana* leaves with *Albugo candida* (white blister rust) causes a reprogramming of host metabolism. Mol Plant Pathol 1: 99–113

Denuit JP, Olivier M, Goffaux M-J, Herman J-L, Goffert J-P, Destain J-P, Frankinet M (2002) Management of nitrogen fertilization of winter wheat and potato crops using the chlorophyll meter for crop nitrogen status assessment. Agronomie 22: 847–853

Entry JA, Cromack K Jr, Hansen E, Waring R (1991) Responses of western coniferous seedlings to infection by *Armillaria ostoyae* under limited light and nitrogen. Phytopathology 81: 89–94

Fry WE, Goodwin SB, Dyer AT, Matuszak JM, Drenth A, Tooley PW, Sujkowski LS, Koh YJ, Cohen BA, Spielman LJ, Deahl KL, Inglis DA, Sandlan KP (1993) Historical and recent migrations of *Phytophthora infestans*: chronology, pathways, and implications. Plant Dis 77: 653–661

Goodwin SB, Drenth A (1997) Origin of the A2 mating type of *Phytophthora infestans* outside Mexico. Phytopathology 87: 992–999

Hakulinen J, Julkunen-Tiitto R, Tahvanainen J (1995) Does nitrogen fertilization have an impact on the trade-off between willow growth and defensive secondary metabolism? Trees 9: 235–240

Herlihy M (1970) Contrasting effects of nitrogen and phosphorous on potato tuber blight. Plant Pathol 19: 65–68

Herms DD, Mattson WJ (1992) The Dilemma of Plants: To Grow or Defend. Q Rev Biol 67: 283–335

Ho G-D, Yang C-H (1999) A single locus leads to resistance of *Arabidopsis thaliana* to bacterial wilt caused by *Ralstonia solanacearum* through a hypersensitive-like response. Phytopathology 89: 673–678

Lamb CJ, Ryals JA, Ward ER, Dixon RA (1992) Emerging strategies for enhancing crop resistance to microbial pathogens. Biotechnology 10: 1436–1445

Lavola A, Julkunen-Tiitto R (1994) The effect of elevated carbon dioxide and fertilization on primary and secondary metabolites in birch, *Betula pendula* (Roth.). Oecologia 99: 315–321

Leser C, Treutter D (2005) Effects of nitrogen supply and pathogen (scab) resistance. Physiol Plant 123: 49–56

Martin T, Oswald O, Graham IA (2002) *Arabidopsis* seedling growth, storage lipid mobilization, and photosynthetic gene expression are regulated by carbon:nitrogen availability. Plant Physiol 128: 472–481

Mittag M, Lee D-H, Hastings JW (1994) Circadian expression of the luciferin-binding protein correlates with the binding of a protein to the 3′ untranslated region of its mRNA. Proc Natl Acad Sci USA 91: 5257–5261

Mittelstraß K, Treutter D, Pleßl M, Heller W, Elstner EF, Heiser I (2006) Modification of primary and secondary metabolism of potato plants by nitrogen application differentially affects resistance to *Phytophthora infestans* and *Alternaria solani*. Plant Biol 8: 653–661

Okushima Y, Koizumi N, Kusano T, Sano H (2000) Secreted proteins of tobacco cultured BY2 cells: identification of a new member of pathogenesis-related proteins. Plant Mol Biol 42: 479–488

Pieterse CMJ, van't Klooster J, van den Berg-Velthuis GCM, Govers F (1995) *NiaA*, the structural nitrate reductase gene of *Phytophthora infestans*: isolation, characterization and expression analysis in *Aspergillus nidulans*. Curr Genet 27: 359–366

Randall TA, Dwyer RA, Huitema E, Beyer K, Cvitanich C, Kelkar H, Ah Fong AMV, Gates K, Roberts S, Yatzkan E, Gaffney T, Law M, Testa A, Torto-Alalibo T, Zahng M, Zheng L, Mueller E, Windass J, Binder A, Birch PRJ, Gisis U, Govers F, Gow NA, Mauch F, van West P, Waugh ME, Yu J, Boller T, Kamoun S, Lam ST, Judelson HS (2005) Large-scale gene discovery in the oomycete *Phytophthora infestans* reveals likely components of phytopathogenicity shared with true fungi. Mol Plant-Microbe Interact 18: 229–243

Restrepo S, Myers KL, del Pozo O, Martin GB, Hart AL, Buell CR, Fry WE, Smart CD (2005) Gene profiling of

a compatible interaction between *Phytophthora infestans* and *Solanum tuberosum* suggests a role for carbonic anhydrase. Mol Plant-Microbe Interact 18: 913–922
Ros B, Thümmler F, Wenzel G (2004) Analysis of differentially expressed genes in a susceptible and moderately resistant potato cultivar upon *Phytophthora infestans* infection. Mol Plant Pathol 5: 191–201
Ros B, Thümmler F, Wenzel G (2005) Comparative analysis of *Phytophthora infestans* induced gene expression in potato cultivars with different levels of resistance. Plant Biol 7: 686–693
Scholes JD, Lee PJ, Horton P, Lewis DH (1994) Invertase understanding changes in the photosynthetic and carbohydrate metabolism of barley leaves infected with powdery mildew. New Phytol 126: 213–222
Schröder M, Hahlbrock K, Kombrinck E (1992) Temporal and spatial patterns of 1,3-β-glucanase and chitinase induction in potato leaves infected by *Phytophthora infestans*. Plant J 2: 161–172
Snoeijers SS, Perez-Garcia A, Joosten MHAJ, De Wit PJGM (2000) The effect of nitrogen on disease development and gene expression in bacterial and fungal plant pathogens. Eur J Plant Pathol 106: 493–506
Stanford A, Bevan M, Northcote D (1989) Differential expression within a family of novel wound-induced genes in potato. Mol Gen Genet 215: 200–208
Swarbrick PJ, Schulze-Lefert P, Scholes JD (2006) Metabolic consequences of susceptibility and resistance (race-specific and broad-spectrum) in barley leaves challenged with powdery mildew. Plant Cell Environ 29: 1061–1076
Tao Y, Xie Z, Chen W, Glazebrook J, Chang HS, Han B, Zhu T, Zou G, Katagiri F (2003) Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. Plant Cell 15: 317–330
Tian ZD, Liu J, Wang BL, Xie CH (2006) Screening and expression analysis of *Phytophthora infestans* induced genes in potato leaves with horizontal resistance. Plant Cell Rep 25: 1094–1103
Tör M, Gordon P, Cuzick A, Eulgem T, Sinapidou E, Mert-Türk F, Can C, Dangl JL, Holub EB (2002) *Arabidopsis SGT1b* is required for defense signaling conferred by several downy mildew resistance genes. Plant Cell 14: 993–1003
Turkensteen LJ (1993) Durable resistance of potatoes against *Phytophthora infestans*. In: Jacobs T, Parlevliet JE (eds) Durability of Disease Resistance. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 115–124
Vos J (1996) Input and offtake of nitrogen, phosphorus and potassium in cropping systems with potato as a main crop and sugar beet and spring wheat as subsidiary crops. Eur J Agron 5: 105–114
Wang B, Liu J, Tian Z, Song B, Xie C (2005) Monitoring the expression patterns of potato genes associated with quantitative resistance to late blight during *P. infestans* infection using cDNA microarrays. Plant Sci 169: 1155–1167
Ward ER, Uknes SJ, Williams SC, Dincher SS, Wiederhold DL, Alexander DC, Ahl-Goy P, Metraux J-P, Ryals JA (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell 3: 1085–1094

Edited by P. Nissen

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.