Transcript and metabolite analysis of the *Trichoderma*-induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana*

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In the present study we have assessed, by transcriptional and metabolic profiling, the systemic defence response of *Arabidopsis thaliana* plants to the leaf pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) induced by the beneficial fungus *Trichoderma asperelloides* T203.

Expression analysis (qPCR) of a set of 137 *Arabidopsis* genes related to *Pst* defence responses showed that T203 root colonization is not associated with major detectable transcriptomic changes in leaves. However, plants challenged with the bacterial pathogen showed quantitative differences in gene expression when pre-inoculated with T203, supporting priming of the plant by this beneficial fungus. Among the defence-related genes affected by T203, lipid transfer protein (LTP)4, which encodes a member of the lipid transfer pathogenesis-related family, is upregulated, whereas the WRKY40 transcription factor, known to contribute to *Arabidopsis* susceptibility to bacterial infection, shows reduced expression. On the other hand, root colonization by this beneficial fungus substantially alters the plant metabolic profile, including significant changes in amino acids, polyamines, sugars and citric acid cycle intermediates. This may in part reflect an increased energy supply required for the activation of plant defences and growth promotion effects mediated by *Trichoderma* species.

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INTRODUCTION

Trichoderma species are beneficial opportunistic fungi which, apart from being very efficient mycoparasites, can interact with roots of diverse plant species leading to induced systemic resistance (ISR) responses to a wide spectrum of pathogens and adverse environmental conditions (Shoreish *et al.*, 2010; Lorito *et al.*, 2010).

Plants, whose roots are colonized by *Trichoderma* species can respond faster and/or more intensively to a pathogen attack following a mechanism known as priming, similar to the mechanism triggered by non-pathogenic rhizobacteria (Verhagen *et al.*, 2004; Shoreish *et al.*, 2010). An increasing number of studies have focused on the molecular features underlying the *Trichoderma*–plant–pathogen interaction.

Trichoderma-related beneficial effects vary with respect to the plant genotype, the fungal strain and its concentration (Segarra *et al.*, 2007; Tucci *et al.*, 2011).

In *Arabidopsis thaliana*, *Trichoderma asperellum* induces systemic resistance against the foliar bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) (Segarra *et al.*, 2009). In this system, *T. asperellum* T34 ISR has been found to be regulated by a jasmonic acid (JA)- and ethylene (Et)-dependent defence signalling pathway.

In recent years, only a few studies have reported on the global transcriptome and proteome changes in plants colonized with *Trichoderma* species. *T. hamatum* 382 systemically induced about 40 genes in tomato plants with functions associated with biotic or abiotic stress, as well as RNA, DNA and protein metabolism (Alfano *et al.*, 2007). A total of 205 differentially expressed proteins, over both roots and shoots, were identified in maize plants inoculated by *T. harzianum* T22 (Shoreish & Harman, 2008). These studies led to the identification of genes and proteins that are likely involved in the beneficial interaction between

Abbreviations: Et, ethylene; GC-MS, gas chromatography-MS; ISR, induced systemic resistance; JA, jasmonic acid; PR, pathogenesis-related; qPCR, quantitative PCR.

A supplementary table of primer sequences is available with the online version of this paper.

Trichoderma species and plants, but did not examine challenge of the primed system with the pathogen. Since at present the *A. thaliana*–*Pst* model is one of the best available for studying plant–pathogen interactions, this model may well be extended to the three-way interaction between plants, *Trichoderma* and the pathogen.

In the present work, we have therefore analysed the systemic response of *A. thaliana* Col-0 plants to three types of interaction: (1) colonization of the root system with the well known biological agent and inducer of defence responses *T. asperelloides* T203 (Yedidia *et al.*, 2003), (2) infection of leaves with *Pst* alone and (3) colonization of roots with *T. asperelloides* T203 followed by infection of leaves with *Pst*.

To obtain a more comprehensive overview of the response, samples were analysed on two levels, i.e. the metabolites (largely limited to primary metabolites) and the transcripts (limited to genes known to respond to *Pst* infection).

Our results show that co-cultivation with *T. asperelloides* T203 protects *Arabidopsis* plants against *Pst* (*hrpA*[−]) infection. No major detectable changes in gene expression were found in leaves in response to colonization of the roots by T203. However, after challenge with the bacterial pathogen, we observed a substantial increase in the number of genes, which showed an augmented expression pattern in plants colonized by T203 compared with plants not colonized, suggesting that T203 pretreatment leads to induced changes upon pathogen infection. Furthermore, we detected altered metabolic signatures of the different interactions. These results are discussed in light of the protective effect of *Trichoderma* species on *A. thaliana*.

METHODS

Fungal and bacterial strains. *T. asperelloides* T-203 (Samuels *et al.*, 2010) from Professor Chet's collection (Hebrew University of Jerusalem, Israel) was grown on potato dextrose agar (PDA; Difco) for 10 days. T203 conidia were harvested by gentle scraping in 5 ml sterile water, counted in a haemocytometer and 10^9 c.f.u. ml^{−1} grown overnight in 100 ml synthetic medium (Yedidia *et al.*, 2003) to allow germination. *P. syringae* pv. *tomato* DC3000 (*Pst hrpA*[−], Roine *et al.*, 1997), received from Professor Jürgen Zeier (Heinrich-Heine University, Germany), was cultured overnight in liquid King's medium B at 28 °C. Bacterial cells were collected by centrifugation (2500 r.p.m.), and resuspended in 10 ml 10 mM MgCl₂.

Plant cultivation and inoculation. For gas chromatography-MS (GC-MS) analysis *A. thaliana* (Col-0) plants were grown for 25 days under long day conditions (16 h light/8 h dark, 120 μmol photons m^{−2} s^{−1}) at 21 °C and 70 % relative humidity, in 2 × autoclaved soil supplemented with or without T203 [10^6 conidia (g soil)^{−1}]. Thirty plants in each group were sprayed with a *Pst* (10^6 c.f.u. ml^{−1}) suspension or an MgCl₂ mock inoculation. Rosette leaves were collected 2 days after *Pst* inoculation at the end of the day by shock-freezing with liquid nitrogen. For gene expression analysis by quantitative PCR (qPCR), *A. thaliana* (Col-0) plants were grown for 21 days under the same conditions indicated above, on rock-wool placed over a 5 l tank supplemented with *Arabidopsis* hydroponic solution (Lanquar *et al.*, 2009). The experiment included four different treatments: (1) untreated plants (control, C); (2) plant

challenged with *Pst* for 48 h (+P); (3) T203 treated plants for 96 h (+T); and (4) plants treated with T203 (48 h) and challenged with *Pst* for 48 h (+T+P). T203 spore suspension was injected into the *Arabidopsis* root growth compartment, resulting in a final concentration of 10^5 germinated spores ml^{−1}. Two days after T203 application, 35 of the plants in each group (control and treated with T203) were sprayed with a *Pst* suspension (10^6 c.f.u. ml^{−1}) or a 10 mM MgCl₂ mock inoculum solution. Rosette leaves were collected 2 days after bacterial challenge.

The protective effect of *Trichoderma* against *Pst* was also validated in a soil system. For the soil system, plant growth conditions, fungal inoculation and bacterial challenge were as described above for GC-MS analysis. Disease reduction was quantified by assessing the *Pst* intrafoliar population [c.f.u. (g fresh weight)^{−1}] as described by Perchev *et al.* (2006). We tested 35 plants for each of the two conditions (+Pst or +T203+Pst in hydroponics and soil) to obtain the c.f.u. in two independent experiments. Boxplots and *t*-tests on the obtained c.f.u. values were done in R. (*t*-test hydr, $P=4 \times 10^{-5}$; *t*-test soil, $P=4 \times 10^{-9}$).

Metabolite analysis. GC-MS analysis was performed as described previously (Lisec *et al.*, 2006). Six replicates, each consisting of 30 pooled plants obtained from two independent experiments, were subjected to GC-MS analysis. Metabolite levels were determined in a targeted fashion using the TargetSearch software package (Cuadros-Inostroza *et al.*, 2009). Metabolites were selected by comparing their retention indices (± 2 s) and spectra (similarity >85 %) against the compounds stored in the Golm-Metabolome-Database (GMD, Kopka *et al.*, 2005), this resulted in 61 metabolites which were kept in the data matrix. Each metabolite is represented by the observed ion intensity of a selected unique ion which allows for a relative quantification between groups. Metabolite data were log₁₀-transformed to improve normality (Steinfath *et al.*, 2008) and normalized to show identical medium peak sizes per sample group. Statistical analyses and graphical representations [Student's *t*-test, ANOVA, Bonferroni-correction, principal component analysis (PCA), boxplots, heatmaps] were performed using the R-software environment 2.13.0 (<http://cran.r-project.org/>).

The PCA was conducted using the 'bpca' algorithm of the pcaMethods package (Stacklies *et al.*, 2007) after a 'pareto' scaling. The data were produced using the heatmap function in R with row-wise scaling and correlation-based clustering. To improve the contrasts between low and high metabolite levels, all observations for one metabolite were divided by their median value and log₂-transformed to centre them around zero.

Expression profiling by qPCR. Expression analysis was performed using an expression profiling platform that covers 137 genes that were selected based on a literature survey for genes that are involved in the defence response against *Pseudomonas* species in *Arabidopsis*. Primer sequences were designed using the QuantPrime programme. All genes and primers are listed in Supplementary Table S1 (available with the online version of this paper).

Total RNA was purified using the RNeasy Mini kit (Qiagen) and DNase I digestion was performed with the Turbo DNA-free kit (Ambion) according to the supplier's instructions. Four micrograms of total RNA was used as template for first-strand cDNA synthesis with RevertAid cDNA synthesis kit (Fermentas). cDNA (20 ng) was used for qPCR with power SYBR green reagent performed on an ABI PRISM 7900HT sequence detection system (Applied Biosystems). Data were analysed with the 7900 v2.0.3 evaluation software (Applied Biosystems). The fold change in the target genes was normalized to *ACTIN2* and *GADPH* reference genes. Fold expression relative to control plants was determined using the $\Delta\Delta CT$ as described by Libault *et al.* (2007). Three biological experiments (with two

independent replicates for each experiment) were performed for each treatment. Comparisons of the gene expression between the different treatments to the control were conducted by using Student's *t*-test. Difference with $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Colonization of *Arabidopsis* roots by *T. asperelloides* T203 decreases *P. syringae* proliferation in leaves

To validate T203-mediated ISR in *Arabidopsis*, *P. syringae* pv. *tomato* DC3000 (*Pst*), was used to challenge leaves of 21-day-old plants 48 h after application of T203 to the root system. An aseptic hydroponic growth system was used with spatial separation of the root-inducing agent from the leaf pathogen. Four days after bacterial challenge, control plants displayed necrotic lesions surrounded by extensively spreading chlorosis, whereas T203 pretreated plants (+T203) showed significantly fewer symptoms. Disease reduction was quantified by assessing *Pst* intrafoliar population (c.f.u. g⁻¹). As shown in Fig. 1, for the hydroponics system, a 54 % reduction in the average bacterial population (c.f.u. g⁻¹) was observed in plants that were pretreated 48 h before the bacterial challenge with T203 (+T203 + *Pst*) compared with non-treated plants (+*Pst*). Similar results were obtained when *Arabidopsis* seedlings were germinated and grown in soil inoculated with T203 spores for 4 weeks (Fig. 1) demonstrating equivalence of the two systems. These data confirm the findings reported for *Arabidopsis* colonized by *T. asperellum* T34 (Segarra *et al.*, 2009).

Gene expression in *Pseudomonas*-infected *Arabidopsis* leaves is affected by *Trichoderma* root colonization

In order to gain insight into systemic changes (changes in the leaf) observed as a result of roots colonized by T203, the expression pattern of 137 genes was followed by qRT-PCR for samples taken from *Arabidopsis* leaves of either untreated plants (C), plants challenged with *Pst* for 48 h (+*Pst*), T203 treated plants for 96 h (+T203) or plants treated with T203 (48 h) and challenged with *Pst* for 48 h (+T203 + *Pst*). These genes include 54 transcription factors, known to respond via changes in expression level to *Pseudomonas* DC3000 infection and genes involved in signalling pathways with respect to resistance to biotic and/or abiotic stress (listed in Supplementary Table S1). Tables 1 and 2 display the genes whose expression was significantly affected ($\geq$ twofold and $P \leq 0.05$) in the different treatments. A relatively low number of genes (eight) was significantly affected in leaves of plants colonized by T203 only (+T203) (Table 1), most of them related to Et/JA signalling (TAT3, *eto3*, *etr1*). Interestingly the gene *HLS1*, involved in auxin signalling and cell growth, is systemically upregulated by T203 root colonization. In contrast, a major difference can be observed when comparing gene expression patterns of plants first treated with T203 and subsequently challenged with *Pst*

Fig. 1. Population of *P. syringae* pv. *tomato* (*Pst*) in *A. thaliana* leaves. Disease reduction was measured in plants grown in hydroponics and soil. For c.f.u. measurement, leaves were collected 48 h after challenge with *Pst*. Boxplots show the distribution of observed c.f.u. values for two sets of 60 randomly selected leaves from two independent experiments. The lower and upper ends of each box represent the 25 and 75 % quantiles, horizontal lines represent the means, small circles indicate possible outliers. In Student's *t*-test, the *P*-value is 4×10^{-5} and 4×10^{-9} for the hydroponics and soil experiments, respectively.

(+T203 + *Pst*) compared with plants directly challenged with *Pst* (+*Pst*) which confirms and extends similar observations made in the cucumber system which demonstrated priming of selected defence genes (Shoresh *et al.*, 2005; Yedidia *et al.*, 2003). T203 root pretreatment strongly activates the expression of the gene *LTP4* which encodes a member of the lipid transfer protein family [PR-14 pathogenesis-related (PR) protein family). Various LTPs have been shown to have *in vitro* antimicrobial activities against fungi and bacteria which result from the interaction of LTPs with biological membranes, possibly leading to membrane permeabilization (Sels *et al.*, 2008). Other PR coding genes (*PR1*, *PR2*) and Et/JA responsive genes (*eir1*, *eto3*, *lox2*) show a massive increase in expression as a result of +T203 + *Pst* treatment versus +*Pst* treatment (Table 1).

Amongst the transcription factors, the Et-responsive transcription factor *ERF13* is strongly induced in the +T203 + *Pst* treatment, further supporting the Et signalling cascades activated by T203 in this system. The *Arabidopsis* *WRKY40* transcription factor is pathogen-induced and encodes a WRKY protein which has a partially redundant negative effect on salicylic acid-mediated

Table 1. Fold-change ratio of responsive genes in *Arabidopsis* leaves

Arabidopsis plants were grown in hydroponic culture and rosette leaves were collected at the same time for each treatment. The experiment included four different treatments: 1, untreated plants (C); 2, plant challenged with *Pst* for 48 h (+P); 3, plants treated with *T. asperelloides* for 96 h (+T); and 4, plants treated with *Trichoderma* (48 h) and challenged with *Pst* for 48 h (+T+P).

Comparison	AGI no.	Fold change ratio*	Gene
+T and C	AT3G49700	6.41	eto3
	AT1G66340	2.19	etr1
	AT4G37580	3.76	hls1
	AT1G75040	4.75	PR5
	AT5G24780	-6.59	VSP
	AT4G37980	4.03	ELI3
	AT5G59310	-22.19	LTP4
	AT2G24850	3.82	TAT3
	AT5G57090	6.01	eir1
	AT3G49700	32.81	eto3
+T+P and C	AT4G02780	3.18	ga1
	AT1G79460	-4.18	ga2
	AT4G39030	2.41	SID1
	AT2G14610	10.17	PR1
	AT3G57260	7.08	PR2
	AT3G45140	7.18	LOX2
	AT2G37040	4.00	PAL1
	AT4G37980	2.26	ELI3
	AT3G15730	2.82	PLDalpha1
	AT3G25070	2.01	RIN4 RPM1
	AT5G44510	14.63	TAO1
	AT1G74710	3.64	SID2
	AT5G59310	10.89	LTP4
	AT1G75830	3.52	PDF1,2
	AT1G66100	3.27	PR13
	AT2G24850	19.02	TAT3
	AT1G66880	21.38	F4N21
	AT1G79460	-4.79	ga2
	AT5G64930	2.74	cpr5
	AT1G02450	-9.27	NIMIN1
+P and C	AT1G75040	8.86	PR5
	AT3G57260	8.41	PR2
	AT5G44420	-19.29	PDF1,2
	AT3G57240	-65.63	BG3
	AT2G37040	2.30	PAL1
	AT3G44300	7.99	NIT2
	AT4G37980	7.05	ELI3
	AT5G05730	4.60	ASA1
	AT4G39980	4.45	DHS1
	AT5G24770	6.43	VSP2
	AT5G44510	8.17	TAO1
	AT1G74710	3.37	SID2
	AT2G38900	106.19	PR6
	AT1G66100	11.22	PR13
	AT2G24850	24.30	TAT3
	AT1G72520	4.83	LOX4
	AT1G66880	12.20	F4N21

*Significant change in gene expression $\geq$ twofold and $P \leq 0.05$.

defence but exerts a positive role in JA-mediated defence (Xu *et al.*, 2006). Constitutive expression of WRKY40 (or WRKY60) results in increased susceptibility of these plants to *Pseudomonas* infection (Xu *et al.*, 2006). T203 root inoculation significantly affects the expression pattern of WRKY40 which is strongly upregulated in the leaves of *Pst*-infected plants but is expressed at a fourfold lower level in T203 pretreated plants (Table 2), suggesting a link between resistance to bacterial infection conveyed by T203 and the expression level of this transcription factor. Other transcription factors significantly affected in T203 treated plants are HIG1/MYB51, a regulator of indolic glucosinolate biosynthesis plant secondary metabolites, which controls responses to biotic challenges (Gigolashvili *et al.*,

Table 2. Fold change ratio of responsive transcription factors in *Arabidopsis* leaves

Arabidopsis plants were grown in hydroponic culture and rosette leaves were collected at the same time for each treatment. The experiment included four different treatments, as described in the legend for Table 1.

Comparison	AGI no.	Gene	Fold-change ratio*
+T and C	AT1G18570	MYB51	6.59
	AT1G73500	MKK9	3.33
	AT1G80840	WRKY40	10.30
	AT2G18170	MPK7	2.98
	AT2G44840	ERT13	3.04
	AT2G38470	WRKY33	3.54
	AT2G40740	WRKY55	3.61
	AT3G50060	MYB77	2.67
	AT4G11070	WRKY41	3.74
	AT1G18570	MYB51	3.08
+T+P and C	AT1G80840	WRKY40	5.22
	AT2G23320	WRKY15	2.26
	AT2G44840	ERT13	12.05
	AT3G01080	WRKY58	7.76
	AT3G50060	MYB77	2.35
	AT5G49520	WRKY48	2.75
	AT5G56580	MKK6	-2.80
	AT1G13960	WRKY4	3.32
	AT1G18570	MYB51	3.55
	AT3G12250	TGA6	2.93
+P and C	AT1G32640	JIN1	6.65
	AT2G44840	ERT13	7.77
	AT1G51660	MKK4	2.88
	AT1G73500	MKK9	4.19
	AT1G80840	WRKY40	22.18
	AT2G18170	MPK7	5.53
	AT2G38470	WRKY33	4.28
	AT2G40740	WRKY55	3.24
	AT3G21220	MKK5	1.68
	AT3G23250	MYB15	14.82
	AT4G26070	MKK1	-2.15
	AT5G49520	WRKY48	4.30

*Significant change in gene expression $\geq$ twofold and $P \leq 0.05$.

2007), and MYB77, a transcription factor which modulates plant responses to auxin, thereby controlling lateral root growth and development under changing environmental conditions.

***T. asperelloides* T203 root colonization changes the primary metabolite signature in *Arabidopsis* leaves**

Changes in primary metabolites of *A. thaliana* leaves were again determined for all four conditions (control, +T203, +Pst and +T203+Pst). The relative levels of 61 known metabolites have been determined in all samples. In a first analysis, all data were subjected to a principal component analysis (PCA; Fig. 2). The PCA scores plot reveals a clear separation of the four different groups (C, +Pst, +T203 and +T203+Pst) with the six biological replicates of each treatment always and, importantly, nicely clustering together, demonstrating on one hand the high reproducibility of the different treatments and on the other hand the significant and differential effect of the treatments on the metabolic level.

Fig. 3 shows a heatmap displaying all metabolite data (six replicates for each condition) again in agreement with the PCA, demonstrating the high level of reproducibility between the biological replicates and the major difference due to the four different treatments.

Even though repression of photosynthesis and induction of sink metabolism seem to be a general response to microbe infection, the effect on sugar levels varies considerably

between different plant–microbe interactions. While bacterial infection causes a pronounced decrease in most of the compounds (yellow–red colour in Fig. 3), much smaller changes are observed in the case of root infection by T203 alone (sugars/sugar alcohols with the exception of glucose, however, are largely unchanged).

The activation of plant defences and growth promotion requires an increased energy supply that ultimately must come from photosynthesis, and probably needs to be accompanied by greater respiratory rates (Bolton *et al.*, 2008; Shores *et al.*, 2010). In a proteomic study of the *T. harzianum* T22–maize system, the most commonly affected differential proteins expressed in *Trichoderma*-inoculated plants were those involved in carbohydrate metabolism, especially those in the glycolytic, TCA or respiratory pathways (Shores & Harman, 2008).

Our data show a decrease in fumaric acid in T203-treated *Arabidopsis* plants. Similar results were reported for *Arabidopsis* plants infected with the plant-growth-promoting and ISR-inducing bacterium WCS358r (Doornbos *et al.*, 2009), where the decrease in Krebs cycle intermediates was related to an increased need for carbon skeletons and increased respiration.

Polyamines (PAs) are organic polycations found in all living organisms. In higher plants, putrescine (Put), spermidine (Spd) and spermine (Spm) are the most abundant PAs and are involved in various developmental processes and adaptation to various stresses (Tonon *et al.*, 2004). It is noteworthy that the level of Put is significantly increased in T203-colonized plants. Sarjala *et al.* (2010) have recently presented data indicating that certain polyamines, especially Put, correlate with the growth increase in pine seedlings promoted by ectomycorrhizal fungi. A similar correlation could thus be of importance for the growth promotion effect induced by *Trichoderma* species in plants.

A clear increase in amino acid levels can be measured in +T203+Pst plants (Fig. 3), suggesting priming of these compounds. Leucine, valine and isoleucine are important precursors for the synthesis of plant defence metabolites such as cyanogenetic glycosides, and glucosinolates (Coruzzi & Last, 2000).

Methionine levels are also increased under +T203+Pst conditions. A biological action of L-methionine as an inducer of plant defence responses has been suggested (Sarosh *et al.*, 2005). In addition, methionine via S-adenosyl methionine (SAM) is a precursor for Et biosynthesis (Bleecker & Kende, 2000), thus supporting the involvement of Et in the ISR.

Glutamate is increasingly discussed as a signalling compound (Forde & Lea, 2007) specifically in the context of nitrogen signalling pathways that enable the plant to monitor and adapt to changes and also affect root structure. An increase in glutamine levels linked to a decrease in glutamate can be seen in both T203-treated plants and plants challenged with the bacteria and colonized by the fungus.

Fig. 2. PCA of 61 metabolites detected in GC–MS analysis of *Arabidopsis* rosette leaves. C, Untreated plants (control); +Pst, plants challenged with *Pst* for 48 h; +T203, plants germinated and grown in *Trichoderma*-inoculated soil; and +T203+Pst, plants germinated and grown in *Trichoderma*-inoculated soil and challenged with *Pst* for 48 h.

Fig. 3. Heatmap representation of the row-wise normalized levels of 61 metabolites in 24 samples (six independent biological replicates for each treatment: C, +Pst, +T203 and +T203 +Pst). Samples were collected at identical time points. To improve the contrast between low and high metabolite levels, all observations for one metabolite were divided by their median value and $\log_2$ -transformed to centre them around zero. *Tartronic acid, 2-methylaminomethyl; beta-D-fructofuranose, beta-D-fructofuranosyl-(2,1)-beta-D-fructofuranose.

The α -amino group of glutamate may be transferred to other amino acids by the action of a wide range of multispecific aminotransferases. In addition, glutamate forms the basis for

the synthesis of proline, considered to protect plants against certain abiotic stresses (Szabados & Savouré, 2010). Proline levels are indeed increased in +T203 plants.

Inoculation of plants with *Trichoderma* has been observed to cause expression of phenylalanine ammonia lyase (PAL) genes (Yedidia *et al.*, 2003 and Table 1). Aromatic amino acids, including phenylalanine, are upregulated in *Trichoderma* pre-treated plants after bacterial challenge. The shikimate pathway provides the basic building blocks for the synthesis of aromatic compounds required for functions as different as UV protection, electron transport, signalling, communication, plant defence, structural components and the wound response (Tzin & Galili, 2010).

Nitrogen signalling intersects with other signalling networks to control plant growth and development. The ability to appropriately allocate and subsequently reuse nitrogen, together with nitrogen uptake, is the major determinant of nitrogen use efficiency (NUE), and hence for optimized plant growth. Several *Trichoderma* strains were shown to improve yields by increasing NUE. Correlation analyses over several strains and nitrogen levels revealed that nearly all of the yield variation could be explained by the nitrogen variation levels in plants (Shoreish *et al.*, 2010; Harman, 2011). The increased level measured for several amino acids in T203-inoculated plants (Fig. 3) could be one of the determinants of the increased NUE, as amino acids are the major form of transported nitrogen. In mycorrhized plants, the plant root cells are assumed to take up amino acids that pass the fungal layer or are produced within the fungus (Damodaran *et al.*, 2010). A similar mechanism cannot be excluded for *Trichoderma*-colonized plants and further research should address this phenomenon which is highly important for *Trichoderma*-induced effects on crop yields.

Conclusions

Expression analysis of a set of genes related to the defence response to *Pst* in *Arabidopsis* has confirmed the priming state of the plant induced by the presence of this particular strain of *Trichoderma* in the roots via Et/JA signalling. Moreover, we show expression modulation of transcription factors, key regulators of defence and growth responses, by *Trichoderma* root colonization. Analysis of the primary metabolome reveals significant priming in the synthesis of amino acids, a phenomenon not previously detected in *Trichoderma*-plant interaction. Amino acid synthesis may directly or indirectly control various aspects of plant growth, development and defence. Therefore, it is remarkable that root colonization by a beneficial fungus known to ameliorate the general physiological state of the plant can substantially alter the metabolic profile by directly modulating the biosynthesis of the building blocks of a wide array of pathways.

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