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Phytochemical profiling of tomato roots following treatments with different microbial inoculants as revealed by IT-TOF mass spectrometry

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Abstract

Background: In light of the growing interest for eco-compatible fertilization, tomato plant roots were treated with four different strains of microorganisms (B1–B4) capable of positively affecting plant growth. The methanolic extracts from treated roots were analysed by reverse phase ultra-high-performance liquid chromatography hyphenated with ion trap time-of-flight high-resolution mass spectrometry to compare their metabolites with that of control plants (B0).

Results: We found, in both treated and control plants, several primary metabolites, such as fatty acids and coumaric acid, and other compounds associated with secondary metabolism pathways such as that of cyclopentaneoctanoic acid (CPOA) or hydroxyoctadecadienoic acid (HODE), and additional molecules which were not characterizable with the available data. A semiquantitative assessment of all metabolites became the basis for further processing the metabolic results by principal component analysis, which highlighted significant differences in the PC1 and PC2 components. The PC1 was particularly affected by the presence of arachidonic acid, myristic acid, and two unidentified metabolites. It effectively differentiated control plants from all bioeffectors treatments, and, in particular, the B4 treatment from the rest (B1–B3). The PC2 was mainly affected by palmitic acid, heptadecanoic acid, two CPOAs, one HODE and two unidentified metabolites. These metabolites successfully differentiated the B0 control from all the bioeffectors treatments, and, especially, showed a difference between B1 and B2.

Conclusions: Our findings suggest that changes in secondary pathways of lipid metabolism may underlie the biostimulation exerted by the four microbial bioeffectors of this study, and that LC–MS coupled by multivariate analysis can easily fingerprint the metabolic alterations induced by bioeffectors in tomato roots.

Background

Owing to the large application of fertilizers, pesticides, and mechanization, crop yields have increased to match the world population demands. However, the extensive usage of agrochemicals has begun to raise an environmental concern. Agricultural science is therefore facing the challenge of providing environmentally sustainable technologies to maintain, if not increase, crop yields without degrading the agricultural ecosystem. In this light, a developing research issue is the use of microbial species, alone or in combination with agricultural and industrial waste byproducts as biostimulants of plant growth (bioeffectors). Microorganisms such as mycorrhizae [1] and, more recently, bacteria [2] have been proved to optimize plant nutrients intake, thereby replacing inorganic fertilization without exhausting N, S and P stocks in heavily exploited soils. It is believed that such species can promote plant development, increase yields and counter stress conditions by acting directly or indirectly on the physiology of plants and, hence, on the stimulation of metabolic pathways [1, 2].

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The search for metabolic variances in plants as a function of either environmental conditions or management treatments has developed rapidly in recent years due to the advancement in sensitivity of analytical techniques. The field of metabolomics is now routine for the identification of primary and secondary metabolites in plant taking advantage of mass spectrometry (MS) [3–6] and for reaching a metabolic fingerprinting in plant tissues by both nuclear magnetic resonance (NMR) spectroscopy [7] and MS [8]. Fingerprinting is often used as a complementary tool to differentiate genetically modified species from pools of modified species [9], or to follow changes in the entire metabolism brought about by a specific treatment [10]. The latter application was already proved to efficiently differentiate mycorrhiza-treated plants from control [10]. However, most studies have been focused on the mechanisms by which microbial treatments affect plants preferentially from a genomic and transcriptomic standpoint [11, 12], thereby indicating that there is still ample margin to develop the metabolomic approach.

In the case of tomato plants (*Lycopersicum esculentum*), it has been reported an extensive characterization of metabolites in peel and flesh tissues of tomato fruits [13], and in response to a lack of micronutrients [14, 15]. While a current consensus holds that phenolic compounds are the main factors involved in adaptability of tomato plants to stress [16, 17], it is not yet clear which metabolic pathways are influenced in roots by microorganisms when they act as biostimulants [18].

The aim of this work was to assess the changes in the chemical profile of methanolic extract of tomato roots by high-resolution electrospray source with ion trap time-of-flight (IT-TOF) detector (ESI-IT-TOF–MS). This information, in turn may be correlated with changes in metabolism which the treated plants underwent when treated with different microbial biostimulants such as *Trichoderma harzianum* [11], Pseudomonas spp. [19], *Bacillus amyloliquefaciens* [2] and *Pseudomonas jessenii* [20] which are raising the interest of research due to their properties, and evaluate which metabolites were the results of the biostimulation.

Methods

Standards and chemicals

Ammonium acetate used as a buffer and all solvents used in gradients were purchased by Sigma-Aldrich (Chromasolv LC–MS quality).

Microbial bioeffectors and plant experiments

A greenhouse experiment was conducted to study the effects of four different bioeffectors on primary and secondary metabolism in tomato roots grown in low phosphorus soil (Table 1).

In this experiment, heavy loam soil (Luvic Cambisol) derived from the top soil of an unfertilized grassland area on the Hohenheim campus with available phosphorus (P) content 20 mg kg^{-1} was used (48°44′42.3″N 8°55′26.7″E; 475 m above sea level; 688 mm av. annual rainfall; 8.8 °C mean annual daily temperature, 24–28 % clay, 67–72 % silt, 4–5 % sand, pH ($CaCl_2$) 6.9, $^{**}C_{org}$, 1 %, $^{**}N_{min}$, 38 kg ha^{-1}; P_{CAL} (Calcium lactate method), 120 mg kg^{-1}). The soil was mixed with 50 % quartz sand (0.2–1.4 mm) weight in weight (W/W) and fertilized for N, K, Mg and P with Ca $(NO_3)_2$, K_2SO_4, $MgSO_4$ and Ca $(H_2PO_4)_2$ to reach 100, 150, 50 and 50 mg g^{-1} substrate dry weight, respectively. After fertilization, 3 litre pots were filled with 2.5 kg substrate (dry weight). *T. harzianum* strain T-22 (B1), *Pseudomonas* sp. (B2) and *B. amyloliquefaciens* FZB42 (FB01 mut1) (B3) were prepared as described in Table 1. *P. jessenii* RU47 (B4) were grown on King's B medium (Merck, Darmstadt, Germany) supplemented with 50 µg ml^{-1} rifampicin, 10 µg ml^{-1} tetracycline,

Table 1 Scheme describing of bioeffector treatments

BEs	Product name	Strains	Initial concentration in the product	Amount of BEs product[a]	Volume of strain stock suspension[b]	Final concentration of BEs[c]
B0	–	No active BE (control)	–	–	–	–
B1	Trianum P	*Trichoderma harzianum* strain T-22	10^9 spores g^{-1}	2.1 g	15 ml	2.5×10^4 spores
B2	Proradix	*Pseudomonas* sp.	6.6×10^{10} CFU g^{-1}	2.5 g	15 ml	2×10^6 CFU
B3	Rhizovital[d]	*Bacillus amyloliquefaciens* FZB42 (FB01 mut1)	2.5×10^{10} CFU ml^{-1}	20.2 ml	15 ml	6×10^6 CFU
B4	–	*Pseudomonas jessenii* RU47	1×10^9 CFU ml^{-1}	–	15 ml	6×10^6 CFU

[a] Resuspended in 500 ml sterile NaCl (0.3 %) (BEs stock suspension)

[b] Volume of strain stock suspension added per pot

[c] Expressed in g^{-1} substrate

[d] *Bacillus amyloliquefaciens* FZB42 was isolated from the commercial product "Rhizovital"

100 μg ml^{-1} ampicillin and 30 μg ml^{-1} chloramphenicol and incubated 48 h at 28 °C. The bacterial cells were harvested from the plates and resuspended in sterile NaCl (0.3 %). The bacterial cells were centrifuged at 12,000*g* for 5 min and the bacterial pellet was washed twice and resuspended in sterile NaCl (0.3 %). The cell suspension of B4 was adjusted to 10^9 cells ml^{-1} using spectrophotometer.

Tomato seeds (cultivar Mobil) were soaked in bioeffectors suspensions for 15 min at room temperature, while control tomato seeds were soaked in sterile NaCl (0.3 %). Treated seeds were directly sown in seedling trays filled with the substrate (soil, quartz and fertilizing nutrients) described above. Eight days after sowing, the seedlings were transferred to 3 l pots with three tomato plantlets per pot. The pots were drenched with 15 ml bioeffectors 10 days after sowing. Control plantlets were watered with the same volume of sterile NaCl (0.3 %). The drenching was repeated 17 days after sowing. Each treatment was conducted with five replicates. The pots were kept in the greenhouse at 28 °C and 30 % humidity. Samples were taken 43 days after seed inoculation and sowing (26 days after second drenching). Root samples were washed with distilled water and treated immediately with liquid nitrogen and stored in glass tubes at −80 °C for 2 days. The samples were lyophilized for 48 h.

Extraction of metabolites

Amounts (10 mg) of freeze-dried roots were transferred in 7-ml glass vials and 2.0 ml of LC–MS grade methanol were added, as reported by several research teams as a viable option to analyse secondary metabolites in plant roots [4, 21, 22]. The sample was sonicated at room temperature for 30 min to favour extraction, and then the residue was allowed to sediment for further 30 min. The supernatant was transferred to another 7-ml vial, while the residue was replenished with 2.0 ml of fresh methanol and sonicated for further 30 min. After the sedimentation of the residue, it was pooled with the previous one. The extract was then evaporated under a nitrogen flow until a volume of approximately 500 μl and then filtered on 0.20-μm cutoff Sartorius MFL syringe filter, and the filtrate was collected in autosampler vials. Finally, the methanol was evaporated from the autosampler vials to dry under nitrogen flow and 200 μl of fresh LC–MS methanol was added.

Ultra-high-performance liquid chromatography

Samples were run on a Shimadzu UHPLC system constituted by two LC-20AD XR UHPLC pumps, a Shimadzu DGU-20A 5R degassing unit, a Shimadzu SIL-20A XR autosampling unit, a Shimadzu SPD-N20A UV DAD detector equipped with a UHPLC cell, and a Shimadzu IT-TOF detector mounting an Electrospray source. A Reprosil-Pur Basic C18 (1.9 μm–100 × 2.0 mm) column was mounted in the UHPLC system and fed at 0.25 ml min^{-1} with solution made of 0.05 M NH_4CH_3COO in LC–MS grade water (A) and in LC–MS grade acetonitrile (B) by the following gradient: 95:5 A:B for 0.1 min; linear gradient to 80:20 in 10 min, then to 50:50 in 20 min and finally to 10:90 in 20 min. The system was kept at 10:90 for 5 min and then re-equilibrated to 95:5 in 10 min for a total of 65 min run cycle. The stationary phase was stabilized at 30 °C. Two replicate LC-MS analyses were carried out for each sample, injecting 10 μl of extracted solution for each chromatographic run.

Mass spectrometry

Mass analysis was carried out in positive and negative mode using the software automatic scan option. Spray voltage was set at 4.5 kV for both modes, and capillary and heat block temperatures were set at 200 °C. An accumulation time of 20 ms and a mass range of 100–700 Dalton was set for both scans, resulting in a loop cycle of 1.80 s. Tandem MS scans were set with CID energy at 50 units, an accumulation time of 40 ms and a mass range of 80–700 for MS^2 and 60 ms and 50–500 for MS^3, respectively. Ion precursor threshold limits used were, respectively, 300,000 for MS^2 and 50,000 for MS^3. All metabolites were semiquantitatively assessed by peak integration of the single ion chromatogram in BPC mode, and furthermore each mass was processed using the Shimadzu bundled software to yield the most likely empirical formula. The programme was given the following parameters: 0–100 C atoms, 0–10 N atoms, 0–300 H atoms, 0–50 O atoms, 0–5 S atoms with a maximum deviation of 10 ppm between calculated and experimental mass. Each empirical formula was compared with all major online metabolites databases (including Max Planck Institute for Plant Physiology, NIST Library and Plantmetabolomics.org, Fiehn Library) to find previously reported structures.

Statistics and principal component analysis

With five operational replicates and two analytical duplicates for each treatment, a set of ten measures was available for each metabolite, all of which displayed standard deviations ranging within 15 % as compared to the average. PCA analysis was performed using XlStat software (v.2012, Addinsoft). One-way ANOVA test was used to evaluate the significance (Tukey test, >95 %) by which the variables, highlighted by PCA loading-plot, differentiated the studied theses.

Results and discussion

Characterization and semiquantitative assessment of metabolites

Through the bundled software masses obtained by high resolution, negative scans were translated into a

restricted number of $C_xH_yO_z$ formulae. Due to the relatively low error of 10 ppm enabled by the Shimadzu IT-TOF instrumentation, we attributed empirical formulae to all detected masses. Oddly, positive scans did not show any plausible peak, probably due to the acidic nature of the metabolites, which hindered ionization in the positive mode. In some cases, tandem MS facilitated formula attribution by either allowing comparison with masses reported in the literature or ruling out equally plausible mother ions that were incompatible with the fragmentation pattern. By this method, the molecular structures of linear fatty acids reported in Table 2 and labelled as L1–14 were obtained by evaluating empirical masses and daughter ions. From a semiquantitative assessment of peak integrations (Table 3), the most abundant metabolites were palmitoleic (L6), linoleic (L8), palmitic (L10) and oleic (L11) acids. Their daughter ions (235, 261, 249, 237) (Table 2) were the result of the characteristic fragmentation of carboxylic acids [23].

An exact match with literature data allowed attribution of coumaric acid (CO) [13]. Other empirical formulae were assigned to cinnamic acid-like structures (CI1–3) by similarity with the CH backbone, although the presence of nitrogen suggested the presence of a primary amide derivative (Table 2). Formation of amides with other amines, namely putrescine and spermidine, is well established in the literature for carboxylic acid metabolites in tomato extracts [16, 17], thus explaining the hydrolysis

Table 2 Metabolites identified in IT-TOF LC–MS of treated and control tomato roots

Rt	$[M-H]^-$	MS/MS	MS/MS/MS	Calculated mass	Formula	Proposed molecule	ID
8.3	194.0466			195.0532	$C_9H_9NO_4$	Cinnamic acid-like 1	CI1
8.4	248.0342			249.0485	$C_8H_{11}NO_8$	Unidentified 1	U1
12.3	194.0461			195.0532	$C_9H_9NO_4$	Cinnamic acid-like 2	CI2
15.0	194.0488			195.0532	$C_9H_9NO_4$	Cinnamic acid-like 3	CI3
24.5	327.2171			328.2250	$C_{18}H_{32}O_5$	HODE-related compound 1	H1
30.1	163.0418	119, 149		164.0507	$C_9H_8O_3$	Coumaric acid-like	CO
33.4	265.1492			266.1518	$C_{15}H_{22}O_4$	Unidentified 2	U2
34.6	311.2206	183, 293	119, 177, 249	312.2301	$C_{18}H_{32}O_4$	HODE-related compound 2	H2
34.9	311.2225	183, 293	119, 177, 249	312.2301	$C_{18}H_{32}O_4$	HODE-related compound 3	H3
35.3	309.1774	183, 293	119	310.1827	$C_{11}H_{26}N_4O_6$	Unidentified 3	U3
35.5	353.1991			354.2056	$C_{20}H_{26}N_4O_2$	Unidentified 4	U4
35.6	326.1916			327.2006	$C_{12}H_{29}N_3O_7$	Unidentified 5	U5
36.0	293.1861	177, 185, 249	149	294.1943	$C_{16}H_{26}N_2O_3$	Cyclopentaneoctanoic acid related 1	CY1
36.6	293.2127	177, 249	149	294.2195	$C_{18}H_{30}O_3$	Cyclopentaneoctanoic acid related 2	CY2
37.0	295.2269			296.2351	$C_{18}H_{32}O_3$	HODE-related compound 4	H4
39.2	337.0569			338.0638	$C_{15}H_{14}O_9$	Unidentified 6	U6
39.4	295.2276	171, 277		296.2351	$C_{18}H_{32}O_3$	HODE-related compound 5	H5
39.9	250.1445	205, 235	148	251.1521	$C_{14}H_{21}NO_3$	Unidentified 7	U7
41.4	301.2168	283, 257		302.4518	$C_{20}H_{30}O_2$	Eicosapentaenoic acid	L1
41.8	277.2171	259, 233		278.2246	$C_{18}H_{30}O_2$	Linolenic acid	L2
42.0	227.2022	209		228.2089	$C_{14}H_{28}O_2$	Myristic acid	L3
42.2	283.2647			284.2715	$C_{18}H_{36}O_2$	Stearic acid	L4
42.4	413.2914			414.2981	$C_{23}H_{42}O_6$	Diacetoxypropyl palmitate (1,3 or 2,3)	L5
42.6	253.2183	235		254.2246	$C_{16}H_{30}O_2$	Palmitoleic acid	L6
43.0	303.2329			304.2402	$C_{20}H_{32}O_2$	Arachidonic acid	L7
43.3	279.2340	261		280.2402	$C_{18}H_{32}O_2$	Linoleic acid	L8
44.4	305.2482			306.2559	$C_{20}H_{34}O_2$	Eicosatrienoic acid	L9
44.3	267.2322	249		268.2402	$C_{17}H_{32}O_2$	Eptadecenoic acid	L10
45.0	255.2318	237		256.2402	$C_{16}H_{32}O_2$	Palmitic acid	L11
45.6	281.2480	263		282.2559	$C_{18}H_{34}O_2$	Oleic acid	L12
46.6	269.2498			270.2559	$C_{17}H_{34}O_2$	Eptadecanoic acid	L13
46.8	517.4082			518.4182	$C_{29}H_{58}O_7$	Unidentified 8	U8
47.3	417.3192			418.3294	$C_{23}H_{46}O_6$	2,8,10,19-tetrahydroxy-18-methyldocosanoic acid	L14

Table 3 Semiquantitative assessment of metabolites extracted from roots of tomato plants treated with different inoculants strains

Metabolite	Treatments					Proposed molecule
	B0	B1	B2	B3	B4	
	Relative abundance (%)					
CI1	0.83	0.82	1.11	1.19	0.72	Cinnamic acid-like 1
CI2	0.87	0.81	0.88	1.04	0.63	Cinnamic acid-like 2
CI3	1.09	1.63	0.92	0.94	1.49	Cinnamic acid-like 3
CO	1.12	1.27	1.40	1.64	1.33	Coumaric acid
CY1	2.52	1.49	1.34	2.43	1.75	Cyclopentaneoctanoic acid related 1
CY2	2.18	0.91	1.58	1.16	2.14	Cyclopentaneoctanoic acid related 2
H1	0.42	0.54	0.47	0.56	0.71	HODE-related compound 1
H2	1.21	0.75	0.49	0.53	0.92	HODE-related compound 2
H3	2.40	2.69	2.82	3.03	3.35	HODE-related compound 3
H4	1.81	0.90	1.28	0.80	1.36	HODE-related compound 4
H5	0.34	0.32	1.01	0.70	0.65	HODE-related compound 5
L1	2.30	3.26	3.67	2.81	3.21	Eicosapentaenoic acid
L2	3.16	4.67	4.11	4.52	4.13	Linolenic acid
L3	2.09	1.56	1.15	1.64	1.24	Myristic acid
L4	4.83	3.75	3.90	3.75	3.04	Stearic acid
L5	1.14	0.85	1.12	1.10	1.36	Diacetoxypropyl palmitate (1,3 or 2,3)
L6	10.15	13.80	18.40	15.63	14.25	Palmitoleic acid
L7	5.68	1.39	1.39	0.96	0.92	Arachidonic acid
L8	12.93	18.14	14.70	16.37	20.22	Linoleic acid
L9	0.91	1.55	1.65	1.42	1.45	Eicosatrienoic acid
L10	1.01	0.90	0.62	0.89	0.51	Eptadecenoic acid
L11	14.66	18.26	13.07	14.18	13.89	Palmitic acid
L12	9.08	7.92	8.67	7.27	7.75	Oleic acid
L13	1.41	0.97	1.04	1.12	0.73	Eptadecanoic acid
L14	1.63	1.26	1.59	1.56	1.08	2,8,10,19-tetrahydroxy-18-methyldocosanoic acid
U1	0.87	0.48	0.23	0.31	0.39	Unidentified 1
U2	4.00	3.25	3.57	4.05	3.08	Unidentified 2
U3	2.67	2.21	2.62	2.97	2.41	Unidentified 3
U4	1.98	1.24	1.76	2.12	1.75	Unidentified 4
U5	1.57	0.22	Traces	Traces	Traces	Unidentified 5
U6	0.34	0.39	0.81	0.41	0.92	Unidentified 6
U7	1.67	1.19	1.63	1.96	1.66	Unidentified 7
U8	1.13	0.60	0.98	0.96	0.98	Unidentified 8

of these secondary metabolites leading to the formation of CI1–3 (Table 2). The semiquantitative distribution of CO and CI metabolites was not highly abundant, nor significantly different in root extracts derived from different bioeffector treatments and from control.

In order to assign a chemical structure to metabolites designated as CY1–2 and H1–5, their empirical mass was compared with all the major metabolites described in online databases. Several matches to previously reported structures were found, all of which traditionally associated with tomato plants metabolism (Table 2). In particular, CY metabolites resulted from the secondary metabolic pathway of cyclopentaneoctanoic acid, which is associated with response to stress and derived from the linoleic acid precursor [24]. Their semiquantitative abundance was average with respect to the measurements of other analytes and varied greatly among B1–4 treatments and control, thereby suggesting that some of the microbial bioeffectors determined a decrease in production of plant antioxidants, as indicated by the lesser amounts of CY1, CY2 or both (Table 3). Conversely, the H1–5 metabolites (Table 2) were found to be associated

with the secondary pathway of a stress response deriving from the hydroxyoctadecadienoic acid (HODE), which may influence lateral root growth. The abundance of these compounds invariably increased for H3 and H5 and decreased for H2 and H4 for all the B1–4 treatments with respect to control. It may be inferred that this secondary pathway is altered by the biostimulation of the bioeffectors, which appeared to favour the production of H3 and H5 against that of H2 and H4. However, the lack of a more detailed characterization of these hormones prevents further speculation on the effects of the microbial species.

Finally, all other compounds for which no plausible matches were found in the databases were left unassigned and designated as U1–8 (Table 2). As a heterogeneous group, they present very different retention times, mass range and abundance at the LC–MS analyses. However, the U5 compound displayed an interesting pattern since its presence decreased dramatically in all extracts of microbially treated plants, until almost disappearance for the B2–4 treatments (Table 3).

PCA analysis

Root extracts from plants treated with bioeffectors and controls were explored and differentiated by principal component analysis. In fact, the PCA score plot (Fig. 1) indicates that two PC1 and PC2 principal components enabled a clear differentiation among treated samples.

PC1 and PC2 represented 35.4 and 34.1 % of the variance explained by each principal component, respectively. The positive axis of PC1 is associated with variables L3, L7, U5 and U7. In particular, U5 and L7 are generally decreasing with the *P. jessenii* (B4) treatment and may thus be used as markers of this particular microbial bioeffector species to differentiate its effect not only from control but also other biostimulants (Fig. 1). Interestingly, the L7 arachidonic acid is a precursor of a number of plant hormones, and its downregulation may indicate a suppression of lipase enzyme that is usually responsible for a stress response [25]. It has been shown that due to this pathway, *Arabidopsis thaliana* mutants are more prone to fungal infection, thereby indicating that the secondary metabolites, such as jasmonic acid, are part of a defensive mechanism [26]. However, the tomato roots treated with all bioeffectors downregulated this pathway, thus suggesting a lesser need for endogenous protection against phytopathogens under such treatments.

Conversely, myristic acid (L3) decreased in tomato roots treated with all B1, B2, B3 and B4 (Fig. 1), highlighting that the effect of these microorganisms can be easily differentiated from control and the induced downregulation affects also other secondary metabolic pathways. Finally, the U7 metabolite is able to further differentiate B1 and B2 from B3 to B4 (Fig. 1).

The PC2 axis of the score plot was positively associated to the U1, U2, CY1, CY2, L11, L13 compounds and

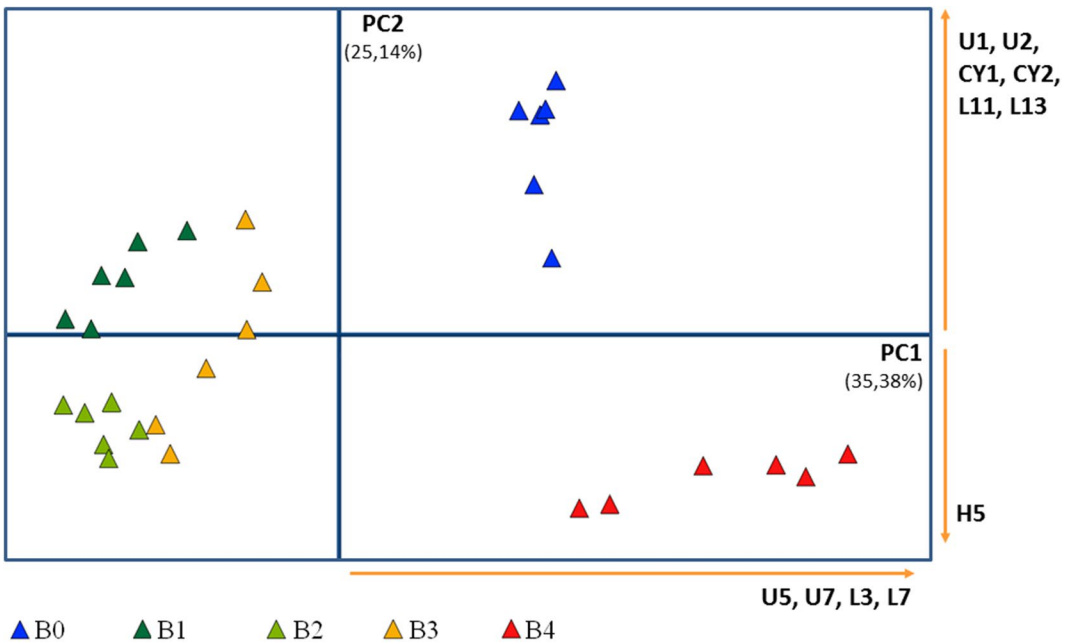

Fig. 1 Principal component analysis (PCA) score *plot* of root extracts from tomato plants treated with *Trichoderma harzianum* (B1 in *dark green*), Proradix (*Pseudomonas* sp.) (B2, in *light green*), *Bacillus amyloliquefaciens* (B3, in *yellow*), *Pseudomonas jesseni* (B4, in *red*) and control (B0, in *blue*). The name and the direction of most significant loading vectors involved in the differentiation among treatments are reported

negatively associated to H5. In particular, CY2 and H5 differentiate from control all roots treated with bioeffectors except B4 for the former and B1 for the latter, which strengthens the hypothesis that the inoculation of plant roots modifies their metabolism. This triggers a decreased response to stress that is ordinarily induced by either a phytopathogen attack or in the absence or scarcity of nutrients [17, 18, 24–28]. Other variables in this axis may be seen as markers to differentiate B2 from control (U1 and U2) and from B1 (CY1, L11 and L13) (Fig. 1). These findings support the hypothesis that, though a general trend to lower their stress response is observed in all treated roots, the metabolic pathways by which this result is accomplished differ significantly among the bioeffectors.

Conclusions

The analytical method presented here successfully represented the chemical profile by methanolic extraction from treated tomato roots and identified with high-resolution MS, through the determination of their empirical formulae. Comparison with metabolite databases in the literature and the use of tandem MS enabled the identification of chemical structures for most of these compounds. Finally, principal component analysis conducted on the semiquantitative data obtained by MS analyses allowed to well differentiate the chemical profiles of inoculated plants from that of control. Moreover, our approach also provided some marker compounds that represented the metabolic responses of different microorganisms. The inoculation of tomato roots with bioeffectors brought about a relevant change in secondary metabolism of tomato plant cells in respect to control, which underlies a remodulation of the biochemical pathways associated with either stress response to phytopathogens, increased salinity or scarcity of nutrients.

Authors' contributions

NE and KS carried out plant growth experiments and bio-effector inoculations. ADM and AN performed extraction of metabolites and chromatography runs. Formulae attribution and principal component analysis were carried out by ADM, AN and AP. All steps were supervised by KS and AP. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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References

1. Al-Karaki GN. Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza. 2000;10:51–4.
2. Qiao JQ, Wu HJ, Huo R, Gao XW, Borriss R. Stimulation of plant growth and biocontrol by *Bacillus amyloliquefaciens* subsp. plantarum FZB42 engineered for improved action. Chem Biol Technol Agr. 2014;1:1–12.
3. Wojakowska A, Piasecka A, García-López PM, Zamora-Natera F, Krajewski P, Marczak Ł, Kachlicki P, Stobiecki M. Structural analysis and profiling of phenolic secondary metabolites of Mexican lupine species using LC–MS techniques. Phytochemistry. 2013;92:71–86.
4. Granica S, Lohwasser U, Jöhrer K, Zidorn C. Qualitative and quantitative analyses of secondary metabolites in aerial and subaerial of Scorzonera hispanica L. (black salsify). Food Chem. 2015;173:321–31.
5. Mohn T, Plitzko I, Hamburger M. A comprehensive metabolite profiling of *Isatis tinctoria* leaf extracts. Phytochemistry. 2009;70:924–34.
6. Stobiecki M, Kachlicki P, Wojakowska A, Marczak Ł. Application of LC/MS systems to structural characterization of flavonoid glycoconjugates. Phytochem Lett. 2015;11:358–67.
7. Le Gall G, Colquhoun IJ, Davis AL, Collins GJ, Verhoeyen ME. Metabolite profiling of tomato (*Lycopersicon esculentum*) using 1H NMR spectroscopy as a tool to detect potential unintended effects following a genetic modification. J Agr Food Chem. 2003;51:2447–56.
8. Pellati F, Orlandini G, Benvenuti S. Simultaneous metabolite fingerprinting of hydrophilic and lipophilic compounds in *Echinacea pallida* by high-performance liquid chromatography with diode array and electrospray ionization-mass spectrometry detection. J Chromatogr A. 2012;1242:43–58.
9. Bino RJ, Hall RD, Fiehn O, Kopka J, Saito K, Draper J, Trethewey RN. Potential of metabolomics as a functional genomics tool. Trends Plant Sci. 2004;9:418–25.
10. Schliemann W, Ammer C, Strack D. Metabolite profiling of mycorrhizal roots of *Medicago truncatula*. Phytochemistry. 2008;69:112–46.
11. Samolski I, Rincón AM, Pinzón LM, Viterbo A, Monte E. The qid74 gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization. Microbiology. 2012;158:129–38.
12. Letzel AC, Pidot SJ, Hertweck C. A genomic approach to the cryptic secondary metabolome of the anaerobic world. Nat Prod Rep. 2013;30:392–428.
13. Moco S, Bino RJ, Vorst O, Verhoeven HA, de Groot J, van Beek TA, Vervoort J, De Vos CR. A liquid chromatography-mass spectrometry-based metabolome database for tomato. Plant Physiol. 2006;141:1205–18.
14. Løvdal T, Olsen KM, Slimestad R, Verheul M, Lillo C. Synergetic effects of nitrogen depletion, temperature, and light on the content of phenolic compounds and gene expression in leaves of tomato. Phytochemistry. 2010;71:605–13.
15. Urbanczyk-Wochniak E, Fernie AR. Metabolic profiling reveals altered nitrogen nutrient regimes have diverse effects on the metabolism of hydroponically-grown tomato (*Solanum lycopersicum*) plants. J Exp Bot. 2005;56:309–21.
16. Larbat R, Paris C, Le Bot J, Adamowicz S. Phenolic characterization and variability in leaves, stems and roots of Micro-Tom and patio tomatoes, in response to nitrogen limitation. Plant Sci. 2014;224:62–73.
17. Larbat R, Le Bot J, Bourgaud F, Robin C, Adamowicz S. Organ-specific responses of tomato growth and phenolic metabolism to nitrate limitation. Plant Biol. 2012;14:760–9.
18. Sung J, Sa Lee, Lee Y, Ha S, Songb B, Kim T, Waters BM, Krishnan HB. Metabolomic profiling from leaves and roots of tomato (*Solanum lycopersicum* L.) plants grown under nitrogen, phosphorus or potassium-deficient condition. Plant Sci. 2015;241:55–64.
19. Parray JA, Jan S, Kamili AN, Qadri RA, Egamberdieva D, Ahmad P. Current perspectives on plant growth-promoting rhizobacteria. J Plant Growth Regul. 2016. 1–26.
20. Patil HJ, Solanki MK. Microbial inoculant: modern era of fertilizers and pesticides. In microbial inoculants in sustainable agricultural productivity. India: Springer; 2016. p. 319–43.

21. Farag MA, Porzel A, Wessjohann LA. Comparative metabolite profiling and fingerprinting of medicinal licorice roots using a multiplex approach of GC–MS, LC–MS and 1D NMR techniques. Phytochemistry. 2012;76:60–72.
22. Chamam A, Sanguin H, Bellvert F, Meiffren G, Comte G, Wisniewski-Dye F, Bertrand C, Prigent-Combaret C. Plant secondary metabolite profiling evidences strain-dependent effect in the *Azospirillum-Oryza sativa* association. Phytochemistry. 2013;87:65–77.
23. Kerwin JL, Wiens AM, Ericsson LH. Identification of fatty acids by electrospray mass spectrometry and tandem mass spectrometry. J Mass Spec. 1996;31:184–92.
24. Delaplace P, Frettinger P, Ghanem ME, Blondiaux A, Bauwens J, Cotton S, De Clerck C, Dewalque A, Guy J, Heuze F, Massoz A, Tassignon T, van Aubel G, du Jardin P, Fauconnier ML. Lipoxygenase pathway and antioxidant system in salt stressed tomato seedlings (*Lycopersicon esculentum* Mill.). Biotechnol Agron Soc. 2009;13:529–36.
25. Lauria S, Casati S, Ciuffreda P. Synthesis and characterization of a new fluorogenic substrate for monoacylglycerol lipase and application to inhibition studies. Anal Bioanal Chem. 2015;407:8163–7.
26. Burow M, Atwell S, Francisco M, Kerwin RE, Halkier BA, Kliebenstein DJ. The glucosinolate biosynthetic gene *aop2* mediates feed-back regulation of jasmonic acid signaling in *Arabidopsis*. Mol Plant. 2015;8:1201–12.
27. Vellosillo T, Martínez M, López MA, Vicente J, Cascón T, Dolan L, Hamberg M, Castresana C. Oxylipins produced by the 9-lipoxygenase pathway in *Arabidopsis* regulate lateral root development and defense responses through a specific signaling cascade. Plant Cell. 2007;19:831–46.
28. Staswick PE, Yuen GY, Lehman CC. Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus pythium irregulare. Plant J. 1998;15:747–54.