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Article

Metabolomics Analysis of *Combretum lanceolatum* Roots in the Presence of Its Endophytic Fungi

Lenard Serrano,[a] Jhuly W. F. Lacerda,[a] Mariana S. Moura,[a] Akbar Ali,[b] Leonardo G. de Vasconcelos,[a] Paulo T. Sousa Junior,[a] Barbara S. Bellete,[c] Marcos A. Soares,[d] Lucas C. C. Vieira[a] and Olívia M. Sampaio[,a]*

[a]*Departamento de Química, Universidade Federal de Mato Grosso, 78060-900 Cuiabá-MT, Brazil*

[b]*Department of Chemistry, Government College University, 38000, Faisalabad, Pakistan*

[c]*Departamento de Química, Universidade Federal de Lavras, 37200-900 Lavras-MG, Brazil*

[d]*Instituto de Biociências, Universidade Federal de Mato Grosso, 78060-900 Cuiabá-MT, Brazil*

The aim of this work was to identify and quantify the metabolites present in the roots of *Combretum lanceolatum* inoculated with its endophytic fungi. The metabolomics was accomplished using the ^{1}H nuclear magnetic resonance (NMR) spectral data and evaluated via rNMR software and Madison Metabolomics Consortium Database (MMCD). The principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) showed that plants inoculated with *Trichoderma spirale* (*Ts*) present differentiation and discrimination over the time compared to control. Seven days after *Ts* fungal inoculation, 15 metabolites were identified at different concentrations comparing to the control plants. The plants inoculated with *Ts* fungus present the metabolites spermidine and pantothenate in higher concentrations and 3-hydroxybutyric acid and β-alanine in lower concentrations compared to control plants, indicating any response to biotic stress. These metabolites are involved in various plant processes, including secondary metabolites biosynthesis, energy metabolism and self-defense. Therefore, this work demonstrates the diversification of primary metabolites composition influenced by endophytes inoculation.

Keywords: plant-fungus interaction, chemical profile, primary metabolism, *Trichoderma spirale*, *Diaporthe phaseolorum*

Introduction

The plant-fungus symbiosis is a harmonic type relationship established between plants and endophytes present within the tissues, especially in the roots of plants.[1] Endophyte plays an asymptomatic relationship with the host plant, leading both morphophysiological and biochemical changes such as the biosynthesis of the primary and secondary metabolites, which improves the microbiota development and resistance of the plant to biotic and abiotic stresses.[2]

The chemical investigation of plant-fungi relationship has been described on several occasions, such as the auxin signaling role of *Trichoderma virens* in plant growth regulation,[3] the increase of aroma compounds in *Aquilaria* spp plants associated with *Fusarium* fungi[4] and the *Rhizophagus irregularis* colonization of *Senecio jacobaea* plants, which caused significant changes in the root metabolome by increasing the chemical defenses.[5] In this context, the study of a plant-fungus relationship, especially on their metabolomics profile, represents an interesting and relevant research area to understand their mutualistic symbiosis and its contribution to plant development.[6-10]

Among analytical methods employed on metabolomics studies,[11,12] the hydrogen nuclear magnetic resonance (^{1}H NMR) technique has been used to study metabolomes because it allows the detection and quantification of primary and secondary metabolites.[13] Recently, the ^{1}H NMR technique has been used for plant and fungus metabolome approach, such as the variation of metabolites involved in carbon metabolism of soybean plants infected by *Sclerotinia sclerotiorum*,[14] the effects of the flavonoids genistein, daidzein, apigenin, and kaempferol on mycotoxin

*e-mail: olivia.sampaio@ufmt.br
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citrinin production by *Monascus* purple-red molds,[15] the influence of environmental conditions on the metabolome of *Solanum lycopersicum*[16] and the chemical profile analysis of ten different *Capsicum annuum* cv. serrano plants cultivated in Mexico.[17]

Combretum lanceolatum is an endemic plant occurring in wetland areas of South America, which has demonstrated relevant biological activities such as antioxidant and anti-hyperglycemic.[18-20] Although *C. lanceolatum* plant has been studied regarding its bioactivity properties, only a little attention has been given towards its phytochemical investigation in the literature.[21,22] In a previous work,[22] our research group described the metabolomic changes in the aerial parts of *C. lanceolatum* plants inoculated with its endophytic fungi *Diaporthe phaseolorum* (*Dp*) and *Trichoderma spirale* (*Ts*). Accordingly, the study demonstrated that *Dp* fungus has significantly affected the plant aerial parts metabolic pathways, improving the biosynthesis of primary metabolites such as threonine, malic acid and *N*-acetyl-mannosamine.[22] Based on those results, the aim of our current work is to analyze the effects of *Dp* and *Ts* fungi on the chemical composition of *C. lanceolatum* roots in order to extend the metabolomics knowledge over the symbiotic interaction between these species.

Experimental

Plant cultivation and endophytic fungal inoculation

C. lanceolatum seeds were collected from Pocone, Mato Grosso state of Brazil, coordinates 16°18'93.2" S and 56°32'29.1" W. A voucher specimen was deposited in herbarium of the Federal University of Mato Grosso (code No. 39.149). The endophytic fungi *Dp* (No. of Access GenBank KF555229) and *Ts* (No. of Access GenBank KF555225) were isolated from the roots of *C. lanceolatum*.[23]

The *C. lanceolatum* seeds disinfection, germination and cultivation as well as the endophytic fungi *Dp* and *Ts* inoculation were performed as previously described.[22] The experiment was divided into three fungal interaction times, immediately after inoculation (0 days), after 1 day and 7 days. For each fungal interaction time, the uninoculated *C. lanceolatum* plant was used as a negative control. The experiment was performed in triplicate manner.

Plant material extraction

Each root sample was frozen under liquid nitrogen, macerated to a fine powder using mortar and pestle, followed by solvent extraction with ethanoic aqueous solution (50% v/v, 4 mL) with formic acid (1%). The extraction procedure was performed employing a Marconi vortex mixer (Piracicaba, SP, Brazil) at 1600 rpm for 30 min and repeated three times for each sample. The extract solutions were filtered with 0.45 μm filters (Millipore, MA, USA) and the solvent was removed under reduced pressure followed by lyophilization. All dry extracts were collected and stored at −80 °C in a Thermo Scientific refrigerator (Long Branch, USA).

^{1}H NMR data acquisition and spectra processing

The ^{1}H NMR analysis was conducted at 4.0 mg mL^{-1} of vegetal material *per* treatment, employing as a solvent deuterium oxide with 0.05 wt.% of sodium 3-(trimethylsilyl) propionate-2,2,3,3-d_4 (TSP) purchased from Sigma-Aldrich (St. Gallen, Switzerland). The TSP was used as both a chemical shift reference (H_δ 0 ppm) and internal standard for quantitative analysis.[24,25] After the solution preparation, samples were taken in the NMR tubes (5 mm) and immediately submitted for ^{1}H NMR spectra acquisition using a Bruker Magnet System AscendTM spectrometer (Fällanden, Switzerland) at 500 MHz, equipped with a 5 mm broadband observe probe.

The ^{1}H NMR spectra were acquired at 25 ºC with 128 transients of 64 K data points, 90° pulse angle, 4.35 s acquisition time and 4 s recycle delay.[22,26] Using a general procedure,[27] ^{1}H NMR spectra pre-processing and multivariate analyses of data for metabolite identification were performed with a few modifications. The spectra were phased and the baseline were corrected using MestReNova software.[28]

Identification and quantification of chemical profile

The NMR data were processed with rNMR software[29] in order to normalize the spectra (with the total area of each spectrum) and generate the spectral matrix,[30] followed by cross-reference analysis employing Madison Metabolomics Consortium Database (MMCD).[31,32]

Statistical analyses

The statistical analyses were performed using the metabolites data obtained through rNMR and MMCD. All multivariate analysis (mean-centering and scaling principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA)), Vulcano plot (threshold limit of $P < 0.05$), unpaired *T*-test and Fold Change (FC > 1.0) were performed with MetaboAnalyst 4.0 online platform.[33,34]

Results and Discussion

Metabolome profiling in plant-fungal association by ^{1}H NMR

The effects of *Dp* and *Ts* fungal synergism on the metabolomic profile of *C. lanceolatum* plants were evaluated after 0, 1 and 7 days of fungal inoculation. The most relevant results were obtained on *Ts*-plant experiment after 7 days of interaction, indicating the presence of *Ts* fungus potentially affected the plant metabolism as compared to the plant control. Additionally, the association of *Dp* fungus to *C. lanceolatum* plant showed no variation in metabolomic profile over the experiment time as compared to the control.

Representative ^{1}H NMR spectra acquired from hydroalcoholic extracts of plant-*Ts* fungi interaction (orange) and plant control plant (gray) after 7 days of interaction are shown in Figure 1. The analysis of ^{1}H NMR spectral data set using rNMR software and MMCD lead to the identification and quantification of 105 primary metabolites (see Supplementary Information section).

The identified metabolites include carbohydrates (threitol, adonitol, fructose-6-phosphate and sorbose), glycerol, ethanolamine and *N*-acetyl-neuramic acid, detected at the chemical shift average 3.00 to 4.50 ppm (Figure 1b). In the aliphatic region (Figure 1c), ^{1}H NMR spectrum of the plant-*Ts* treated samples demonstrated differences compared to the plant control, related to the compounds methionine, glutathione and spermidine. Although the signals were observed with lower intensity in the aromatic region (Figure 1a) compared with those in the carbohydrate (H_δ 5.50-3.00 ppm) and aliphatic (H_δ 3.00-0.50 ppm) regions, formaldehyde was presented in higher concentrations in inoculated plants as compared to the control.

Furthermore, the fungal association induces different metabolomic variations towards the plant aerial parts and roots, since the interaction of *Dp* fungus with *C. lanceolatum* plants significantly affected the metabolic profile, increasing the primary metabolites such as threonine, malic acid and *N*-acetyl-mannosamine.[22] Based on these results, the *Ts* fungus interaction represents a more relevant influence on the biosynthesis of primary compounds in the plant roots, improving the plant's development and secondary metabolism pathways.

Statistical multivariate analysis

The ^{1}H NMR spectral data (chemical shift and intensity) were used as a matrix in the multivariate analyses. The principal component analysis (PCA) was used to analyze the NMR data set and loading plots were used to detect the spectral areas (metabolites) responsible for separation in the data.[35] Data set obtained from the analysis of plant-*Ts* fungi interaction after 7 days of inoculation, the first two components model could explain 74.9%. Among the PCs, combination of PC1 and PC2 can give well-separated two clusters for plant-*Ts* fungi interaction as compared to the control plants (Figure 2a).

The multivariate analysis of spectral data proved to be a useful tool in order to discriminate between treated plants (with *Dp* and *Ts* fungi) and control plants. From the PCA score plot, it is obvious that *Ts* fungus induced metabolic perturbations in *C. lanceolatum* roots after 7 days of inoculation. The samples originating from the *Ts*-fungi interaction and control plants could be observed as clusters in different areas of the plots. The first component, which explains 42.4% of the total variance, includes the metabolites 1,4-diaminebutane, *muco*-inositol and trimethylamine. At the opposite position on PC1, we can find the metabolites 6-phosphogluconic acid, glyceraldehyde-3-phosphate and choline.

To investigate the effects of plant-fungi interaction over the experimental time, partial least squares-discriminant analysis (PLS-DA) models were performed employing two components to demonstrate metabolic differences between the plant control and *Ts*-fungi interaction groups. The score plot of the two firstly components (PC1 and PC2) for PLS-DA model of plant-*Ts* fungi interaction and plant control after 7 days of interaction showed separation of the classes, mainly in the first component (Figure 2b). The PC1 and PC2 explain the total variance with 71.2% contribution and Q^2 parameter obtained was 0.68 ($R^2X = 0.98$, $R^2Y = 0.99$). The control and *Ts* fungi interaction groups clustered on positive and negative sides, respectively, along the component 1 axis. The PLS-DA loadings were analyzed and the compounds that affected the metabolic profile variation were identified. The most significant metabolic variation found between control and *Ts* fungi interaction extracts included 3- hydroxybutyric acid, trimethylamine and alanine for the control group and glycolate, 6-phosphogluconic acid and sorbitol for *Ts* fungi interaction.

Furthermore, the differences observed between the metabolomic profiles of *Ts* fungi interaction and plant control are due to the entire metabolome and not due to the major contribution of a few differentially abundant metabolites. Considering these metabolites are involved in many biosynthetic pathways, including the secondary metabolite synthesis, the variations observed on primary metabolites concentration through endophytic fungal association potentially influence the biosynthesis of important compounds for plant development.[36]

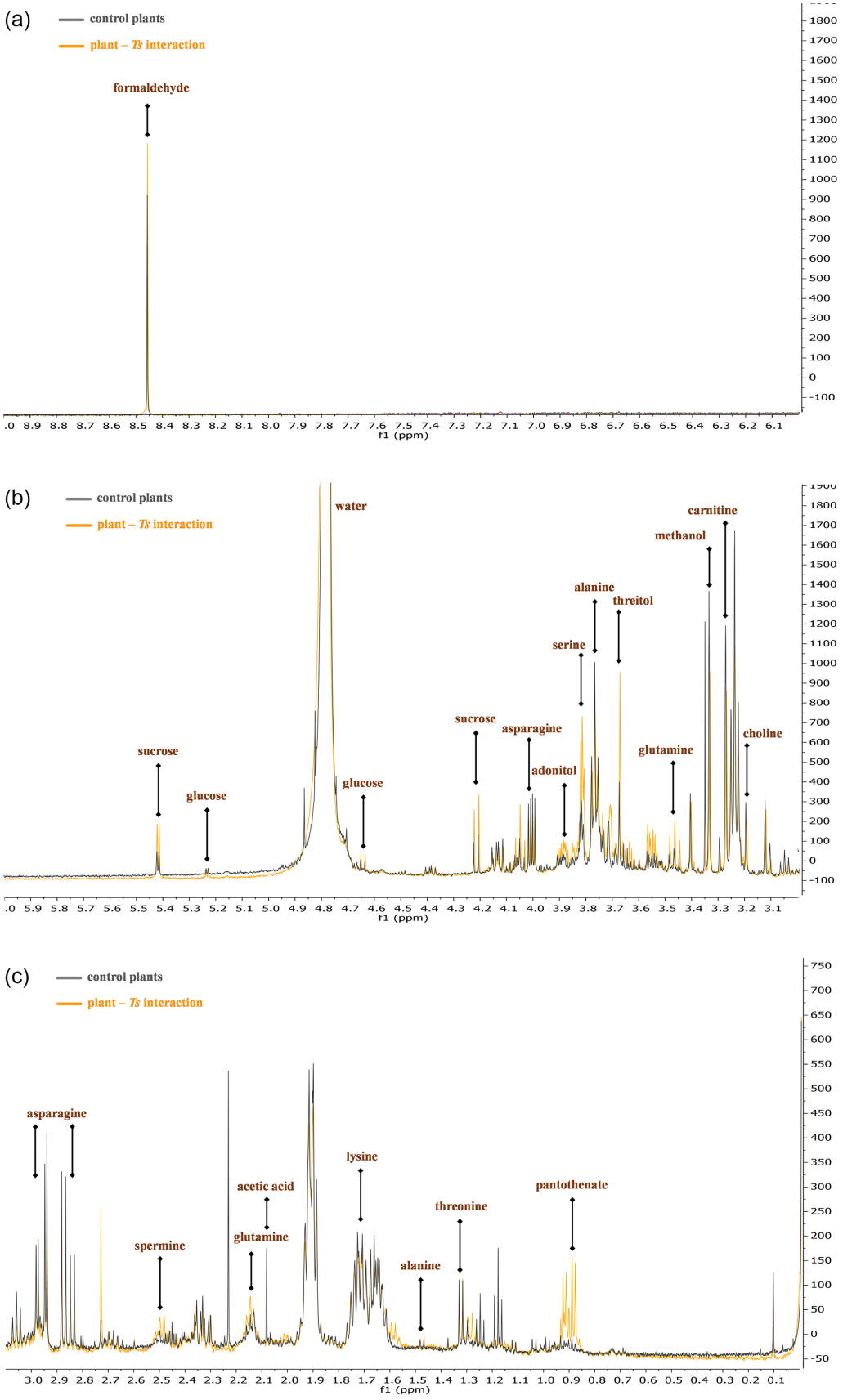

Figure 1. Representative 1D ^{1}H NMR spectra of hydroalcoholic extracts of plant-*Ts* fungi interaction (orange) and plant control plant (gray) after 7 days of interaction. Aromatic region (a), carbohydrate region (b) and aliphatic region (c).

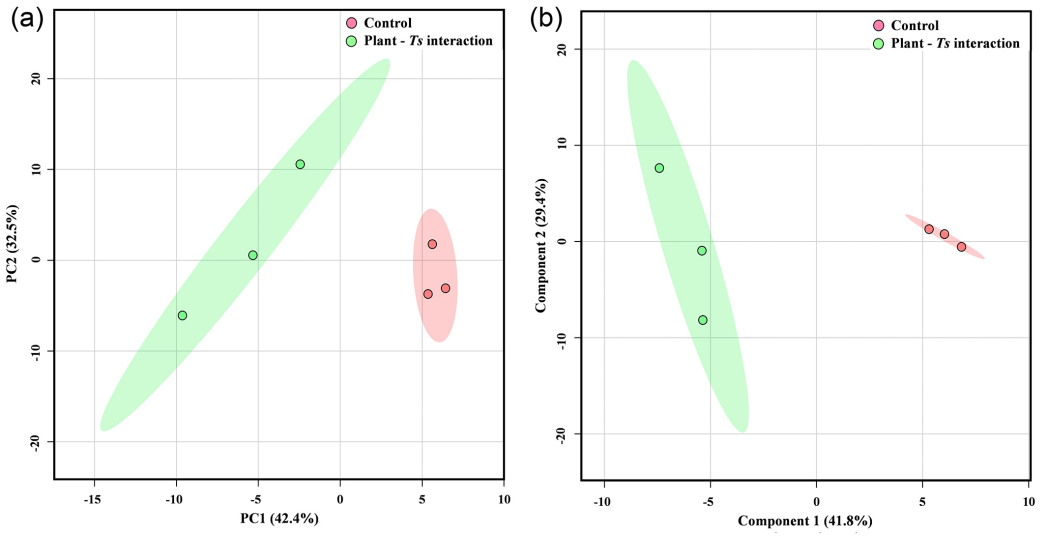

Figure 2. Scores plots of PCA (a) and PLS-DA (b) of the hydroalcoholic extracts of plant-*Ts* fungi interaction (green) and plant control plants (red) after 7 days of interaction.

Identification of significant metabolites and determination of fold changes

Aiming to identify the primary metabolites that are present at different concentration in comparison with fungal inoculated plants (FIP) and control plants (CP), fold changes ($\log_2$(FC)) of the concentration ratio of FIP by CP and related *p* values were determined for each treatment. The calculations of fold changes and *T*-tests were performed with MetaboAnalyst. When comparing *Ts* inoculated plants to control plant samples, a total of 27 ions presented significant accumulation differences ($p \leq 0.05$ and fold change ≥ 1.0) over the interaction time (0, 1 and 7 days), of which 12 metabolites presented an increase, and 15 metabolites a decrease, in their accumulation (Table 1). Volcano plot analyses for the metabolomic profile of *C. lanceolatum-Dp* interaction were not performed since the group discrimination were not observed in multivariate analysis.

At the initial interaction time of the *C. lanceolatum-Ts* experiment, there was observed modification on six metabolites concentrations. Based on the fold changes results, 2,3-diphosphoglyceric acid ($\log_2$(FC) = 2.77), sorbose ($\log_2$(FC) = 1.23) and threitol ($\log_2$(FC) = 1.11) were present at higher concentrations in FIP than in CP. These metabolites are related to energetic sugar metabolism, directly involved in the glycolysis pathway through 2,3-diphosphoglyceric acid,[37] being extended to sorbose and threitol, which are natural sugars.[38,39] Cysteine ($\log_2$(FC) = −1.42) and the carbohydrates fructose ($\log_2$(FC) = −1.20) and α-galactose-1-phosphate ($\log_2$(FC) = −1.02) were present at lower concentration in FIP than in CP, indicating the reduction of cysteine concentration potentially be related to the synthesis of methionine and glutathione.[40]

After one day, plants were inoculated with *Ts* fungus, α-lactose ($\log_2$(FC) = 1.67) and galactono-1,4-lactone ($\log_2$(FC) = 1.08) were present at higher concentrations in plant-*Ts* treatment group. Galactono-1,4-lactone is an intermediate compound in the galactose pathway, that is the main route towards galactose catabolism and ascorbic acid formation.[41] The galactose catabolism may be induced in the interaction between endophyte and plant, which explains the reduced level of arabinose ($\log_2$(FC) = −1.18), which is quickly consumed in the presence of galactose or lactose according to the Leloir pathway.[42] Furthermore, pyruvate ($\log_2$(FC) = −1.14) and succinate ($\log_2$(FC) = −1.14) present at lower concentration in FIP than in CP. These compounds are important metabolites in the glycolytic pathway in plants or fungi metabolism and are also related to acetate and mevalonate pathways for the biosynthesis of polyketides, fatty acids, terpenoids or steroids, as well as to biosynthesis of aliphatic amino acids, building blocks of proteins or secondary metabolites, such as alkaloids.[43-46]

The diversity of chemical components resulting from the interaction of plant fungi has increased over time. With the increase in reaction time, there were observed more metabolites in the extract of the plant inoculated with *Ts* fungus. After 7 days of inoculation, 15 compounds were detected as changes in the metabolic profile. Spermidine ($\log_2$(FC) = 2.95) and pantothenate ($\log_2$(FC) = 2.55) were present at significantly higher concentration in FIP than in CP after 7 days of plant-*Ts* interaction. Spermidine is a polyamine compound derived from 1,4-diaminobutane ($\log_2$(FC) = −1.82) that is related to abiotic stress relieve[47]

Table 1. Identified metabolites with differential accumulation in *Ts*-plant association with its fold changes ($p \leq 0.05$, $\log_2(FC) \geq 1.0$ or ≤ -1) over interaction time

Metabolite	Interaction time					
	0 day		1 day		7 days	
	$\log_2$(FC)	*p*-Value	$\log_2$(FC)	*p*-Value	$\log_2$(FC)	*p*-Value
2,3-Diphosphoglyceric acid	2.77	0.001	–	–	–	–
Sorbose	1.23	0.041	–	–	–	–
Threitol	1.11	0.023	–	–	–	–
α-Galactose-1-phosphate	–1.02	0.030	–	–	–	–
Fructose	–1.20	0.043	–	–	–	–
Cysteine	–1.42	0.037	–	–	–	–
Galactono-1,4-lactone	–	–	1.08	0.049	–	–
α-Lactose			1.67	0.046	–	–
Arabinose	–	–	–1.18	0.010	–	–
Pyruvate	–	–	–1.14	0.032	–	–
Succinate	–	–	–1.14	0.033	–	–
N-Carbamoyl-methyl-imino-diacetic acid	–	–	–1.12	0.036	–	–
Spermidine	–	–	–	–	2.95	4.1×10^{-4}
Pantothenate	–	–	–	–	2.55	0.008
Glyceraldehyde-3-phosphate	–	–	–	–	1.79	0.027
Threitol	–	–	–	–	1.60	0.001
Serine	–	–	–	–	1.59	0.012
Formaldehyde	–	–	–	–	1.56	0.004
Sorbitol	–	–	–	–	1.06	5.1×10^{-4}
Acetic acid	–	–	–	–	–1.05	0.006
Saccharate	–	–	–	–	–1.21	7.4×10^{-4}
meso-Erythritol	–	–	–	–	–1.26	0.001
Glutathione oxidized	–	–	–	–	–1.36	0.003
Trimethylamine	–	–	–	–	–1.38	7.4×10^{-4}
1,4-Diaminobutane	–	–	–	–	–1.82	0.004
β-Alanine	–	–	–	–	–4.31	5.0×10^{-5}
3-Hydroxybutyric acid	–	–	–	–	–4.40	0.0002

and increase of antioxidant proprieties, phytohormone levels and adenosine triphosphate (ATP) concentration, which represents a parameter for energy metabolism quantification in plants and fungus.[48] In addition, serine was detected at a higher concentration in the treated plants ($\log_2(FC) = 1.59$). This amino acid is involved in the glycine-betaine metabolism and the glyoxylate pathway, but the main route for its formation is by 3-phosphoglycerate transformation.[49] It has multiple functions in osmoprotectant and as a building block of several other metabolites such as tryptophan, glutathione, porphyrins and fatty acids, which play an important role in defense, anabolic action and energy metabolism.[50]

According to pantothenate levels increasing, there was observed a significant reduction of β-alanine ($\log_2(FC) = -4.31$) and 3-hydroxybutyric acid ($\log_2(FC) = -4.40$) concentrations. Plants and fungi produce pantothenate through the condensation of β-alanine with pantoate, which is obtained in two steps from an intermediate in branched-chain amino acid biosynthesis. Pantothenate is the main precursor of coenzyme A (CoA),[51] which is involved in innumerable reactions of central metabolism, as fatty acid oxidation, and biosynthesis of glycolipids and sterols, as well as secondary metabolic pathways, including those for polyketides, non-ribosomal protein synthesis, flavonoids, and lignin.[52,53] In plants, 3-hydroxybutyric acid acts as a regulatory molecule that most likely influences the expression of genes involved in deoxyribonucleic acid (DNA) methylation.[54] These results indicate that the plant-fungus interaction potentially modify the biosynthesis of secondary metabolites.

Glyceraldehyde-3-phosphate present higher concentration in FIP ($\log_2(FC) = 1.79$) over 7 days of interaction. This metabolite is an intermediate of glycolysis and gluconeogenesis and also the precursor for deoxyxylulose phosphate pathway, which leads to the formation of terpenoid and steroid metabolites.[55] Moreover, there were observed an increase levels of threitol ($\log_2(FC) = 1.60$) and sorbitol ($\log_2(FC) = 1.06$) and reduction of *meso*-erythritol ($\log_2(FC) = -1.26$), which

metabolites are related to energy metabolism, carbon source and signaling molecules in plants.[56]

Therefore, the differences observed in the metabolomic profile of *C. lanceolatum* inoculated with *Ts* compared to uninoculated plants demonstrated the variations on biochemical pathways, inducing or inhibiting the synthesis of some primary metabolites as carbohydrates and amino acids.

Conclusions

In conclusion, this work demonstrate the metabolomic profiling of *C. lanceolatum* plant roots inoculated with its endophytic fungi. The metabolomic approach was based on ^{1}H NMR spectral data and analysis performed employing rNMR software and MMCD database. Multivariate analysis showed that roots inoculated with *Ts* fungus presents significantly class differentiation and discrimination over 7 days of interaction as compared to roots of control, indicating the chemical profile of the plant-fungus association is variable and time-dependent. Based on the fold change analysis, it was observed that *Ts* fungus affected the metabolic profile of the roots by inhibiting or improving the biosynthesis of primary metabolites such as spermidine, pantothenate, β-alanine and 3-hydroxybutyric acid, which are involved in various plant processes such as secondary metabolites biosynthesis, energy metabolism, carbon source and self-defense. Therefore, we demonstrated that the interaction between roots and endophytic fungi results in the plant's metabolomic profile changes, which suggest the influence of fungal association for plant development.

Supplementary Information

Supplementary information (multivariate analysis and table of compounds annotation and concentration) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

The manuscript was written through contributions of all authors. O.M.S, B.S.B., P.T.S.J., L.C.C.V., M.A.S. were responsible for study design; L.S., J.W.F.L., L.C.C.V., L.G.V. for sample collection; L.S., A.A., L.C.C.V., B.S.B., O.M.S. for data analysis; L.S., L.S.M, M.A.S., O.M.S. for statistical analysis. All authors contributed to manuscript revision and gave final approval for publication.

References

1. Yan, L.; Zhu, J.; Zhao, X.; Shi, J.; Jiang, C.; Shao, D.; *Appl. Microbiol. Biotechnol.* **2019**, *103*, 3327. [Crossref]
2. Salvatore, M. M.; Andolfi, A.; Nicoletti, R.; *Agriculture* **2020**, *10*, 488. [Crossref]
3. Contreras-Cornejo, H. A.; Macías-Rodríguez, L.; Cortés-Penagos, C.; López-Bucio, J.; *Plant Physiol.* **2009**, *149*, 1579. [Crossref]
4. Sen, S.; Dehingia, M.; Talukdar, N. C.; Khan, M.; *Sci. Rep.* **2017**, *7*, 44406. [Crossref]
5. Hill, E. M.; Robinson, L. A.; Abdul-Sada, A.; Vanbergen, A. J.; Hodge, A.; Hartley, S. E.; *J. Chem. Ecol.* **2018**, *44*, 198. [Crossref]
6. Heviefo, G. A.; Nyamador, S. W.; Nyamador, S. W.; Datinon, B. D.; Glitho, I. A.; Tamò, M.; *Int. J. Biol. Chem. Sci.* **2020**, *14*, 1448. [Crossref]
7. Zhou, J.; Li, X.; Huang, P.-W.; Dai, C.-C.; *Microbiol. Res.* **2018**, *206*, 99. [Crossref]
8. Salomé-Abarca, L. F.; Mandrone, M.; Sanna, C.; Poli, F.; van der Hondel, C. A. M. J. J.; Klinkhamer, P. G. L.; Choi, Y. H.; *Phytochemistry* **2020**, *176*, 112402. [Crossref]
9. Sebastiana, M.; Gargallo-Garriga, A.; Sardans, J.; Pérez-Trujillo, M.; Monteiro, F.; Figueiredo, A.; Maia, M.; Nascimento, R.; Silva, M. S.; Ferreira, A. N.; Cordeiro, C.; Marques, A. P.; Sousa, L.; Malhó, R.; Peñuelas, J.; *Sci. Rep.* **2021**, *11*, 8576. [Crossref]
10. Mazzei, P.; Cozzolino, V.; Piccolo, A.; *J. Agric. Food Chem.* **2018**, *66*, 2580. [Crossref]
11. Feng, Z.; Ding, C.; Li, W.; Wang, D.; Cui, D.; *Food Chem.* **2020**, *310*, 125914. [Crossref]
12. Ghatak, A.; Chaturvedi, P.; Weckwerth, W. In *Plant Genetics and Molecular Biology*, vol. 164; Springer: Cham, 2018, p. 187. [Crossref]
13. Valentino, G.; Graziani, V.; D'Abrosca, B.; Pacifico, S.; Fiorentino, A.; Scognamiglio, M.; *Molecules* **2020**, *25*, 1444. [Crossref]
14. de Oliveira, C. S.; Lião, L. M.; Alcantara, G. B.; *Phytochemistry* **2019**, *167*, 112099. [Crossref]
15. He, S.; Wang, Y.; Xie, J.; Gao, H.; Li, X.; Huang, Z.; *Food Res. Int.* **2020**, *137*, 109532. [Crossref]
16. Abreu, A. C.; Fernández, I.; *Molecules* **2020**, *25*, 3738. [Crossref]
17. Villa-Ruano, N.; Ramírez-Meraz, M.; Méndez-Aguilar, R.; Zepeda-Vallejo, L. G.; Álvarez-Bravo, A.; Pérez-Hernández, N.; Becerra-Martínez, E.; *Food Res. Int.* **2019**, *119*, 785. [Crossref]

18. Dechandt, C. R. P.; Siqueira, J. T.; de Souza, D. L. P.; Araujo, L. C. J.; da Silva, V. C.; de Sousa Jr., P. T.; Andrade, C. M. B.; Kawashita, N. H.; Baviera, A. M.; *Rev. Bras. Farmacogn.* **2013**, *23*, 291. [Crossref]
19. Dechandt, C. R. P.; de Souza, D. L. P.; Siqueira, J. T.; Pereira, M. P.; de Assis, R. P.; da Silva, V. C.; de Sousa Jr., P. T.; Brunetti, I. L.; Andrade, C. M. B.; Kawashita, N. H.; Baviera, A. M.; *J. Pharm. Res.* **2014**, *4*, 2340. [Crossref]
20. Siqueira, J. T.; Batistela, E.; Pereira, M. P.; da Silva, V. C.; de Sousa Jr., P. T.; Andrade, C. M. B.; Kawashita, N. H.; Bertolini, G. L.; Baviera, A. M.; *Pharm. Biol.* **2016**, *54*, 1671. [Crossref]
21. Araujo, L. C. J.; da Silva, V. C.; Dall'Oglio, E. L.; de Sousa Jr., P. T.; *Biochem. Syst. Ecol.* **2013**, *49*, 37. [Crossref]
22. Lacerda, J. W. F.; Siqueira, K. A.; Vasconcelos, L. G.; Bellete, B. S.; Dall'Oglio, E. L.; Sousa Jr., P. T.; Faraggi, T. M.; Vieira, L. C. C.; Soares, M. A.; Sampaio, O. M.; *Chem. Biodiversity* **2021**, *18*, e2100350. [Crossref]
23. de Siqueira, K. A.; Brissow, E. R.; dos Santos, J. L.; White, J. F.; Santos, F. R.; de Almeida, E. G.; Soares, M. A.; *Symbiosis* **2017**, *71*, 211. [Crossref]
24. Caligiani, A.; Acquotti, D.; Palla, G.; Bocchi, V.; *Anal. Chim. Acta* **2007**, *585*, 110. [Crossref]
25. Maes, P.; Monakhova, Y. B.; Kuballa, T.; Reusch, H.; Lachenmeier, D. W.; *J. Agric. Food Chem.* **2012**, *60*, 2778. [Crossref]
26. Escudero, N.; Marhuenda-Egea, F. C.; Ibanco-Cañete, R.; Zavala-Gonzalez, E. A.; Lopez-Llorca, L. V.; *Metabolomics* **2014**, *10*, 788. [Crossref]
27. Sevastos, A.; Kalampokis, I. F.; Panagiotopoulou, A.; Pelecanou, M.; Aliferis, K. A.; *Pestic. Biochem. Physiol.* **2018**, *148*, 50. [Crossref]
28. Willcott, M. R.; *MestRe Nova*, v.9.0; Department of Chemistry, Rice University, USA, 2009.
29. rNMR-Open Source Software for NMR Data Analysis, http://rnmr.nmrfam.wisc.edu, accessed in July 2022.
30. Lewis, I. A.; Schommer, S. C.; Markley, J. L.; *Magn. Reson. Chem.* **2009**, *47*, S123. [Crossref]
31. Anderson, M. E.; Li, J.; Schulte, C. F.; Westler, W. M.; Eghbalnia, H. R.; Sussman, M. R.; Markley, J. L.; *Nat. Biotechnol.* **2008**, *26*, 162.
32. Cui, Q.; Lewis, I. A.; Hegeman, A. D.; Anderson, M. E.; Li, J.; Schulte, C. F.; Westler, W. M.; Eghbalnia, H. R.; Sussman, M. R.; Markley, J. L.; *Nat. Biotechnol.* **2008**, *26*, 162. [Crossref]
33. MetaboAnalyst, https://www.metaboanalyst.ca/, accessed in July 2022.
34. Chong, J.; Soufan, O.; Li, C.; Caraus, I.; Li, S.; Bourque, G.; Wishart, D. S.; Xia, J.; *Nucleic Acids Res.* **2018**, *46*, W486. [Crossref]
35. Casanova, L. M.; Rodrigues, L. M.; de Aguiar, P. F.; Tinoco, L. W.; *J. Nat. Prod.* **2020**, *83*, 243. [Crossref]
36. Aharoni, A.; Galili, G.; *Curr. Opin. Biotechnol.* **2011**, *22*, 239. [Crossref]
37. Lin, D.; Zhang, L.; Mei, J.; Chen, J.; Piao, Z.; Lee, G.; Dong, Y.; *Plant Biol.* **2019**, *21*, 585. [Crossref]
38. Kutty, N. N.; Ghissing, U.; Mitra, A.; *Plant Mol. Biol.* **2021**, *106*, 533. [Crossref]
39. Noiraud, N.; Maurousset, L.; Lemoine, R.; *Plant Physiol. Biochem.* **2001**, *39*, 717. [Crossref]
40. Rausch, T.; Wachter, A.; *Trends Plant Sci.* **2005**, *10*, 503. [Crossref]
41. Tao, J.; Hao, Z.; Huang, C.; *AoB Plants* **2020**, *12*, ID plaa055. [Crossref]
42. Németh, Z.; Kulcsár, L.; Flipphi, M.; Orosz, A.; Aguilar-Pontes, M. V.; de Vries, R. P.; Karaffa, L.; Fekete, E.; *Fungal Genet. Biol.* **2019**, *123*, 53. [Crossref]
43. Zografos, A. L.; Anagnostaki, E. E. In *From Biosynthesis to Total Synthesis*; John Wiley & Sons, Inc: Hoboken, NJ, 2016.
44. White, S. W.; Zheng, J.; Zhang, Y.-M.; Rock, C. O.; *Annu. Rev. Biochem.* **2005**, *74*, 791. [Crossref]
45. Rawlings, B. J.; *Nat. Prod. Rep.* **1997**, *14*, 523. [Crossref]
46. Bromke, M.; *Metabolites* **2013**, *3*, 294. [Crossref]
47. Catalá, R.; López-Cobollo, R.; Álvaro Berbís, M.; Jiménez-Barbero, J.; Salinas, J.; *Sci. Adv.* **2021**, *7*, ID eabd9296. [Crossref]
48. Zhou, T.; Wang, P.; Gu, Z.; Ma, M.; Yang, R.; *Food Chem.* **2020**, *309*, 125759. [Crossref]
49. Igamberdiev, A. U.; Kleczkowski, L. A.; *Front. Plant Sci.* **2018**, *9*, 318. [Crossref]
50. Ros, R.; Muñoz-Bertomeu, J.; Krueger, S.; *Trends Plant Sci.* **2014**, *19*, 564. [Crossref]
51. Aslam, A.; Zhao, S.; Lu, X.; He, N.; Zhu, H.; Malik, A. U.; Azam, M.; Liu, W.; *Biomolecules* **2021**, *11*, 628. [Crossref]
52. Coxon, K. M.; Chakauya, E.; Ottenhof, H. H.; Whitney, H. M.; Blundell, T. L.; Abell, C.; Smith, A. G.; *Biochem. Soc. Trans.* **2005**, *33*, 743. [Crossref]
53. Parthasarathy, A.; Savka, M. A.; Hudson, A. O.; *Front. Plant Sci.* **2019**, *10*, 921. [Crossref]
54. Mierziak, J.; Burgberger, M.; Wojtasik, W.; *Biomolecules* **2021**, *11*, 402. [Crossref]
55. Phulara, S. C.; Chaturvedi, P.; Gupta, P.; *Appl. Environ. Microbiol.* **2016**, *82*, 5730. [Crossref]
56. Yang, J.; Zhu, L.; Cui, W.; Zhang, C.; Li, D.; Ma, B.; Cheng, L.; Ruan, Y. L.; Ma, F.; Li, M.; *Hortic. Res.* **2018**, *5*, 71. [Crossref]

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