Modified distribution in the polyphenolic profile of rosemary leaves induced by plant inoculation with an arbuscular mycorrhizal fungus

Running title: Polyphenolic changes in rosemary leaves induced by mycorrhizas

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Abstract

BACKGROUND: Rosemary forms an arbuscular mycorrhizal (AM) symbiosis with a group of soilborne fungi belonging to the phylum *Glomeromycota*, which can modify the plant metabolome responsible for the antioxidant capacity and other health beneficial properties of Rosemary.

RESULTS: The effect of inoculating rosemary plants with an AM fungus on their growth via their polyphenolic fingerprinting was evaluated after analyzing leaf extracts from non-inoculated and inoculated rosemary plants by ultra-high performance liquid chromatography-high resolution mass spectrometry. . Plant growth parameters indicated that mycorrhizal inoculation significantly increased plant height and biomass. Chemical modifications in the plant polyphenolic profile distribution were found after a principal components analysis (PCA) loading plots study. Four compounds hosting strong antioxidant properties: ferulic acid, asiatic acid, carnosol, and vanillin were related to mycorrhizal rosemary plants while caffeic and chlorogenic acids had a higher influence in non-mycorrhizal plants.

CONCLUSION: Mycorrhization was found to stimulate growth in order to obtain a higher biomass of plant leaves in short time and avoiding chemical fertilization, while analytical results demonstrate that there is an alteration in the distribution of polyphenols in plants colonized by the symbiotic fungus, which can be related to an improvement in nutritional properties with future industrial significance.

KEYWORDS: *Rosmarinus officinalis*; Polyphenolic fingerprinting; High resolution mass spectrometry; Orbitrap; *Rhizoglomus irregulare*; symbiosis

Introduction

Rosemary (*Rosmarinus officinalis* L.) shrubs are ubiquitous in Mediterranean environments and intensively cultivated in nurseries for revegetation of degraded land, sustainable landscaping, medicinal and culinary purposes. The species belongs to the Lamiaceae family and, among other herbs, it is a valuable plant due to its high content in active ingredients with therapeutic, aromatic and organoleptic properties.[1] Those characteristics make the crop very attractive for pharmacological applications, as well as for nutraceutical and food industries. The effects on human health of bioactive compounds from plants has been a major research topic in the last decades focusing on their functional skills.[2] Concerning rosemary, a high content in leaves of phenolic acids, flavonoids, essential oil, triterpenic acids and triterpenic alcohols can be responsible for stimulating the nervous and the circulatory systems[3] and for providing anticancer effects.[4]

As most vascular plant species in all geographical terrestrial areas,[5] rosemary forms an arbuscular mycorrhizal (AM) symbiosis with a group of soilborne fungi belonging to the phylum *Glomeromycota*.[6] In this mutualistic association, the fungus benefits from the plant by acquiring photosynthates delivered by the host while it helps the plant absorbing nutrients from the soil through an extended root system via the fungal extraradical mycelium. The arbuscular mycorrhizal relationship, besides facilitating nutrient uptake by plants and significantly stimulating plant growth,[7] has proven to increase tolerance against abiotic stresses such as drought, salinity and soil

toxicity,[8] and to outgrow the disease damage caused by plant pathogens.[7] The plant's physiology is positively altered by the symbiosis with quantified changes in root exudation[9] and in the composition of secondary metabolites,[10] often related to plant defense mechanisms.[11]

The symbiosis forms spontaneously in natural ecosystems where native AM fungal propagules are present in undegraded soils. When plants are industrially produced in intensive nursery agronomic systems on free-soil substrates, they lack mycorrhizas in their roots unless they were artificially inoculated with selected AM fungi. Accordingly, the plant metabolomics composition in active compounds will most probably not be the same than the one expected to be found in a plant with a fully established mycorrhizal root system. An experimental set up was designed in order to confirm the latter hypothesis.

Polyphenols are aromatic secondary metabolites ubiquitously spread through the plant kingdom comprising more than eight thousand substances with highly diverse structures. The main reasons for the interest in polyphenols deals with the recognition of their antioxidant properties, the great abundance in the diet, and the important role in the prevention of various diseases.[12–14] Furthermore, polyphenols, which also constitute the active substance found in many medicinal plants, modulate the activity of a wide range of enzymes and cell receptors.[15] They are also playing an important role in plant defense mechanisms being involved in the interaction between pathogens and the plants.[1,16,17]

The analysis of polyphenols in plant materials is relatively complex due to the great variety of compounds that can be present, which differ in polarity and size (from simple phenolic acids to tannins), but also because many of these compounds are found at low concentration levels. Liquid chromatography coupled to mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS) is the most effective technique for the structural characterization and determination of polyphenols in a great variety of sample matrices.[18,19] Recently, high resolution mass spectrometry (HRMS) and accurate mass measurements have gained popularity due to their great ability to provide more comprehensive information concerning the exact molecular mass, elemental composition and detailed molecular structure of a given compound, being today one of

the better options when dealing with the characterization and determination of polyphenols in plant derived products.[19,20]

In the present work, the polyphenolic profile in leaves of greenhouse grown mycorrhizal and non-mycorrhizal rosemary plants was evaluated by UHPLC-HRMS in a quadrupole-Orbitrap mass spectrometer. Rosemary plant leaves from both mycorrhizal and non-mycorrhizal rosemary plants were periodically collected at different time periods, and bioactive compounds extracted using methanol followed by a simple solid-phase extraction (SPE) clean-up step with C_{18} cartridges. After UHPLC-HRMS analysis of the obtained plant extracts, a targeted polyphenolic approach using a target accurate mass database list comprising 55 characterized polyphenols was employed, and the obtained polyphenolic profiles were then subjected to exploratory principal component analysis (PCA) to establish patterns showing the effect of plant inoculation with an arbuscular mycorrhizal fungus on rosemary plant polyphenolic distribution.

Materials and Methods

Samples and sample treatment

The effect of inoculating rosemary plants with AM fungi on the growth of rosemary plants via their polyphenolic fingerprinting was evaluated as follows:

Rosemary rooted cuttings were used in the experimental set up. Before planting, fifteen leaves from ten cuttings, chosen at random and labelled as RT0, were collected and refrigerated until their analysis. At the same time, one hundred cuttings were planted in 1.5 L containers filled with a substrate mixture of autoclaved sandy soil (120ºC, 1hour), quartz sand and sphagnum peat (3:2:1; v/v). Half of the plants, labelled as RTM, were inoculated with *Rhizoglomus irregulare* (syn. *Glomus intraradices*) (Glomeromycota:Glomaraceae, a selected AM fungus, registered in the European bank for the Glomeromycota as BEG 72, while fifty non-inoculated control plants were labelled as RTC. The fungus had been isolated from a citrus nursery[21] and has proven to be effective at stimulating plant growth of different plant species and at favoring their establishment in diverse environments.[22,23]

To achieve the mycorrhizal inoculation, 10 grams per plant of bulk inoculum containing more than 1000 spores per gram were placed below the root system at transplanting RTM plants. The inoculum was obtained from the rhizosphere of a leek (*Allium porrum* L.) plant heavily colonized with *R. irregulare*.The colonization rate measured by the grid-line intersect method described by Giovannetti and Mosse[24] after clarifying and staining a root sample using the procedure described by Koske and Gemma [25] was 85%.. RTC plants received each 10 ml of filtrate free of mycorrhizal propagules from a solution obtained after stirring 100 g of inoculum in 1 L autoclaved (120º, 20 min) distilled water during 30 min, in order to provide the inoculum acompanying microbiota to non-inoculated plants. Plants were kept in a greenhouse under controlled conditions (18 $\pm$ 5 ºC, 14 hours light). They were watered when needed and no fertilization was ever applied.

Leaves from both RTC and RTM plants were periodically collected at different time periods: six weeks (RTC1 and RTM1), twelve weeks (RTC2 and RTM2), eighteen weeks (RTC3 and RTM3), twenty four weeks (RTC4 and RTM4) and thirty weeks (RTC5 and RTM5) after inoculation. Fifteen leaves from ten plants per treatment were collected every time and immediately frozen until analysis. Shoot height was recorded in ten plants per treatment taken at random every six weeks until the end of the experiment, when plants were cut and dried at 60 ºC and shoot dry weight was measured. Data obtained for both parameters was statistically analyzed by one-way ANOVA and means compared by Tukey's test.

Root samples were also excised from eight plants per treatment in order to estimate the mycorrhizal root colonization extent. Root samples were clarified and stained,[25] and the percentage of mycorrhizal root was measured.[24]

Sample extraction was performed as follows: 0.1 g of leaf samples from ten plants were suspended in 6 mL of LC-MS grade methanol and mechanically extracted by employing Ultraturrax T25 basic (Ika-Werke, Staufen, Germany). The extract solution was then processed by solid-phase extraction (SPE) for clean-up using Discovery® DSC-18 SPE (500 mg, 6 mL) cartridges obtained from Supelco (Darmstadt, Germany), that were previously activated-conditioned with LC-MS grade methanol.

The first mL was discarded, and then a portion of 1.5-2 mL of extract solution was transferred into 2 mL injection vials. The extracts were then stored at -4 ^{o}C until their analysis with the proposed UHPLC-HRMS method.

Additionally, a chemometric quality control (QC) sample was prepared by mixing 50 µL of each sample extract.

Chemicals

Unless otherwise stated, all reagents were of analytical grade. Fifty-five polyphenolic standards belonging to different families (phenolic acids, benzoic acids, cinnamic acids, phenolic aldehydes, phenolic terpenes, flavones, flavanols, proanthocyanidins and stilbenes), all of them obtained from Sigma-Aldrich (Steinhein, Germany), were employed to build a user target accurate mass database for TraceFinder^{TM} software. Stock standard solutions of studied polyphenolic standards (~1000 mg/L) were prepared in methanol in amber-glass vials. Intermediate working solutions were prepared weekly from these stock standard solutions by appropriate dilution with water. All stock solutions were stored at 4 ^{o}C for not more than one month.

Formic acid (98-100 %) was provided by Merck (Darmstadt, Germany). LC-MS grade acetonitrile, methanol and water were purchased from Sigma-Aldrich.

UHPLC-HRMS Polyphenolic Fingerprinting Study

Instrumental conditions

UHPLC separation was performed on an Accela liquid chromatography system (Thermo Fisher Scientific, San José, CA, USA), equipped with a quaternary pump, an autosampler and a column oven. An Accucore C_{18} reversed-phase (150 × 2.1 mm, 2.6 µm particle size) fused-core column (Thermo Fisher Scientific) was used for the proposed method. Gradient separation was created from solvent A (0.1 % formic acid aqueous solution) and solvent B (acetonitrile) as follows: 0-1 min, isocratic elution at 10% B; 1-20 min, linear gradient elution from 10 to 95% B; 20-27 min, at 95% B; 27-28 min, back to initial conditions; and from 28 to 33 min, at 10% B for column re-

equilibration. The mobile phase flow rate was 300 µL/min. Column was kept at room temperature and the injection volume used was 2 µL.

The UHPLC instrument was coupled to a Q-Exactive Orbitrap HRMS system (Thermo Fisher Scientific) equipped with a heated electrospray ionization source (HESI-II) operated in negative ionization mode. Nitrogen was used as a sheath gas, sweep gas, and auxiliary gas at flow rates of 50, 0 and 10 a.u. (arbitrary units), respectively. HESI-II heater temperature at 350 ^{o}C and capillary voltage at -2.5 kV were applied. Instrument capillary temperature was set at 320 ^{o}C, and an S-Lens RF level of 50 V was used. Q-Exactive Orbitrap HRMS system was tuned and calibrated using commercially available Thermo Fisher calibration solution every three days. The HRMS instrument was operated in full scan mode with a *m/z* range from 100 to 1,500 at a mass resolution of 70,000 full width at half-maximum (FWHM) at *m/z* 200, an automatic gain control (AGC) target (the number of ions to fill the C-Trap) of 10^6 and a maximum injection time (IT) of 200 ms. The data was acquired using data-dependent scan, using a full scan followed by a product ion scan for those ions above a threshold intensity value of 10^5. The selected ions were isolated with a 0.5 *m/z* window and fragmented by applying stepped normalized collision energies (NCE) of 17.5, 35 and 52.5 eV in the higher-collision dissociation (HCD) cell. At this stage, a mass resolution of 17,500 FWHM at *m/z* 200, with an AGC target at $2x10^5$ and a maximum IT of 200 ms were employed.

Data analysis

Polyphenolic fingerprints for each analyzed rosemary plant were obtained as follows:

HRMS raw data was processed using TraceFinder™ 3.3 software (Thermo Fisher Scientific) by applying a user target accurate mass database list comprising 55 characterized polyphenols. Parameters including chromatographic retention time, accurate mass errors, isotope pattern matches and product ion spectra were used for identification and confirmation purposes. Data matrices with rosemary polyphenolic fingerprints were then built by employing the peak area values of the characterized

polyphenols detected in the samples by TraceFinder™ software at different retention time values (depending if it was the native polyphenols or one of their derivative). Thus, the dimension of the polyphenolic fingerprinting matrix was 123 (samples and QCs) × 194 (peak area signal variables).

Stand Alone Chemometrics Software (SOLO) from Eigenvector Research was used for PAC calculations.[26] A detailed description of the theoretical background of this chemometric method is given elsewhere.[27]

Autoscale pretreatment with respect to the overall polyphenolic concentration was applied to provide similar weighs to all the samples. Scatter plots of scores and loadings of the principal components (PCs) were used to investigate the structure of maps of samples and variables, respectively. The plot of scores showing the distribution of the samples on the PCs revealed patterns that may be correlated to sample characteristics, such as inoculated or non-inoculated samples. The study of the distribution of variables from the loading plot provided information dealing with their correlations as well as dependences of polyphenols on rosemary plant properties.

Results and discussion

Growth parameters measured indicated that mycorrhizal inoculation with *R. irregulare* significantly increased plant height and shoot biomass (Figures 1 and 2). RTM plants were already significantly higher than RTC plants six weeks after inoculation. By the end of the experiment, thirty weeks after mycorrhizal inoculation, the root colonization extent achieved by the symbiotic fungus in RTM plants was 89 $\pm$ 4 % (mean $\pm$ SD) while no mycorrhizal colonization was observed in RTC plants. Previous results already demonstrated growth stimulation due to mycorrhizal colonization of rosemary plants under greenhouse and field conditions as well as a higher production of essential oils.[28] Those results obtained after inoculation with an AM fungus may be related to chemical variations in the plant metabolomics, which can be associated to modifications in the distribution as well as the contents of plant bioactive compounds such as polyphenols.

UHPLC-HRMS Polyphenolic Fingerprinting

. In the present work, the polyphenolic fingerprints of control rosemary plants (non-inoculated) as well as those inoculated with *R. irregulare* were studied at different time periods while the plants were growing after the inoculation process.As an example, Figure 3 shows the total ion chromatogram (TIC) for sample RT0-5. For polyphenolic fingerprinting, a threshold signal of 10^5 was set in the screening software to consider that a compound could be present in a sample, and several confirmation parameters such as accurate mass measurements (mass error lower than 5 ppm) and isotope pattern fit (higher than 85%) were used to confirm the presence of the compound. Additionally, chromatographic retention times and product ion spectra were employed to ensure the identity among the 55 native polyphenols studied. After raw data processing with TraceFinder™ a report was provided for each rosemary plant extract analyzed (see Table 1 as an example).. It should be commented that TraceFinder™ software is only assigning a match when an expected *m/z* value within the accurate mass database list is found in the sample, but isobaric compounds are not differentiated. In those cases, and when standards are available, assignment was also performed from the chromatographic retention time. As an example, Figure 3 shows the extracted chromatogram for carnosol (expected *m/z* value at 329.1758 for the $[M-H]^-$ ion) in the RT0-5 rosemary plant control sample, as well as the HRMS spectrum showing the isotopic cluster (100% isotope pattern match with the theoretical one, and an accurate mass measurement error of 0.97 ppm). In some cases, the obtained reports showed the detection of the same polyphenolic compound (same expected *m/*z value) at different chromatographic retention times within the analyzed samples. This was due to the presence in the sample of both the native polyphenol and some of their derivatives (i.e., glycosylated derivatives and other adducts). These derivative compounds were chromatographically separated from the native polyphenol, but could suffer in-source collision dissociation (CID) fragmentation in the ESI source yielding the ion corresponding to the native compound, and consequently providing a match by TraceFinder™ at a different retention time than that of the native polyphenol.

Exploratory principal component analysis

The data matrix to be analyzed included the rosemary plant polyphenolic profiles from the peak areas provided by TraceFinder™ software at the different retention times assigned (native polyphenols and their derivatives). The dimension of the data set was 123 samples × 194 variables. This matrix was then subjected to a non-supervised exploratory PCA method. Data was autoscaled with respect to the overall polyphenolic signal to provide similar weights to all the samples. PCA results showed that 4 PCs allowed to explain most of the data variance observed within the analyzed samples. Figure 4 shows the score plots of PC1 *vs* PC2. As can be seen, QCs appeared grouped in the center of the plot, demonstrating the good repeatability and robustness of the chemometric method employed and the feasibility of the PCA results. As a first noticeable result, all the rosemary control plants (RT0 samples) were grouped at the right side of the plot, and completely separated from the other samples. As previously commented in the experimental section, after sampling the RT0 samples, rosemary plants were divided in two groups: RTM samples (inoculated with *R. irregulare*) and RTC samples (non-inoculated controls). Although the scatter plot suggested a poor discrimination of samples a deeper study revealed some interesting patterns. For example, it can be observed that PC2 can be related to the growing process of the plants for both RTM and RTC samples, since samples collected six weeks (RTM1 and RTC1) and twelve weeks after inoculation (RTM2 and RTC2) tend to appear at the lowest part of the plot according to PC2, while samples are distributed at the top of the plot (higher values of PC2) when the plant is growing, as can be seen for RTM5 and RTC5 plants which were sampled thirty weeks after inoculation. However, a more interesting result can be extracted when comparing the control samples (RTC) with those inoculated (RTM) all collected at the same time (i.e. RTM1 *vs* RTC1, RTM2 *vs* RTC2, etc.). It can be observed that samples from inoculated rosemary plants (RTM samples) tend to be located further to the left side in the scatter plot than the corresponding non-inoculated ones (RTC samples). This behavior was more clearly observed after longer sampling time periods (see, for instance, samples RTM4 *vs* RTC4 or RTM5 *vs* RTC5). Hence, PC1 seems to be related to changes in plants due to mycorrhizal inoculation.

Results confirmed that physiological responses recorded in rosemary plants due to the symbiotic association were related to chemical modifications in the plant metabolome, and specifically in the polyphenolic profile distribution. In order to see more clearly this behavior, PCA models were built by using only the UHPLC-HRMS polyphenolic fingerprints of rosemary plant samples from both treatments collected at the same time. The highest differentiation, as expected, was observed for samples collected twenty four weeks (RTC4 and RTM4) and thirty weeks (RTC5 and RTM 5) after the inoculation. Figure 5 shows the scatter plots of PC1 *vs* PC3 for (a) RTM4 and RTC4 and (b) RTM5 and RTC5 samples. A clear discrimination between inoculated (RTM4 and RTM5) and non-inoculated (RTC4 and RTC5) samples was obtained.

The study of PCA loading plots, representing the distribution of composition variables with the PCs, in combination with scores showed those polyphenolic signals contributing to the differentiation among inoculated and non-inoculated plant samples. Figure 6 shows the biplots (both score and loading plots) of PC1 *vs* PC3 for (a) RTM4 and RTC4 samples and (b) RTM5 and RTC5 samples. In this figure, triangles are depicting all the polyphenolic variables contributing to the separation of the addressed samples. However, many of these variables were not native polyphenolic compounds but derivatives or isomeric forms that were not completely identified (not the aim of the present contribution). In Figure 6, the names of the variables that are identified and confirmed from chemical standards available are given. From this study, it was concluded that several polyphenolic compounds hosting strong antioxidant properties such as ferulic acid,[29] asiatic acid,[30] carnosol,[31] and vanillin[32] were clearly related to mycorrhizal rosemary plant samples, while other polyphenolic compounds such as caffeic and chlorogenic acids have a higher influence in the non-inoculated plants. These results are in accordance with those previously reported by López-Ráez et al.[33] in tomato roots; the levels of ferulic acid increased after the establishment of a mycorrhizal colonization by two AM fungi, *Glomus intraradices* and *Glomus mosseae*, while the levels of chlorogenic and caffeic acids decreased. Work reported by Rivero et al.[10] also demonstrated a rearrangement of metabolites but again in mycorrhizal tomato roots. A review article by Pedone-Bonfim et al.[34] concluded that in most situations the

arbuscular mycorrhizal association favors the accumulation of secondary metabolites that can potentially act as bioactive compounds, but it does not mention any analytical results concerning phenolic compounds obtained from leaves of rosemary plants. Other polyphenols such as rosmanol, rosmarinic acid, genkwanin, D-(-)-quinic acid and cirsimaritin seem to play also an important role in the sample classification. It is nevertheless not so clear, at this stage of the study, if they are more or less related to the effect the inoculation with AM fungi exerts on rosemary plants.

Conclusions

The process of artificially inoculating rosemary plants with effective arbuscular mycorrhizal fungi in nurseries can lead to several benefits according to the results exposed. Mycorrhization will clearly stimulate growth in order to obtain a higher biomass of plant leaves in short time and reducing chemical fertilization, while analytical results demonstrate that there is an alteration in the distribution of polyphenols in plants colonized by the symbiotic fungus. The industrial significance of obtaining higher levels of compounds in leaves with therapeutic and nutritional properties will be the basis of future work.

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Figure captions

Figure 1. Rosemary growth measured as shoot height at different time periods after inoculation with Rhizoglomus irregulare. Data are means + SD of ten replicates (n=10) analyzed by one-way ANOVA. Different letters next to the point marks indicate significant differences between treatments after Tukey s test (pd 0,05).

Figure 2. Rosemary plant biomass measured as shoot dry weight at harvest, 30 weeks after plant inoculation with *Rhizoglomus irregulare*. Data are means + SD of ten replicates (n=10) analyzed by one-way ANOVA. Different letters next to bars indicate significant differences between treatments after Tukey s test (pd0.05).

Figure 3. UHPLC-HRMS total ion chromatogram (TIC), extracted ion chromatogram for carnosol, and HRMS spectrum of carnosol in the rosemary control plant RT0-5.

Figure 4. PCA score plot of PC1 *vs* PC2 when using UHPLC-HRMS chromatographic fingerprints of all the analyzed rosemary plants and QCs.

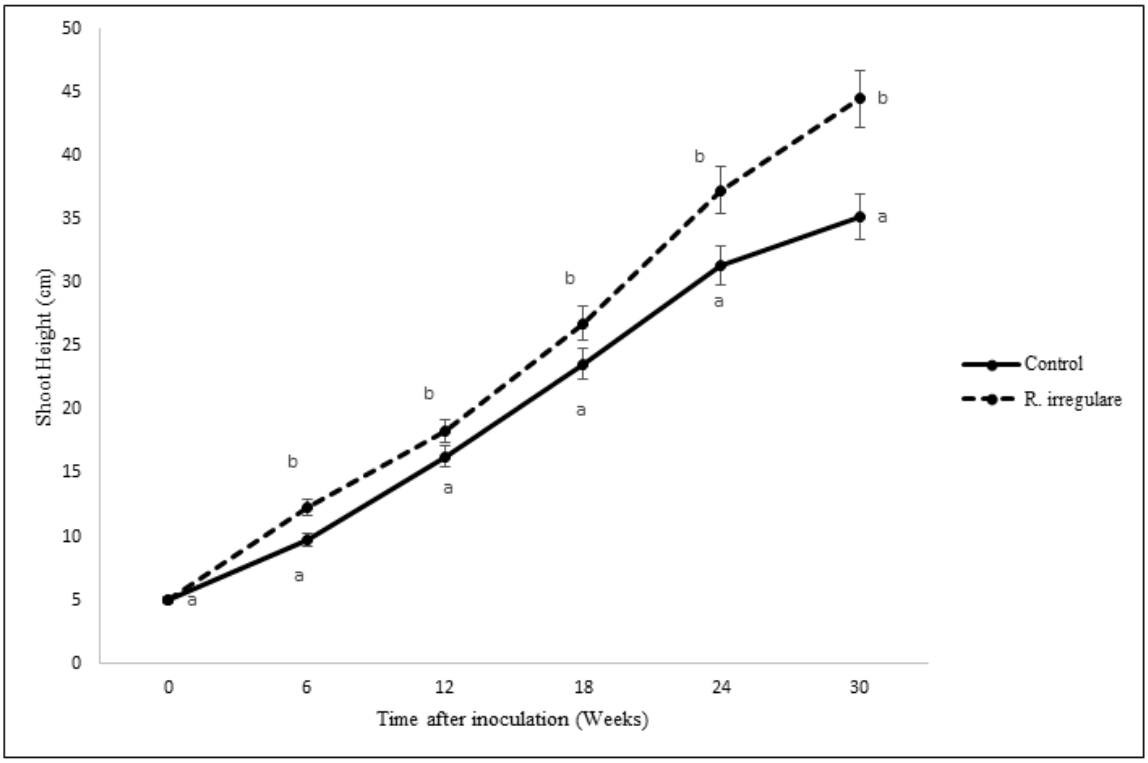
Figure 5. PCA score plots of PC1 *vs* PC3 for (a) RTM4 and RTC4 and (b) RTM5 and RTC5 samples.

Figure 6. Biplots of PC1 *vs* PC3 for (a) RTM4 and RTC4 and (b) RTM5 and RTC5 samples. Diamonds depicts the samples and triangles the polyphenolic variables.

Table 1: TraceFinder™ report for rosemary plant sample RT0-5 when using a user target accurate mass database list of 55 characterized polyphenols.

Found	Comfirmed	Target Name	+/-	Area	RT (Meas.)	Formula	Expected m/z	Measured m/z	Delta m/z	Isotopic Pattern Score

										(%)
●	1 out of 1	D-(-)-Quinic acid	-	2.51E+07	1.14	C7H12O6	191.0561	191.05644	1.79	100
●	1 out of 1	3,4-Dihydroxybenzaldehyde	-	6.87E+06	5.43	C7H6O3	137.0244	137.02432	-0.57	100
●	1 out of 1	4-Hydroxybenzoic acid	-	6.87E+06	5.43	C7H6O3	137.0244	137.02432	-0.57	100
●	1 out of 1	Homovanillic acid	-	9.84E+05	5.43	C9H10O4	181.0506	181.05087	1.51	100
●	1 out of 1	Syringaldehyde	-	9.84E+05	5.43	C9H10O4	181.0506	181.05087	1.51	100
●	1 out of 1	Veratric acid	-	9.84E+05	5.43	C9H10O4	181.0506	181.05087	1.51	100
●	1 out of 1	p-coumaric acid	-	1.14E+06	6.88	C9H8O3	163.0401	163.04007	-0.19	100
●	1 out of 1	4-o-Caffeoylquinic acid (cryptochlorogenic acid)	-	1.61E+06	7.11	C16H18O9	353.0878	353.08795	0.43	99
●	1 out of 1	Chlorogenic acid	-	1.61E+06	7.11	C16H18O9	353.0878	353.08795	0.43	99
●	1 out of 1	Homogentisic acid	-	1.43E+06	7.20	C8H8O4	167.0350	167.03493	-0.32	100
●	1 out of 1	Vanillic acid	-	1.43E+06	7.20	C8H8O4	167.0350	167.03493	-0.43	100
●	1 out of 1	Caffeic acid	-	1.12E+07	7.35	C9H8O4	179.0350	179.03516	0.87	100
●	1 out of 1	Quercitrin hydrate	-	2.20E+06	10.69	C21H20O11	447.0933	447.09369	0.87	100
●	1 out of 1	Nepetin-7-glucoside	-	4.18E+07	11.12	C22H22O12	477.1038	477.10425	0.94	100
●	1 out of 1	Hesperidin	-	9.90E+06	11.17	C28H34O15	609.1825	609.18347	1.60	100
●	1 out of 1	Ferulic acid	-	6.06E+06	11.23	C10H10O4	193.0506	193.05083	1.18	100
●	1 out of 1	Rosmarinic acid	-	6.03E+08	11.64	C18H16O8	359.0772	359.0777	1.39	100
●	1 out of 1	Vanillin	-	5.66E+05	11.64	C8H8O3	151.0401	151.04005	-0.30	100
●	1 out of 1	Ethyl gallate	-	6.43E+06	11.65	C9H10O5	197.0455	197.04523	-1.39	100
●	1 out of 1	Syringic acid	-	6.43E+06	11.65	C9H10O5	197.0455	197.04523	-1.39	100
●	1 out of 1	Umbelliferon	-	2.81E+07	11.65	C9H6O3	161.0244	161.02411	-1.81	100
●	1 out of 1	Homoplantaginin	-	2.88E+07	12.01	C22H22O11	461.1089	461.10947	1.23	100
●	1 out of 1	Trans-Cinnamic acid	-	1.72E+05	12.25	C9H8O2	147.0452	147.04507	-0.85	100
●	1 out of 1	Morin hydrate	-	1.41E+05	13.38	C15H10O7	301.0354	301.03555	0.51	100
●	1 out of 1	Quercetin	-	1.41E+05	13.38	C15H10O7	301.0354	301.03555	0.51	100
●	1 out of 1	Luteolin-7-O-b-D-glucuronide	-	4.01E+07	13.77	C23H20O13	503.0831	503.08377	1.33	100
●	1 out of 1	Fisetin	-	6.37E+06	13.78	C15H10O6	285.0405	285.04050	0.13	100
●	1 out of 1	Kaempferol	-	6.37E+06	13.78	C15H10O6	285.0405	285.04050	-0.01	100
●	1 out of 1	procyanidine B2	-	6.72E+05	14.80	C30H26O12	577.1352	577.13574	1.03	100
●	1 out of 1	Rosmanol	-	1.02E+08	14.80	C20H26O5	345.1707	345.17093	0.66	100
●	1 out of 1	Cirsimaritin	-	2.66E+07	15.57	C17H14O6	313.0718	313.07202	0.71	100
●	1 out of 1	Genkwanin	-	1.22E+07	17.44	C16H12O5	283.0612	283.06125	0.17	100
●	1 out of 1	Carnosol	-	8.32E+08	18.46	C20H26O4	329.1758	329.17618	1.15	100
●	1 out of 1	Asiatic acid	-	4.24E+06	18.86	C30H48O5	487.3429	487.34338	0.99	100
●	1 out of 1	Anemosapogein	-	3.74E+06	20.35	C30H48O4	471.3480	471.34821	0.44	100
●	1 out of 1	Carnosic acid	-	1.08E+08	20.88	C20H28O4	331.1915	331.19193	1.28	100
●	1 out of 1	Betulinic acid	-	1.35E+07	22.45	C30H46O3	453.3374	453.33801	1.35	85
●	1 out of 1	Ursolic acid	-	1.35E+07	22.45	C30H46O3	453.3374	453.33801	1.35	85



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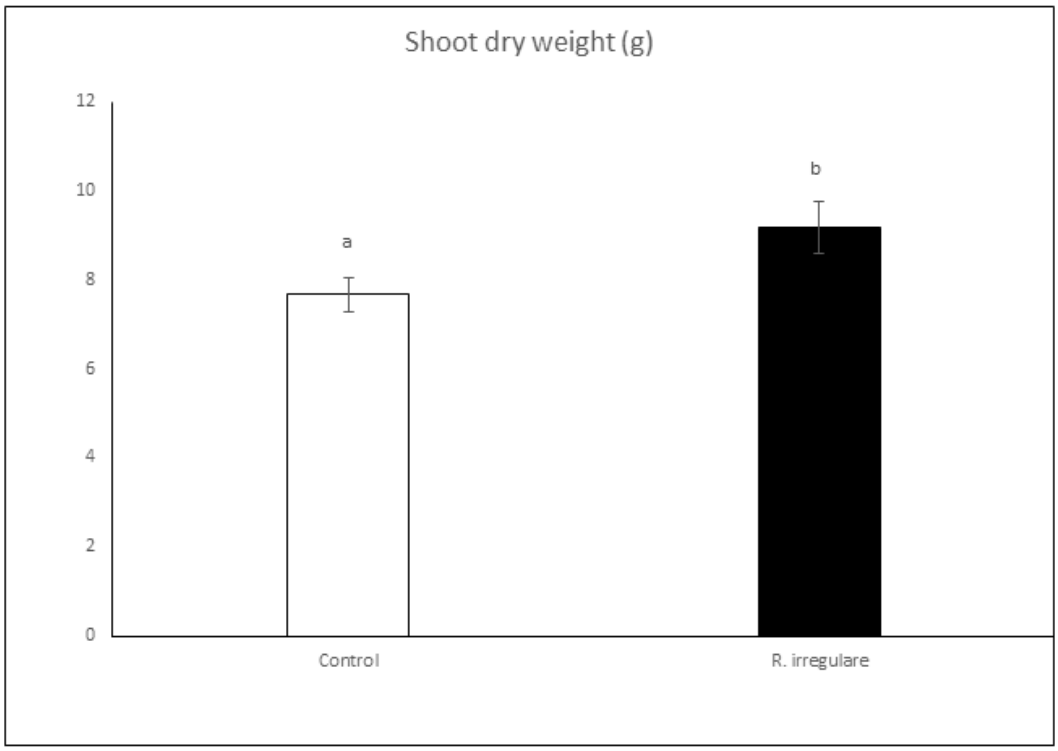

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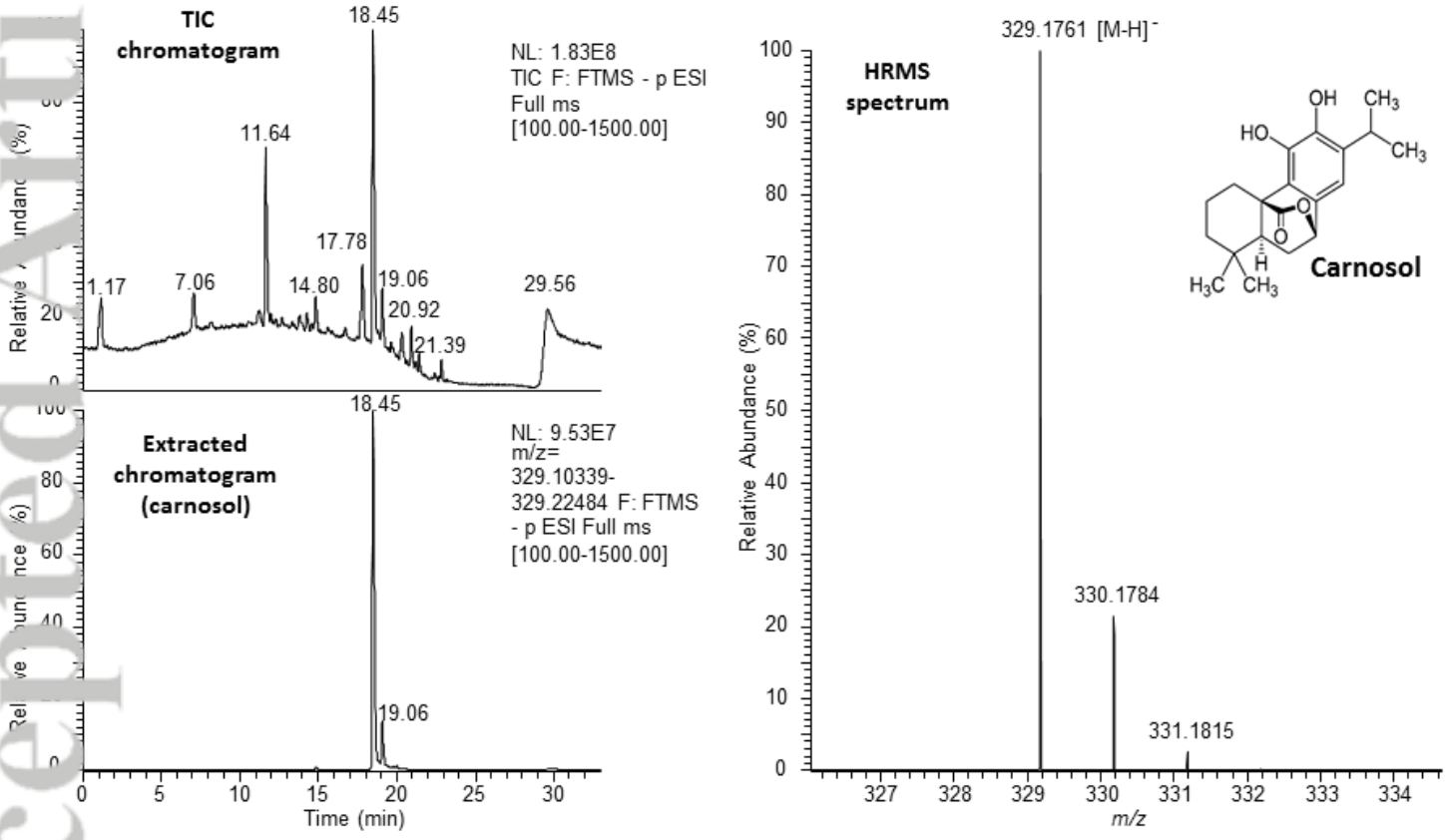

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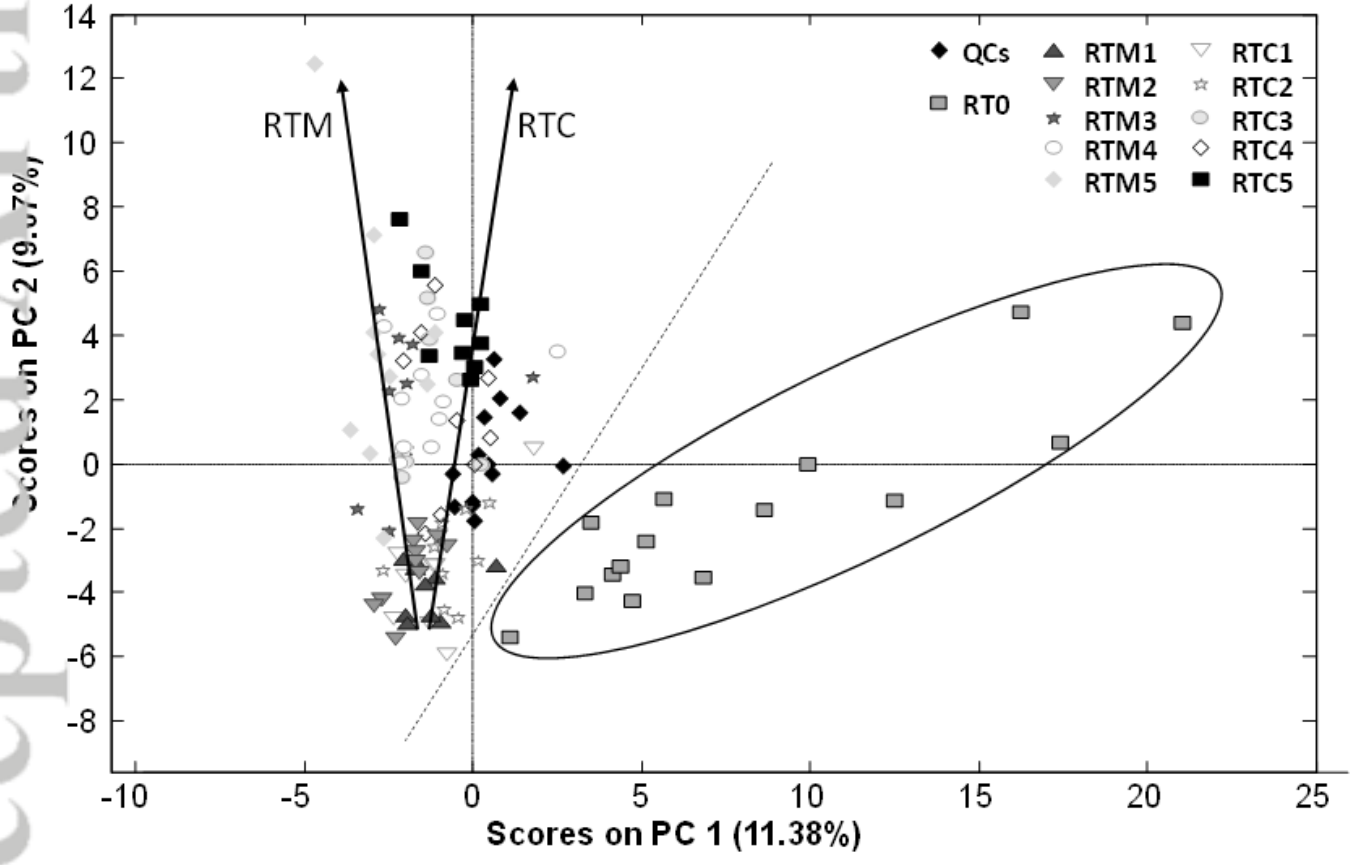

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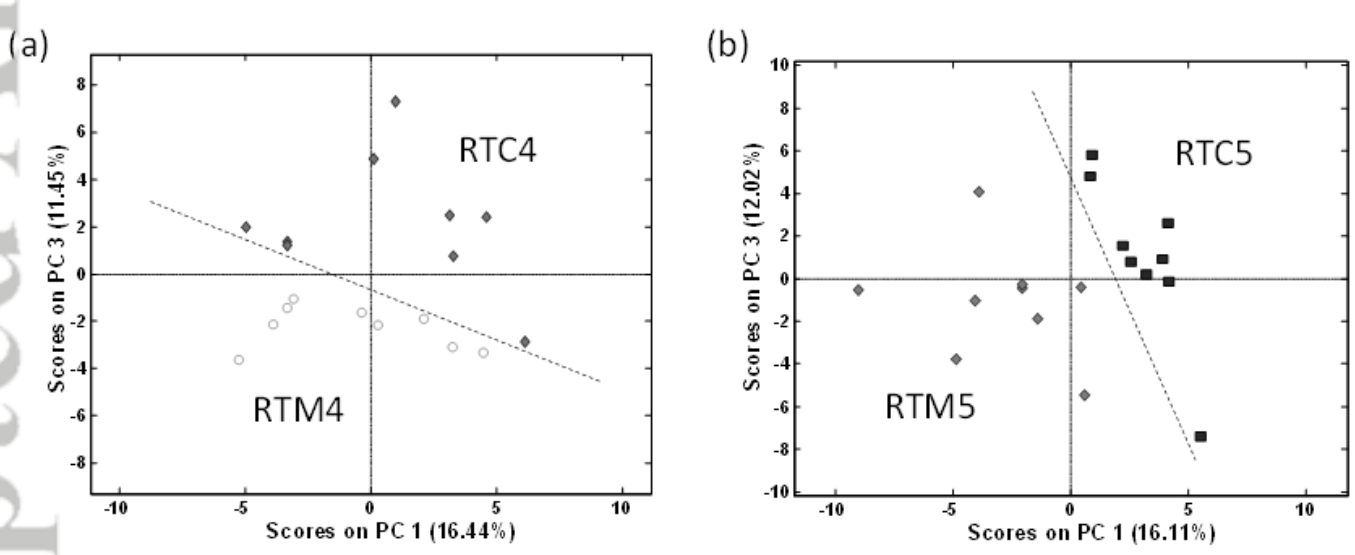

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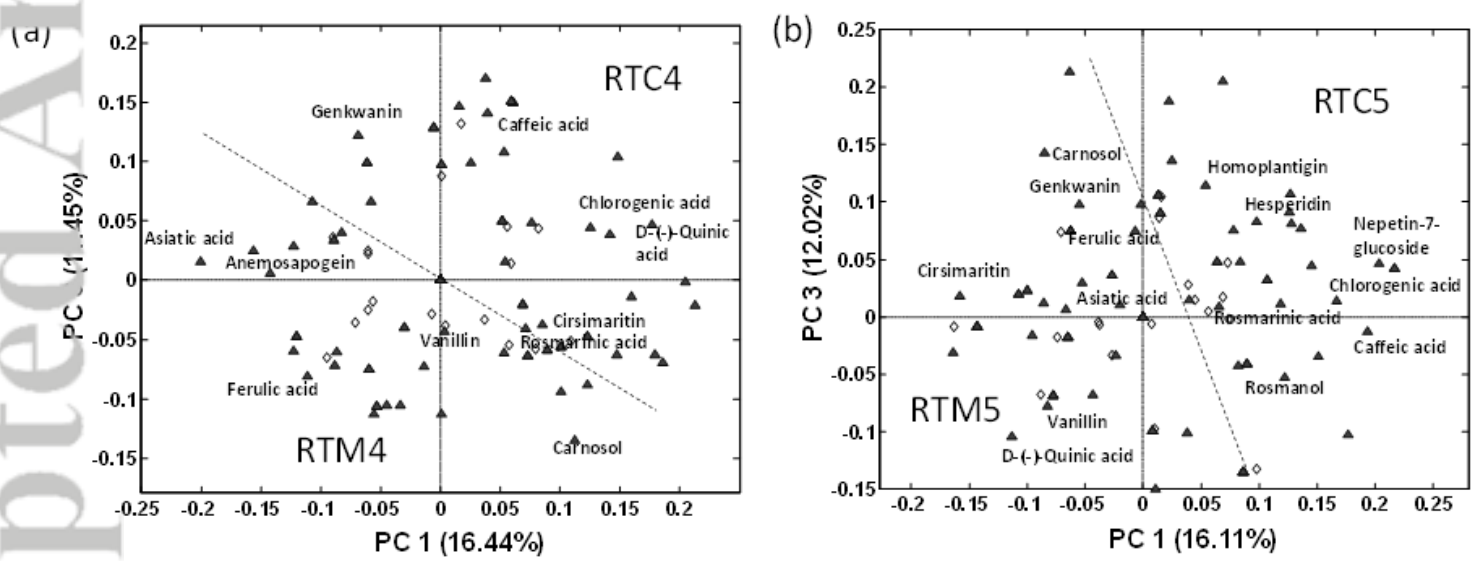

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