# Modulation of plant chemistry by rhizosphere bacteria

Jeon, J.

# Chapter 4

# Steering the Broccoli shoot metabolome by root-colonizing *Paraburkholderia* species: impacts on growth and defense

Je-Seung Jeon, Natalia Carreno-Quintero, Ric De Vos,
Jos M. Raaijmakers and Desalegn W. Etalo

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## Abstract

Beneficial rhizobacteria can promote plant growth and induce resistance. Recent advances in analytical chemistry allow to study the impact of rhizobacteria on primary and secondary metabolism in plants and how these changes associate with the plant phenotypic changes. In this study, a new strategy integrating GC/LC-MS-based metabolomics was firstly employed to elucidate changes in primary and secondary metabolite networks in the shoots of two Broccoli cultivars upon root colonization by three *Paraburkholderia* species, *P. graminis* (*Pbg*), *P. hospita* (*Pbh*), and *P. terricola* (*Pbt*). Results showed that *Pbt* was a poor colonizer of the roots of Malibu cultivar and did not promote plant growth in contrast to other combinations of the two Broccoli cultivars and *Paraburkholderia* species. *Pbh* and *Pbt* induced resistance in Malibu cultivar to infections by the bacterial leaf pathogen *Xanthomonas campestris*. Subsequent metabolomics exhibited that soluble sugar levels were much higher in leaves of Malibu cultivar most likely providing resources for the biosynthesis of phenylpropanoid downstream metabolites such as hydroxycinammates, flavonoids, and stilbenoids, metabolites with antimicrobial activities. In addition to enhancement of key precursors for growth and secondary metabolite biosynthesis, treatment of *Paraburkholderia* also induced metabolite remobilization. The metabolite remobilization involved both suppression of resource competing metabolite pathways such as amino acids and rechanneling of existing primary metabolite-derivatives and other secondary metabolites to other metabolite pathways. Flavonoids, hydroxycinnamates, stilbenoids, coumarins and lignins showed substantial accumulation upon treatments with the *Paraburkholderia* species, metabolites with direct antimicrobial effects and that can act as a physical barrier against pathogenic bacteria such as *Xanthomonas campestris*. The integrated primary and secondary metabolome profiling conducted in this study further suggests that rhizobacteria could avert the negative impact of defense priming on the host fitness by generating substantial amounts of soluble sugars and by remobilizing other metabolites to compensate for the high energy and carbon skeleton demand associated with growth and defense priming.

**Keywords:** broccoli metabolites; *Paraburkholderia*; beneficial rhizobacteria; non-targeted metabolomics; secondary metabolites, primary metabolites.

## Introduction

Plants are fine-tuned factories of more than 100,000 secondary metabolites (Wink, 2010). Many plant species exhibit pharmaceutical activities, providing valuable scaffolds for the development of new pharmaceuticals (Cragg & Newman, 2013; Bauer & Brönstrup, 2014). For the plant itself, secondary metabolites are involved in adaptation to environmental changes (Bourgaud *et al.*, 2001) including tolerance to biotic stresses such as insect herbivory and pathogen infections (Bennett & Wallsgrove, 1994; Rattan, 2010; Boulogne *et al.*, 2012). Therefore, steering plant chemistry to elevate specific metabolites has been a major direction in plant breeding. Recent studies have shown that rhizobacteria, i.e. bacteria colonizing the surface and internal plant root tissue, can enhance plant growth, induce systemic resistance and alter changes in plant chemistry, providing new avenues to enhance the levels of specific plant secondary metabolites of interest.

Plants photosynthetically fix carbon and metabolize it for growth and defense. Both processes appear incompatible due to limited resources and is referred to as the "defense-growth trade-off" (Huot *et al.*, 2014). However, several studies have shown that specific rhizobacteria can parallelly induce both growth and defense. For instance, our previous studies demonstrated that *Pseudomonas fluorescens* SS101 enhanced biomass of Arabidopsis as well as resistance against a bacterial leaf pathogen and insect herbivory (van de Mortel *et al.*, 2012; Cheng *et al.*, 2017). Similar results were observed in *Oryza sativa* (Chamam *et al.*, 2013), *Panax ginseng* (Gao *et al.*, 2015), *Piper betle* (Lavania *et al.*, 2006), *Pisum sativum* (Mabrouk *et al.*, 2007), and *Salvia officinalis* (Ghorbanpour *et al.*, 2016) upon inoculation with various rhizobacterial genera. However, how specific rhizobacteria induce defense and enhance growth simultaneously is not well understood. Here, we studied the impact of root-colonizing *Paraburkholderia* species on growth and defense of two Broccoli cultivars. Broccoli (*Brassica olearacea* var. italica) is a crop plant consumed worldwide, known to have high value natural compounds including glucosinolates and flavonoids (Naguib *et al.*, 2012). The Broccoli cultivars used in present study were Coronado and Malibu, bred to possess high and lower levels of glucosinolates (glucoiberin, glucoraphanin and glucobrassicin), respectively. *Paraburkholderia* is a diverged monophyletic clade from the genus *Burkholderia* (Sawana *et al.*, 2014). A number of rhizospheric and endophytic *Paraburkhoderia* species, in particular *Paraburkholderia phytofirmans* PsJN, *P. fungorum* and *P. graminis,* can promote growth of maize, strawberry and Arabidopsis (Ledger *et al.*, 2016; Mitter *et al.*, 2017; Rahman *et al.*, 2018) and suppress pathogen infections (Timmermann *et al.*, 2017; Carrión *et al.*, 2018). Also various *Paraburkholderia* species are typically found in the mycosphere consuming organic acids released from the fungi and using the hyphae as 'highways' for translocation (Nazir *et al.*, 2009). The *Paraburkholderia* species used in our work presented here are *Paraburkholderia graminis* (*Pbg*), *P. hospita* (*Pbh*), and *P. terricola* (*Pbt*) which exhibited plant protection against the fungal root pathogen *Rhizoctonia solani.* For *P. graminis*, we further showed that the production of sulfurous volatiles was a key mechanism in disease

suppression (Carrión *et al.*, 2018).

In the present study, we monitored the effects of different root-colonizing *Paraburkholderia* species on phenotypes of two Broccoli cultivars, in particular growth promotion and defense against the bacterial leaf pathogen *Xanthomonas campestris*. At two different time points during the *Paraburkholderia*-Broccoli interactions we conducted untargeted metabolomics to map the systemic changes in primary and secondary metabolomism in the shoots of Broccoli. Our results show that, in the partnerships of *Paraburkholderia* species with the two Broccoli cultivars, there were common and specific signatures in both primary and secondary metabolism. The results suggest that the enhanced accumulation of soluble sugars in shoots of Broccoli cultivars upon *Paraburkholderia* root colonization translate into distinct changes in secondary metabolism that in turn associate with distinctive changes in plant growth and defense. The integrated strategy adopted in this study enhanced our fundamental understanding of metabolic fluxes associated with plant growth and defense.



## Materials and Methods

### Bacterial strains and culture conditions

The rhizobacterial strains *Paraburkholderia graminis* (*Pbg*), *P. hospita* (*Pbh*), and *P. terricola* (*Pbt*) used in this study were originally isolated from rhizospheric soil of *Beta vulgaris* grown in *Rhizoctonia solani* suppressive soil (Carrión *et al.*, 2018). Culture of *Paraburkholderia* species were maintained in Luria Bertani (LB)-medium (Lennox, Carl Roth) at 25 °C. After incubation for 16 hours, bacteria cells were spun down to make bacteria pellets. These pellets were then washed three times with 10 mM $MgSO_4$ and resuspended in 10 mM $MgSO_4$ to a final density of $OD_{600}$ = 1.0 (~$10^9$ cells per ml).

### Plant Materials and growth conditions

Seeds of two Broccoli cultivars (*Brassica olearacea* var. italica), Coronado and Malibu, were kindly provided by Bejo Seeds (Warmenhuizen, The Netherlands). The seeds were surface sterilized for 30 minutes by immersing them in 1% (v/v) sodium hypochlorite amended with 0.1 % (v/v) of Tween 20, and rinsed three times with ample sterile distilled water. Thereafter, five seeds were sown on 100 X 100 mm square petri dishes containing 50 ml of half-strength Murashige and Skoog (0.5 X MS) agar media with 0.5% sucrose (w/v) and 1.2% plant agar (w/v). Five days after germination and vegetative growth in petri dishes, the root tips of the seedlings from the two Broccoli cultivars were inoculated with 2 µl cell suspension (±$10^9$ cells per ml) of each three *Paraburkholderia* species. Plants treated with 2 µl 10 mM $MgSO_4$ served as controls. The plates with the control and inoculated plants were then sealed and incubated in a climate chamber (21 °C / 21 °C day/night temperature; 180 µmol light $m^{-2}s^{-1}$ at plant level during 16 h/d; 70% relative humidity) until harvest (11 days post inoculation).

Temporal changes in shoot fresh biomass were measured every two days until harvest.

**Rhizobacteria root colonization assay**

Bacterial root colonization was determined at 6 and 11 dpi for each of the three *Paraburkholderia* species on each of the two Broccoli cultivars. Briefly, treated roots were collected at 6 and 11 dpi and placed in sterile 50 mL Falcon tube and its biomass was measured. Then the root samples were vortexed (60 s) in 10 mM $MgSO_4$, sonicated (60 s), and again vortexed (15 s) to resuspend the bacteria adhering to the root. The suspensions were serial dilution plated onto PSA plates containing 100 µg $ml^{-1}$ delvocid (DSM) to inhibit fungal growth. Plates were incubated at 25 ℃ in the dark for 3 days, colonies were counted and the number of colony-forming units (cfu) per gram of root fresh weight was calculated.

**Plant phenotyping**

Fresh biomass of the Broccoli shoots was measured to determine the effect of the rhizobacteria on plant growth. For Broccoli, shoot fresh biomass from the respective treatments was weighed every two day after bacterial inoculation until the last harvest at 11 dpi. The average weight of 5 Broccoli seedlings was considered as one independent biological replicate. The roots were carefully removed from the MS-agar and washed with distilled water to eliminate adhering agar, blotted dry on filter paper and their fresh weight was recorded.



**Induced resistance assays**

To assess the impact of *Paraburkholderia* species on induced resistance, the two Broccoli cultivars were inoculated with the three *Paraburkholderia* species and grown for 11 days. Thereafter, leaves were inoculated with the bacterial leaf pathogens *Xanthomonas campestris* pv. Armoraciae P4216 (*Xc*a) and *Xanthomonas campestris* pv. Campestris P4014 (*Xc*c). To do that, *Xc*a and *Xc*c were cultured in LB-medium at 25 ℃. After 16 hours, bacterial cells were washed following similar procedure for *Paraburkholderia* as described above. A 2 µl suspension of *Xc*a or *Xc*c (~1 X $10^9$ cell per ml) was inoculated on the first true leaf of the Broccoli seedlings after scratching the leaf surface with sterile 20 µl pipet tips. Ten days after pathogen challenge, disease severity of the shoots was assessed by determining the migration of the lesion from the inoculation spot to the other parts of the shoot following an ordinal scale from 0 - 5 as shown in supplementary **Fig S2**. Severity values were converted to 0 to 100 Disease severity index (DSI) using the equation (Kobriger & Hagedorn, 1983). DSI (%) = ∑(severity class x no. plants in class)/(total no. of plants x the highest class No.)

**Plant metabolomics**

*Sample collection*

Shoots from the control plants and plants treated with three *Paraburkholderia* species were harvested at 6 and 11 dpi. For each plant cultivar x *Paraburkholderia* combination, 4 biological replicates of 5 plants each were considered. Briefly, shoots were snap frozen in liquid nitrogen and ground to fine powder under continuous cooling and kept at -80 °C until further use.

*Polar primary metabolite extraction and analysis*

Polar primary metabolite sample preparation was performed as described by (Weckwerth *et al.*, 2004; Carreno-Quintero *et al.*, 2012). Briefly, a total of 1.4 mL of methanol containing ribitol (0.2mg/mL) as an internal standard was added to a 2 mL Eppendorf tube containing a total of 200 mg Broccoli leaf powder. After vortexing (10 s) and shaking in a thermomixer at 950 rpm for 10 min, the samples were centrifuged at maximum speed for 10 min. 500 uL of the supernatant was transferred to a new 2 mL Eppendorf tube and 370 µl of chloroform and 750 µl of distilled water were added. The mixture was vigorously mixed by vortexing and centrifuging for 10 min at maximum speed (14,000 rpm). 50 µl of the upper polar phase was transferred to an insert in a 2 mL vial. The solvent was then vacuum dried (speedvac) for 16 h at room temperature and sealed under an argon atmosphere. The dried samples were derivatized online as described by Lisec *et al*. (2006) using a Combi PAL autosampler (CTC Analytics). Initially, 12.5 µl methoxyamine (20 mg $mL^{-1}$ pyridine) was added to each of the samples and incubated for 30 min at 40 °C under agitation. The samples were then derivatized for one hour by adding 17.5 µl of *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA). An alkane mixture (C11-C21 and C24-C33) was added to determine the retention indices of metabolites. The derivatized samples were analyzed by a GC-TOF-MS system consisting of an Optic 3 high-performance injector (ATAS) and an Agilent 6890 gas chromatograph (Agilent Technologies) coupled to a Pegasus III time-of-flight mass spectrometer (Leco Instruments).

2 uL of each sample was subjected to the injector at 70 °C using a split flow of 19 mL $min^{-1}$. The chromatographic separation was performed using a VF-5ms capillary column (Varian; 30 m x 0.25 mm x 0.25 mm) including a 10-m guardian column with helium as carrier gas at a flow rate of 1 mL $min^{-1}$. The temperature was isothermal for 2 min at 70 °C, followed by a 10 °C $min^{-1}$ ramp to 310 °C, and was held for 5 min. The transfer line temperature was set at 270 °C. The column effluent was ionized by electron impact at 70 eV. Mass spectra were acquired at 20 scans $s^{-1}$ within a mass-to-charge ratio range of 50 to 600 at a source temperature of 200 °C. A solvent delay of 295 s was set. The detector voltage was set to 1,400 V.

*Semi-polar secondary metabolite extraction and analysis*

For extraction of semi-polar secondary metabolites, 300 µL of 99.89% methanol containing 0.13% (v/v) formic acid was added to 100 mg plant material in 2 ml round bottom Eppendorf tubes, and sonicated for 15 min followed by centrifugation for 15 min at 20,000 X *g*. The supernatants containing predominantly the semi-polar metabolites were transferred to 96-well filter plate (AcroPrepTM, 350 µL, 0.45µm, PALL), vacuum filtrated to the 96-deep-well autosampler plates (Waters) using a Genesis Workstation (Tecan Systems).

An UltiMate 3000 U-HPLC system (Dionex) was used to create a 45 minutes linear gradient of 5-35% acetonitrile in 0.1% formic acid (FA) in water at a flow rate of 0.19 ml $min^{-1}$. 5 µl of each extract was injected and compounds were separated on a Luna C18 column (2.0 X 150 mm, 3µm; Phenomenex) maintained at 40 °C (De Vos *et al.*, 2007). The detection of compounds eluting from the column was performed with a Q-Exactive Plus Orbitrap FTMS mass spectrometer (Thermo Scientific). Full scan MS data were generated with electrospray in switching positive/negative ionization mode at a mass resolution of 35,000 (FWHM at m/z 200) in a range of *m/z* 95-1350. Subsequent MS/MS experiments for identification of selected metabolites were performed with separate positive or negative electrospray ionization at a normalized collision energy of 27 and a mass resolution of 17,500. The ionization voltage was optimized at 3.5 kV for positive mode and 2.5 kV for negative mode; capillary temperature was set at 250 °C; the auxiliary gas heater temperature was set to 220 °C; sheath gas, auxiliary gas and the sweep gas flow were optimized at 36, 10 and 1 arbitrary units, respectively. Automatic gain control was set a $3^{e6}$ and the injection time at 100 ms. External mass calibration with formic acid clusters was performed in both positive and negative ionization modes before each sample series.



**Data processing and analysis**

*GC-TOF-MS*

Raw data was primarily processed by Chroma TOF software 2.0 (Leco Instruments) and MassLynx software (Waters), and subjected to MetAlign software to extract and align the mass signal whose signal-to-noise ratio larger than 3 as described by Carreno-Quintero *et al*. (2012). The mass features were considered as a signal if they were detected in at least 3 biological replicates. Mass features fragments originating from the same metabolites were clustered together by MSClust software into 138 representative primary metabolites. Further, data transformation and scaling were performed in GeneMaths XT 1.6 (www.applied-maths.com). This then used for hierarchical cluster analysis using Pearson's correlation coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA). To identify metabolites, the reconstructed mass spectra file was introduced to NIST MS search software (v 2.2) with Wiley spectral libraries and in-house library, followed by comparison with

retention indices determined by a series of alkanes. Metabolite annotations were manually curated. The mass intensity of the representative metabolites was normalized by the internal standard, ribitol.

*LC-MS*

Peak picking, baseline correction, and mass signal alignment of the LC-MS data was performed using Metalign software (Lommen, 2009). The mass features were considered as a signal if they were detected in at least 3 biological replicates of a treatment with signal intensity 3 times of the noise value. Then, mass features originating from the same metabolites were clustered based on retention window and their correlation across all measured samples, using MSClust software (Tikunov *et al.*, 2012). After this, so-called centrotypes, representing reconstructed putative metabolites mass spectra were selected, of which relative abundance was represented by the Measured Ion Count (MIC) representing the sum of the ion count values (corrected by their membership) for all measured cluster ions in a given sample. The samples were batch corrected to reduce batch effect of large series of samples during the LCMS analysis according to (Wehrens *et al.*, 2016). ANOVA and fold changes > 2.0 were applied to identify mass signals that were significantly changed between the treatments. Data transformation and scaling was performed in GeneMaths XT 1.6 (www.applied-maths.com). Transformed and scaled values were used for hierarchical cluster analysis using Pearson's correlation coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

Annotation of differential metabolites was performed based on selection of pseudomolecule ions from the masses in the MSClust-reconstructed metabolites, first by matching their accurate masses plus retention times to previously reported metabolites present in Arabidopsis and Broccoli on the same LC-MS system and similar chromatographic conditions. Second, if compounds were not yet present in this experimentally obtained database, detected masses were matched with compound libraries, including Metabolomics Japan (www.metabolomics.jp), the Dictionary of Natural Products (http://dnp.chemnetbase.com), KNApSAcK (http://kanaya.naist.jp/KNApSAcK), and Metlin (http:// metlin.scripps.edu/) using a maximum deviation of observed mass from calculated of 5 ppm. The identity of potential candidate metabolites was further verified using Magma online tool (Ridder *et al.*, 2013) that compares the Insilco fragmentation patterns of a given metabolites to the experimentally obtained fragmentation patterns.

**Statistical analysis**

The relative changes in shoot biomass, root biomass in the combinations of two Broccoli cultivars and *Paraburkholderia* species was analyzed with R Studio software (Version 3.6.1). First, the normality and homogeneity of variance of the data was assessed and when the two assumptions were not met the data was transformed using Box-Cox or log transformation

using a package MASS. Differences were tested by two-way analysis of variance (ANOVA). A Tukey-HSD test was used to separate group mean values when the ANOVA was significant at $p < 0.05$. The ANOVA table is shown in Supplementary Material, **Table S1**. Differences in phenotypic parameters between the rhizobacterial treatments and non-treated controls were assessed by Student's *t*-Test.

## Results

### *Paraburkholderia* species promote growth of Broccoli

In general, root tip inoculation of the Broccoli cultivars with different *Paraburkholderia* species led to changes in leaf color (deep green leaves), shoot biomass, root biomass and root architecture (**Fig 1a**). Percent change in biomass was used as a standard measure to assess the growth-promoting effects of the *Paraburkholderia* species in two Broccoli cultivars. The percent change in biomass was calculated by dividing the biomass difference between rhizobacteria-treated plants and the control to the biomass of the control plants. Two-way analysis of variance was conducted to assess the influence of the two independent variables (*Paraburkholderia* species and Broccoli cultivars) on shoot and root biomass. The *Paraburkholderia* species included three levels (*Pbg*, *Pbh* and *Pbt*) and the Broccoli cultivars consisted of two levels (Coronado and Malibu). Furthermore, two-tailed student's *t*-test was used to assess the impact of the *Paraburkholderia* species inoculation on shoot and root biomass when compared to the control plants.

Results showed that all but *Pbt*-Malibu interaction resulted in significant increase in shoot biomass when compared to the control plants (**Fig1b**). All three *Paraburkholderia* species significantly increased the root biomass in both Broccoli cultivars when compared to the control (**Fig 1c**). In general, the relative impact of *Paraburkholderia* species was up to 3 times higher for root biomass than for shoot biomass (**Fig 1b** and **1c**). The two-way ANOVA showed highly significant interactions between *Paraburkholderia* species and Broccoli cultivars regarding the percent change in shoot and root biomass (Supplementary **Table S1**). Overall, for cultivar Coronado the percent change in shoot biomass was not significantly different between the three *Paraburkholderia* species. Whereas, in Malibu the percent change in shoot biomass was significantly higher for *Pbg* and *Pbh* inoculated plants as compared to *Pbt*. Furthermore, inoculation of plants with *Pbh* led to a significantly higher increase in shoot biomass in Malibu than in cultivar Coronado. When it comes to percent change in root biomass, only inoculation of *Pbt* showed significant differences between the two Broccoli cultivars. Over a period of 11 days, both *Pbg* and *Pbh* inoculated Broccoli cultivars showed significantly higher shoot and root biomass from 7 dpi onwards. Whereas, *Pbt* inoculated plants showed significantly higher shoot biomass in Coronado cultivar from 9 dpi onward. As indicated above, the shoot biomass of Malibu cultivar inoculated with *Pbt* was not significantly different from the untreated plants (**Fig 1d**).

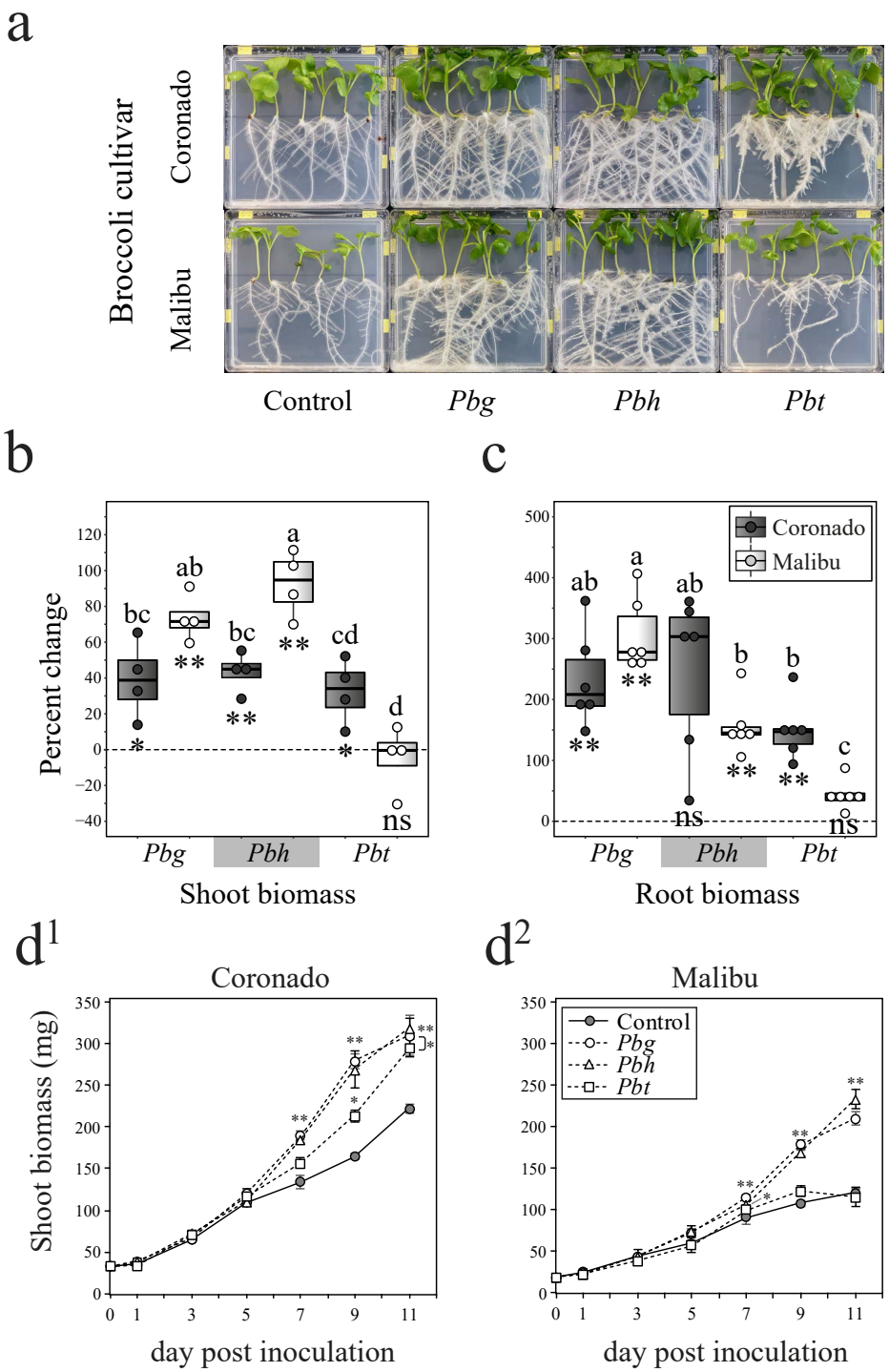

a
Broccoli cultivar
Coronado
Malibu
Control
Pbg
Pbh
Pbt
b
Percent change
Shoot biomass
c
Coronado
Malibu
Root biomass
d1
Coronado
Shoot biomass (mg)
day post inoculation
d2
Malibu
Control
Pbg
Pbh
Pbt
day post inoculation

**Fig 1.**

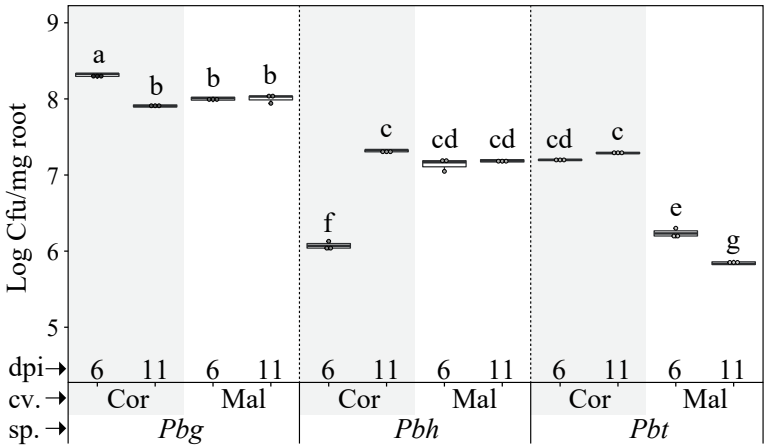



**Fig 2**. Root colonization ability of three *Paraburkholderia* species on two Broccoli cultivars at 6 and 11 dpi. Means of 3 replicates are shown. Different letters indicate significant differences based on three-ways ANOVA (Broccoli cultivars and Bacteria species, $P < 0.05$). Broccoli cultivars (Cor: Coronado, Mal: Malibu) and *Paraburkholderia* species, *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*.



**Relation between root colonization and plant growth promotion**

Root colonization of the three *Paraburkholderia* species was assessed for the two Broccoli cultivars at the early and late seedling growth stages. The data was log-transformed as the data did not meet both criteria for homogeneity of variance and normality. Three-way analysis of variance was conducted on the interaction effect of *Paraburkholderia* species, Broccoli cultivars and time after inoculation on root colonization. The *Paraburkholderia* species included three levels (*Pbg*, *Pbh* and *Pbt*), the Broccoli cultivars included two levels (Coronado and Malibu) and time after inoculation consisted of two levels (6 dpi and 11 dpi). There was highly significant three-way interaction effect on root colonization

◀◀◀ **Fig 1**. Biomass and phenotypic changes in Broccoli cultivars in response to root tip inoculation with three *Paraburkholderia* species. (a) Pictures of MS agar plate with two Broccoli cultivars (Coronado and Malibu) at 11 days post inoculation with the three *Paraburkholderia* species. (b) Percent changes in shoot biomass (mean ± standard error, n = 4) and (c) Percent changes in root biomass (mean ± standard error, n = 6) of two broccoli cultivars inoculated with the *Paraburkholderia* species. *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola.* Different letters show significant difference between the treatments (Two-way ANOVA, Tukey's HSD *post hoc* test, $P < 0.05$). Asterisks denote statistical differences between non-bacteria treated control (two-tailed Student's t test): * $P< 0.05$; ** $P< 0.01$. (d) Temporal changes in shoot biomass of two Broccoli cultivars ($d^1$: Coronado and $d^2$: Malibu) inoculated with the *Paraburkholderia* species. Asterisks denote statistical differences (two-tailed Student's t test): * $P< 0.05$; ** $P< 0.01$. *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*.

(Supplementary **Table S3**). In general, compared to the other two *Paraburkhoderia* species, *Pbg* showed significantly higher root colonization in both cultivars at both time points (**Fig 2**) In addition, *Pbg* showed significantly higher root colonization in Coronado cultivar at early time point, while in Malibu the root colonization by *Pbg* was not significantly different between the two time points. In contrast, *Pbh* showed significantly lower root colonization at 6 dpi on Coronado cultivar. However, at the later time point, it showed significant increase in root colonization. Whereas, its root colonization was not significantly different between the two time points in Malibu cultivar. On the other hand, *Pbt* showed significantly lower root colonization in Malibu cultivar at both time points. Furthermore, *Pbt* on Malibu showed significant decline in root colonization at the later time point. Hence, ineffective growth promotion in combination of Malibu-*Pbt* seemed to have a strong correlation with cultivar specific lower root colonization capacity of *Pbt*.



### ***Paraburkholderia*** **species altered primary and secondary metabolism of Broccoli shoot**

Considering the extent of species and cultivar-dependent variations in root colonization and plant growth promotion, we investigated the systemic effect of the different *Paraburkholderia* species on the shoot metabolome of the two Broccoli cultivars at 6 and 11 dpi. We specifically looked into the association between primary and secondary metabolism affected by the *Paraburkholderia* species. GC-MS- and LC-MS-based non-targeted metabolomics were used to profile polar primary metabolites and semi-polar secondary metabolites in shoot extracts, respectively. The data was subjected to ANOVA with correction for multiple testing (Benjamini-Hochberg) and metabolites that were significantly different ($P < 0.05$ and fold change >2) between at least two treatments were used for multivariate analysis. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to reduce the dimensionality of the data and explore specific patterns between the different plant–rhizobacterial interactions.

#### *Effect of Paraburkholderia on shoot primary metabolism*

GC-MS-based non-targeted metabolomics demonstrated that out of 138 polar metabolites, 68 metabolites were significantly different at least between two treatments in shoots of the two Broccoli cultivars upon inoculation with three rhizobacterial species at 6 and 11 dpi. At 6 dpi, PCA indicated that the first three principal components (PC) explained 67.7% of the total variance (**Fig 3a1**). The first PC (PC1), explained 34.8% of the total variance and corresponded to either enhanced or reduced metabolites in the Broccoli cultivars by the *Paraburkholderia* treatment when compared to the controls (**Fig 3b1,** Clusters **1**, **5, 8** and **9**). Out of the three *Paraburkhoderia* species, root tip inoculation by *Pbg* had the greatest impact on shoot primary metabolism of both Broccoli cultivars. Inoculation with *Pbh* and *Pbt* resulted in changes in the shoot primary metabolome in a cultivar-dependent manner. For *Pbh*, the impact on shoot primary metabolome was more extensive for Malibu than for

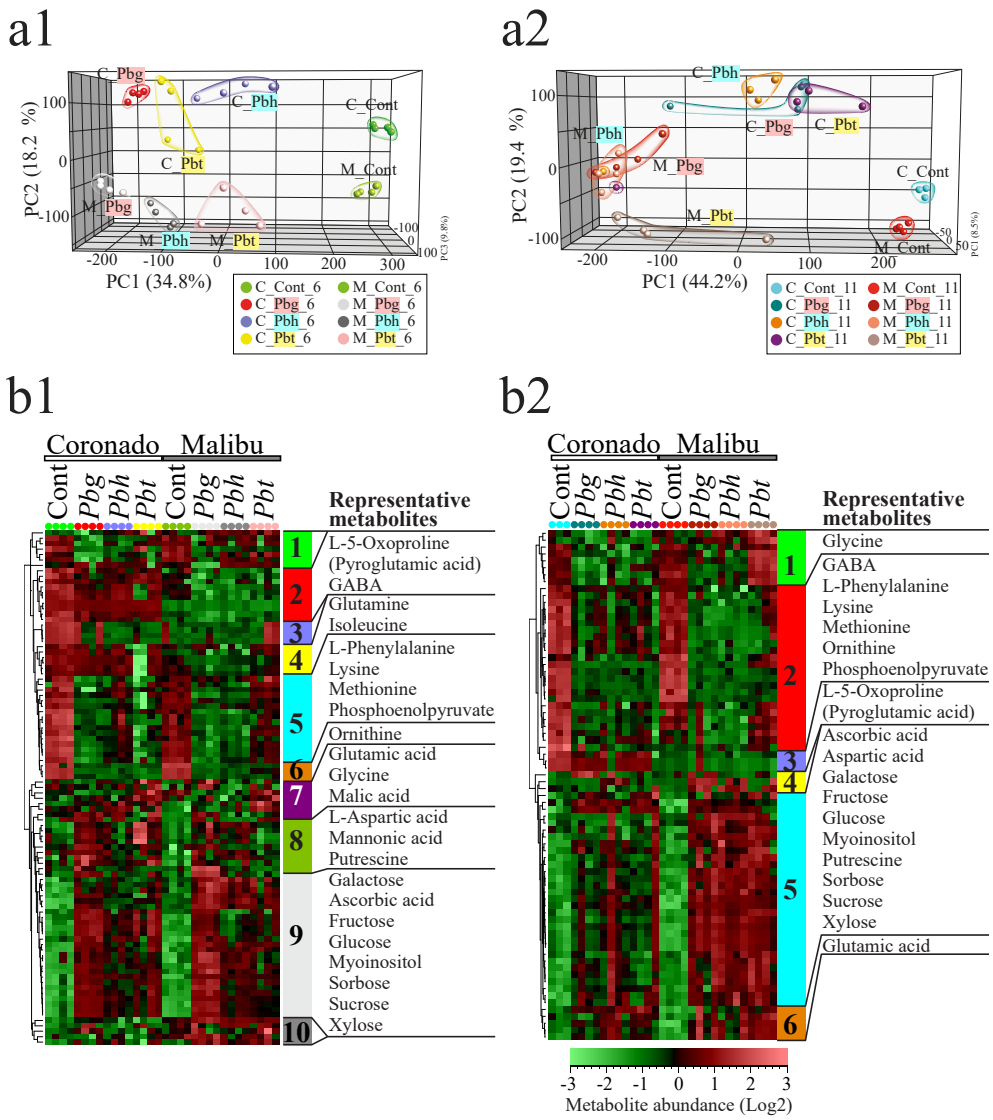



**Fig 3**. Rhizobacteria-mediated changes in shoot primary metabolites in two Broccoli cultivars. (**a**) Principal Component Analysis (PCA) and (**b**) Hierarchical Cluster Analysis (HCA) based on differentially regulated metabolites of the samples at 6 dpi (**1**) and 11 dpi (**2**). In the HCA, metabolite clusters are indicated by different colors. Information on the representative metabolites of each clusters is given on the right side, if the metabolites are annotated. (**c**) Impact of *Paraburkholderia* species on sugar generation and utilization of two Broccoli cultivars. Broccoli cultivars (Cor: Coronado, Mal: Malibu), Cont.: non-rhizobacteria treated control, *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*.

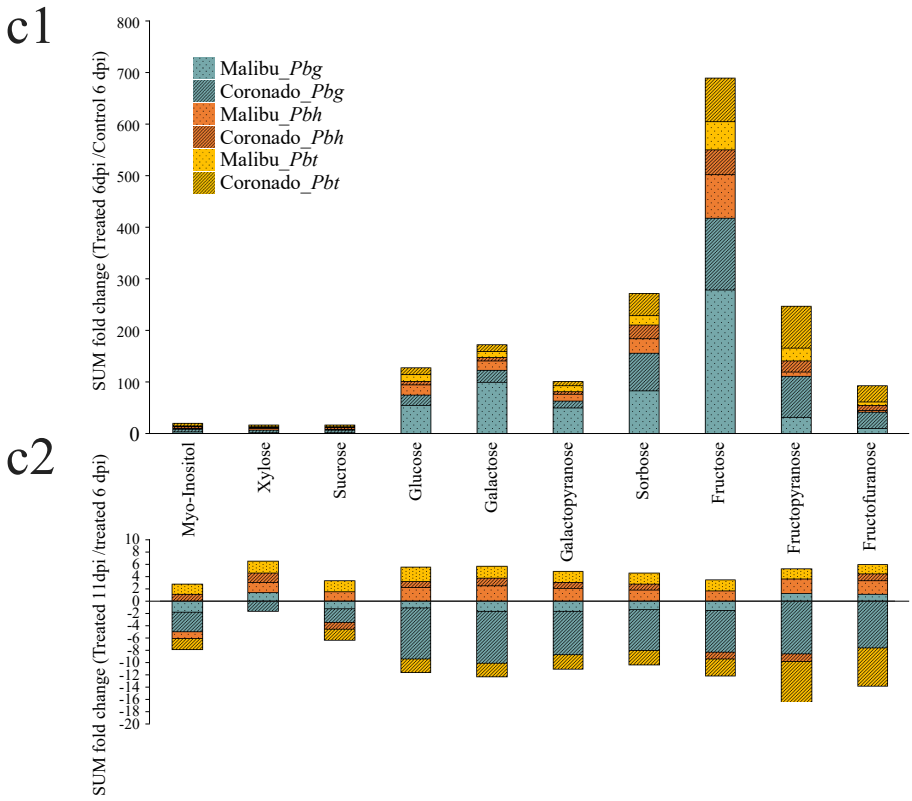



**Fig 3**. (continued)

Coronado, while for *Pbt* more extensive changes in the shoot primary metabolome were observed for Coronado (**Fig 3a1**). The major changes in primary metabolism induced by *Paraburkholderia* included accumulation of sugars (Cluster **9**) and depletion of amino acids (Cluster **5**, phenylalanine, lysine, methionine) and of the phosphate ester, phosphoenolpyruvate (PEP), a key intermediate in glycolysis and gluconeogenesis.

Some of the representative metabolites in cluster **8** that showed accumulation in all interactions, except in the ineffective *Pbt*-Malibu interaction, include aspartic acid, mannonic acid and putrescine. The second principal component (PC2) explained 18.2% of the total variance and associated with metabolites that showed variation between the two cultivars. Furthermore, treatment of the two cultivars with *Pbg* and *Pbh* widened the inherent variation in the level of some of the metabolites between the two Broccoli cultivars. (**Fig 3b1** Clusters **2** and **3**). Amino acids such as glutamine, oxoproline (pyroglutamic acid), GABA (γ-aminobutyric acid) and isoleucine were intrinsically higher in Coronado when compared to Malibu.

At the later seedling growth stage (11 dpi), inoculation of the Broccoli cultivars with the three *Paraburkholderia* species continued to have substantial impact on shoot primary metabolism.

PCA showed that the first three principal component explained 72.0% of the total variance (**Fig 3a2**). Here, the impact of all three *Paraburkholderia* species on the shoot metabolome was Broccoli cultivar dependent and was greater in Malibu (**Fig 3a2**). The first principal component (PC1) explained 44.2% of the total variance and corresponded to metabolites that were enhanced (**Fig 3b2** Cluster **5**) or reduced (Cluster **2**) in the *Paraburkholderia* treatments. The depleted Broccoli shoot metabolites upon inoculation with the three rhizobacterial species encompassed amino acids such as lysine, phenylalanine, methionine, the non-proteinogenic amino acids ornithine and GABA, as well as the important metabolic intermediate phosphoenolpyruvate (PEP). In all but the ineffective partnership between *Pbt*-Malibu, the important intermediate phosphoenolpyruvate, showed 11-14 fold decreases (Supplementary excel, **Table S6**). On the other hand, sugars and other metabolites, including ascorbic acid and aspartic acid, represent the induced metabolites by the *Paraburkholderia* treatments when compared to the control plants (Cluster **5**). Sugars showed greater abundance in Malibu cultivar treated with *Paraburkholderia* than in Coronado. However, at 11 dpi, sugars in *Paraburkholderia*-treated plants showed dramatic depletion in Coronado cultivar as compared to 6 dpi. Whereas in Malibu cultivar the temporal variation in the level of the sugars was less pronounced (**Fig 3b2** Cluster **5,** Supplementary **Fig S3** and **S4**). The PC2, representing 19.4% of the total variance, was associated with metabolites in cluster **1** including glycine that were depleted in all treatment combinations except in the controls and in the ineffective partnership between *Pbt* and Malibu (**Fig 3b2** Cluster **1**). Oxoproline and some other metabolites shown in cluster **3** were intrinsically abundant in the shoots of Coronado.



*Paraburkholderia's impact on Broccoli primary metabolism is associated with soluble sugars*

As sugars are the primary drivers of plant growth, we looked into their temporal dynamics, particularly related to sugar generation and utilization in the shoots of the two Broccoli cultivars with or without the influence of the *Paraburkholderia* species. The fold change in sugar level between *Paraburkholderia* treated and control plants at 6dpi was used as a measure of sugar generation. While the temporal fold change in sugar level of treated plants from 6 to 11 dpi was used as a measure of sugar utilization. In control plants, sugars showed no significant change between the two Broccoli cultivars at 6 dpi (supplementary **Fig S3**). In contrast, treatment with the *Paraburkholderia* species showed unprecedented impact on the sugar generation in shoots of both Broccoli cultivars resulting in significant increases in the level of fructose and its derivatives, glucose, sorbose, galactose and galactopyranose at 6 dpi. Moreover, the magnitude of sugar generation showed remarkable difference between the *Paraburkholderia* species-Broccoli cultivar combinations (**Fig 3c1**). *Pbg* showed the highest sugar generation when compared to *Pbh* and *Pbt* and its sugar generation ability was significantly higher in Malibu cultivar. The ineffective partnership between *Pbt* and Malibu had the least significant impact on sugar generation. Similarly, the sugar utilization also showed noticeable differences among the *Paraburkholderia* species-Broccoli cultivar

combinations. In Coronado, *Pbg* inoculation led to greater sugar utilization when compared to cultivar Malibu. The ineffective partnership between *Pbt* and Malibu showed reduced sugar utilization when compared to the effective partnership of *Pbt* with Coronado (**Fig 3c2**).

*Effects of Paraburkholderia on shoot secondary metabolism*

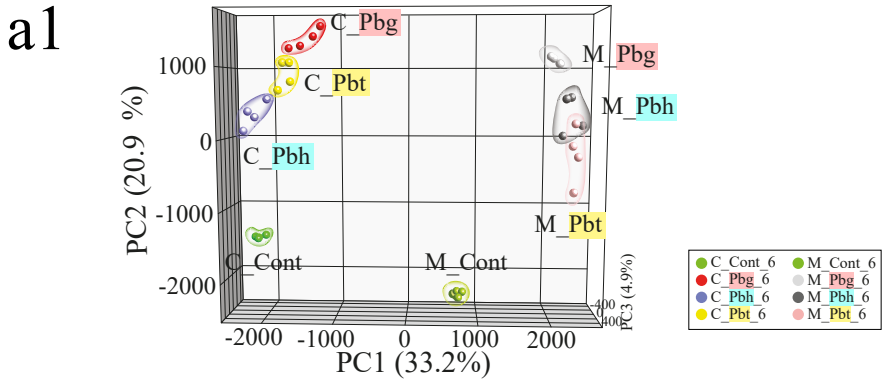
From the total of 1868 metabolites detected in positive or negative ionization mode, 1386 metabolites were significantly different between at least two treatments. PCA of the metabolites at 6 dpi demonstrated distinct clustering of the samples based on the *Paraburkholderia* species-Broccoli cultivar combination (**Fig 4a1**). The first PC explained 33.2% of the total variation and was associated with metabolites that were intrinsically abundant in one of the two Broccoli cultivars (Clusters **3**, **5** and **12**). Treatment of the two Broccoli cultivars with the *Paraburkhoderia* species also widened the variation in the level of the metabolites that were intrinsically different between the two Broccoli cultivars. Metabolites that were intrinsically abundant in Coronado included aliphatic glucosinolates such as 2-methylbutyl glucosinolate and glucoiberverin as well as the aromatic glucosinolates glucotropaeolin and gluconasturtiin. The levels of aliphatic glucosinolates 2-methylbutyl glucosinolate and glucoiberverin were 147 and 209 times higher in Coronado when compared to Malibu, respectively (Cluster **3**). In Malibu, on the other hand, a number of metabolites from the phenylpropanoid pathway were intrinsically abundant (Clusters **5** and **12**). The second principal component (PC2) explained 20.9% of the total variance and was associated with metabolites that were reduced (**Fig 4b1** Clusters **2** and **4**) or induced (Clusters **7** and **11**) by the *Paraburkholderia* treatments. Inoculation of *Pbg* had the greatest impact on the shoot secondary metabolome profile of both Broccoli cultivars, whereas the ineffective partnership between *Pbt* and Malibu had less pronounced impact on the shoot metabolome. Metabolites in cluster **2,** comprising amino acids such as arginine, asparagine, tryptophan and N-acetylated glutamic acid/fucosamine, showed greater reduction in their abundance upon treatment with *Paraburkholderia* species. Cluster **4** encompassed metabolites that were more abundant in Malibu than Coronado and included ascorbic acid ethyl ester, N-acetyl-tryptophan, and terpenoids such as S-furanopetasitin and sonchuionoside C. The metabolites in clusters **7** and **11** were induced by all the *Paraburkholderia* species and were dominated by phenylpropanoids. In Malibu, inoculation of *Pbg* led to greater accumulation of phenylpropanoids such as flavonoids glycosides (kaempferol-di/tri-(feruloyl/coumaroyl) glycosides and robinin), hydroxycinnamates (ferulic acid and its derivatives, caffeic acid derivatives such as chlorogenic acid) and an indole acetic acid derivative such as indole-3-acetic-acid-O-glucuronide when compared to the other two *Paraburkholderia* species. PC3 explained 4.9% of the total variance and was represented by *Pbg*-triggered (Clusters **8** and **10**) or *Pbt*-induced (Cluster **13**) metabolites in both Broccoli cultivars. *Pbg*-induced metabolites in cluster **8** consisted of the flavonoid kaempferol 3-sophorotrioside. Whereas *Pbt*-triggered metabolites in cluster **13** included the hydroxycinnamate 1,2-bis-O-sinapoyl-beta-D-glucoside and resveratrol-sulfoglucoside, a stilbenoid.

Similarly, at 11dpi, inoculation with *Paraburkholderia* species led to substantial changes in the shoot metabolite profiles of the two Broccoli cultivars (**Fig 4a2** and **4b2**). In the PCA, the first three PCs explained 51.1% of the total variance. The first PC, explaining 29.1% of the total variance is associated with metabolites that accumulated or were reduced in response to *Paraburkholderia* and the change in these group of metabolites was more pronounced in Malibu cultivar (**Fig 4b2** Clusters **1**, **2**, **3**, **4** and **5**: up, **10** and **11**: down). The induced metabolites in the above-mentioned clusters included flavonoids i.e. flavonol-di/tri-glycosides, kaempferol-di/tri-glycosides (feruloyl/caffeoyl/coumaroyl), robinin, medicarpin-O-glucoside-malonate, as well as hydroxycinnamates i.e. ferulic acid, caffeic acid and various derivatives of these metabolites. Furthermore, coumarins such as eupatoriochromene and mahaleboside and mevalonate, a precursor of mevalonate pathways that goes to terpenoid biosynthesis was also induced by the *Paraburkholderia* treatment. The reduced metabolites in both Broccoli cultivars included amino acids such as arginine, asparagine and acetyl/valyl conjugated amino acids i.e. valyl-methionine, and N-acetylglutamic acid (Cluster **10**). Meanwhile, metabolites in cluster **11** were also reduced by the *Paraburkhoderia* treatment and these metabolites were intrinsically abundant in Coronado cultivar. Some of the metabolites in cluster **11** included sulfur-containing metabolites such as the aliphatic glucosinolates 2-methylbutyl glucosinolate and glucoiberverin, derivatives of sulfurous amino acids including leucyl-cysteine and methionyl-isoleucine, as well as precursor or breakdown products of glucosinolates, for instance, 6-methylthiohexanaldoxime and 3-methylsulfinylpropyl isothiocyanate. The second PC (PC2) explained 23.8% of the total variance and was associated with metabolites that were intrinsically more abundant in cultivar Malibu (**Fig 4b2** Clusters **6**, **7**, **8** and **9**). Metabolites in clusters **6**, **7**, **8** and **9** showed significant depletion in all effective partnerships between the *Paraburkholderia* species and Broccoli cultivars. Tryptophan, a building block for indolic glucosinolate and the growth hormone indole-3-acetic acid, N-acetylated amino acid including N-acetyl phenylalanine/tryptophan, terpenoids i.e. S-furanopetasitin and sonchuionoside C, and sulforaphane an isothiocyanate are some of the metabolites in these cluster worth mentioning. PC3 explained 6.2% of the total variance and was associated with yet unknown metabolites that showed *Pbg* specific alteration in both Broccoli cultivars (Clusters **13**).



### *Paraburkholderia* induces systemic resistance against the bacterial leaf pathogen *Xanthomonas campestris* in a cultivar-dependent manner

As shown above, the two Broccoli cultivars exhibited inherent differences in their shoot chemistry (**Fig 4**). Furthermore, treatment of the plant roots with different *Paraburkholderia* species led to substantial alteration of the shoot metabolome including metabolome signatures specific to the individual combination of *Paraburkholderia* species and Broccoli cultivar (**Fig 4**). Based on this, we hypothesized that the inherent and induced differences in shoot chemistry between the two cultivars could contribute to a differential defense response against leaf pathogens. To address this hypothesis, treated and control plants of the two



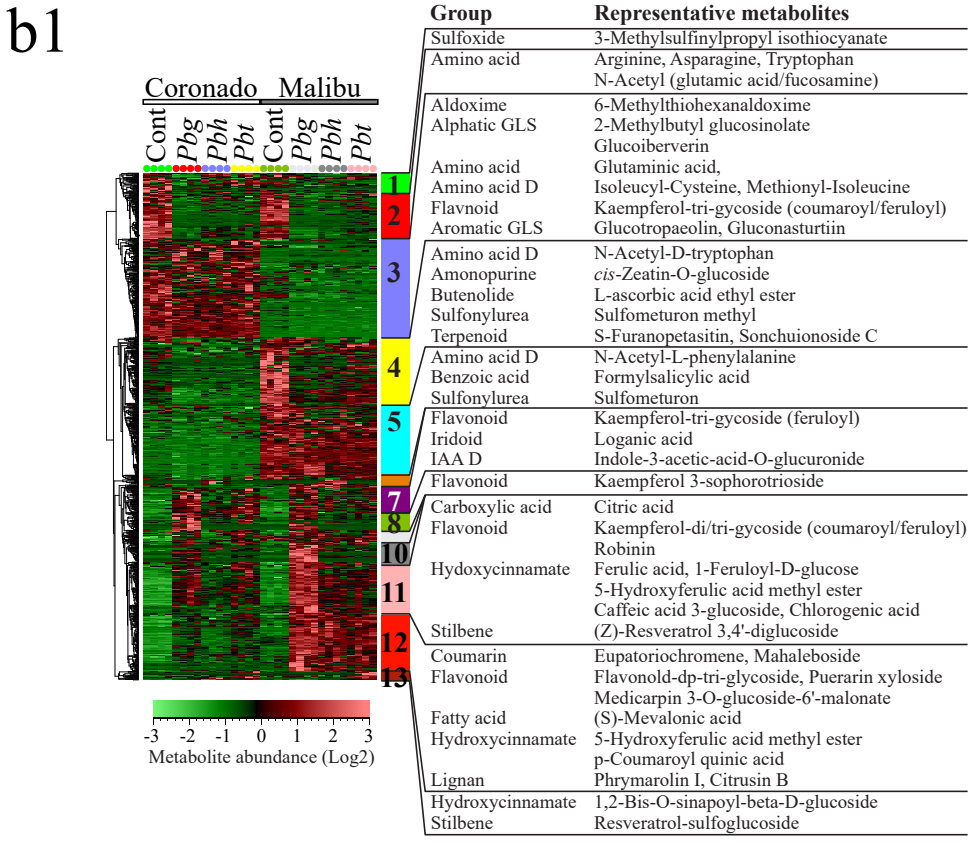
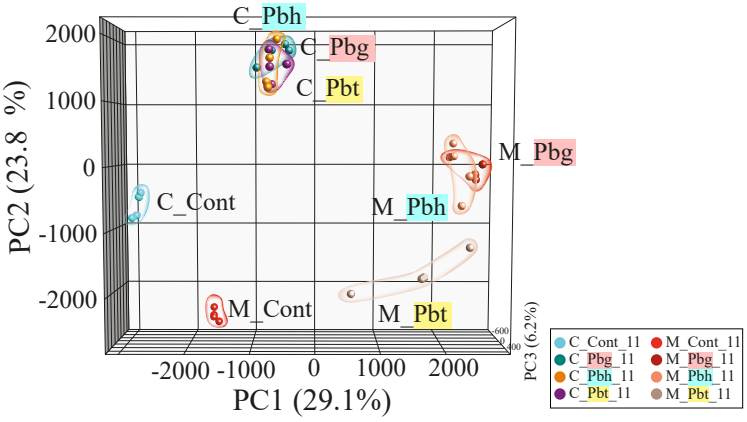
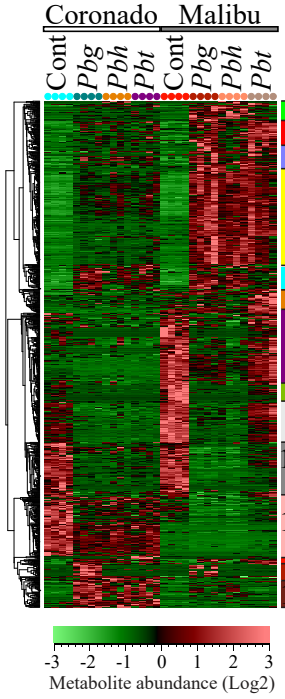




**Fig 4**. Rhizobacteria-mediated changes in the shoot secondary metabolites in Broccoli cultivars. (**a**) Principal component analysis (PCA) and (**b**) Hierarchical cluster analysis (HCA) based on differentially regulated metabolites of the samples at 6 dpi (**1**) and 11 dpi (**2**). In the HCA, metabolite clusters are indicated by different colors. Information on the representative metabolites of each clusters is given on the right side, if the metabolites are annotated. Broccoli cultivars (Cor: Coronado, Mal: Malibu), Cont.: non-rhizobacteria treated control, *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*. * GLS=glucosinolate, ** D=derivative.

a2




b2




| Group | Representative metabolites |
|---|---|
| Flavonoid | Kaempferol 3-sophorotrioside |
| Sulfonylurea | Sulfometuron methyl |
| Carboxylic acid | Citric acid |
| Hydroxycinnamate | 5-O-Caffeoylshikimic acid |
| | Trimethoxycinnamic acid |
| Stilbene | (Z)-Resveratrol 3,4'-diglucoside |
| | Resveratrol-sulfoglucoside |
| Hydroxycinnamate | 5-Hydroxyferulic acid methyl ester |
| IAA D* | Indole-3-acetic-acid-O-glucuronide |
| Coumarin | Eupatoriochromene, Mahaleboside |
| Flavonoid | Flavonol-di/tri-glycosides |
| | Kaempferol-di/tri-glycosides |
| | Kaempferol-tri-glycosides (feruloyl/caffeoyl/coumaroyl) |
| | Kaempferol 3-sophorotrioside |
| | Puerarin xyloside, Robinin |
| | Medicarpin 3-O-glucoside-6'-malonate |
| Fatty acid | (S)-Mevalonic acid |
| Hydroxycinnamate | Ferulic acid, 1-Feruloyl-D-glucose |
| | 2-Feruloyl-1,2'-disinapoylgentiobiose |
| | 5-Hydroxyferulic acid methyl ester |
| | Caffeic acid 3-glucoside |
| | Chlorogenic acid |
| | p-Coumaroyl quinic acid |
| Lignan | Citrusin B |
| Aromatic GLS** | Gluconasturtiin |
| Iridoid | Loganic acid |
| Amino acid D | N-Acetyl-L-phenylalanine |
| Sulfoxide | Sulforaphane |
| Amino acid | Tryptophan |
| Amino acid D | N-Acetyl-D-tryptophan |
| Amonopurine | *cis*-Zeatin-O-glucoside |
| Sulfonylurea | Sulfometuron |
| Terpenoid | S-Furanopetasitin, Sonchuionoside C |
| Amino acid | Arginine, Asparagine |
| Amino acid D | Valyl-Methionine |
| | N-Acetylglutamic acid |
| Aldoxime | 6-Methylthiohexanaldoxime |
| Aliphatic GLS | 2-Methylbutyl glucosinolate |
| | Glucoiberverin |
| Amino acid D | Leucyl-Cysteine, Methionyl-Isoleucine |
| Sulfoxide | 3-Methylsulfinylpropyl isothiocyanate |
| Benzoic acid | Formylsalicylic acid |

**Fig 4.** (Continued)

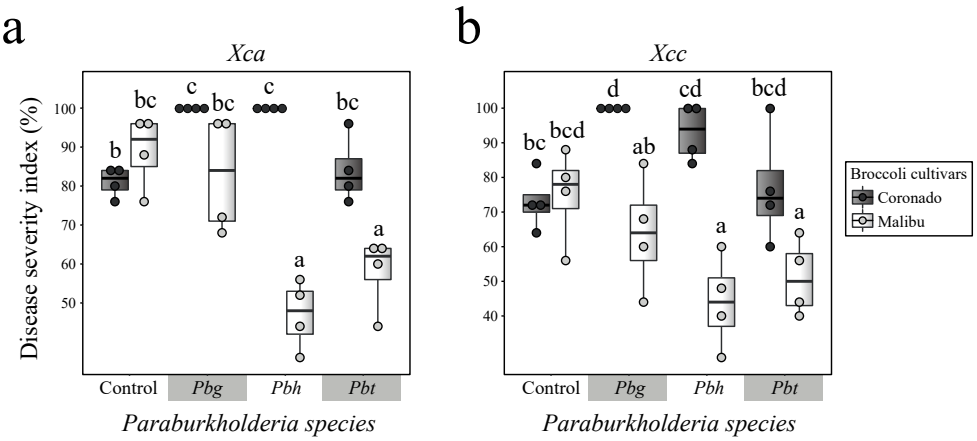



**Fig 5**. Impact of root-colonizing *Paraburkholderia* species on defense of Broccoli cultivars against two bacterial leaf pathogens. Disease severity index of two broccoli cultivars treated with either one of the three *Paraburkholderia* species and challenged with two bacterial leaf pathogens (a) *Xanthomonas campestris* pv. armoraciae P4216 (*Xc*a) and (b) *Xanthomonas campestris* pv. campestris P4014 (*Xcc*). Broccoli cultivars (Cor: Coronado, Mal: Malibu), Control: non-treated control, *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*. Different letters show statistically significant differences between the treatments (Two-way ANOVA, Tukey's HSD *post hoc* test, $P < 0.05$).



cultivars were challenged with two bacterial leaf pathogens, i.e. *Xanthomonas campestris* pv. armoraciae P4216 (*Xca*) and *Xanthomonas campestris* pv. campestris P4014 (*Xcc*).

Regression analysis was employed to examine the interaction effect of two independent variables (*Paraburkholderia* species and Broccoli cultivars) on disease severity of the two bacterial pathogens using the "betareg" package in R. The *Paraburkholderia* species included three levels (*Pbg*, *Pbh* and *Pbt*) and the Broccoli cultivars consisted of two levels (Coronado and Malibu). There was a highly significant interaction effect of the *Paraburkholderia* species and Broccoli cultivars on disease severity for both *Xanthomonas* pathovars (Supplementary **Table S4**). No significant inherent variation in disease severity was observed between the two Broccoli cultivars when they were challenged with the two bacterial pathogens (**Fig 5**). However, treatment of the roots of the two Broccoli cultivars with the *Paraburkholderia* species led to a reduction or an enhancement of disease severity. For example, treatment with *Pbg* and *Pbh* enhanced disease severity in Coronado cultivar challenged by both bacterial pathogens, whereas *Pbh* and *Pbt* significantly reduced disease severity in cultivar Malibu challenged by each of the two bacterial pathogens (**Fig 5**).

## Discussion

Our results showed that root-colonizing *Paraburkholderia* species altered shoot primary and secondary metabolism of Broccoli seedlings, promoted growth and induced systemic defense

against bacterial leaf pathogens. The magnitude of the alteration of these traits is dependent on the *Paraburkholderia* species and Broccoli cultivar combinations. The widely accepted "defense-growth tradeoff" concept asserts that activation of plant defense comes at the expense of plant growth due to resource limitations (Huot *et al.*, 2014). Here we showed that rhizobacteria treatment of plant roots promotes growth and at the same time primes the plant defense against biotic stress factors. Rhizobacteria defense priming is considered to have lower cost when compared to activation of direct defense (Martinez-Medina *et al.*, 2016). However, judging the cost of defense priming by only assessing some key physiological process such as seed production, number of flowers, pollen quality and number, and plant growth does not necessarily explain the energy and carbon costs associated with defense priming. Hence, to understand the underlying mechanisms by which rhizobacteria promote growth and prime the plant's defense without compromising plant fitness, it requires a comprehensive investigation of the host metabolome network.

Root colonization by rhizobacteria is an important trait required for establishing beneficial effect on plant growth and health (Lugtenberg *et al.*, 2001; Compant *et al.*, 2010). In line with this, our study showed an association between root colonization ability of the *Paraburkholderia* species, their impact on the host metabolome and their ability to promote plant growth. For example, *Pbh* showed significantly reduced root colonization for cultivar Coronado at 6 dpi and its impact on both primary and secondary metabolism was the lowest when compared to *Pbg* and *Pbt* treated plants. Interestingly, at 11dpi, *Pbh* exhibited enhanced root colonization which coincided with an elevated impact on the shoot metabolome, comparable to that of plants treated with *Pbg* and *Pbt* (**Fig 3** and **4** PCA). Similarly, *Pbt* showed significantly reduced root colonization for cultivar Malibu at both 6 and 11dpi with no significant growth promotion effect and the least impact on the shoot primary and secondary metabolism as compared to plants inoculated with *Pbg* and *Pbh*. The two Broccoli cultivars used in this study showed inherent differences in their shoot metabolome profile. Phenylpropanoids were more abundant in Malibu and glucosinolate and other sulfur-containing compounds showed higher abundance in Coronado. Assuming this variation in shoot chemistry may be reflected in differences in root chemistry, these intrinsic differences between the two cultivars might have influenced the root colonization ability of *Paraburkholderia* species and the effectiveness of the partnerships. Although the root exudate profile of the two Broccoli cultivars was not assessed in our study, exudate composition of plant species has a major impact on microbiome assembly and functions in the rhizosphere (Philippot *et al.*, 2013; Pérez-Jaramillo *et al.*, 2016; Hu *et al.*, 2018; Lundberg & Teixeira, 2018; Pérez-Jaramillo *et al.*, 2018; Stringlis, Ioannis A *et al.*, 2018; Huang *et al.*, 2019). Assessing the root exudate profile of the two broccoli cultivars in combination with bioassay-guided fractionation of the exudates will be instrumental to identify metabolite signatures that govern the differences in root colonization by the *Paraburkholderia* species and the effectiveness of their partnership with different Broccoli cultivars.

The *Paraburkholderia* species exerted a substantial impact on the host primary and secondary metabolism at both early and late stages of seedling growth. Their biggest impact on primary metabolism was reflected in soluble sugar generation and utilization and both parameters showed significant variation across the *Paraburkholderia* species - Broccoli cultivar combinations (**Fig 3c**). All combinations, except *Pbt*-Malibu, resulted in effective partnership, i.e. plant growth promotion. Effective partnerships showed higher soluble sugar generation at the early stage of seedling growth and high sugar utilization at the later stage of seedling growth, whereas the ineffective partnership between Malibu and *Pbt* showed reduced sugar generation and utilization. Soluble sugars are fuel for growth and for the biosynthesis of secondary metabolites involved in defense (Herrmann & Weaver, 1999; Hartmann & Trumbore, 2016). Moreover, soluble sugars such as galactose, glucose, sorbose, fructose, sucrose and xylose have been reported to be effective chemotaxis agents for bacteria (Adler *et al.*, 1973; Ordal *et al.*, 1979). Their increased production under effective partnership between plants and rhizobacteria could provide both the plants and rhizobacteria with fuel for energy production to sustain growth and secondary metabolite production. In general, low sugar concentration promotes 'source' activities such as photosynthesis, nutrient mobilization and export, while high sugar level enhances 'sink' activities such as growth and sugar storage (Rolland *et al.*, 2006). Hence, we postulate that the enhancement of the sugar levels is a key mechanism that determines the effectiveness of the partnership between rhizobacteria and plants. Furthermore, under effective partnership, there was significantly higher depletion (>11-fold) of PEP, a key substrate for the TCA cycle and the shikimate pathway (**Fig 6**), whereas PEP depletion was only about 2-fold under ineffective partnership. Furthermore, greater depletion of GABA under the effective partnership suggests catabolism of GABA to succinyl semialdehyde followed by its conversion to succinate to feed the greater demand of pyruvate in the TCA cycle. Key intermediates in the TCA cycle such as citric acid and malic acid showed increased abundance under effective partnership. In the TCA cycle, citrate is converted to malate and used in the mitochondria for energy production (Fernie *et al.*, 2004). Hence, these observations most likely meet the greater demand for carbon and energy occurring during enhanced growth and defense priming in the effective *Paraburkholderia*-Broccoli interactions.

For the soluble sugars, *Paraburkholderia* species showed their biggest impact on fructose abundance. *Pbg*-Malibu interaction showed the highest root colonization and had the highest impact on shoot fructose level (~>280 folds). In contrast, *Pbt*-Malibu and *Pbh*-Coronado interactions showed the lowest root colonization levels and had the lowest impact on shoot fructose generation at the early stages of seedling growth. Interestingly, the biggest impact on shoot fructose abundance by *Pbg* also showed the biggest impact on secondary metabolism in general and phenolic compounds accumulation in particular when compared to *Pbh* and *Pbt* at 6 dpi (**Fig 6**). This suggests that the effects of elevated levels of soluble sugars are not only limited to plant growth but also extend to secondary metabolites biosynthesis. For example, fructose is the primary substrates for fructose-6-phospate, a key substrate for the

biosynthesis of both PEP and erythros-4-phosphate (**Fig 6**). These two intermediates are channeled to the shikimate pathway that bridges carbohydrate metabolism to biosynthesis of aromatic primary and secondary metabolites (Herrmann & Weaver, 1999). The shikimate pathway provides all the important precursors for the biosynthesis of phenylpropanoids including hydroxycinnamates, flavonoids, stilbenoids, coumarins and lignins that showed significant accumulation in plants treated with *Paraburkholderia* species (**Fig 4b** and **6**). A study on the *pho3* mutant of Arabidopsis that accumulates soluble sugars to high levels, showed large increases in the expression of transcriptional factors and enzymes involved in anthocyanin biosynthesis (Lloyd & Zakhleniuk, 2004). Another study also showed greater accumulation of fructose (~10 fold) and flavonoids in quinoa cotyledons in response to UV-B radiation (Hilal *et al.*, 2004). Considering our results and the aforementioned findings by other studies, we postulate that one of the key mechanisms by which rhizobacteria modulate host secondary metabolism is by soluble sugar generation.

In addition to enhancement of key precursors for growth and secondary metabolite biosynthesis, treatment of *Paraburkholderia* also induced metabolite remobilization. The metabolite remobilization involved both suppression of resource competing metabolite pathways such as amino acids and rechanneling of existing primary metabolite-derivatives and other secondary metabolites to other metabolite pathways. For example, aromatic glucosinolate, amino acids and their derivatives, some terpenoids showed more depletion in effective partnerships (**Fig 6** and **Fig 4b2**). Hence, remobilization of the existing metabolites towards targeted metabolite pathways could be an additional strategy used by rhizobacteria to reduce the cost of *de novo* biosynthesis of metabolites.



Our results also showed that rhizobacteria-mediated reorganization of the host metabolome landscape not only affects plant growth but also the defense response of Broccoli cultivars to bacterial leaf pathogens. In general, inoculation of the *Paraburkholderia* species showed greater suppression of pathogen proliferation in cultivar Malibu that intrinsically has more phenolic compounds (**Fig 5**). The phenylpropanoid pathway appears to be the central target by the *Paraburkholderia* species and was altered to a greater extent in Malibu than in Coronado (**Fig 6**). All metabolite classes belonging to this pathway including flavonoids, hydroxycinnamates, stilbenoids, coumarins and lignins showed substantial accumulation upon treatments with the *Paraburkholderia* species (**Fig 6**). These metabolites were reported to have direct antimicrobial effects and/or act as a physical barrier against pathogenic microorganisms (Dixon *et al.*, 2002; Cvikrova *et al.*, 2006). Hydroxycinnamic acids and flavonoid were shown to negatively affect the disease symptom development in Chinese cabbage challenged by *Xanthomonas campestris* pv. campestris (*Xcc*) (Islam *et al.*, 2018). Of all the *Paraburkholderia* species-Broccoli cultivars combinations, treatment of roots of cultivar Coronado with *Pbg* resulted in higher disease severity (**Fig 5**). However, we could not find a specific metabolic signature that corresponds to the increased pathogen susceptibility of Coronado treated with *Pbg*.

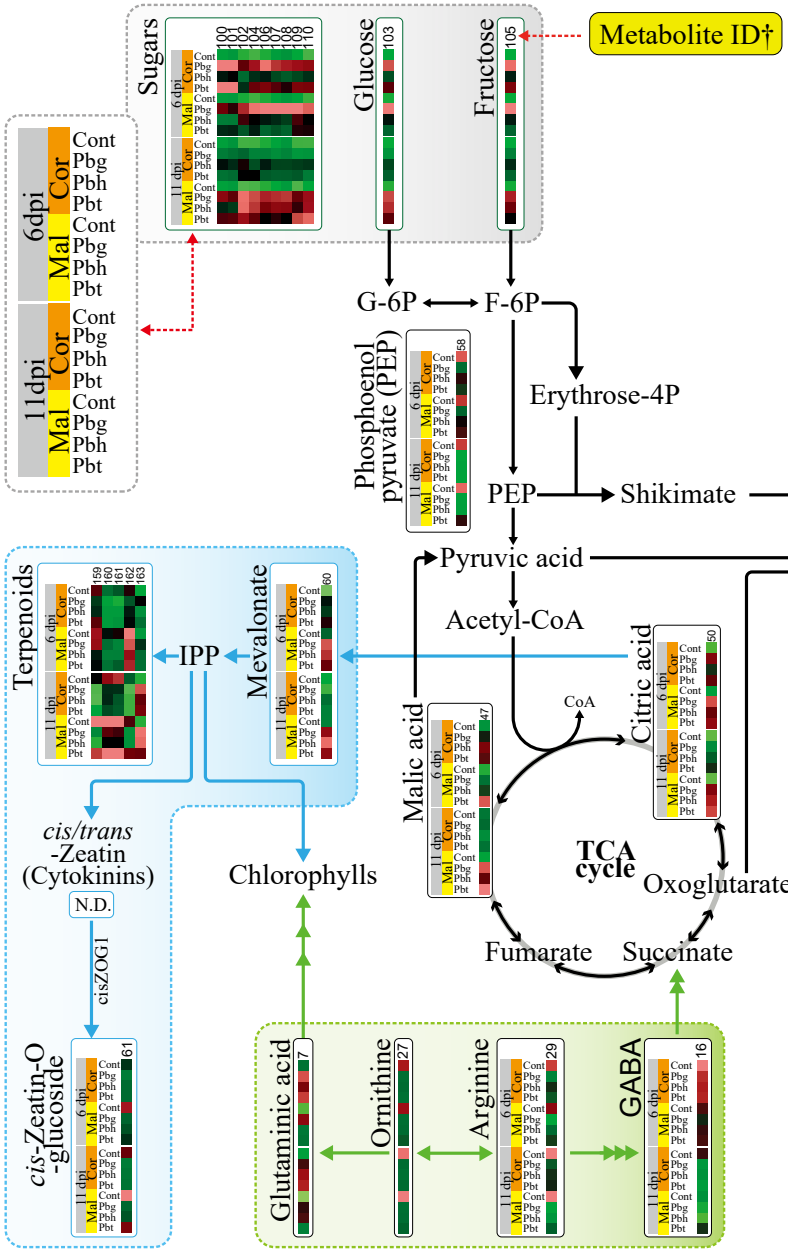
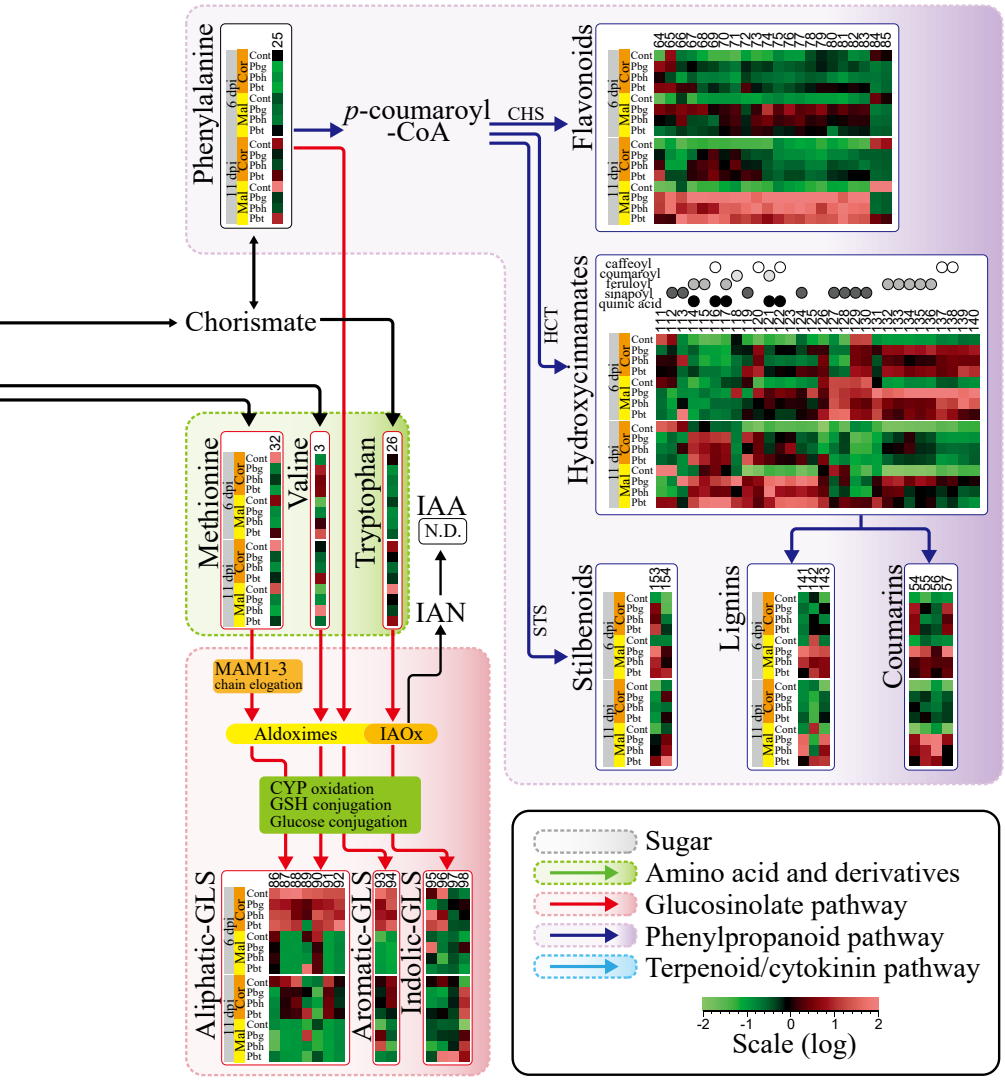



**Fig 6**. Alteration of core primary and secondary metabolite pathways by *Paraburkholderia* species in Broccoli. The metabolite pathways are organized as modules inside different colored boxes and the abundance of the significantly altered metabolites is represented by the heat map where each cells representing the abundance of a metabolite of a sample for *Paraburkholderia* species-Broccoli cultivar and time combinations. The metabolite ID corresponding to each metabolites is indicated at the top of the heat map and detailed information on the identity of the individual metabolites is provided in the supplementary excel **Table S9**. G-6P (Glucose 6-phosphate), F-6P (Fructose 6-phosphate), CHS




(Chalcone synthase), cisZOG1 (Cis-zeatin O-glucosyltransferase 1), CYP (Cytochrome P450), GABA (γ-aminobutyric acid), GLS (Glucosinolates), GSH (Glutathione), HCT (hydroxycinnamoyl-CoA shikimate/quinate hydroxy-cinnamoyl transferase), IAA (indole-3-acetic acid), IAN (indole-3-acetonitrile), IAOx (indole-3-acetaldoxime), IPP (isopentenyl pyrophosphate), MAM (methylthioalkylmalate synthase), and STS (stilbene synthase). Broccoli cultivars (Cor: Coronado, Mal: Malibu), Cont.: non-rhizobacteria treated control, *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*. Multiple-headed arrows indicate hidden intermediate processes in the pathways.

Defense priming is not a low-cost defensive measure as postulated but could cost substantial amounts of energy and carbon resources. This is shown by the massive accumulation of phenylpropanoids and other defensive compounds in plants primed by *Paraburkholderia* species even before the plants were challenged with the bacterial leaf pathogens. The integrated primary and secondary metabolome profiling of primed plants suggests that rhizobacteria could avert the negative impact of defense priming on the host fitness by generating massive amount of soluble sugars and remobilizing other metabolites to accommodate for the high energy and carbon skeleton demand associated with growth and defense priming. This could indicate that defense costs can be regulated if resources are not limiting and aligns with studies that showed the inevitability of defense-growth trade-off occurs primarily under resource-limiting conditions (Bergelson & Purrington, 1996; van Dam & Baldwin, 1998).

The magnitude of the impact of the rhizobacteria on plant growth, metabolism and defense priming showed an association with the level of root colonization by the rhizobacteria. On the other hand, the plant genotype and its intrinsic chemical composition could affect the ability of the rhizobacteria to colonize the host root and could determine the impact rhizobacteria have on different phenotypic traits of the host. Hence, breeding of plant for different traits should consider its impact on the host chemistry and its associated effect on the recruitment of beneficial microbes for the plant functioning. Furthermore, investigating the underlying mechanism of rhizobacteria-mediated generation of soluble sugars in plants could be instrumental for breeding programs that aim to produce high yielding and stress-resilient crops. Hence, further studies on beneficial bacterial traits involved in the modulation of the host metabolism could provide information on the underlying mechanisms of rhizobacteria-mediated alteration of host metabolism, growth and defense priming.



## Acknowledgements

Seeds of two Broccoli cultivars (*Brassica olearacea* var. italic), Coronado and Malibu, were kindly provided by Bejo seed company (Warmenhuizen, The Netherlands). We are grateful to Bert Schipper and Henriëtte Vaneekelen for their help with LC MS analysis and pre-processing of metabolomics data.

**Supplementary materials**

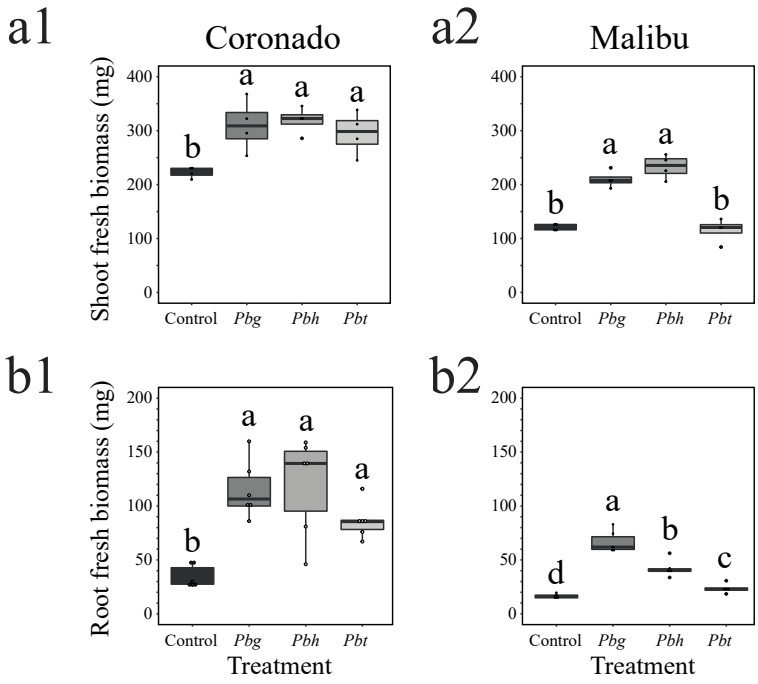



**Fig S1**. Absolute changes in shoot (**a**) and root (**b**) biomass of Broccoli cultivars Coronado (**1)** and Malibu (**2)** at 11 dpi with *Paraburkholderia* species. *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola.* Different letters show significant statistical difference between the treatments (One-way ANOVA, Tukey's HSD *post hoc* test, $P < 0.05$).

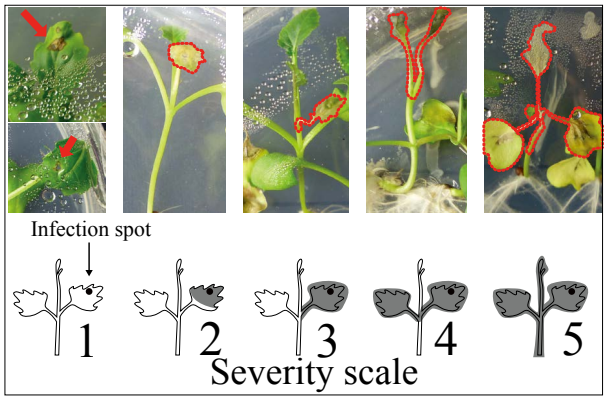



**Fig S2**. Disease severity scale. For the severity (**c**), each Broccoli seedlings from four biological replicates were individually scored (n = 20). Disease severity was scored by determining the migration of the lesion from the inoculation spot to the other parts of the shoot following an ordinal scale from 1- 5, where 1 = no necrosis or migration, 2 = full infection of the treated leaf, 3 = migration to the leafstalk of the treated leaf, 4 = infection of the neighboring leaf, and 5 = infection of the entire seedling.

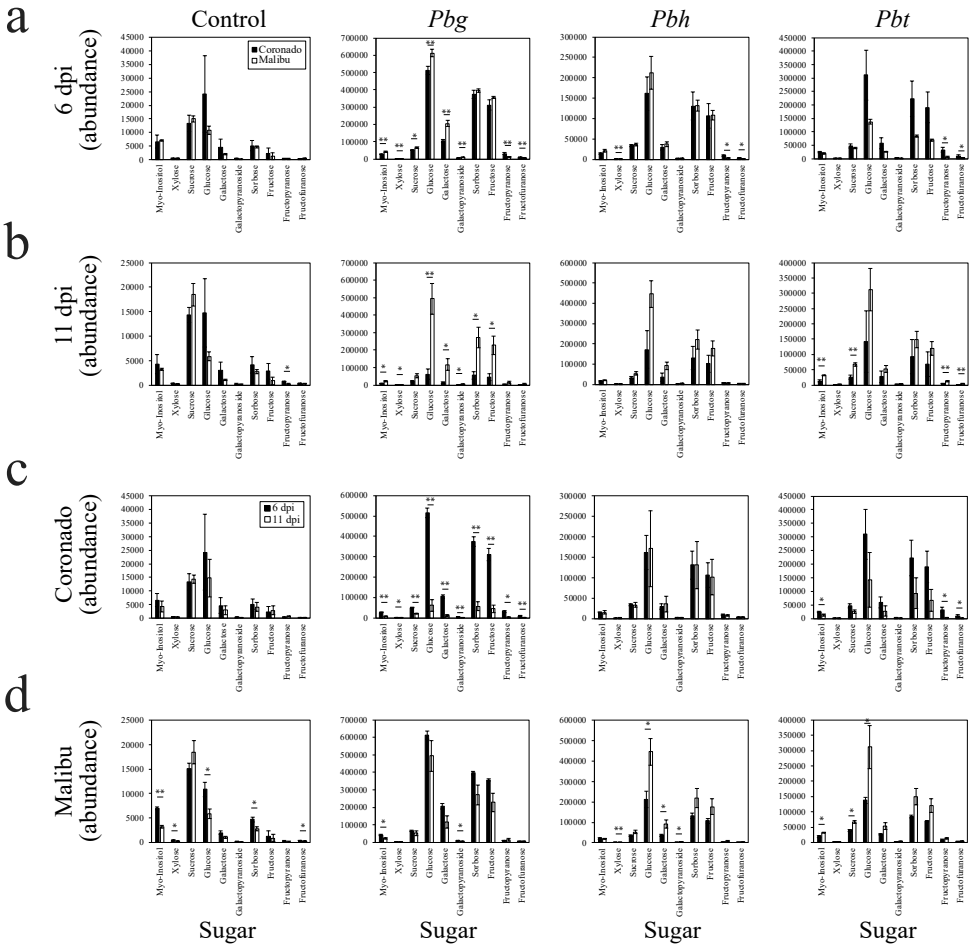



**Fig S3**. Absolute abundance of soluble sugars in two Broccoli cultivars upon *Paraburkholderia* inoculation at two time points (6 and 11 dpi). Comparative analysis of the of abundance of soluble sugars between the two cultivars at 6 dpi (**a)** and at 11 dpi (**b**). Comparative analysis of the abundance of soluble sugars at two time points in Coronado (**c**) and Malibu (**d**) cultivars. *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*. Asterisks denote significant statistical differences between Broccoli cultivars, Coronado and Malibu (two tailed Student's t test): * $P < 0.05$; ** $P < 0.01$.

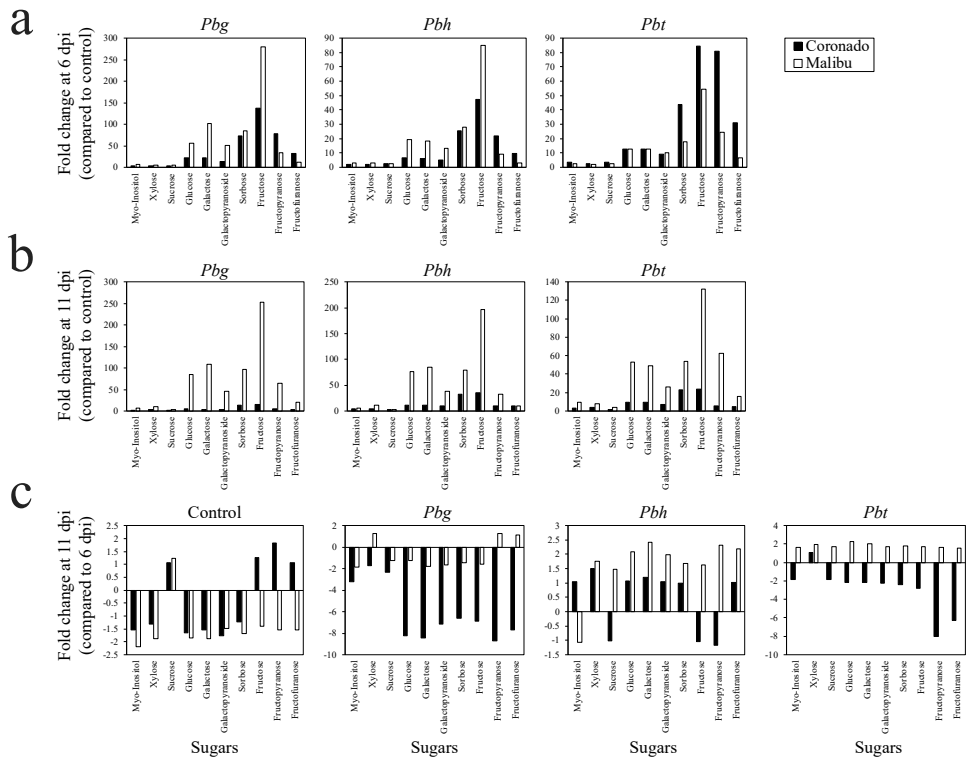



**Fig S4**. *Paraburkholderia* species-mediated changes in shoot soluble sugar abundance in two Broccoli cultivars, Coronado and Malibu, at 6 dpi (**a**), 11 dpi (**b**), and relative fold change between the two time points (**c**). In (**a**) and (**b**), fold change was calculated by dividing the abundance of each soluble sugars in treated plants to that of the non-treated control. *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*.

**Table S1.** Analysis of variance ANOVA (type II) of percent changes in shoot and root biomass in two Broccoli cultivars inoculated with three *Paraburkholderia* genera at 11 dpi.

| Sample | Factor | Sum Sq | Df | F value | Pr(>F) | |
|---|---|---|---|---|---|---|
| Shoot fresh biomass relative change | Broccoli cultivars | 1425 | 1 | 4.882 | 0.040333 | * |
| | Bacteria species | 12949 | 2 | 22.182 | 1.39E-05 | *** |
| | Broccoli cultivars: Bacteria species | 8561 | 2 | 14.665 | 0.000166 | *** |
| | Residuals | 5254 | 18 | | | |
| Root fresh biomass relative change | Broccoli cultivars | 1210.18 | 2 | 19.711 | 3.42E-06 | *** |
| | Bacteria species | 142.42 | 1 | 4.6394 | 0.039404 | * |
| | Broccoli cultivars: Bacteria species | 400.55 | 2 | 6.5241 | 0.004441 | ** |
| | Residuals | 920.94 | 30 | | | |

Significance codes: *** 0, ** 0.001, * 0.01.



**Table S2.** Population density of three *Paraburkholderia* species on roots of two Broccoli cultivars. *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricol*a.

| Broccoli cultivar | Rhizobacteria | Population density (Cfu/mg roots) | |
|---|---|---|---|
| | | 6 dpi | 11 dpi |
| Coronado | *Paraburkholderia graminis* | $2.05 \pm 0.11 \times 10^{8}$ | $8.10 \pm 0.28 \times 10^{7}$ |
| | *P. hospita* | $1.18 \pm 0.09 \times 10^{6}$ | $2.06 \pm 0.08 \times 10^{7}$ |
| | *P. terricola* | $1.58 \pm 0.03 \times 10^{7}$ | $1.95 \pm 0.04 \times 10^{7}$ |
| Malibu | *P. graminis* | $1.00 \pm 0.05 \times 10^{8}$ | $1.02 \pm 0.07 \times 10^{8}$ |
| | *P. hospita* | $1.40 \pm 0.15 \times 10^{7}$ | $1.53 \pm 0.06 \times 10^{7}$ |
| | *P. terricola* | $1.72 \pm 0.16 \times 10^{6}$ | $7.02 \pm 0.35 \times 10^{5}$ |

Cfu: Colony forming unit
*Values represent the average of 3 replicates ± SE of three replicates

**Table S3.** Analysis of variance ANOVA (type II) of root colonization of three *Paraburkholderia* species in two Broccoli cultivars at two time points (6 dpi, 11 dpi). Data was log transformed using the package MASS in R.

| Factor | Sum Sq | Df | F value | Pr(>F) | |
|---|---|---|---|---|---|
| Day post inoculation | 0.0887 | 1 | 41.745 | 1.11E-06 | *** |
| Broccoli cultivar | 0.7069 | 1 | 332.569 | 1.44E-15 | *** |
| Bacteria species | 13.4135 | 2 | 3155.343 | < 2.2e-16 | *** |
| Day post inoculation: Broccoli cultivar | 0.4061 | 1 | 191.066 | 6.35E-13 | *** |
| Day post inoculation: Bacteria species | 1.3366 | 2 | 314.421 | < 2.2e-16 | *** |
| Broccoli cultivar: Bacteria species | 4.353 | 2 | 1023.972 | < 2.2e-16 | *** |
| Day post inoculation: Broccoli cultivar: Bacteria species | 0.9733 | 2 | 228.966 | 2.33E-16 | *** |
| Residuals | 0.051 | 24 | | | |

Signif. codes: 0 =***, 0.001 = **, 0.01 = *



**Table S4**. Beta regression analysis of disease severity in twoBroccoli cultivars (Coronado and Malibu) primed with *Paraburkholderia* species and treated with*Xanthomonas, a* Broccoli leaf pathogen. Different *Paraburkholderia* species were used to induced systemic resistance. *Xca*: *Xanthomonas campestris* pv. Armoraciae P4216 and *Xcc*: *X. campestri*s pv. Campestris P4014.

| Pathovar | model term | df1 | df2 | F.ratio | p.value |
|---|---|---|---|---|---|
| *Xca* | Rhizobacteria | 3 | Inf | 11.74 | <.0001 |
| | Broccoli cultivar | 1 | Inf | 64.893 | <.0001 |
| | Rhizobacteria:Broccoli cultivar | 3 | Inf | 20.627 | <.0001 |
| *Xcc* | Rhizobacteria | 3 | Inf | 3.811 | 0.0096 |
| | Broccoli cultivar | 1 | Inf | 49.535 | <.0001 |
| | Rhizobacteria:Broccoli cultivar | 3 | Inf | 7.604 | <.0001 |

**Table S5.** *Paraburkholderia* species-mediated changes in shoot polar primery metabolites (GC-MS) in two Broccoli cultivars (Coronado and Malibu) at 6 dpi. The tabulated data corresponds to the hierarchical cluster analysis (HCA) showen in **Fig 3b1** of the manuscript. The fold changecorresponding to each metabolites was calculated by dividing the abundance of each metabolites in Paraburkholderia treated plants to the non-treated control. *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*.

| Cluster Number | Mapping Number | RT(m) | Mass | RI | | Annotation | Fold change | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Obse-rved | in DB | | Coronado | | | Malibu | | |
| | | | | | | | *Pbg* | *Pbh* | *Pbt* | *Pbg* | *Pbh* | *Pbt* |
| 1 | | 16.6 | 73 | 1848 | 1846 | 4-(2-Aminoethyl)-2-methoxyphenol, 3TMS derivative | -8.54 | -6.92 | -1.48 | -8.19 | -5.17 | -5.01 |
| 2 | | 13.0 | 55 | 1529 | 1529 | Hexamethylene diacrylate | -1.58 | -1.8 | -1 | -5.82 | -5.37 | -3.47 |
| 2 | 18 | 12.8 | 73 | 1520 | 1522 | L-5-Oxoproline | -1.4 | -1.38 | -1.43 | -3.07 | -2.81 | -2.28 |
| 2 | 16 | 12.9 | 73 | 1523 | 1525 | GABA | -1.23 | -1.26 | -1.25 | -1.74 | -1.64 | 0.67 |
| 4 | 10 | 9.8 | 73 | 1286 | 1286 | Isoleucine | -2.16 | -2.82 | -3.21 | 0.49 | 0.25 | 0.43 |
| 4 | | 11.2 | 174 | 1387 | 1386 | Ethyl 2,4-Dimethylbenzoate | -1.16 | -1.21 | -1.7 | 0.4 | -2.84 | 0.69 |
| 4 | 12 | 15.7 | 73 | 1767 | 1768 | Glutamine | -1.36 | -1.32 | -2.65 | -3.67 | -3.38 | -2.24 |
| 5 | 25 | 14.1 | 73 | 1623 | 1624 | L-Phenylalanine | -1.94 | -1.6 | -1.93 | -1.31 | -1.47 | 1.01 |
| 5 | | 8.5 | 73 | 1192 | | Methyl 7-Trimethylsilylheptanoate | -5.11 | -2.09 | -2.81 | -3.99 | -2.64 | -2.32 |
| 5 | 31 | 17.2 | 73 | 1910 | 1912 | Lysine | -3.61 | -2.87 | -4.21 | -2.54 | -2.89 | -2.23 |
| 5 | | 13.5 | 73 | 1573 | | Trimethyl[(2E/Z)-6-(2-thienyl)-2,6-heptadienyl]silane | -2.52 | -1.94 | -3.12 | -2.53 | -1.95 | -1.64 |
| 5 | | 12.0 | 73 | 1453 | 1452 | N,N-Diethyl 6-oxoheptamide | -16.07 | -4.09 | -16.43 | -18.08 | -10.83 | -10.08 |
| 5 | 32 | 12.8 | 176 | 1514 | 1515 | Methionine | -2.85 | -2.19 | -3.51 | -3.09 | -2.9 | -1.85 |
| 5 | | 13.7 | 73 | 1591 | 1595 | Diethyl (2R/S,3R)-2-Allyl-3-(trimethylsiloxy)succinate | -3.45 | -2.42 | -3.28 | -4.1 | -3.7 | -2.73 |
| 5 | | 14.6 | 73 | 1669 | 1673 | 7,8-Dehydro-8-deoxocurcumen-11-ol | -3.05 | -2.05 | -3.23 | -3.1 | -2.56 | -1.96 |
| 5 | 58 | 9.5 | 73 | 1263 | 1263 | Phosphoenolpyruvate | -3.81 | -2.05 | -2.63 | -3.48 | -2.32 | -1.76 |
| 6 | 27 | 16.1 | 73 | 1805 | | DL-Ornithine | -12.52 | -10.61 | -13.78 | -11.85 | -8.33 | -6.05 |
| 7 | 47 | 12.3 | 73 | 1479 | 1480 | Malic acid | 1.31 | 1.57 | 1.48 | 1.12 | 1.48 | 1.71 |
| 7 | 22 | 10.0 | 73 | 1299 | 1300 | Glycine | -1.08 | 1.23 | 1.03 | 0.77 | 1.01 | 1.28 |
| 7 | 6 | 13.9 | 73 | 1611 | 1612 | Glutamic acid | 1.28 | 1.14 | 1.13 | 1.24 | -1.35 | 0.99 |
| 8 | 4 | 11.6 | 73 | 1420 | 1420 | L-Aspartic acid | 2.3 | 1.67 | 4.18 | 2.96 | 2.67 | 1.97 |
| 8 | 146 | 17.9 | 73 | 1977 | 1981 | Mannonic acid, lactone | 1.96 | -1.24 | 4.08 | 1.59 | 0.87 | 0.62 |
| 8 | | 9.4 | 73 | 1259 | 1260 | TMS 4-DI-TMS-AMINOBUTYRATE | 3.22 | 1.55 | 1.7 | 1.68 | 1.86 | 1.76 |
| 8 | 8 | 15.3 | 73 | 1729 | 1731 | Putrescine | 1.44 | 1.09 | 1.42 | 1.18 | 1.02 | 0.95 |
| 9 | 45 | 16.5 | 73 | 1839 | 1863 | Ascorbic acid | 1.5 | 1.34 | 1.46 | 1.85 | 1.49 | 1.71 |
| 9 | | 16.9 | 191 | 1877 | 1877 | 2-Amino-3-cyano-4-(2'-furyl)-4-thioxobut-2-ene | 28.8 | 5.29 | 24.23 | 20.2 | 2.46 | 4.7 |
| 9 | 101 | 16.1 | 73 | 1801 | 1802 | D-(-)-Fructopyranose (isomer 2) | 78.58 | 22.07 | 80.8 | 26.95 | 10.85 | 15.98 |
| 9 | 100 | 16.0 | 73 | 1795 | 1792 | D-(-)-Fructofuranose (isomer 1) | 32.09 | 9.51 | 31.1 | 15.76 | 5.46 | 7.45 |
| 9 | 102 | 14.3 | 73 | 1640 | 1634 | Xylose | 3.71 | 2.08 | 2.64 | 4.77 | 2.75 | 2.96 |
| 9 | 105 | 16.7 | 73 | 1863 | 1865 | Fructose | 138.02 | 47.19 | 84.47 | 157.82 | 40.86 | 37.71 |
| 9 | 109 | 18.8 | 73 | 2074 | 2077 | Myo-Inositol | 4.06 | 2.18 | 3.69 | 6.29 | 2.58 | 3.45 |
| 9 | 110 | 23.3 | 73 | 2606 | 2611 | Sucrose | 3.8 | 2.52 | 3.57 | 4.89 | 2.76 | 2.86 |
| 9 | 106 | 16.6 | 73 | 1854 | 1854 | Sorbose | 73.61 | 25.66 | 43.78 | 77.87 | 21.94 | 20.4 |
| 9 | 107 | 18.2 | 73 | 2010 | 2010 | D-Galactopyranoside | 14.23 | 5.01 | 9.38 | 23.53 | 5.31 | 5.29 |
| 9 | 108 | 17.1 | 73 | 1899 | 1898 | d-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1E)- | 22.64 | 6.32 | 12.58 | 44.36 | 6.79 | 6.93 |
| 9 | 103 | 16.9 | 73 | 1879 | 1881 | Glucose | 21.19 | 6.66 | 12.81 | 25.26 | 6.97 | 7.46 |

**Table S6.** *Paraburkholderia* species-mediated changes in shoot polar primery metabolites (GC-MS) in two Broccoli cultivars (Coronado and Malibu) at 11 dpi. The tabulated data corresponds to the hierarchical cluster analysis (HCA) showen in **Fig 3b2** of the manuscript. The fold changecorresponding to each metabolites was calculated by dividing the abundance of each metabolites in Paraburkholderia treated plants to the non-treated control. *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*.

| Cluster Number | Mapping Number | RT(m) | Mass | RI | | Annotation | Fold change | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Obse-rved | in DB | | Coronado | | | Malibu | | |
| | | | | | | | *Pbg* | *Pbh* | *Pbt* | *Pbg* | *Pbh* | *Pbt* |
| 1 | 22 | 10.0 | 73 | 1299 | 1300 | Glycine | -1.16 | -1.84 | -1.29 | -2.4 | -1.22 | -1.14 |
| 2 | 32 | 12.8 | 176 | 1514 | 1515 | Methionine | -2.37 | -3.18 | -2.1 | -2.59 | -3.39 | -1.95 |
| 2 | | 13.5 | 73 | 1573 | | Trimethyl[(2E/Z)-6-(2-thienyl)-2,6-heptadienyl]silane | -2.22 | -2.21 | -2.41 | -2.06 | -2.12 | -1.4 |
| 2 | | 14.6 | 73 | 1669 | 1673 | 7,8-Dehydro-8-deoxocurcumen-11-ol | -2.27 | -2.16 | -2.36 | -2.31 | -2.49 | -1.7 |
| 2 | | 12.0 | 73 | 1453 | 1452 | N,N-Diethyl 6-oxoheptamide | -11.7 | -4.59 | -3.09 | -11.19 | -10.7 | -4.25 |
| 2 | | 13.7 | 73 | 1591 | 1595 | Diethyl (2R/S,3R)-2-Allyl-3-(trimethylsiloxy)succinate | -2.14 | -2.12 | -2.35 | -2.7 | -2.95 | -2.89 |
| 2 | 25 | 14.1 | 73 | 1623 | 1624 | L-Phenylalanine | -1.05 | -1.17 | -0.83 | -2.72 | -2.86 | -1.63 |
| 2 | 58 | 9.5 | 73 | 1263 | 1263 | Phosphoenolpyruvate | -14.18 | -11.91 | -12.75 | -11.43 | -11.3 | -2.13 |
| 2 | 31 | 17.2 | 73 | 1910 | 1912 | Lysine | -2.71 | -2.55 | -2.75 | -3.47 | -3.4 | -2.92 |
| 2 | 27 | 16.1 | 73 | 1805 | | DL-Ornithine | -10.86 | -14.9 | -10.33 | -23.84 | -22.03 | -13.68 |
| 2 | | 16.6 | 73 | 1848 | 1846 | 4-(2-Aminoethyl)-2-methoxyphenol, 3TMS derivative | -6.71 | -10.66 | -10.6 | -11.9 | -13.78 | -8.23 |
| 2 | | 8.5 | 73 | 1192 | | Methyl 7-Trimethylsilylheptanoate | -7.85 | -3.95 | -3.55 | -2.19 | -3.16 | -1.29 |
| 2 | 16 | 12.9 | 73 | 1523 | 1525 | GABA | -2.38 | -2.31 | -2.44 | -1.13 | -1.27 | 1.31 |
| 3 | | 13.0 | 55 | 1529 | 1529 | Hexamethylene diacrylate | -9.3 | -13.75 | -12.34 | 1.09 | 1.07 | 1.44 |
| 3 | 18 | 12.8 | 73 | 1520 | 1522 | L-5-Oxoproline | -2.49 | -2.15 | -2.3 | 1.03 | -1.11 | 1.01 |
| 5 | 8 | 15.3 | 73 | 1729 | 1731 | Putrescine | 1.42 | 1.36 | 1.32 | 4.01 | 3.62 | 2.83 |
| 5 | | 9.4 | 73 | 1259 | 1260 | TMS 4-DI-TMS-AMINOBUTYRATE | 3.61 | 2.89 | 3.65 | 2.61 | 2.84 | 2.16 |
| 5 | 4 | 11.6 | 73 | 1420 | 1420 | L-Aspartic acid, 2TMS derivative | 1.74 | 2.07 | 1.77 | 3.03 | 4.77 | 3.4 |
| 5 | 110 | 23.3 | 73 | 2606 | 2611 | Sucrose | 1.63 | 2.47 | 1.91 | 2.84 | 2.89 | 3.65 |
| 5 | 102 | 14.3 | 73 | 1640 | 1634 | Xylose | 2.13 | 3.11 | 2.88 | 10.9 | 10.76 | 7.98 |
| 5 | 105 | 16.7 | 73 | 1863 | 1865 | Fructose | 20.05 | 45.02 | 30.13 | 252.76 | 196.15 | 132.07 |
| 5 | 109 | 18.8 | 73 | 2074 | 2077 | Myo-Inositol | 1.26 | 2.26 | 2.03 | 7.05 | 6.12 | 9.87 |
| 5 | 106 | 16.6 | 73 | 1854 | 1854 | Sorbose | 11.13 | 25.71 | 18.28 | 97.3 | 79.09 | 53.56 |
| 5 | 107 | 18.2 | 73 | 2010 | 2010 | D-Galactopyranoside, methyl 2,3,4-tris-O-(trimethylsilyl)-, acetate | 1.99 | 5.2 | 4.14 | 45.56 | 38.49 | 25.88 |
| 5 | 103 | 16.9 | 73 | 1879 | 1881 | Glucose | 2.57 | 7.06 | 5.83 | 84.07 | 75.88 | 53.04 |
| 5 | 108 | 17.1 | 73 | 1899 | 1898 | d-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1E)- | 2.68 | 7.65 | 5.92 | 107.78 | 84.51 | 48.95 |
| 5 | 45 | 16.5 | 73 | 1839 | 1863 | Ascorbic acid | 1.19 | 1.39 | 0.91 | 2.13 | 2.33 | 2.55 |
| 6 | 6 | 13.9 | 73 | 1611 | 1612 | Glutamic acid | 1.05 | 1.12 | 1.22 | 1.66 | 1.57 | 1.69 |
| 6 | 5 | 12.7 | 73 | 1511 | 1512 | ASPARTIC ACID-TRITMS | -1.47 | 1.1 | 1.04 | 2.22 | 1.78 | 2.46 |
| 6 | 101 | 16.1 | 73 | 1801 | 1802 | D-(-)-Fructopyranose (isomer 2) | 9.02 | 18.82 | 10.12 | 64.34 | 32.8 | 62.32 |
| 6 | 100 | 16.0 | 73 | 1795 | 1792 | D-(-)-Fructofuranose (isomer 1) | 4.18 | 9.7 | 4.95 | 20.31 | 10.47 | 15.98 |

**Table S7**. *Paraburkholderia* species-mediated changes in shoot semi-polar secondary metabolites (LC-MS) in two Broccoli cultivars (Coronado and Malibu) at 6 dpi. The tabulated data corresponds to the hierarchical cluster analysis (HCA) showen in **Fig 4b1** of the manuscript. The fold changecorresponding to each metabolites was calculated by dividing the abundance of each metabolites in Paraburkholderia treated plants to the non-treated control. *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*.

| Cluster number | Mapping number | RT (m) | m/z | Mass (D) | Compound | Formula | Δ ppm to DB | Classification | data base | Fold change | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Coronado | | | Malibu | | |
| | | | | | | | | | | *Pbg* | *Pbh* | *Pbt* | *Pbg* | *Pbh* | *Pbt* |
| 1 | 157 | 17.46 | [M+H]+ | 164.019562 | 3-Methylsulfinylpropyl isothiocyanate | C5H9NOS2 | -1.64 | Sulfoxide | HMDB | -3.4 | -2.3 | -3.0 | -1.2 | -1.4 | -1.4 |
| 2 | | 3.00 | [M+H]+ | 114.054932 | 1-Pyrroline-2-carboxylic acid | C5H7NO2 | -0.39 | Pyrroline | HMDB | -1063.2 | -146.0 | -16.1 | -1425.4 | -1410.1 | -23.4 |
| 2 | 111 | 20.19 | [M-H]- | 536.176819 | 4-Demethylsimmondsin 2'-(E)-ferulate | C25H31NO12 | -0.99 | Coumaric acid | HMDB | -1.8 | -1.4 | -1.5 | -1.4 | -1.4 | -1.4 |
| 2 | 29 | 1.63 | [M+H]+ | 175.118820 | Arginine | C6H14N4O2 | -0.89 | Amino acid | KEGG | -3.3 | -2.0 | -2.4 | -4.6 | -2.2 | -1.7 |
| 2 | | 1.80 | [M-H]- | 173.104156 | Arginine | C6H14N4O2 | -1.55 | Amino acid | KEGG | -3.0 | -1.6 | -2.3 | -4.6 | -2.3 | -1.8 |
| 2 | 30 | 1.97 | [M-H]- | 131.045929 | Asparagine | C4H8N2O3 | -2.25 | Amino acid | KEGG | -3.7 | -2.3 | -2.8 | -3.8 | -2.3 | -2.1 |
| 2 | 85 | 23.64 | [M-H]- | 609.144470 | Flavonoid di glycoside or (C12634), (C05625), (C17563) | C27H30O16 | -2.72 | Flavonoid | KEGG | -5.1 | -4.4 | -4.2 | -5.9 | -4.1 | -3.2 |
| 2 | 84 | 23.63 | [M+H]+ | 611.158447 | Flavonoid di glycoside or (C05625), (C19796) | C27H30O16 | -3.65 | Flavonoid | KEGG | -7.3 | -5.7 | -7.8 | -9.3 | -4.7 | -3.8 |
| 2 | 42 | 7.21 | [M-H]- | 138.055878 | Gabaculine | C7H9NO2 | -1.06 | Benzoic acid | KEGG | -1.2 | -1.1 | 1.0 | -2.6 | -2.6 | -1.2 |
| 2 | 148 | 3.12 | [M+H]+ | 286.138916 | Glycylprolylhydroxyproline | C12H19N3O5 | -2.90 | Oligopeptide | HMDB | -2.6 | -1.7 | -1.2 | -3.4 | -2.3 | -1.1 |
| 2 | 145 | 2.98 | [M-H]- | 204.087799 | N-Acetyl-D-fucosamine | C8H15NO5 | 0.37 | Monosaccharide | KEGG | -5.5 | -4.7 | -4.2 | -8.6 | -7.1 | -5.4 |
| 2 | 28 | 2.95 | [M-H]- | 188.056519 | N-Acetylglutamic acid | C7H11NO5 | 0.39 | Glutamic acid | HMDB | -5.0 | -3.8 | -4.5 | -5.3 | -4.1 | -3.7 |
| 2 | 26 | 7.80 | [M-H]- | 203.082779 | Tryptophan | C11H12N2O2 | 0.76 | Amino acid | KEGG | -3.3 | -2.2 | -2.5 | -2.2 | -2.0 | -1.2 |
| 2 | 149 | 28.71 | [M-H]- | 593.208496 | Zizybeoside II | C25H38O16 | -0.38 | Oligosaccharide | KEGG | -2.2 | -1.9 | -1.8 | -3.2 | -2.9 | -2.3 |
| 3 | 170 | 21.27 | [M-H]- | 278.107025 | 2-Acetamido-3-hexylsulfonylpropanoic acid | C11H21NO5S | 1.08 | | PubChem | 1.1 | -1.0 | -1.0 | -1.4 | -1.3 | -1.5 |
| 3 | 88 | 20.01 | [M-H]- | 388.074219 | 2-Methylbutyl glucosinolate | C12H23NO9S2 | 0.24 | Aliphatic GLS | Metlin, KEGG | -2.1 | -2.1 | -1.5 | -1.7 | 1.2 | 1.4 |
| 3 | 176 | 29.31 | [M+H]+ | 162.094620 | 6-Methylthiohexanaldoxime | C7H15NOS | -0.35 | | KEGG | -1.1 | -1.1 | -1.9 | -2.5 | -3.5 | -1.8 |
| 3 | 92 | 10.13 | [M-H]- | 406.030487 | Glucoiberverin | C11H21NO9S3 | -0.09 | Aliphatic GLS | KEGG | -1.1 | -1.2 | 1.5 | 3.9 | 1.9 | 1.6 |
| 3 | 93 | 23.98 | [M-H]- | 422.058563 | Gluconasturtiin | C15H21NO9S2 | 0.09 | Other GLS | KEGG | -1.4 | -1.3 | -1.2 | 1.7 | 1.7 | 1.7 |
| 3 | 94 | 15.94 | [M-H]- | 408.042725 | Glucotropaeolin | C14H19NO9S2 | -0.24 | Other GLS | KEGG | 1.0 | -1.3 | 1.1 | -1.4 | -1.3 | -1.2 |
| 3 | 7 | 2.00 | [M-H]- | 146.045837 | Glutaminic acid | C5H9NO4 | -0.60 | Amino acid | KEGG | 1.8 | 1.4 | 1.7 | 2.1 | 1.4 | 1.4 |
| 3 | 13 | 4.25 | [M+H]+ | 235.110428 | Isoleucyl-Cysteine, Leucyl-Cysteine | C9H18N2O3S | -2.81 | Dipeptide | HMDB | -2.5 | -1.7 | -1.1 | 1.1 | 1.1 | 1.2 |
| 3 | 53 | 3.31 | [M+H]+ | 246.169876 | Isovalerylcarnitine | C12H23NO4 | -0.41 | Carnitine | KEGG | -1.5 | -1.4 | -1.2 | -1.1 | -1.5 | -1.2 |
| 3 | 80 | 20.47 | [M-H]- | 947.247437 | Kaempferol 3-(2-feruloylsophoroside) 7-glucoside | C43H48O24 | 1.23 | Flavonoid | HMDB | 1.0 | -1.3 | 1.2 | 1.8 | 1.7 | 1.2 |
| 3 | 65 | 20.90 | [M+H]+ | 919.249146 | Kaempferol 3-O-[6-(4-coumaroyl)-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside] | C42H46O23 | -1.23 | Flavonoid | KEGG | -1.1 | -1.5 | -1.0 | 2.0 | 1.7 | 1.4 |
| 3 | | 20.91 | [M-H]- | 917.235291 | Kaempferol 3-O-[6-(4-coumaroyl)-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside] | C42H46O23 | -0.47 | Flavonoid | KEGG | -1.2 | -1.6 | 1.0 | 2.1 | 2.0 | 1.8 |
| 3 | 17 | 2.96 | [M+H]+ | 263.141815 | Methionyl-Isoleucine | C11H22N2O3S | -2.18 | Dipeptide | HMDB | -1.6 | -1.6 | -1.6 | -1.3 | -1.3 | -1.2 |
| 3 | 112 | 33.28 | [M-H]- | 385.150146 | Methylsyringin | C18H26O9 | -0.68 | Phenolic glycoside | HMDB | -1.4 | -1.3 | -1.3 | -2.3 | -2.0 | -1.7 |
| 3 | 44 | 33.29 | [M+H]+ | 225.111435 | Olivetolic acid | C12H16O4 | -3.29 | Benzoic | KEGG | -1.6 | -1.6 | -1.5 | -3.0 | -2.2 | -2.1 |
| 3 | 52 | 6.20 | [M-H]- | 305.087860 | Starch acetate | C12H18O9 | 0.18 | Carboxylic acid | HMDB | -1.2 | -1.3 | 1.0 | -1.0 | -1.5 | -1.0 |

**Table S7.** (Continued)

| Cluster number | Mapping number | RT (m) | m/z | Mass (D) | Compound | Formula | Δ ppm to DB | Classification | data base | Fold change | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Coronado | | | Malibu | | |
| | | | | | | | | | | *Pbg* | *Pbh* | *Pbt* | *Pbg* | *Pbh* | *Pbt* |
| 4 | 172 | 3.87 | [M-H]- | 276.988342 | 2-C-Methyl-D-erythritol 2,4-cyclodiphosphate | C5H12O9P2 | -0.30 | | KEGG | -1.8 | -1.1 | -1.2 | -1.6 | -1.9 | -2.2 |
| 4 | | 33.64 | [M-H]- | 333.145691 | 3'-N'-Acetylfusarochromanone | C17H22N2O5 | 0.29 | Benzopyran | HMDB | 2.0 | -1.0 | 1.8 | -1.1 | -1.1 | 1.3 |
| 4 | 165 | 43.28 | [M-H]- | 629.261658 | Brassica napus non-fluorescent chlorophyll catabolite 3 | C34H38N4O8 | -0.05 | Tetrapyrrole | HMDB | -4.3 | -2.7 | -4.0 | -5.5 | -5.7 | -3.5 |
| 4 | 61 | 16.87 | [M-H]- | 380.156128 | cis-Zeatin-O-glucoside | C16H23N5O6 | -3.67 | Fattyacyl glycoside | KEGG | -2.0 | -1.5 | -1.5 | -3.3 | -2.8 | -2.2 |
| 4 | 35 | 12.05 | [M-H]- | 222.077301 | Dioxacarb | C11H13NO4 | 0.35 | Benzene | KEGG | -1.7 | -1.3 | -1.3 | -1.7 | -1.6 | -1.3 |
| 4 | 46 | 26.20 | [M-H]- | 203.056168 | L-ascorbic acid ethyl ester | C8H12O6 | 0.22 | Butenolide | metlin | -2.5 | -1.5 | -2.4 | -11.8 | -4.7 | -4.9 |
| 4 | 23 | 27.97 | [M+H]+ | 247.107407 | N-Acetyl-D-tryptophan | C13H14N2O3 | -1.09 | Amino acid | KEGG | -2.0 | -1.8 | -1.6 | -1.8 | -1.8 | -1.4 |
| 4 | 164 | 12.04 | [M+H]+ | 224.091324 | Salsolinol 1-carboxylate | C11H13NO4 | -1.83 | Quinoline | HMDB | -1.9 | -1.5 | -1.4 | -2.1 | -1.7 | -1.4 |
| 4 | 160 | 19.07 | [M-H]- | 431.191284 | S-Furanopetasitin | C24H32O5S | 3.51 | Terpenoid | HMDB | -49.1 | -5.3 | -4.7 | -2.0 | -3.0 | -2.2 |
| 4 | 161 | 20.25 | [M+H]+ | 387.200470 | Sonchuionoside C | C19H30O8 | -2.26 | Terpenoid | HMDB | -1.7 | -1.4 | -1.4 | -1.6 | -1.5 | -1.3 |
| 4 | 24 | 4.14 | [M+H]+ | 384.113953 | Succinyladenosine | C14H17N5O8 | -2.70 | Purine nucleoside | HMDB | -2.3 | -1.6 | -1.4 | -2.0 | -1.9 | -1.3 |
| 4 | 156 | 34.23 | [M-H]- | 363.075562 | Sulfometuron methyl | C15H16N4O5S | -3.48 | Sulfonylurea | KEGG | -3.0 | -2.6 | -3.3 | -6.5 | -3.2 | -1.5 |
| 5 | 36 | 17.69 | [M-H]- | 329.087708 | 1-O-Vanilloyl-beta-D-glucose | C14H18O9 | -0.35 | Phenolic glycoside | KEGG | -2.1 | -1.7 | -1.7 | -1.6 | -1.9 | -1.7 |
| 5 | 21 | 23.98 | [M+H]+ | 252.085983 | 4-Hydroxy-3-methoxy-cinnamoylglycine | C12H13NO5 | -2.75 | Amino acid | KEGG | -1.6 | -1.5 | -1.3 | 1.0 | -1.1 | -1.0 |
| 5 | 41 | 24.57 | [M-H]- | 165.019363 | Formylsalicylic acid or (C14096), (C14100) | C8H6O4 | 0.24 | Benzoic acid | KEGG | 1.0 | -1.2 | 1.1 | -1.2 | -1.0 | 1.2 |
| 5 | 20 | 23.98 | [M-H]- | 206.082062 | N-Acetyl-L-phenylalanine | C11H13NO3 | -0.79 | Phenylalanine | KEGG, Metlin | -1.4 | -1.3 | -1.2 | 1.1 | -1.1 | 1.0 |
| 5 | 155 | 27.17 | [M-H]- | 349.059875 | Sulfometuron | C14H14N4O5S | -3.86 | Sulfonylurea | KEGG | -1.2 | -1.1 | -1.0 | -1.1 | -1.3 | -1.1 |
| 7 | 63 | 23.89 | [M+H]+ | 225.148117 | (6S,9R)-Vomifoliol | C13H20O3 | -1.59 | Fatty acyl glycoside | KEGG | 1.7 | 1.5 | 1.5 | 1.6 | 1.4 | 1.4 |
| 7 | 120 | 21.34 | [M-H]- | 335.077667 | 5-O-Caffeoylshikimic acid | C16H16O8 | 1.32 | Coumaric acid | Metlin, KEGG | 4.8 | 4.5 | 5.3 | 3.9 | 2.7 | 2.6 |
| 7 | 72 | 33.14 | [M-H]- | 577.156250 | Flavonoid-di-glycoside or (C16981), (C12628), (C12627) | C27H30O14 | -0.13 | Flavonoid | KEGG | 2.4 | 2.2 | 3.2 | 2.2 | 2.5 | 2.5 |
| 7 | 147 | 4.77 | [M-H]- | 375.093384 | Furaneol 4-(6-malonylglucoside) | C15H20O11 | 0.26 | O-Glycosyl comp. | HMDB | 1.4 | 1.1 | 1.4 | 1.4 | 1.4 | 1.5 |
| 7 | 48 | 23.68 | [M-H]- | 350.088165 | Indole-3-acetic-acid-O-glucuronide | C16H17NO8 | 0.07 | O-Glucuronides | HMDB | 6.4 | 1.8 | 3.3 | 3.1 | 1.9 | 1.8 |
| 7 | 67 | 17.00 | [M-H]- | 963.241943 | Kaempferol 3-O-hydroxyferuloylsophoroside 7-O-glucoside | C43H48O25 | 1.27 | Flavonoid | PubChem | 1.5 | 1.2 | 1.6 | 2.7 | 1.9 | 1.9 |
| 7 | 163 | 20.31 | [M+H]+ | 377.144623 | Loganic acid | C16H24O10 | 1.19 | Iridoid | KEGG | 5.1 | 1.9 | 2.9 | 2.8 | 2.0 | 1.9 |
| 7 | | 2.40 | [M-H]- | 133.014084 | Malic acid | C4H6O5 | -1.05 | Hydroxy acid | KEGG | 1.8 | 1.9 | 1.9 | 1.6 | 1.4 | 1.9 |
| 8 | 59 | 23.68 | [M+H]+ | 323.122986 | Binapacryl | C15H18N2O6 | -2.44 | Dinoseb | KEGG | 6.2 | 1.9 | 3.0 | 3.3 | 2.1 | 2.0 |
| 8 | 64 | 15.09 | [M+H]+ | 773.210571 | Kaempferol 3-sophorotrioside | C33H40O21 | -3.76 | Flavonoid | KEGG | 6.7 | 4.9 | 6.4 | 12.9 | 9.4 | 6.4 |
| 9 | 124 | 39.91 | [M-H]- | 929.272949 | 2-Feruloyl-1,2'-disinapoylgentiobiose | C44H50O22 | 0.92 | Phenylpropanoid | HMDB | 1.1 | -1.0 | 1.3 | 1.3 | 1.3 | 1.3 |
| 9 | 175 | 33.63 | [M+H]+ | 335.159943 | 3'-N'-Acetylfusarochromanone | C17H22N2O5 | -0.61 | Benzopyran | HMDB | 3.1 | 1.9 | 1.5 | 1.0 | -1.3 | -1.1 |

**Table S7.** (Continued)

| Cluster number | Mapping number | RT (m) | m/z | Mass (D) | Compound | Formula | Δ ppm to DB | Classification | data base | Fold change | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Coronado | | | Malibu | | |
| | | | | | | | | | | *Pbg* | *Pbh* | *Pbt* | *Pbg* | *Pbh* | *Pbt* |
| 11 | 153 | 21.46 | [M-H]- | 551.176697 | (Z)-Resveratrol 3,4'-diglucoside | C26H32O13 | -0.58 | Stilbenoid | HMDB | 1.7 | 1.6 | 2.0 | 2.0 | 1.7 | 1.6 |
| 11 | 132 | 18.43 | [M-H]- | 355.103210 | 1-Feruloyl-D-glucose | C16H20O9 | -0.60 | Phenylpropanoid | KEGG | 1.8 | 1.6 | 1.8 | 2.1 | 1.7 | 1.6 |
| 11 | 37 | 26.28 | [M-H]- | 415.124878 | 2-Hydroxybenzaldehyde O-[xylosyl-(1->6)-glucoside] | C18H24O11 | 0.70 | Phenolic glycoside | HMDB | 3.0 | 2.3 | 3.2 | 2.7 | 1.8 | 1.9 |
| 11 | 125 | 43.09 | [M+H]+ | 239.090393 | 3,4,5-Trimethoxycinnamic acid | C12H14O5 | -4.20 | Phenylpropanoid | HMDB | 2.3 | 1.5 | 2.4 | 2.6 | 1.4 | 1.7 |
| 11 | 43 | 13.33 | [M-H]- | 299.077576 | 4-Hydroxybenzoate-O-glucoside | C13H16O8 | 1.18 | Benzoic acid | KEGG | 1.3 | 1.2 | 1.3 | 1.8 | 1.5 | 1.8 |
| 11 | 135 | 12.94 | [M+H]+ | 211.059418 | 5-Hydroxyferulic acid methyl ester I | C10H10O5 | -3.12 | Phenylpropanoid | KEGG | 2.1 | 1.6 | 2.0 | 2.7 | 1.7 | 1.7 |
| 11 | 54 | 18.44 | [M+H]+ | 177.054489 | 7-Methoxycoumarin or (C03081) | C10H8O3 | -0.49 | Coumarin | KEGG | 2.0 | 1.9 | 1.9 | 2.6 | 1.7 | 1.7 |
| 11 | 38 | 12.10 | [M+H]+ | 138.054703 | Anthranilic acid | C7H7NO2 | -1.98 | Aromatic acid | KEGG | 1.9 | 1.3 | 1.9 | 2.4 | 1.5 | 1.8 |
| 11 | 138 | 11.42 | [M-H]- | 341.087616 | Caffeic acid 3-glucoside | C15H18O9 | -0.61 | Phenylpropanoid | KEGG | 2.1 | 2.0 | 2.0 | 2.6 | 2.0 | 1.7 |
| 11 | 122 | 9.97 | [M-H]- | 353.087585 | Chlorogenic acid | C16H18O9 | -0.68 | Phenylpropanoid | KEGG | 2.3 | 1.7 | 2.4 | 2.0 | 1.7 | 1.5 |
| 11 | | 9.92 | [M+H]+ | 355.101624 | Chlorogenic acid | C16H18O9 | -2.12 | Phenylpropanoid | KEGG | 2.3 | 1.7 | 2.2 | 1.9 | 1.6 | 1.3 |
| 11 | | 15.86 | [M-H]- | 353.087616 | Chlorogenic acid or Coumarin: (C01527) or (C08996) | C16H18O9 | -0.59 | Phenylpropanoid | KEGG | 3.1 | 2.5 | 3.2 | 2.1 | 1.8 | 1.5 |
| 11 | 123 | 15.81 | [M+H]+ | 355.101654 | Chlorogenic acid or Coumarin: (C01527) or (C08996) | C16H18O9 | -2.03 | Phenylpropanoid | KEGG | 2.6 | 2.1 | 2.7 | 2.1 | 1.8 | 1.6 |
| 11 | 50 | 2.95 | [M-H]- | 191.019562 | citric acid | C6H8O7 | -0.85 | Tricarboxylic acid | KEGG | 3.9 | 2.7 | 3.5 | 3.4 | 2.4 | 2.7 |
| 11 | 136 | 12.93 | [M-H]- | 371.097992 | Dihydroferulic acid 4-O-glucuronide | C16H20O10 | -1.02 | Phenylpropanoid | HMDB | 2.4 | 1.8 | 2.1 | 2.9 | 1.9 | 2.0 |
| 11 | 133 | 18.43 | [M+H]+ | 195.064651 | Ferulic acid | C10H10O4 | -2.69 | Phenylpropanoid | KEGG | 1.7 | 1.5 | 1.8 | 2.2 | 1.6 | 1.6 |
| 11 | 51 | 2.54 | [M-H]- | 207.014572 | Garcinia acid | C6H8O8 | -0.33 | Carboxylic acid | HMDB | 11.1 | 8.5 | 27.6 | 41.4 | 12.8 | 8.9 |
| 11 | 104 | 2.04 | [M-H]- | 225.061401 | Glucoheptonic acid | C7H14O8 | -0.99 | Glycoside | Metlin | 4.9 | 3.0 | 4.1 | 6.2 | 3.2 | 2.9 |
| 11 | 68 | 26.16 | [M-H]- | 725.194031 | Isoschaftoside 4'-glucoside or (HMDB40878) | C32H38O19 | 0.80 | Flavonoid | HMDB | 2.9 | 1.8 | 2.5 | 3.8 | 2.1 | 2.2 |
| 11 | 83 | 20.03 | [M+H]+ | 611.159851 | Kaempferol 3-O-beta-D-sophoroside or (C05625), (C17563) | C27H30O16 | -1.35 | Flavonoid | KEGG | 1.2 | 1.1 | 1.4 | 1.6 | 1.5 | 1.4 |
| 11 | 81 | 20.46 | [M+H]+ | 949.258301 | Kaempferol tri glycoside + feruloyl | C43H48O24 | -2.66 | Flavonoid | HMDB | -1.0 | -1.3 | 1.2 | 2.0 | 1.8 | 1.6 |
| 11 | 139 | 15.97 | [M-H]- | 325.092926 | p-Coumaroyl-D-glucose or (C04415), (C05158) | C15H18O8 | 0.01 | Phenylpropanoid | KEGG | 2.3 | 2.0 | 2.7 | 4.0 | 4.1 | 2.3 |
| 11 | 140 | 15.99 | [M+H]+ | 165.054581 | Phenylpyruvic acid or (C12621), (C00811) | C9H8O3 | 0.03 | Phenylpropanoid | KEGG | 2.4 | 2.0 | 2.5 | 4.4 | 3.7 | 2.2 |
| 11 | 79 | 17.75 | [M+H]+ | 935.242615 | Quercetin 3-[p-coumaroyl-(->6)-glucosyl-(1->2)-glucosyl-(1->2)-glucoside], Kaempferol 3-O-caffeoyl-sophoroside 7-O-glucoside | C42H46O24 | -2.74 | Flavonoid | HMDB | 1.0 | -1.2 | 1.1 | 2.3 | 1.9 | 1.8 |
| 11 | 69 | 29.93 | [M-H]- | 739.210144 | Robinin | C33H40O19 | 1.38 | Flavonoid | KEGG | 2.3 | 1.8 | 2.4 | 1.8 | 1.3 | 1.6 |
| 11 | 57 | 12.94 | [M+H]+ | 193.048950 | Scopoletin, or (C10290), (C18077), (C01938) | C10H8O4 | -3.24 | Coumarin | KEGG | 2.4 | 1.9 | 2.1 | 2.9 | 1.8 | 1.8 |
| 12 | 60 | 3.85 | [M-H]- | 147.066345 | (S)-Mevalonic acid | C6H12O4 | 0.14 | Hydroxy fatty acid | KEGG | 2.1 | 2.0 | 2.3 | 1.7 | 1.6 | 1.3 |
| 12 | 137 | 13.27 | [M-H]- | 341.087585 | 1-Caffeoyl-beta-D-glucose or (C10431) | C15H18O9 | -0.70 | Phenylpropanoid | KEGG | 2.4 | 2.1 | 2.1 | 3.0 | 2.3 | 2.1 |
| 12 | 131 | 23.26 | [M-H]- | 195.029892 | 3,4-Dihydroxyphenylpyruvate | C9H8O5 | -0.16 | Phenylpropanoid | KEGG | 2.7 | 1.8 | 2.1 | 2.5 | 1.7 | 1.9 |
| 12 | 99 | 19.52 | [M-H]- | 401.109009 | 4-Hydroxy-5-(3',5'-dihydroxyphenyl)-valeric acid-O-glucuronide | C17H22O11 | 0.18 | Glucuronide | HMDB | 1.6 | 1.5 | 1.6 | 2.0 | 1.8 | 1.8 |

**Table S7**. (Continued)

| Cluster number | Mapping number | RT (m) | m/z | Mass (D) | Compound | Formula | Δ ppm to DB | Classification | data base | Fold change | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Coronado | | | Malibu | | |
| | | | | | | | | | | *Pbg* | *Pbh* | *Pbt* | *Pbg* | *Pbh* | *Pbt* |
| 12 | 74 | 22.70 | [M+H]+ | 341.138000 | 5-Deoxykievitone or (C09832), (C18023), (C09753), (C15510) | C20H20O5 | -1.10 | Flavonoid | KEGG | 2.4 | 1.9 | 2.3 | 3.0 | 2.4 | 2.0 |
| 12 | 134 | 14.64 | [M+H]+ | 211.059479 | 5-Hydroxyferulic acid methyl ester II | C10H10O5 | -2.83 | Phenylpropanoid | KEGG | 2.4 | 1.8 | 2.1 | 2.9 | 1.8 | 1.9 |
| 12 | 143 | 27.39 | [M-H]- | 567.207886 | Citrusin B | C27H36O13 | -0.76 | Phenylpronanoid | HMDB | 2.6 | 1.8 | 2.7 | 2.3 | 1.9 | 1.9 |
| 12 | 55 | 27.40 | [M+H]+ | 219.101242 | Eupatoriochromene | C13H14O3 | -1.52 | Coumarin | KEGG | 2.6 | 1.7 | 2.4 | 2.0 | 1.5 | 1.6 |
| 12 | 82 | 20.06 | [M-H]- | 609.145386 | Flavonoid-di-glycoside or (C12634), (C05625), (C18942)) | C27H30O16 | -1.21 | Flavonoid | KEGG | 1.3 | 1.1 | 1.6 | 1.7 | 1.7 | 1.5 |
| 12 | 73 | 38.75 | [M-H]- | 561.160889 | Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucoside | C27H30O13 | -0.78 | Flavonoid | KEGG | 3.9 | 3.4 | 4.6 | 3.7 | 4.0 | 3.7 |
| 12 | 70 | 35.31 | [M-H]- | 723.213989 | Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside | C33H40O18 | -0.32 | Flavonoid | KEGG | 2.2 | 1.9 | 2.5 | 2.0 | 1.9 | 1.8 |
| 12 | 71 | 38.58 | [M-H]- | 723.213928 | Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside tri glycoside | C33H40O18 | -0.41 | Flavonoid | KEGG | 1.9 | 1.6 | 2.0 | 1.9 | 1.8 | 1.7 |
| 12 | 76 | 39.79 | [M-H]- | 531.150269 | Flavonol 3-O-D-xylosylglucoside | C26H28O12 | -1.04 | Flavonoid | KEGG | 11.7 | 7.3 | 14.0 | 18.4 | 12.7 | 8.5 |
| 12 | 78 | 17.76 | [M-H]- | 933.231506 | Kaempferol 3-O-caffeoyl-sophoroside 7-O-glucoside/ Quercetin 3-[p-coumaroyl-(->6)-glucosyl-(1->2)-glucosyl-(1->2)-glucoside] | C42H46O24 | 0.94 | Flavonoid | HMDB | 1.1 | -1.1 | 1.2 | 2.4 | 2.1 | 1.9 |
| 12 | 56 | 33.73 | [M+H]+ | 325.091370 | Mahaleboside | C15H16O8 | -1.30 | Coumarin | HMDB | 3.2 | 2.8 | 3.7 | 5.0 | 5.3 | 4.2 |
| 12 | 75 | 34.36 | [M-H]- | 517.134094 | Medicarpin 3-O-glucoside-6'-malonate | C25H26O12 | -1.99 | Flavonoid | KEGG | 26.1 | 18.8 | 30.3 | 28.1 | 24.0 | 15.0 |
| 12 | 121 | 38.74 | [M+H]+ | 339.106934 | p-Coumaroyl quinic acid | C16H18O8 | -1.60 | Phenylpronanoid | KEGG | 3.5 | 3.0 | 4.2 | 3.8 | 3.7 | 3.0 |
| 12 | 141 | 33.28 | [M-H]- | 487.125000 | Phrymarolin I | C24H24O11 | 0.77 | Lignan | KEGG | 13.1 | 3.8 | 8.4 | 67.3 | 48.7 | 20.0 |
| 12 | 77 | 33.75 | [M-H]- | 547.145325 | Puerarin xyloside | C26H28O13 | -0.73 | Flavonoid | KEGG | 3.5 | 2.9 | 4.2 | 4.4 | 4.5 | 3.7 |
| 12 | 150 | 22.62 | [M-H]- | 135.045029 | Simple phenolic or (C05613), (C07086), (C07215) or (C01454) | C8H8O2 | -0.70 | Phenolica aicd | KEGG | 3.4 | 2.2 | 2.3 | 2.3 | 1.7 | 1.7 |
| 13 | 119 | 37.81 | [M-H]- | 591.171875 | 1,2-Bis-O-sinapoyl-beta-D-glucoside | C28H32O14 | -0.08 | Phenylpronanoid | KEGG | 1.4 | 1.3 | 1.8 | -1.4 | -1.1 | -1.0 |
| 13 | 154 | 31.87 | [M-H]- | 469.080780 | Resveratrol-sulfoglucoside | C20H22O11S | -0.48 | Stilbenoid | Metlin, HMDB | 1.2 | 3.7 | 4.9 | 1.8 | 1.7 | 2.5 |

**Table S8.** *Paraburkholderia* species-mediated changes in shoot semi-polar secondary metabolites (LC-MS) in two Broccoli cultivars (Coronado and Malibu) at 11 dpi. The tabulated data corresponds to the hierarchical cluster analysis (HCA) showen in **Fig 4b2** of the manuscript. The fold changecorresponding to each metabolites was calculated by dividing the abundance of each metabolites in Paraburkholderia treated plants to the non-treated control. *Pbg*: *Paraburkholderia graminis*, *Pbh*: *P. hospita*, and *Pbt*: *P. terricola*.

| Cluster number | Mapping number | RT(m) | m/z | Mass (D) | Compound | Formula | Δ ppm to DB | Classification | data base | Fold change | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Coronado | | | Malibu | | |
| | | | | | | | | | | *Pbg* | *Pbh* | *Pbt* | *Pbg* | *Pbh* | *Pbt* |
| 1 | 64 | 15.09 | [M+H]+ | 773.210571 | Kaempferol 3-sophorotrioside | C33H40O21 | -3.76 | Flavonoid | KEGG | 3.5 | 4.4 | 6.3 | 22.8 | 22.6 | 16.0 |
| 1 | 118 | 23.80 | [M+H]+ | 438.238007 | N1,N10-Dicoumaroylspermidine | C25H31N3O4 | -1.66 | Phenylpronanoid | HMDB | -1.1 | -1.7 | 1.0 | 10.7 | 2.3 | 5.3 |
| 1 | 156 | 34.23 | [M-H]- | 363.075562 | Sulfometuron methyl | C15H16N4O5S | -3.48 | sulfonylurea | KEGG | -1.6 | 1.0 | -1.5 | 1.2 | 1.4 | 6.9 |
| 2 | 153 | 21.46 | [M-H]- | 551.176697 | (Z)-Resveratrol 3,4'-diglucoside | C26H32O13 | -0.58 | Stilbenoid | HMDB | 1.6 | 1.7 | 1.6 | 1.7 | 1.8 | 2.2 |
| 2 | 125 | 43.09 | [M+H]+ | 239.090393 | 3,4,5-Trimethoxycinnamic acid | C12H14O5 | -4.20 | Phenylpronanoid | HMDB | 3.1 | 2.8 | 3.3 | 7.9 | 5.7 | 7.6 |
| 2 | 120 | 21.34 | [M-H]- | 335.077667 | 5-O-Caffeoylshikimic acid | C16H16O8 | 1.32 | Phenylpronanoid | Metlin, KEGG | 7.4 | 7.5 | 7.8 | 8.5 | 7.7 | 7.4 |
| 2 | 38 | 12.10 | [M+H]+ | 138.054703 | Anthranilic acid | C7H7NO2 | -1.98 | aromatic acid | KEGG | 1.9 | 1.8 | 2.1 | 3.4 | 2.9 | 4.8 |
| 2 | 50 | 2.95 | [M-H]- | 191.019562 | citric acid | C6H8O7 | -0.85 | Carboxylic acid | KEGG | 2.2 | 2.9 | 3.3 | 4.8 | 5.4 | 6.1 |
| 2 | | 2.40 | [M-H]- | 133.014084 | Malic acid | C4H6O5 | -1.05 | Hydroxy acid | KEGG | 1.6 | 1.4 | 2.0 | 2.3 | 2.3 | 2.4 |
| 2 | 154 | 31.87 | [M-H]- | 469.080780 | Resveratrol-sulfoglucoside | C20H22O11S | -0.48 | Stilbenoid | Metlin, HMDB | 1.8 | 1.1 | 1.6 | 1.6 | 1.7 | 2.9 |
| 3 | 134 | 14.64 | [M+H]+ | 211.059479 | 5-Hydroxyferulic acid methyl ester II | C10H10O5 | -2.83 | Phenylpronanoid | KEGG | 4.2 | 4.4 | 3.5 | 6.8 | 5.2 | 4.7 |
| 3 | 59 | 23.68 | [M+H]+ | 323.122986 | Binapacryl | C15H18N2O6 | -2.44 | Dinoseb | KEGG | 7.0 | 6.9 | 7.8 | 10.3 | 7.9 | 8.7 |
| 3 | 48 | 23.68 | [M-H]- | 350.088165 | Indole-3-acetic-acid-O-glucuronide | C16H17NO8 | 0.07 | o-glucuronides | HMDB | 5.8 | 5.9 | 6.2 | 10.8 | 6.7 | 10.1 |
| 4 | 63 | 23.89 | [M+H]+ | 225.148117 | (6S,9R)-Vomifoliol | C13H20O3 | -1.59 | Fattyacyl glycoside | KEGG | 2.4 | 2.6 | 2.2 | 2.9 | 2.6 | 2.8 |
| 4 | 60 | 3.85 | [M-H]- | 147.066345 | (S)-Mevalonic acid | C6H12O4 | 0.14 | Hydroxy fatty acid | KEGG | -1.1 | 1.2 | 1.2 | 1.6 | 2.0 | 1.6 |
| 4 | 137 | 13.27 | [M-H]- | 341.087585 | 1-Caffeoyl-beta-D-glucose or (C10431) | C15H18O9 | -0.70 | Phenylpronanoid | KEGG | 3.0 | 4.2 | 2.8 | 5.9 | 4.8 | 4.1 |
| 4 | 132 | 18.43 | [M-H]- | 355.103210 | 1-Feruloyl-D-glucose | C16H20O9 | -0.60 | Phenylpronanoid | KEGG | 2.1 | 2.4 | 2.1 | 3.6 | 3.1 | 3.0 |
| 4 | 124 | 39.91 | [M-H]- | 929.272949 | 2-Feruloyl-1,2'-disinapoylgentiobiose | C44H50O22 | 0.92 | Phenylpronanoid | HMDB | 1.4 | 1.5 | 1.8 | 2.9 | 2.3 | 2.7 |
| 4 | 37 | 26.28 | [M-H]- | 415.124878 | 2-Hydroxybenzaldehyde O-[xylosyl-(1->6)-glucoside] | C18H24O11 | 0.70 | Phenolic glycoside | HMDB | 3.0 | 4.6 | 4.2 | 5.4 | 4.6 | 5.1 |
| 4 | 131 | 23.26 | [M-H]- | 195.029892 | 3,4-Dihydroxyphenylpyruvate | C9H8O5 | -0.16 | Phenylpronanoid | KEGG | 2.2 | 3.8 | 2.8 | 3.7 | 3.5 | 5.8 |
| 4 | 74 | 22.70 | [M+H]+ | 341.138000 | 5-Deoxykievitone or (C09832), (C18023), (C09753), (C15510) | C20H20O5 | -1.10 | Flavonoid | KEGG | 3.5 | 3.4 | 3.5 | 6.8 | 5.7 | 4.4 |
| 4 | 135 | 12.94 | [M+H]+ | 211.059418 | 5-Hydroxyferulic acid methyl ester I | C10H10O5 | -3.12 | Phenylpronanoid | KEGG | 2.7 | 2.9 | 2.7 | 5.7 | 4.3 | 4.3 |
| 4 | 54 | 18.44 | [M+H]+ | 177.054489 | 7-Methoxycoumarin or (C03081) | C10H8O3 | -0.49 | Coumarin | KEGG | 2.4 | 2.8 | 2.6 | 4.0 | 3.9 | 3.2 |
| 4 | 138 | 11.42 | [M-H]- | 341.087616 | Caffeic acid 3-glucoside | C15H18O9 | -0.61 | Phenylpronanoid | KEGG | 2.4 | 3.0 | 2.5 | 5.1 | 4.4 | 4.0 |
| 4 | | 9.92 | [M+H]+ | 355.101624 | Chlorogenic acid | C16H18O9 | -2.12 | Phenylpronanoid | KEGG | 2.8 | 3.0 | 3.0 | 4.5 | 3.4 | 3.8 |
| 4 | 122 | 9.97 | [M-H]- | 353.087585 | Chlorogenic acid | C16H18O9 | -0.68 | Phenylpronanoid | KEGG | 2.8 | 3.0 | 2.9 | 4.3 | 3.2 | 4.0 |

**Table S8.** (Continued)

| Cluster number | Mapping number | RT(m) | m/z | Mass (D) | Compound | Formula | Δ ppm to DB | Classification | data base | Fold change | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Coronado | | | Malibu | | |
| | | | | | | | | | | *Pbg* | *Pbh* | *Pbt* | *Pbg* | *Pbh* | *Pbt* |
| 4 | 123 | 15.81 | [M+H]+ | 355.101654 | Chlorogenic acid or Coumarin: (C01527) or (C08996) | C16H18O9 | -2.03 | Phenylpronanoid | KEGG | 2.7 | 3.4 | 2.7 | 3.5 | 2.8 | 3.6 |
| 4 | | 15.86 | [M-H]- | 353.087616 | Chlorogenic acid or Coumarin: (C01527) or (C08996) | C16H18O9 | -0.59 | Phenylpronanoid | KEGG | 2.8 | 3.5 | 2.9 | 3.6 | 2.8 | 3.9 |
| 4 | 143 | 27.39 | [M-H]- | 567.207886 | Citrusin B | C27H36O13 | -0.76 | Phenylpronanoid | HMDB | 4.3 | 4.5 | 5.3 | 4.6 | 3.5 | 4.0 |
| 4 | 136 | 12.93 | [M-H]- | 371.097992 | Dihydroferulic acid 4-O-glucuronide | C16H20O10 | -1.02 | Phenylpronanoid | HMDB | 3.0 | 3.3 | 3.1 | 6.6 | 5.0 | 5.4 |
| 4 | 55 | 27.40 | [M+H]+ | 219.101242 | Eupatoriochromene | C13H14O3 | -1.52 | Coumarin | KEGG | 4.3 | 3.7 | 4.8 | 5.0 | 3.9 | 3.9 |
| 4 | 133 | 18.43 | [M+H]+ | 195.064651 | Ferulic acid | C10H10O4 | -2.69 | Phenylpronanoid | KEGG | 2.2 | 2.4 | 2.1 | 3.6 | 3.1 | 2.8 |
| 4 | 82 | 20.06 | [M-H]- | 609.145386 | Flavonoid-di-glycoside (C12634) or (C05625), (C18942) | C27H30O16 | -1.21 | Flavonoid | KEGG | 1.7 | 1.8 | 2.2 | 4.4 | 4.2 | 3.9 |
| 4 | 72 | 33.14 | [M-H]- | 577.156250 | Flavonoid-di-glycoside (C16981) or (C12628), (C12627) | C27H30O14 | -0.13 | Flavonoid | KEGG | 4.9 | 5.5 | 7.0 | 19.1 | 17.7 | 14.9 |
| 4 | 73 | 38.75 | [M-H]- | 561.160889 | Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucoside | C27H30O13 | -0.78 | Flavonoid | KEGG | 6.7 | 8.3 | 10.3 | 16.0 | 18.2 | 12.8 |
| 4 | 70 | 35.31 | [M-H]- | 723.213989 | Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside | C33H40O18 | -0.32 | Flavonoid | KEGG | 2.6 | 2.8 | 3.0 | 4.5 | 4.1 | 4.2 |
| 4 | 71 | 38.58 | [M-H]- | 723.213928 | Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside tri glycoside | C33H40O18 | -0.41 | Flavonoid | KEGG | 2.5 | 2.8 | 2.4 | 3.2 | 2.8 | 2.5 |
| 4 | 76 | 39.79 | [M-H]- | 531.150269 | Flavonol 3-O-D-xylosylglucoside | C26H28O12 | -1.04 | Flavonoid | KEGG | 12.1 | 35.8 | 47.3 | 127.5 | 119.5 | 96.3 |
| 4 | 104 | 2.04 | [M-H]- | 225.061401 | Glucoheptonic acid | C7H14O8 | -0.99 | Glycoside | Metlin | 2.0 | 2.6 | 3.1 | 6.3 | 5.9 | 4.4 |
| 4 | 68 | 26.16 | [M-H]- | 725.194031 | Isoschaftoside 4'-glucoside or Schaftoside 4'-glucoside | C32H38O19 | 0.80 | Flavonoid | HMDB | 4.7 | 5.6 | 5.4 | 13.1 | 7.4 | 10.5 |
| 4 | 80 | 20.47 | [M-H]- | 947.247437 | Kaempferol 3-(2-feruloylsophoroside) 7-glucoside | C43H48O24 | 1.23 | Flavonoid | HMDB | 1.2 | 1.5 | 1.6 | 3.6 | 3.1 | 2.9 |
| 4 | 65 | 20.90 | [M+H]+ | 919.249146 | Kaempferol 3-O-[6-(4-coumaroyl)-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside] | C42H46O23 | -1.23 | Flavonoid | KEGG | 1.1 | 1.0 | 1.4 | 4.3 | 4.3 | 2.8 |
| 4 | | 20.91 | [M-H]- | 917.235291 | Kaempferol 3-O-[6-(4-coumaroyl)-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside] | C42H46O23 | -0.47 | Flavonoid | KEGG | 1.0 | 1.1 | 1.3 | 4.5 | 4.8 | 3.4 |
| 4 | 83 | 20.03 | [M+H]+ | 611.159851 | Kaempferol 3-O-beta-D-sophoroside or (C05625), (C17563) | C27H30O16 | -1.35 | Flavonoid | KEGG | 2.1 | 1.9 | 2.5 | 4.3 | 3.9 | 3.6 |
| 4 | 78 | 17.76 | [M-H]- | 933.231506 | Kaempferol 3-O-caffeoyl-sophoroside 7-O-glucoside or (HMDB37938) | C42H46O24 | 0.94 | Flavonoid | HMDB | 1.1 | 1.4 | 1.5 | 5.2 | 4.9 | 4.5 |
| 4 | 67 | 17.00 | [M-H]- | 963.241943 | Kaempferol 3-O-hydroxyferuloylsophoroside 7-O-glucoside | C43H48O25 | 1.27 | Flavonoid | PubChem | 1.6 | 2.3 | 2.3 | 5.4 | 4.4 | 4.9 |
| 4 | 81 | 20.46 | [M+H]+ | 949.258301 | Kaempferol tri glycoside + feruloyl | C43H48O24 | -2.66 | Flavonoid | HMDB | 1.3 | 1.5 | 1.7 | 2.8 | 3.1 | 3.0 |
| 4 | 56 | 33.73 | [M+H]+ | 325.091370 | Mahaleboside | C15H16O8 | -1.30 | Coumarin | HMDB | 4.5 | 5.8 | 9.8 | 40.6 | 46.2 | 22.5 |
| 4 | 75 | 34.36 | [M-H]- | 517.134094 | Medicarpin 3-O-glucoside-6'-malonate | C25H26O12 | -1.99 | Flavonoid | KEGG | 7.5 | 38.7 | 59.3 | 476.8 | 401.1 | 264.0 |
| 4 | 121 | 38.74 | [M+H]+ | 339.106934 | p-Coumaroyl quinic acid | C16H18O8 | -1.60 | Phenylpronanoid | KEGG | 6.3 | 6.9 | 9.9 | 15.4 | 17.1 | 11.4 |
| 4 | 77 | 33.75 | [M-H]- | 547.145325 | Puerarin xyloside | C26H28O13 | -0.73 | Flavonoid | KEGG | 5.2 | 9.6 | 11.8 | 27.6 | 27.4 | 20.6 |
| 4 | 79 | 17.75 | [M+H]+ | 935.242615 | Quercetin 3-[p-coumaroyl-(->6)-glucosyl-(1->2)-glucosyl-(1->2)-glucoside], Kaempferol 3-O-caffeoyl-sophoroside 7-O-glucoside | C42H46O24 | -2.74 | Flavonoid | HMDB | 1.2 | 1.5 | 1.7 | 4.8 | 3.6 | 3.9 |
| 4 | 69 | 29.93 | [M-H]- | 739.210144 | Robinin | C33H40O19 | 1.38 | Flavonoid | KEGG | 2.9 | 3.0 | 3.2 | 4.5 | 3.4 | 4.5 |
| 4 | 57 | 12.94 | [M+H]+ | 193.048950 | Scopoletin or (C10290), (C18077), (C01938) | C10H8O4 | -3.24 | Coumarin | KEGG | 3.1 | 3.3 | 3.3 | 7.4 | 5.5 | 5.4 |

**Table S8.** (Continued)

| Cluster number | Mapping number | RT(m) | m/z | Mass (D) | Compound | Formula | Δ ppm to DB | Classification | data base | Fold change | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Coronado | | | Malibu | | |
| | | | | | | | | | | *Pbg* | *Pbh* | *Pbt* | *Pbg* | *Pbh* | *Pbt* |
| 4 | 150 | 22.62 | [M-H]- | 135.045029 | Benzylformate or (C07086), (C07215) or (C01454) | C8H8O2 | -0.70 | Phenolica aicds | KEGG | 2.6 | 4.5 | 2.9 | 3.1 | 3.2 | 4.6 |
| 5 | 119 | 37.81 | [M-H]- | 591.171875 | 1,2-Bis-O-sinapoyl-beta-D-glucoside | C28H32O14 | -0.08 | Phenylpronanoid | KEGG | 2.3 | 1.8 | 2.5 | 2.7 | 2.9 | 2.9 |
| 5 | 175 | 33.63 | [M+H]+ | 335.159943 | 3'-N'-Acetylfusarochromanone | C17H22N2O5 | -0.61 | Benzopyran | HMDB | 17.2 | 4.5 | 11.5 | 7.5 | 6.4 | 2.8 |
| 5 | | 33.64 | [M-H]- | 333.145691 | 3'-N'-Acetylfusarochromanone | C17H22N2O5 | 0.29 | Benzopyran | HMDB | 16.8 | 4.7 | 10.7 | 5.9 | 4.0 | 2.9 |
| 5 | 99 | 19.52 | [M-H]- | 401.109009 | 4-Hydroxy-5-(3',5'-dihydroxyphenyl)-valeric acid-O-glucuronide | C17H22O11 | 0.18 | Glucuronide | HMDB | 2.2 | 2.4 | 1.7 | 2.4 | 2.2 | 1.9 |
| 5 | 147 | 4.77 | [M-H]- | 375.093384 | Furaneol 4-(6-malonylglucoside) | C15H20O11 | 0.26 | o-glycosyl comp. | HMDB | 1.5 | 1.3 | 1.5 | 2.6 | 2.2 | 1.9 |
| 5 | 93 | 23.98 | [M-H]- | 422.058563 | Gluconasturtiin | C15H21NO9S2 | 0.09 | other GLS | KEGG | -1.2 | -1.1 | -1.3 | 4.7 | 6.4 | 2.5 |
| 5 | 163 | 20.31 | [M+H]+ | 377.144623 | Loganic acid | C16H24O10 | 1.19 | Iridoid | KEGG | 16.6 | 10.7 | 11.5 | 21.0 | 20.1 | 12.0 |
| 7 | 21 | 23.98 | [M+H]+ | 252.085983 | 4-Hydroxy-3-methoxy-cinnamoylglycine | C12H13NO5 | -2.75 | Amino acid | KEGG | -1.8 | -2.2 | -2.0 | -1.9 | -1.9 | -1.1 |
| 7 | 148 | 3.12 | [M+H]+ | 286.138916 | Glycylprolylhydroxyproline | C12H19N3O5 | -2.90 | Oligopeptides | HMDB | -1.2 | -1.9 | -1.4 | -1.6 | -2.3 | -1.0 |
| 7 | 20 | 23.98 | [M-H]- | 206.082062 | N-Acetyl-L-phenylalanine | C11H13NO3 | -0.79 | Phenylalanine | KEGG, Metlin | -1.9 | -2.1 | -1.9 | -2.0 | -2.0 | -1.2 |
| 7 | 158 | 19.33 | [M+H]+ | 178.035156 | Sulforaphane | C6H11NOS2 | -1.83 | Sulfoxides | HMDB | -17.3 | -2.9 | -2.8 | -12.0 | -8.4 | -1.7 |
| 8 | 165 | 43.28 | [M-H]- | 629.261658 | Brassica napus non-fluorescent chlorophyll catabolite 3 | C34H38N4O8 | -0.05 | Tetrapyrrole | HMDB | -11.2 | -10.9 | -11.0 | -29.9 | -37.4 | -6.8 |
| 9 | 36 | 17.69 | [M-H]- | 329.087708 | 1-O-Vanilloyl-beta-D-glucose | C14H18O9 | -0.35 | Benzoic acid | KEGG | -6.0 | -4.8 | -3.7 | -5.9 | -4.7 | -2.2 |
| 9 | 61 | 16.87 | [M-H]- | 380.156128 | cis-Zeatin-O-glucoside | C16H23N5O6 | -3.67 | Fatty acyl glycoside | KEGG | -3.5 | -3.3 | -3.1 | -7.2 | -6.1 | -2.5 |
| 9 | 35 | 12.05 | [M-H]- | 222.077301 | Dioxacarb | C11H13NO4 | 0.35 | Benzenes | KEGG | -2.6 | -2.6 | -2.7 | -3.9 | -3.1 | -1.7 |
| 9 | 85 | 23.64 | [M-H]- | 609.144470 | Flavonoid di glycoside (C12634) or (C05625), (C17563), (C19796)) | C27H30O16 | -2.72 | Flavonoid | KEGG | -6.1 | -6.4 | -6.9 | -18.6 | -17.6 | -4.4 |
| 9 | 84 | 23.63 | [M+H]+ | 611.158447 | Flavonoid di glycoside (C12634) or (C05625), (C17563), (C19796) | C27H30O16 | -3.65 | Flavonoid | KEGG | -5.4 | -5.8 | -6.4 | -13.8 | -15.1 | -3.0 |
| 9 | 42 | 7.21 | [M-H]- | 138.055878 | Gabaculine | C7H9NO2 | -1.06 | Benzoic acid | KEGG | -3.5 | -2.6 | -2.6 | -4.7 | -5.4 | -1.2 |
| 9 | 23 | 27.97 | [M+H]+ | 247.107407 | N-Acetyl-D-tryptophan | C13H14N2O3 | -1.09 | Amino acid | KEGG | -3.0 | -3.1 | -3.7 | -3.8 | -3.9 | -1.6 |
| 9 | 44 | 33.29 | [M+H]+ | 225.111435 | Olivetolic acid | C12H16O4 | -3.29 | Benzoic derivative | KEGG | -2.2 | -2.2 | -2.1 | -4.0 | -5.4 | -1.9 |
| 9 | 164 | 12.04 | [M+H]+ | 224.091324 | Salsolinol 1-carboxylate | C11H13NO4 | -1.83 | Quinoline | HMDB | -2.5 | -2.5 | -2.6 | -3.4 | -3.1 | -1.6 |
| 9 | 160 | 19.07 | [M-H]- | 431.191284 | S-Furanopetasitin | C24H32O5S | 3.51 | Terpenoid | HMDB | -2.2 | -2.1 | -2.6 | -2.2 | -3.2 | 1.1 |
| 9 | 161 | 20.25 | [M+H]+ | 387.200470 | Sonchuionoside C | C19H30O8 | -2.26 | Terpenoid | HMDB | -1.7 | -1.8 | -1.9 | -1.9 | -2.1 | -1.1 |
| 9 | 24 | 4.14 | [M+H]+ | 384.113953 | Succinyladenosine | C14H17N5O8 | -2.70 | Purine nucleoside | HMDB | -3.1 | -2.2 | -2.2 | -3.1 | -2.5 | -1.4 |
| 9 | 155 | 27.17 | [M-H]- | 349.059875 | Sulfometuron | C14H14N4O5S | -3.86 | Sulfonylurea | KEGG | -1.7 | -1.8 | -1.5 | -1.9 | -2.2 | -1.1 |
| 9 | 26 | 7.80 | [M-H]- | 203.082779 | tryptophan | C11H12N2O2 | 0.76 | Amino acid | KEGG | -1.6 | -2.9 | -2.0 | -3.9 | -4.9 | -2.3 |
| 9 | 149 | 28.71 | [M-H]- | 593.208496 | Zizybeoside II | C25H38O16 | -0.38 | Oligosaccharides | KEGG | -2.6 | -3.2 | -2.8 | -5.1 | -5.3 | -2.2 |
| 10 | | 3.00 | [M+H]+ | 114.054932 | 1-Pyrroline-2-carboxylic acid | C5H7NO2 | -0.39 | Pyrrolines | HMDB | -1091.0 | -1044.4 | -1099.5 | -1494.1 | -1572.9 | -1495.0 |
| 10 | 172 | 3.87 | [M-H]- | 276.988342 | 2-C-Methyl-D-erythritol 2,4-cyclodiphosphate | C5H12O9P2 | -0.30 | | KEGG | -4.3 | -2.7 | -3.8 | -8.0 | -4.6 | -1.7 |
| 10 | 111 | 20.19 | [M-H]- | 536.176819 | 4-Demethylsimmondsin 2'-(E)-ferulate | C25H31NO12 | -0.99 | Phenylpronanoid | HMDB | -2.3 | -2.0 | -1.9 | -3.1 | -3.0 | -1.4 |
| 10 | 29 | 1.63 | [M+H]+ | 175.118820 | Arginine | C6H14N4O2 | -0.89 | Amino acid | KEGG | -3.0 | -2.5 | -2.4 | -6.7 | -5.2 | -3.1 |

**Table S8**. (Continued)

| Cluster number | Mapping number | RT(m) | m/z | Mass (D) | Compound | Formula | Δ ppm to DB | Classification | data base | Fold change | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Coronado | | | Malibu | | |
| | | | | | | | | | | *Pbg* | *Pbh* | *Pbt* | *Pbg* | *Pbh* | *Pbt* |
| 10 | | 1.80 | [M-H]- | 173.104156 | Arginine | C6H14N4O2 | -1.55 | Amino acid | KEGG | -2.9 | -2.5 | -2.2 | -8.1 | -5.4 | -4.0 |
| 10 | 30 | 1.97 | [M-H]- | 131.045929 | Asparagine | C4H8N2O3 | -2.25 | Amino acid | KEGG | -2.5 | -2.9 | -2.7 | -4.3 | -4.0 | -4.9 |
| 10 | 112 | 33.28 | [M-H]- | 385.150146 | Methylsyringin | C18H26O9 | -0.68 | Phenylpronanoid | HMDB | -2.1 | -2.1 | -1.9 | -3.2 | -4.2 | -1.6 |
| 10 | 145 | 2.98 | [M-H]- | 204.087799 | N-Acetyl-D-fucosamine | C8H15NO5 | 0.37 | Monosaccharide | KEGG | -4.6 | -6.2 | -6.6 | -8.4 | -8.0 | -5.3 |
| 10 | 28 | 2.95 | [M-H]- | 188.056519 | N-Acetylglutamic acid | C7H11NO5 | 0.39 | Glutamic acid | HMDB | -4.0 | -5.3 | -4.8 | -9.2 | -7.2 | -7.4 |
| 10 | 34 | 7.09 | [M-H]- | 161.968781 | Raphanusamic acid | C4H5NO2S2 | -0.88 | Alpha amino acid | Metlin, HMDB | -1.6 | -2.0 | -1.7 | -3.4 | -2.7 | -2.0 |
| 10 | 52 | 6.20 | [M-H]- | 305.087860 | Starch acetate | C12H18O9 | 0.18 | Carboxylic acid | HMDB | -3.0 | -2.9 | -2.4 | -2.2 | -2.0 | 1.0 |
| 10 | 33 | 10.80 | [M-H]- | 247.111847 | Valyl-Methionine/ Methionyl-Valine | C10H20N2O3S | -1.38 | Dipeptide | HMDB | -3.1 | -2.5 | -2.3 | -8.7 | -3.8 | -2.8 |
| 11 | 170 | 21.27 | [M-H]- | 278.107025 | 2-acetamido-3-hexylsulfonylpropanoic acid | C11H21NO5S | 1.08 | | PubChem | -1.5 | -1.5 | -1.6 | -2.2 | -1.9 | -1.3 |
| 11 | 88 | 20.01 | [M-H]- | 388.074219 | 2-Methylbutyl glucosinolate | C12H23NO9S2 | 0.24 | Aliphatic GLS | Metlin, KEGG | -2.2 | -1.7 | -2.0 | -2.4 | -2.2 | -1.2 |
| 11 | 157 | 17.46 | [M+H]+ | 164.019562 | 3-Methylsulfinylpropyl isothiocyanate | C5H9NOS2 | -1.64 | Sulfoxide | HMDB | -5.3 | -2.2 | -2.8 | -3.8 | -4.7 | -1.4 |
| 11 | 176 | 29.31 | [M+H]+ | 162.094620 | 6-Methylthiohexanaldoxime | C7H15NOS | -0.35 | | KEGG | -1.7 | -1.7 | -2.9 | -1.7 | -2.0 | 1.7 |
| 11 | 92 | 10.13 | [M-H]- | 406.030487 | glucoiberverin | C11H21NO9S3 | -0.09 | Aliphatic GLS | KEGG | -1.4 | -1.6 | -1.0 | 2.1 | 3.2 | 2.1 |
| 11 | 13 | 4.25 | [M+H]+ | 235.110428 | Isoleucyl-Cysteine, Leucyl-Cysteine | C9H18N2O3S | -2.81 | Dipeptide | HMDB | -2.6 | -2.0 | -1.8 | -1.3 | -1.7 | 1.1 |
| 11 | 53 | 3.31 | [M+H]+ | 246.169876 | Isovalerylcarnitine | C12H23NO4 | -0.41 | Carnitine | KEGG | -1.5 | -1.8 | -1.5 | -2.0 | -1.7 | -1.0 |
| 11 | 17 | 2.96 | [M+H]+ | 263.141815 | Methionyl-Isoleucine | C11H22N2O3S | -2.18 | Dipeptide | HMDB | -3.3 | -1.9 | -2.7 | -1.4 | -1.9 | 1.2 |
| 14 | 41 | 24.57 | [M-H]- | 165.019363 | Formylsalicylic acid or (C14100) | C8H6O4 | 0.24 | Benzoic acid | KEGG | 4.4 | 2.2 | 2.4 | 1.3 | 1.2 | -1.3 |
| 14 | 7 | 2.00 | [M-H]- | 146.045837 | Glutaminic acid | C5H9NO4 | -0.60 | Amino acid | KEGG | 1.6 | 1.8 | 1.9 | 2.3 | 2.4 | 1.8 |

**Table S9.** Polar primary and semi-polar secondary metabolites classes and their metabolites ID used in **Fig 6** of the manuscript. GC: Gas chromatography- mass spectrometry, LC: Liquid chromatography- mass spectrometry.

| Mapping # | Compound | Tool |
|---|---|---|
| | Amino acid | |
| 1 | Serine | GC |
| 2 | Threonine | GC |
| 3 | Valine | LC |
| 4 | L-Aspartic acid | GC |
| 5 | ASPARTIC ACID-TRITMS | GC |
| 6 | Glutamic acid | GC |
| 7 | Glutaminic acid | LC |
| 8 | Putrescine | GC |
| 9 | Tryptophan or (C07839), (C14916) | LC |
| 10 | Isoleucine | GC |
| 11 | Proline | GC |
| 12 | Glutamine | GC |
| 13 | Isoleucyl-Cysteine | LC |
| 14 | 5-Hydroxyindoleacetylglycine | LC |
| 15 | L-Asparagine | GC |
| 16 | GABA | GC |
| 17 | Methionyl-Isoleucine | LC |
| 18 | L-5-Oxoproline | GC |
| 19 | L-Serine | GC |
| 20 | N-Acetyl-L-phenylalanine | LC |
| 21 | 4-Hydroxy-3-methoxy-cinnamoylglycine | LC |
| 22 | Glycine | GC |
| 23 | N-Acetyl-D-tryptophan | LC |
| 24 | Succinyladenosine | LC |
| 25 | L-Phenylalanine | GC |
| 26 | tryptophan | LC |
| 27 | Ornithine | GC |
| 28 | N-Acetylglutamic acid | LC |
| 29 | Arginine | LC |
| 30 | Asparagine | LC |
| 31 | Lysine | GC |
| 32 | Methionine | GC |
| 33 | Valyl-Methionine | LC |
| 34 | Raphanusamic acid | LC |
| | Benzene, Benzoic acid | |
| 35 | Dioxacarb | LC |
| 36 | 1-O-Vanilloyl-beta-D-glucose | LC |
| 37 | 2-Hydroxybenzaldehyde O-[xylosyl-(1->6)-glucoside] | LC |
| 38 | Anthranilic acid | LC |
| 39 | Apiosylglucosyl 4-hydroxybenzoate | LC |
| 40 | dihydroxybenzoic acid glucoside | LC |
| 41 | Formylsalicylic acid | LC |
| 42 | Gabaculine | LC |
| 43 | 4-Hydroxybenzoate-O-glucoside | LC |
| 44 | Olivetolic acid | LC |
| | Butenolide | |
| 45 | Ascorbic acid | GC |
| 46 | L-ascorbic acid ethyl ester | LC |
| | Carboxylic acid | |
| 47 | Malic acid | GC |
| 48 | Indole-3-acetic-acid-O-glucuronide | LC |
| 49 | Indole-3-acetaldehyde or (C10663), (C06345) | LC |
| 50 | Citric acid | LC |
| 51 | Garcinia acid | LC |
| 52 | Starch acetate | LC |
| | Carnitines | |
| 53 | Isovalerylcarnitine | LC |
| | Coumarin | |
| 54 | 7-Methoxycoumarin or (C03081) | LC |
| 55 | Eupatoriochromene | LC |
| 56 | Mahaleboside | LC |
| 57 | Scopoletin or (C10290), (C18077), (C01938) | LC |
| | Ester | |
| 58 | Phosphoenolpyruvate | GC |
| 59 | Binapacryl | LC |
| | Fatty acid, Fatty acyl glycoside | |
| 60 | (S)-Mevalonic acid | LC |
| 61 | cis-Zeatin-O-glucoside | LC |
| 62 | Ethyl 7-epi-12-hydroxyjasmonate glucoside | LC |
| 63 | (6S,9R)-Vomifoliol | LC |
| | Flavonoid | |
| 64 | Kaempferol 3-sophorotrioside | LC |
| 65 | Kaempferol 3-O-[6-(4-coumaroyl)-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside] | LC |
| 66 | Kaempferol 3-(2'''-sinapoylsophoroside) 7-glucoside | LC |
| 67 | Kaempferol 3-O-hydroxyferuloylsophoroside 7-O-glucoside | LC |
| 68 | Isoschaftoside 4'-glucoside/ Schaftoside 4'-glucoside | LC |
| 69 | Robinin | LC |
| 70 | Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside | LC |
| 71 | Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucosyl-(1->2)-beta-D-glucoside tri glycoside | LC |
| 72 | Flavonoid-di-glycoside_ Kaempferitrin (C16981)/ Vitexin 2''-rhamnoside (C12628)/ Apigenin 7-O-neohesperidoside (C12627) | LC |
| 73 | Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucoside | LC |
| 74 | 5-Deoxykievitone/ 6-Prenylnaringenin (C09832)/ Flavaprenin (C18023)/ Glepidotin B (C09753)/ 4-Glyceollidin (C15510) | LC |
| 75 | Medicarpin 3-O-glucoside-6'-malonate | LC |
| 76 | Flavonol 3-O-D-xylosylglucoside | LC |
| 77 | Puerarin xyloside | LC |
| 78 | Kaempferol 3-O-caffeoyl-sophoroside 7-O-glucoside/ Quercetin 3-[p-coumaroyl-(->6)-glucosyl-(1->2)-glucosyl-(1->2)-glucoside] | LC |

**Table S9**. (Continued)

| Mapping # | Compound | Tool |
|---|---|---|
| 79 | Quercetin 3-[p-coumaroyl-(->6)-glucosyl-(1->2)-glucosyl-(1->2)-glucoside], Kaempferol 3-O-caffeoyl-sophoroside 7-O-glucoside | LC |
| 80 | Kaempferol 3-(2-feruloylsophoroside) 7-glucoside | LC |
| 81 | Kaempferol tri glycoside + feruloyl | LC |
| 82 | Flavonoid-di-glycoside:(C12634) or (C05625), (C18942)) | LC |
| 83 | Kaempferol 3-O-beta-D-sophoroside or (C05625), (C17563) | LC |
| 84 | Flavonoid di glycoside: (C12634) or (C05625), (C17563), (C19796) | LC |
| 85 | Flavonoid di glycoside: (C12634) or (C05625), (C17563), (C19796)) | LC |
| | Glucosinolate | |
| 86 | Glucoraphanin | LC |
| 87 | hexyl glucosinolate (3-Methylpentyl glucosinolate/ 4-Methylpentyl glucosinolate) | LC |
| 88 | 2-Methylbutyl glucosinolate | LC |
| 89 | 4-Methylthiobutyl-desulfoglucosinolate | LC |
| 90 | Glucoalyssin | LC |
| 91 | Glucoiberin | LC |
| 92 | Glucoiberverin | LC |
| 93 | Gluconasturtiin | LC |
| 94 | Glucotropaeolin | LC |
| 95 | 4-Hydroxyglucobrassicin | LC |
| 96 | 4-methoxyglucobrassicin | LC |
| 97 | Desulfoglucobrassicin | LC |
| 98 | Glucobrassicin | LC |
| | Glucuronide | |
| 99 | 4-Hydroxy-5-(3',5'-dihydroxyphenyl)-valeric acid-O-glucuronide | LC |
| | Glycoside | |
| 100 | D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 1) | GC |
| 101 | D-(-)-Fructopyranose | GC |
| 102 | Xylose | GC |
| 103 | Glucose | GC |
| 104 | Glucoheptonic acid | LC |
| 105 | Fructose | GC |
| 106 | Sorbose | GC |
| 107 | D-Galactopyranoside, methyl 2,3,4-tris-O-(trimethylsilyl)-, acetate | GC |
| 108 | d-Galactose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1E)- | GC |
| 109 | Myo-Inositol | GC |
| 110 | Sucrose | GC |
| | Hydroxycinnamate | |
| 111 | 4-Demethylsimmondsin 2'-(E)-ferulate | LC |
| 112 | Methylsyringin | LC |
| 113 | Sinapine | LC |
| 114 | 5-O-Feruloylquinic acid | LC |
| 115 | 6-Feruloylglucose 2,3,4-trihydroxy-3-methylbutylglycoside | LC |
| 116 | 3-O-Caffeoyl-4-O-methylquinic acid | LC |
| 117 | O-Feruloylquinate | LC |

| Mapping # | Compound | Tool |
|---|---|---|
| 118 | N1,N10-Dicoumaroylspermidine | LC |
| 119 | 1,2-Bis-O-sinapoyl-beta-D-glucoside | LC |
| 120 | 5-O-Caffeoylshikimic acid | LC |
| 121 | p-Coumaroyl quinic acid | LC |
| 122 | Chlorogenic acid | LC |
| 123 | Chlorogenic acid or Coumarin: (C01527) or (C08996) | LC |
| 124 | 2-Feruloyl-1,2'-disinapoylgentiobiose | LC |
| 125 | 3,4,5-Trimethoxycinnamic acid | LC |
| 126 | Urolithin A-3-O-glucuronide | LC |
| 127 | 1,2,2'-Trisinapoylgentiobioside | LC |
| 128 | 3',6-Disinapoylsucrose | LC |
| 129 | 1-O-Sinapoyl beta-D-glucoside or 4-O-beta-D-Glucosyl-sinapate | LC |
| 130 | Sinapic acid | LC |
| 131 | 3,4-Dihydroxyphenylpyruvate | LC |
| 132 | 1-Feruloyl-D-glucose | LC |
| 133 | Ferulic acid | LC |
| 134 | 5-Hydroxyferulic acid methyl ester II | LC |
| 135 | 5-Hydroxyferulic acid methyl ester I | LC |
| 136 | Dihydroferulic acid 4-O-glucuronide | LC |
| 137 | 1-Caffeoyl-beta-D-glucose or (C10431) | LC |
| 138 | Caffeic acid 3-glucoside | LC |
| 139 | p-Coumaroyl-D-glucose or (C04415), (C05158) | LC |
| 140 | Phenylpyruvic acid or (C12621), (C00811) | LC |
| | Lignan | |
| 141 | Phrymarolin I | LC |
| 142 | Syringin | LC |
| 143 | Citrusin B | LC |
| | Methoxyphenol | |
| 144 | 4-Heptyloxyphenol | LC |
| | Monosaccharide | |
| 145 | N-Acetyl-D-fucosamine | LC |
| | Oraganic acid | |
| 146 | Mannonic acid, lactone | GC |
| | O-glycosyl compound | |
| 147 | Furaneol 4-(6-malonylglucoside) | LC |
| | Oligopeptide | |
| 148 | Glycylprolylhydroxyproline | LC |
| | Oligosaccharide | |
| 149 | Zizybeoside II | LC |
| | Phenolic acid | |
| 150 | Simple phenolic: (C05613) or (C07086), (C07215), (C01454) | LC |
| | Quinoline | |
| 151 | Quinoline | LC |
| | Saccharide | |
| 152 | (3S,7E,9S)-9-Hydroxy-4,7-megastigmadien-3-one 9-glucoside | LC |
| | Stilbenoid | |
| 153 | (Z)-Resveratrol 3,4'-diglucoside | LC |
| 154 | Resveratrol-sulfoglucoside | LC |
| | Sulfonylurea | |
| 155 | Sulfometuron | LC |
| 156 | Sulfometuron methyl | LC |

**Table S9**. (Continued)

| Mapping # | Compound | Tool |
|---|---|---|
| | Sulfoxide | |
| 157 | 3-Methylsulfinylpropyl isothiocyanate | LC |
| 158 | Sulforaphane | LC |
| | Terpenoid | |
| 159 | (4R,5S,7R,11S)-11,12-Dihydroxy-1(10)-spirovetiven-2-one 11-glucoside | LC |
| 160 | S-Furanopetasitin | LC |
| 161 | Sonchuionoside C | LC |
| 162 | 10-Deoxygeniposidic acid | LC |
| 163 | Loganic acid | LC |
| | Others | |
| 164 | Salsolinol 1-carboxylate | LC |
| 165 | Brassica napus non-fluorescent chlorophyll catabolite 3 | LC |
| 166 | 1-Methyl-4-(1-methyl-2-propenyl)-benzene/ alpha-Ionene/ 1,2,3,4,Tetrahydro-1,5,7-trimethylnapthalene/ 5,7alpha-Dihydro-1,4,4,7a-tetramethyl-4H-indene | LC |
| 167 | 2-(3-Phenylpropyl)tetrahydrofuran | LC |
| 168 | 2,5-Dihydro-2,4,5-trimethyloxazole/ 2-Acetylpyrrolidine | LC |
| 169 | 2,6-Dimethoxy-4-propylphenol | LC |
| 170 | 2-acetamido-3-hexylsulfonylpropanoic acid | LC |
| 171 | 2-Amino-2-deoxyisochorismate | LC |
| 172 | 2-C-Methyl-D-erythritol 2,4-cyclodiphosphate | LC |
| 173 | O-Demethylpuromycin | LC |
| 174 | pantothenic acid (vitamin B5) | LC |
| 175 | 3'-N'-Acetylfusarochromanone | LC |
| 176 | 6-Methylthiohexanaldoxime | LC |