SUPPLEMENTARY MATERIAL

NMR-based metabolic study of leaves of three species of Actinidia with different degrees of susceptibility to Pseudomonas syringae pv actinidiae

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ABSTRACT

Bacterial canker of Actinidia, caused by the bacterium Pseudomonas syringae pv. actinidiae (Psa), is the most serious disease of these plants worldwide. Leaves of three species of Actinidia, namely A. chinensis var. chinensis, A. chinensis var. deliciosa and A. arguta, having different degrees of tolerance to Psa, were analyzed by Nuclear Magnetic Resonance spectroscopy. Aqueous extracts of leaves were studied and several metabolites, classified as organic acids, amino acids, carbohydrates, phenols and other metabolites, were identified by 1D and 2D NMR experiments and quantified. The metabolic profiles of these species were compared through univariate statistical analysis ANOVA and multivariate PCA. Levels of metabolites with known antibacterial activity, such as caffeic and chlorogenic acids, were observed to be higher in the A. arguta samples. Moreover, these metabolites have different Pearson correlation patterns among the three Actinidia species, suggesting a difference at the phenylpropanoid biosynthetic pathway.
EXPERIMENTAL

Species features.

'Hayward' of the species *Actinidia chinensis* var. *deliciosa* (A. Chev.) A. Chev. selected in 1920 in Auckland, (New Zealand) produces fruit of good quality that store well, but it is medium susceptible to Psa, depending on prevailing climatic conditions and specific strains of the pathogen.

'Hort16A' of the species *Actindia chinensis* Planch. var. *chinensis* was a type of kiwifruit which upon its introduction to the market about 15 years ago attracted consumers due to its yellow flesh, distinct sweet flavour and early harvest period. However, the cultivar resulted to be very sensitive to Psa and therefore it was chosen for this trial.

'Arguta 87-14-15' of the species *Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq. is dioecious and it shows medium vigor. The cultivar was chosen for the current study because it represents a species which is less sentient to Psa (Datson et al., 2015; Vanneste, 2017), a fact which was confirmed also during observations carried out in the field collection of CREA-OFA in Rome.

Field

The experimental orchard was established in the 2012 in the farm of CREA Research Centre for Olive, Citrus, and Fruit Trees – Rome in Latium Region (Italy). The area is characterized by Mediterranean climate with high summer temperatures, mild winters, an annual rainfall of 700 mm concentrated in Autumn and Spring. The soil is classified as clay loam with normal mineral dotation of macro and micro elements.

One year old vines grafted on D1 seedling of *A. chinensis* var. *deliciosa*, selected and propagated in vitro by Vitroplant srl., were spaced 5.0 meters between the row and 3.0 meters on the row, trained as T-bar, with each plant having a straight trunk reaching at the top of the support at a height of 1.60 m from which two main opposite permanent side branches (cordon) depart. Fruiting canes are grown on this framework and can be easily renewed each year by pruning.

Water requirements were met by a localized irrigation system with two drippers for each plant distributing a volume of 5,000 m³ per hectare.

Fertilization provided the following units of macro-elements per hectare: 120 kg of nitrogen, 80 kg of phosphorus pentoxide and 120kg of potassium oxide.

Weed control was done by mechanical tillage in the alleyway and herbicide distribution along the rows.

To prevent Psa outbreak, a protective spray containing copper oxychloride at 0.3% (copper active ingredient) was applied regularly once after harvest, at leaf fall and before bud burst, respectively and if necessary, also before flowering.
During the plant growth, the orchard was routinely monitored to assess the eventual presence of Psa infected trees, but none were present at the time of the sample collection.

**Sampling.** Eight young leaves for each examined species were collected from different plants of the examined cultivars. The leaves were collected from the central parts of different one-year-old shoots at the end of May. After sampling, the materials were put in polyethylene bags to avoid water loss until the laboratory preparation of the sample (4 hours).

**Sample preparation.** Homogeneous portions of *Actinidia* leaves (about 0.6 g of fresh weight) were frozen in liquid nitrogen, finely powdered and extracted according to the modified Bligh-Dyer methodology (Capuani et al., 1992; Miccheli et al., 1991) with methanol/chloroform/water 2:2:1 final volumetric ratio. This extraction procedure was employed since it allows to separate low weight compounds on the basis of their polarity without degradation of high weight macromolecules such as polysaccharides and proteins. The samples were kept at 277 K for 1 hour and then centrifuged for 20 minutes at 10000 x g at the same temperature. The upper hydroalcoholic phase and the lower organic one were carefully separated, dried and stored at 193 K until NMR analysis.

**NMR experiments.** Each dry hydroalcoholic extract was dissolved in 0.6 mL of D$_2$O containing 3-(trimethylsilyl)-propionic-2,2,3,3-d$_4$ acid sodium salt 2 mM as chemical shift and concentration reference.

All solvents and standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

All spectra were recorded at 298 K on a Bruker AVANCE III spectrometer operating at the proton frequency of 400.13 MHz and equipped with a Bruker multinuclear z-gradient inverse probehead.

$^1$H spectra were acquired employing the presat pulse sequence for solvent suppression with 128 transients, a spectral width of 6000 Hz and 64K data points for an acquisition time of 5.5 s. The recycle delay was set to 9.5 s in order to achieve complete resonance relaxation between successive scanions.

Bidimensional TOCSY, HSQC and HMBC spectra were acquired on a selection of samples in order to univocally assign $^1$H and $^{13}$C resonances. TOCSY experiments were acquired with spectral width of 6000 Hz in both dimensions, a data matrix of 8K x 256 points, mixing time of 150 ms and relaxation delay of 2 s.

HSQC experiments were performed with a spectral width of 6000 and 25000 Hz for the proton and carbon respectively, a data matrix of 8K x 256 points, an average $^1$J$_{C-H}$ of 145 Hz and recycle delay of 2 s.

Several HMBC spectra were acquired with a spectral width of 6000 and 25000 Hz for the proton and carbon respectively, a data matrix of 8K x 256 points, long-range constants $^3$J$_{C-H}$ of 3, 6 and 10 Hz and recycle delay of 2 s.

The signals that could be clearly identified and had no overlap with neighbouring resonances were integrated for each sample and quantification was performed by comparison of the signal integral with the reference.
signal, and quantities were expressed in mg/g of fresh weight. The resulting data set was studied by
univariate and multivariate statistical analysis tools for the evaluation of statistical differences between
Actinidia leaves to identify the molecules responsible for the discrimination.

**Univariate Analysis**

Univariate One-Way ANOVA was performed with Sigmaplot 12.0 software (Systat Software Inc., San José,
California). Shapiro-Wilk test was performed on each variable to assess their normality prior one-way
ANOVA analysis. On the variables positive to ANOVA, a Holm-Sidak, an all pairwise multiple comparison
test, was applied to determine which categories were discriminated by these metabolites (p<0.05).

**Multivariate Analysis**

Multivariate PCA data analyses were performed on the data matrix with the Unscrambler ver. 9.8 software
(Camo Software AS, Oslo, Norway). Data were mean centered since the variables with the largest response
could dominate the PCA analysis, and they were also autoscaled to equalize the importance of the
variation of each variable.

**Pearson correlation Analysis**

Pearson correlation (Pearson product moment correlation) was carried out using Sigma stat 12.0 software to
observe the correlation between the quantified metabolites. The threshold value for correlation significance,
calculated for a population of 8 samples, was $r = \pm 0.707$. Therefore, in the discussion only correlations with $r$
values $< -0.707$ and $r > 0.707$ will be considered.
Figure S1: Superimposition of the $^1$H NMR spectra of the aqueous extracts of kiwifruit leaves of *Actinidia arguta* (upper, black), *A. chinensis* var. *deliciosa* (middle, red) and *A. chinensis* var. *chinensis* (bottom, green).
Figure S2: $^1$H NMR spectrum of the aqueous extracts of kiwifruit leaves of *Actinidia arguta* with the assignment of the major metabolites.
**Figure S3:** Bidimensional NMR $^{1}$H-$^{1}$H TOCSY spectrum of *Actinidia arguta* kiwifruit leaf hydroalcoholic extract at 298 K

**Figure S4:** Bidimensional NMR $^{1}$H-$^{13}$C HSQC spectrum of *Actinidia arguta* kiwifruit leaf hydroalcoholic extract at 298 K
Figure S5: Bidimensional NMR $^1$H-$^{13}$C HMBC spectrum of *Actinidia arguta* kiwifruit leaf hydroalcoholic extract at 298 K
| Compound                  | Assignment | $^1$H (ppm) | Multiplicity | $^{13}$C (ppm) |
|---------------------------|------------|-------------|--------------|----------------|
| **Amino acids**           |            |             |              |                |
| Valine (Val)              | $\alpha$-CH | 3.62        | m            | 63.86          |
|                           | $\beta$-CH | 2.29        | m            | 31.89          |
|                           | $\gamma$-CH | 1.05        | d            | 20.75          |
|                           | $\gamma'$-CH | 0.99        | d            | 19.41          |
| Isoleucine (Ile)          | $\alpha$-CH | 3.69        | m            | 63.04          |
|                           | $\beta$-CH | 1.99        | m            | 38.71          |
|                           | $\gamma$-CH | 1.25        | m            | 27.01          |
|                           | $\gamma'$-CH | 1.49        | m            | 17.38          |
|                           | $\delta$-CH | 1.02        | d            | 13.85          |
|                           | $\delta'$-CH | 0.95        | t            |                |
| Leucine (Leu)             | $\alpha$-CH | 3.74        | m            | 56.21          |
|                           | $\beta$-CH | 1.73        | m            | 42.60          |
|                           | $\gamma$-CH | 1.69        | m            | 26.81          |
|                           | $\delta$-CH | 0.97        | t            | 24.23          |
|                           | $\delta'$-CH | 0.96        | t            | 23.81          |
| Alanine (Ala)             | $\alpha$-CH | 3.77        | q            | 53.55          |
|                           | $\beta$-CH | 1.47        | d            | 19.02          |
| Threonine (Thr)           | $\alpha$-CH | 3.57        | d            | 63.46          |
|                           | $\beta$-CH | 4.24        | m            | 68.91          |
|                           | $\gamma$-CH | 1.31        | d            | 22.30          |
| Glutamic acid (Glu)       | $\alpha$-CH | 3.74        | t            | 57.64          |
|                           | $\beta$-CH | 2.08        | dt           | 29.82          |
|                           | $\gamma$-CH | 2.34        | t            | 36.35          |
| Aspartic acid (Asp)       | $\alpha$-CH | 3.90        | dd           | 55.08          |
|                           | $\beta$-CH | 2.71        | dd           | 39.47          |
|                           | $\beta'$-CH | 2.80        | dd           | 39.33          |
| Asparagine (Asn)          | $\alpha$-CH | 3.99        | dd           | 54.13          |
|                           | $\beta$-CH | 2.84        | dd           | 37.35          |
|                           | $\beta'$-CH | 2.94        | dd           | 37.43          |
| $\gamma$-aminobutyric acid (GABA) | $\alpha$-CH | 3.21        | t            | 37.04          |
|                           | $\beta$-CH | 1.91        | m            | 26.37          |
|                           | $\gamma$-CH | 3.03        | t            | 42.21          |
| Tyrosine (Tyr)            | $\alpha$-CH | 3.93        | dd           | 58.98          |
|                           | $\beta$-CH | 3.15        | dd           | 38.27          |
|                           | $\beta'$-CH | 3.05        | dd           | 38.27          |
|                           | $C_2$,6H-ring | 7.20        | d            | 133.48         |
|                           | $C_3$,5H-ring | 6.90        | d            | 118.89         |
| **Organic Acids**         |            |             |              |                |
| Acetic acid (AA)          | CH$_3$     | 1.92        | s            | 25.98          |
| Compound                  | CH-5 | CH-3 | CH-4 | CH-6 | CH-6' | CH-2 | CH-2' | Value   |
|--------------------------|------|------|------|------|-------|------|-------|---------|
| Quinic acid (QA)         | 4.12 | 4    | 3.53 | 2.05 | 2.03  | 1.94 | 1.85  | q m dd   |
| Malic acid (MA)          |      |      |      |      |       |      |       | 73.03  |
| Citric acid (CA)         |      |      |      |      |       |      |       | 66.00  |
| Fumaric acid (Fuma)      |      |      |      |      |       |      |       | 78.50  |
| Lactic acid (LA)         |      |      |      |      |       |      |       | 43.34  |
| α-hydroxybutyric acid (a-HIB) |      |      |      |      |       |      |       | 40.22  |
| Formic acid (FA)         |      |      |      |      |       |      |       | 40.22  |

| Compound                  | CH-5 | CH-3 | CH-4 | CH-6 | CH-6' | CH-2 | CH-2' | Value   |
|--------------------------|------|------|------|------|-------|------|-------|---------|
| Carbohydrates             |      |      |      |      |       |      |       |         |
| α-Glucose (α-G)           |      |      |      |      |       |      |       |         |
| CH-1                      | 5.25 | 3.55 | 3.72 | 3.42 | 3.84  | 3.73 | 3.90  | 93.10   |
| CH-1 Glucose              |      |      |      |      |       |      |       |         |
| CH-2                      | 4.69 | 3.26 | 3.50 | 3.42 | 3.48  | 3.74 | 3.91  | 96.97   |
| CH-3                      |      |      |      |      |       |      |       |         |
| CH-4                      |      |      |      |      |       |      |       |         |
| CH-5                      |      |      |      |      |       |      |       |         |
| CH-6                      |      |      |      |      |       |      |       |         |
| CH-6'                     |      |      |      |      |       |      |       |         |
| CH-2                      |      |      |      |      |       |      |       |         |
| CH-2 Galactose            | 5.01 | 3.82 | 3.89 | 3.98 | 3.95  | 3.73 | 3.67  | 101.08  |
| CH-3                      |      |      |      |      |       |      |       |         |
| CH-4                      |      |      |      |      |       |      |       |         |
| CH-5                      |      |      |      |      |       |      |       |         |
| CH-6                      |      |      |      |      |       |      |       |         |
| CH-6'                     |      |      |      |      |       |      |       |         |
| CH-2 Fructose             |      |      |      |      |       |      |       |         |
| C-2 F                     |      |      |      |      |       |      |       |         |
| CH-3 F                    | 4.22 |      |      |      |       |      |       |         |

| Compound                  | Value   |
|--------------------------|---------|
| α-HIB                    | 71.37   |
| β-HIB                    | 22.90   |
| 2-CH₃                    | 29.34   |
| α-GLUCOSE (α-G)           | 71.49   |
| β-GLUCOSE (β-G)           | 72.49   |
| Trisaccharide (T)         | 73.49   |

| Compound                  | CH-5 | CH-3 | CH-4 | CH-6 | CH-6' | CH-2 | CH-2' | Value   |
|--------------------------|------|------|------|------|-------|------|-------|---------|
| Formic acid (FA)         |      |      |      |      |       |      |       | 79.00   |

| Compound                  | CH-5 | CH-3 | CH-4 | CH-6 | CH-6' | CH-2 | CH-2' | Value   |
|--------------------------|------|------|------|------|-------|------|-------|---------|
| Malic acid (MA)          |      |      |      |      |       |      |       | 138.00  |
| Citric acid (CA)         |      |      |      |      |       |      |       |         |
| Fumaric acid (Fuma)      |      |      |      |      |       |      |       |         |
| Lactic acid (LA)         |      |      |      |      |       |      |       |         |
| α-hydroxybutyric acid (a-HIB) |      |      |      |      |       |      |       |         |
| Formic acid (FA)         |      |      |      |      |       |      |       |         |

| Compound                  | CH-5 | CH-3 | CH-4 | CH-6 | CH-6' | CH-2 | CH-2' | Value   |
|--------------------------|------|------|------|------|-------|------|-------|---------|
| Carbohydrates             |      |      |      |      |       |      |       |         |
| α-Glucose (α-G)           |      |      |      |      |       |      |       |         |
| CH-1 Glucose              | 5.25 | 3.55 | 3.72 | 3.42 | 3.84  | 3.73 | 3.90  | 93.10   |
| CH-2                      | 4.69 | 3.26 | 3.50 | 3.42 | 3.48  | 3.74 | 3.91  | 96.97   |
| CH-3                      |      |      |      |      |       |      |       |         |
| CH-4                      |      |      |      |      |       |      |       |         |
| CH-5                      |      |      |      |      |       |      |       |         |
| CH-6                      |      |      |      |      |       |      |       |         |
| CH-6'                     |      |      |      |      |       |      |       |         |
| CH-2                      |      |      |      |      |       |      |       |         |
| CH-2 Galactose            | 5.01 | 3.82 | 3.89 | 3.98 | 3.95  | 3.73 | 3.67  | 101.08  |
| CH-3                      |      |      |      |      |       |      |       |         |
| CH-4                      |      |      |      |      |       |      |       |         |
| CH-5                      |      |      |      |      |       |      |       |         |
| CH-6                      |      |      |      |      |       |      |       |         |
| CH-6'                     |      |      |      |      |       |      |       |         |
| CH-2 Fructose             |      |      |      |      |       |      |       |         |
| C-2 F                     |      |      |      |      |       |      |       |         |
| CH-3 F                    | 4.22 |      |      |      |       |      |       |         |

| Compound                  | Value   |
|--------------------------|---------|
| α-HIB                    | 71.37   |
| β-HIB                    | 22.90   |
| 2-CH₃                    | 29.34   |
| α-GLUCOSE (α-G)           | 71.49   |
| β-GLUCOSE (β-G)           | 72.49   |
| Trisaccharide (T)         | 73.49   |
| Formic acid (FA)         | 79.00   |
|                | CH-4 | CH-5 | CH₂-6 |        |        |        |
|----------------|------|------|-------|--------|--------|--------|
|                | 4.05 | 3.88 | 3.77-3.82 | dd     | m      | 79.69  |
| Sucrose (S)    |      |      |        |        |        |        |
| CH-1           | 5.42 |      |        | d      |        | 93.22  |
| CH-2           | 3.59 |      |        | dd     |        | 72.11  |
| CH-3           | 3.79 |      |        | dd     |        | 73.54  |
| CH-4           | 3.48 |      |        | dd     |        | 70.26  |
| CH-5           | 3.85 |      |        | m      |        | 73.38  |
| CH₂-6          | 3.82 |      |        | m      |        | 61.18  |
| CH₂-1'         | 3.69 |      |        | d      |        | 62.44  |
| C-2            |      |      |        |        | m      | 104.85 |
| CH-3'          | 4.22 |      |        | m      |        | 77.45  |
| CH-4'          | 4.06 |      |        | m      |        | 75.04  |
| CH-5'          | 3.90 |      |        | m      |        | 82.44  |
| CH₂-6'         | 3.82 |      |        | m      |        | 63.38  |
| α-Xylose (α-X) |      |      |        |        |        |        |
| CH-1           | 5.19 |      |        | d      |        | 94.03  |
| CH-2           | 3.52 |      |        | m      |        | 74.27  |
| CH-3           | 3.68 |      |        | m      |        | 75.67  |
| CH-4           | 3.64 |      |        | m      |        | 72.13  |
| CH₂-5          | 3.55 |      |        | m      |        | 63.55  |
| β-Xylose (β-X) |      |      |        |        |        |        |
| CH-1           | 4.57 |      |        | d      |        | 99.45  |
| CH-2           | 3.21 |      |        | m      |        | 77.92  |
| CH-3           | 3.43 |      |        | m      |        | 78.68  |
| CH-4           | 3.61 |      |        | m      |        | 72.09  |
| CH₂-5          | 3.31 – 3.90 | |  | dd     |        | 67.88  |
| Fructose (F)   |      |      |        |        |        |        |
| CH-1           | 3.69 |      |        | m      |        | 62.94  |
| C-2            |      |      |        |        | m      | 104.23 |
| CH-3           | 4.22 |      |        | d      |        | 77.24  |
| CH-4           | 4.06 |      |        | m      |        | 75.04  |
| CH-5           | 3.90 |      |        | m      |        | 83.37  |
| CH-6           | 3.82 |      |        | m      |        | 63.88  |

Other Compounds

|                | -CH= | -CH- | -CH₂- |        |        |        |
|----------------|------|------|-------|--------|--------|--------|
| Chlorogenic     | 7.67 | 7.22 | 7.14  |        |        |        |
| acid (CGA)      |      |      |       | dd     |        | 148.56 |
| CH-2           | 6.96 |      |       | d      |        | 148.77 |
| CH-6           | 6.41 |      |       | dd     |        | 117.83 |
| CH-5           | 5.32 |      |       | d      |        | 125.33 |
| =CH-CO₂⁻       | 3.88 |      |       | m      |        | 117.28 |
| CH-2’          | 4.24 |      |       | m      |        | 73.78  |
| CH-3’          | 2.22, 2.07 | |  | m      |        | 75.68  |
| CH-4’          | 2.09, 2.24 | |  | m      |        | 73.52  |
| CH₂-1’         |      |      |       | m      |        | 40.07  |
| CH₂-5’         |      |      |       | m      |        | 41.16  |
| Choline (Chn)  | N⁺(CH₃)₃ | 3.21 | | s      |        |        |
| Caffeic acid    | 7.30 | 7.18 | 7.09  |        |        |        |
| (Caff)          |      |      |       | dd     |        | 143.33 |
| CH-2           | 6.95 |      |       | d      |        | 117.14 |
| CH-6           | 6.43 |      |       | d      |        | 124.12 |
| CH-5           |      |      |       | d      |        | 118.85 |
| =CH-CO₂⁻       |      |      |       | d      |        | 124.31 |
| Metabolite            | Position | δ (ppm) | J (Hz) | Mass (Da) |
|-----------------------|----------|---------|--------|-----------|
| Kampferitrine (Kampf) | 2',6'    | 7.79    | d      | 130.8     |
|                       | 3',5'    | 6.92    | d      | 115.5     |
|                       | 8        | 6.79    | d      | 94.6      |
|                       | 6        | 6.45    | d      | 99.5      |
|                       | 1''      | 5.55    | d      | 98.4      |
|                       | 1''      | 5.30    | d      | 101.9     |
|                       | 2''      | 3.98    | s      | 70.1      |
|                       | 2''      | 3.84    | s      | 69.9      |
|                       | 3''      | 3.63    | m      | 70.3      |
|                       | 3''      | 3.40-3.49 | m  | 71.1      |
|                       | 4''      | 3.31    | m      | 71.8      |
|                       | 4''      | 3.15    | m      | 70.2      |
|                       | 5''      | 3.17    | m      | 70.9      |
|                       | 6''      | 0.86    | d      | 17.5      |
|                       | 6'''     | 1.16    | d      | 18.5      |
| Afzelin (Afz)         | 2',6'    | 7.72    | d      | 131.8     |
|                       | 3',5'    | 6.93    | d      | 115.9     |
|                       | 8        | 6.38    | d      | 94.9      |
|                       | 6        | 6.19    | d      | 100.1     |
|                       | 1''      | 5.35    | d      | 103.5     |
|                       | 2''      | 4.23    | s      | 72.1      |
|                       | 3''      | 3.72    | m      | 72.0      |
|                       | 4''      | 3.40    | m      | 72.9      |
|                       | 5''      | 3.34    | m      | 71.9      |
|                       | 6''      | 0.92    | d      | 17.7      |
| Epicatechin (EC)      | CH-2'    | 6.95    | d      | 117.09    |
|                       | CH-5'    | 6.75    | d      | 118.52    |
|                       | CH-6'    | 6.68    | dd     | 120.59    |
|                       | CH-8     | 6.12    | d      | 95.51     |
|                       | **CH-6** | **6.14** | d   | 101.9     |
|                       | CH-2''   | 4.56    | d      | 81.12     |
|                       | CH-3''   | 3.97    | m      | 70.45     |
|                       | CH₂-4''  | 2.49-2.82 | dd | 31.16     |

**Table 1** Metabolites identified in the $^1$H NMR spectrum of the aqueous extracts of kiwifruit leaves. In bold are evidenced the resonances chosen for metabolite quantification; s: singlet, d: doublet, t: triplet, q: quadruplet, dd: doublet of doublets, m: multiplet.
**Figure S6:** Pearson’s correlation map of *Actinidia arguta* metabolites. Positive correlations are in orange (p<0.05) and red (p<0.01) while negative ones are in cyan (p<0.05) and blue (p<0.01).

**Figure S7:** Pearson’s correlation map of *Actinidia chinensis* var. *deliciosa* metabolites. Positive correlations are in orange (p<0.05) and red (p<0.01) while negative ones are in cyan (p<0.05) and blue (p<0.01).
**Figure S8**: Pearson’s correlation map of *Actinidia chinensis* var. *chinensis* metabolites. Positive correlations are in orange (p<0.05) and red (p<0.01) while negative ones are in cyan (p<0.05) and blue (p<0.01).

|   | Thr | Ala | GABA | Glu | Asp | Aon | Tyr | F  | R  | S  | LA | QA | a-HIB | CA | MA | FumA | FA | CGA | EC | Kam | Alz | Caf |
|---|-----|-----|------|-----|-----|-----|-----|----|----|----|----|----|------|----|----|------|----|-----|----|-----|-----|-----|
| Thr |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| Ala |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| GABA|     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| Glu |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| Asp |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| Aon |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| Tyr |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| F   |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| R   |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| S   |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| LA  |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| QA  |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| a-HIB|     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| CA  |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| MA  |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| FumA|     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| FA  |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| CGA |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| EC  |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| Kam |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| Alz |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |
| Caf |     |     |      |     |     |     |     |    |    |    |    |    |      |    |    |      |    |     |    |     |     |     |