NMR-Based Metabolomic Comparison of Brassica oleracea (Var. italica): Organic and Conventional Farming

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Abstract: Brassicaceae family provides several crops which are worldwide known for their interesting phytochemical profiles, especially in terms of content of glucosinolates. These secondary metabolites show several beneficial effects toward consumers’ health, and several studies have been conducted to identify cultivation factors affecting their content in crops. One of the agronomic practices which is attracting growing interest is the organic one, which consists in avoiding the use of mineral fertilizers as well as pesticides. The aim of this study is to define the metabolic profile of Brassica oleracea (var. italica) and to compare the samples grown using organic and conventional fertilization methods. The hydroalcoholic and organic extracts of the samples have been analyzed by NMR spectroscopy. Forty-seven metabolites belonging to the categories of organic acids, amino acids, carbohydrates, fatty acids, sterols, and other molecules have been identified. Thirty-seven metabolites have been quantified. Univariate and multivariate PCA analyses allowed to observe that the organic practice influenced the nitrogen transport, the carbohydrate metabolism, the glucosinolate content and the phenylpropanoid pathway in B. oleracea (var. italica).

Keywords: NMR; metabolomics; Brassica oleracea (var. italica); organic and conventional practices; glucosinolates
Figure S1: $^1$H spectrum of *Brassica Oleracea* var *Italica* hydroalcoholic extract
Figure S2: $^1$H spectrum of *Brassica Oleracea* var *Italia* chloroform extract
Figure S3: $^1$H-$^1$H TOCSY spectrum of *Brassica Oleracea var Italica* hydroalcoholic extract
Figure S4: $^1$H-$^3$H TOCSY spectrum of *Brassica Oleracea var Italica* chloroform extract
Figure S5: $^1$H-$^{13}$C HSQC spectrum of *Brassica Oleracea var Italica* hydroalcoholic extract
Figure S6: $^1$H-$^{13}$C HSQC spectrum of Brassica Oleracea var Italica chloroform extract
Figure S7: $^1$H-$^{13}$C HMBC spectrum of *Brassica Oleracea var Italica* hydroalcoholic extract
Figure S8: $^1$H-$^{13}$C HMBC spectrum of *Brassica Oleracea* var *Italica* chloroform extract
| Compound          | Assignment | $\delta^1$H (ppm) | Multiplicity | $\delta^{13}$C (ppm) |
|-------------------|------------|-------------------|--------------|----------------------|
| **Amino acids**   |            |                   |              |                      |
| Alanine (Ala)     | $\beta$-CH$_3$ | 1.49              | d            | 16.8                 |
|                   | $\alpha$-CH | 3.78              | m            | 51.1                 |
| Arginine (Arg)    | $\alpha$-CH | 3.78              | t            | 54.6                 |
|                   | $\beta$-CH$_2$ | 1.90              | m            | 28.0                 |
|                   | $\gamma$-CH$_2$ | 1.71              | m            | 24.4                 |
|                   | $\delta$-CH$_2$ | 3.21              | t            | 41.0                 |
| Asparagine (Asn)  | $\beta$-CH | 2.87              | dd           | 36.5                 |
|                   | $\beta'$-CH | 2.96              | dd           | 36.5                 |
|                   | $\alpha$-CH | 4.01              | m            | 52.6                 |
| Aspartate (Asp)   | $\beta$-CH | 2.77              | dd           | 37.1                 |
|                   | $\beta'$-CH | 2.87              | dd           | 37.1                 |
|                   | $\alpha$-CH | 3.92              | m            | 52.7                 |
| $\gamma$-Aminobutyric acid | $\beta$-CH$_2$ | 1.97              | m            | 24.5                 |
|                   | $\gamma$-CH$_2$ | 2.42              | t            | 40.1                 |
|                   | $\alpha$-CH$_2$ | 3.03              | t            | 35.2                 |
| Glycine (Gly)     | $\alpha$-CH$_2$ | 3.56              | s            | 43.6                 |
| Glutamate (Glu)   | $\beta,\beta'$-CH | 2.09              | m            | 29.7                 |
|                   | $\gamma$-CH$_2$ | 2.34              | t            | 36.2                 |
|                   | $\alpha$-CH | 3.73              | t            | 57.6                 |
| Glutamine (Gln)   | $\beta$-CH$_2$ | 2.11              | m            | 28.9                 |
|                   | $\gamma$-CH$_2$ | 2.44              | m            | 33.8                 |
|                   | $\alpha$-CH | 3.78              | t            | 56.9                 |
| Histidine (His)   | $\beta$-CH$_2$ | 3.16              | dd           | 30.2                 |
|                   | $\beta'$-CH$_2$ | 3.23              | dd           | 30.2                 |
|                   | $\alpha$-CH | 3.98              | dd           | 57.3                 |
|                   | CH-5        | 7.09              | d            | 120.0                |
|                   | CH-2        | 7.90              | d            | 138.2                |
| Residue       | δ-CH₃ | γ'-CH₃ | γ'-CH | β-CH | α-CH |
|--------------|-------|--------|-------|------|------|
| Isoleucine (Ile) | 0.93  | 1.02   | 1.24  | 1.48 | 3.68 |
| Leucine (Leu)  | CH₃   | 0.94   | 0.96  | 1.71 | 3.73 |
| Lysine (Lys)   | α-CH  | 3.73   | 1.89  | 1.43 | 1.71 |
| Phenylalanine (Phe) | β-CH₂ | 3.12   | 3.27  | 3.98 | 7.32 |
| Threonine (Thr) | γ-CH₃ | 1.33   | 3.59  | 4.24 | 7.74 |
| Triptophan     | CH-4  | 7.74   | 7.54  | 7.29 | 7.36 |
| Tyrosine (Tyr) | CH-2,6| 7.18   | 6.88  | 7.18 | 7.38 |
| Valine (Val)   | γ-CH₃ | 0.99   | 1.04  | 1.09 | 3.68 |
|                | γ'-CH₃|        |       |      |      |

**Notes:**
- Δ and γ refer to the carbon atom in question.
- CH₃ and CH refer to the methyl and hydrogen groups attached to the carbon atom.
- The values represent chemical shifts in parts per million (ppm) relative to the standard.
- The symbols t, d, and m denote the type of magnetic coupling.
|                  | β-CH  | α-CH  |   |   |   |
|------------------|-------|-------|---|---|---|
| Organic Acids    | 2.25  | 3.60  | m | m | 29.7 |
|                  |       |       |   |   | 61.1 |
| Acetic acid (AA) | αCH\textsubscript{3} | 1.92  | s |   | 25.9 |
| Formic acid (FA) | HCOO\textsuperscript{-} | 8.45  | s |   | 173.77 |
| Fumaric acid     | α,β-HC=CH | 6.52  | s |   | 137.9 |
| (FumA)           |       |       |   |   |   |
| Isovaleric acid  | CH\textsubscript{3}, CH\textsubscript{3}' | 0.91  | d |   | 24.6 |
|                  | CH    | 1.94  | m |   | 28.9 |
|                  | CH\textsubscript{2} | 2.04  | d |   | 50.1 |
| Malic acid (MA)  | β-CH  | 2.67  | dd|   | 43.4 |
|                  | β'-CH | 2.42  | dd|   | 43.4 |
|                  | α-CH  | 4.38  | dd|   | 71.2 |
| Pyruvic acid (PA)| CH\textsubscript{3} | 2.34  | s |   | 39.2 |
| Succinic acid (SA)| α, β-CH\textsubscript{2} | 2.39  | s |   | 34.8 |
|                  | C1,4  | /     | / |   | 182.9 |
| Carbohydrates    | CH-1  | 5.26  | d |   | 95.1 |
|                  | CH-2  | 3.78  | m |   |   |
|                  | CH-3  | 3.98  | d |   |   |
|                  | CH-4  | 3.78  | m |   |   |
|                  | CH-5  | 4.08  | t |   |   |
|                  | CH\textsubscript{6,6}' | 3.73  | m |   |   |
|                  | CH-1  | 4.59  | d |   | 97.3 |
|                  | CH-2  | 3.50  | dd|   | 72.9 |
|                  | CH-3  | 3.62  | dd|   | 73.8 |
|                  | CH-4  | 3.97  | d |   | 69.7 |
|                  | CH-5  | 3.72  | m |   | 76.1 |
|                  | CH\textsubscript{6,6}' | 3.82  | m |   | 72.2 |
| α-Glucose (α-G)  | CH-1  | 5.25  | d |   | 93.1 |
|                  | CH-6  | 3.55  | m |   | 72.5 |
|                  | CH-3  | 3.72  | m |   | 73.8 |
|                  | CH-5  | 3.42  | m |   | 70.7 |
|   | CH-2   | 3.84 | CH-2-6   | 3.73–3.90 | m   | 72.5 | m   | 96.9 |
|---|--------|------|---------|-----------|-----|------|-----|------|
|   |        |      |         |           |     |      |     |      |
| β-Glucose (β-G) | CH-1   | 4.69 |        |           | d   | 96.9 | m   | 75.2 |
|   | CH-5   | 3.26 |        |           | m   | 76.8 | m   | 70.7 |
|   | CH-3   | 3.50 |        |           | m   | 74.6 | m   | 74.6 |
|   | CH-4   | 3.42 |        |           | m   | 74.6 | m   | 74.6 |
|   | CH-2   | 3.48 | 3.74–3.91|         | m   | 74.6 | m   | 74.6 |
| Fructose (F) | CH-1   | 3.57 |        |           | m   | 63.6 | /   | 102.6|
|   | C2     |      | /       |           | /   |       |     |      |
|   | CH-3   | 4.23 |        |           | d   | 76.4 | /   | 102.6|
|   | CH-4   | 4.10 |        |           | m   | 75.4 | m   | 75.4 |
|   | CH-5   | 3.82 |        |           | m   | 81.6 | m   | 81.6 |
|   | CH-6   | 3.79 |        |           | m   | 63.2 |     |      |
| Myo-inositol | CH-1,3 | 3.54 | dd |           |     | 73.3 |     |      |
|   | CH-2   | 4.07 | t     |           |     | 73.1 |     |      |
|   | CH-4,6 | 3.61 | t     |           |     | 71.9 |     |      |
|   | CH-5   | 3.29 | t     |           |     | 75.1 |     |      |
| Sucrose (S) | CH-1   | 5.42 | d     |           |     | 93.2 | m   | 72.1 |
|   | CH-2   | 3.59 | dd    |           |     | 73.1 |     |      |
|   | CH-3   | 3.79 | dd    |           |     | 73.5 |     |      |
|   | CH-4   | 3.48 | dd    |           |     | 70.3 |     |      |
|   | CH-5   | 3.85 | m     |           |     | 73.4 |     |      |
|   | CH2-6  | 3.82 | m     |           |     | 61.2 |     |      |
|   | CH2-1' | 3.69 | d     |           |     | 62.44|     |      |
|   | C-2    |      | /     |           | /   | 104.8|     |      |
|   | CH-3'  | 4.22 | m     |           |     | 77.4 |     |      |
|   | CH-4'  | 4.06 | m     |           |     | 75.0 |     |      |
|   | CH-5'  | 3.90 | m     |           |     | 82.4 |     |      |
|   | CH2-6' | 3.82 | m     |           |     | 63.4 |     |      |
| α-Xylose (α-X) | CH-1   | 5.17 | d     |           |     | 93.1 | m   | 72.5 |
|   | CH-6   | 3.80 | m     |           |     | 72.5 |     |      |
|   | CH-3   | 3.70 | m     |           |     | 73.8 |     |      |
|   | CH-5   | 3.45 | m     |           |     | 70.7 |     |      |
|                  | CH-2 | 3.35 | m   | 72.5 |
|------------------|------|------|-----|------|
| CH₂-6            | 3.73-3.90 | m   | 96.9 |

|                | CH-1 | 4.56 | d   | 97.0 |
|----------------|------|------|-----|------|
| CH-5            | 3.87 | m    | 74.6|
| CH-3            | 3.69 | m    | 76.8|
| CH-4            | 3.36 | m    | 70.7|
| CH-2            | 3.18 | m    | 75.2|

β-Xylose (β-X)

|                | CH-2 | 3.35 | m   | 72.5 |
|----------------|------|------|-----|------|
| CH-6            | 3.73-3.90 | m   | 96.9 |

|                | CH-1 | 4.56 | d   | 97.0 |
|----------------|------|------|-----|------|
| CH-5            | 3.87 | m    | 74.6|
| CH-3            | 3.69 | m    | 76.8|
| CH-4            | 3.36 | m    | 70.7|
| CH-2            | 3.18 | m    | 75.2|

Miscellaneous

|                | CH-2 | 3.77 | s   | 126.6|
|----------------|------|------|-----|------|
| CH₂-3           | 7.76 | d    | 114.9|
| CH₂-4           | 7.29 | t    | 125.7|
| CH₂-5           | 7.21 | t    | 122.7|
| CH₂-6           | 7.78 | d    | 121.8|
| CH₂-8           | 4.21 | dd   | 31.9 |
| CH₁'            | 4.85 | d    | 82.2 |
| CH₂'            | 3.26 | m    | 72.8 |
| CH₃'            | 3.41 | m    | 78.2 |
| CH₄'            | 3.29 | m    | 69.9 |
| CH₅'            | 3.36 | m    | 81.0 |
| CH₆'            | 3.62; 3.88 | m   | 61.4 |

|                | CH₂-1 | 2.75 | t   | 31.6 |
|----------------|------|------|-----|------|
| CH₂-2           | 1.92 | m    | 25.5|
| CH₂-3           | 1.87 | m    | 21.5|
| CH₂-4           | 2.82 | t    | 53.0|
| CH₃             | 2.64 | s    | 36.6|
| CH₁'            | 4.85 | d    | 82.2|
| CH₂'            | 3.26 | m    | 72.8|
| CH₃'            | 3.41 | m    | 78.2|
| CH₄'            | 3.29 | m    | 69.9|
| CH₅'            | 3.36 | m    | 81.0|
| CH₆'            | 3.62; 3.88 | m   | 61.4|

|                | CH-2 | 7.21 | s   | 124.7|
|----------------|------|------|-----|------|
| CH-4            | 7.33 | d    | 112.2|

|                | CH-2 | 3.21 | s   | 56.2 |
|----------------|------|------|-----|------|
| CH₂-3           | 7.77 | d    | 129.5|
| CH₂-4           | 7.29 | t    | 130.7|
| CH₂-5           | 7.21 | t    | 130.7|
| CH₂-6           | 7.78 | d    | 129.5|
| CH₂-8           | 4.21 | dd   | 31.9 |
| CH₁'            | 4.85 | d    | 82.2 |
| CH₂'            | 3.26 | m    | 72.8 |
| CH₃'            | 3.41 | m    | 78.2 |
| CH₄'            | 3.29 | m    | 69.9 |
| CH₅'            | 3.36 | m    | 81.0 |
| CH₆'            | 3.62; 3.88 | m   | 61.4 |

|                | CH₂-1 | 2.75 | t   | 31.6 |
|----------------|------|------|-----|------|
| CH₂-2           | 1.92 | m    | 25.5|
| CH₂-3           | 1.87 | m    | 21.5|
| CH₂-4           | 2.82 | t    | 53.0|
| CH₃             | 2.64 | s    | 36.6|
| CH₁'            | 4.85 | d    | 82.2|
| CH₂'            | 3.26 | m    | 72.8|
| CH₃'            | 3.41 | m    | 78.2|
| CH₄'            | 3.29 | m    | 69.9|
| CH₅'            | 3.36 | m    | 81.0|
| CH₆'            | 3.62; 3.88 | m   | 61.4|

|                | CH-2 | 7.21 | s   | 124.7|
|----------------|------|------|-----|------|
| CH-4            | 7.33 | d    | 112.2|
| Compound          | CH-5 | CH-6 | CH-7 | CH-8 |
|-------------------|------|------|------|------|
|                   | 7.08 | 7.01 | 7.64 | 4.79 |
| Sinapine          |      |      |      |      |
| CH2-10            | 4.83 |      |      |      |
| CH2-11            | 3.56 |      |      |      |
| CH-2,6            | 6.9  |      |      |      |
| CH-7              | 7.70 |      |      |      |
| CH-8              | 6.47 |      |      |      |
| Trigonelline (Trg)|      |      |      |      |
| N-CH3             | 4.42 |      |      |      |
| CH-4              | 8.07 |      |      |      |
| CH-3,5            | 8.82 |      |      |      |
| CH-1              | 9.11 |      |      |      |
| Lipids and Sterols|      |      |      |      |
| Stearic Acid (SFA)|      |      |      |      |
| CH3               | 0.88 |      |      |      |
| n-CH2             | 1.27 |      |      |      |
| CH2-CH2-CO2^-     | 1.62 |      |      |      |
| CH2-CO2^-         | 2.31 |      |      |      |
| Oleic Acid (W-9)  |      |      |      |      |
| CH3               | 0.88 |      |      |      |
| n-CH2             | 1.27 |      |      |      |
| CH2-CH=CH         | 2.03 |      |      |      |
| CH=CH             | 5.35 |      |      |      |
| CH2-CH2-CO2^-     | 1.62 |      |      |      |
| CH2-CO2^-         | 2.31 |      |      |      |
| Linoleic Acid (W-6)|      |      |      |      |
| CH3               | 0.86 |      |      |      |
| n-CH2             | 1.36 |      |      |      |
| CH2-CH=CH         | 2.04 |      |      |      |
| CH=CH             | 5.37 |      |      |      |
| =CH-CH2-CH=       | 2.76 |      |      |      |
| CH2-CH2-CO2^-     | 2.06 |      |      |      |
|                                      | CH₂-CO₂⁻ | 2.31 | t   | 34.1 |
|--------------------------------------|----------|------|-----|------|
| Linolenic Acid (W-3)                |          |      |     |      |
| CH₃                                  | 0.95     | t    | 14.3|
| n-CH₂                                | 1.37     | m    | 29.22|
| CH₂-CH=CH                            | 2.04     | m    | 27.2|
| CH=CH                                | 5.36     | m    | 130.4; 128.3|
| =CH-CH₂-CH=                          | **2.82** | t    | 26.9|
| CH₂-CH₂-CO₂⁻                         | 2.03     | m    | 24.6|
| CH₂-CO₂⁻                             | 2.30     | t    | 33.9|
| **Monoacylglycerol (MAG)**           |          |      |     |      |
| CH₂                                  | 3.65-3.55| dd   | 65.4|
| CH₂                                  | 4.05-4.15| dd   | 70.3|
| CH                                   | 3.82     | m    | 75.1|
| **Triacylglycerol (TG)**             |          |      |     |      |
| CH                                   | 5.13-5.21| m    | 77.4|
| CH₂                                  | 4.15-4.29| dd   | 68.2|
| **Campesterol (Cmp)**                |          |      |     |      |
| CH₂-1                                | 1.08, 1.85| m   | 37.2|
| CH₂-2                                | 1.51, 1.84| m   | 31.5|
| CHOH-3                               | 3.52     | m    | 71.8|
| CH₂-4                                | 2.28     | m    | 42.4|
| CH-6                                 | 5.34     | m    | 121.8|
| CH₂-7                                | 1.52, 1.98| m  | 32.0|
| CH-8                                 | 1.46     | m    | 31.8|
| CH-14                                | 0.99     | m    | 56.7|
| CH₂-15                               | 1.57     | m    | 24.2|
| CH₂-16                               | 1.26, 1.85| m  | 28.4|
| **CH₃-18**                           | **0.70** | s    | 12.2|
| CH₃-25                               | 1.01     | s    | 19.1|
| **β-Sitosterol (β-ST)**              |          |      |     |      |
| CH₂-1                                | 1.08, 1.85| m  | 37.2|
| CH₂-2                                | 1.51, 1.84| m  | 31.5|
| CHOH-3                               | 3.52     | m    | 71.8|
| CH₂-4                                | 2.28     | m    | 42.4|
| Metabolite  | Chemical Shift(s) | Multiplicity | Integration |
|------------|------------------|--------------|-------------|
| CH-6       | 5.34             | m            | 121.8       |
| CH₂-7      | 1.52, 1.98       | m            | 32.0        |
| CH-8       | 1.46             | m            | 31.8        |
| CH-14      | 0.99             | m            | 56.7        |
| CH₂-15     | 1.57             | m            | 24.2        |
| CH₂-16     | 1.26, 1.85       | m            | 28.4        |
| **CH₃-18** | **0.68**         | s            | **12.2**    |
| CH₃-25     | 1.01             | s            | 19.1        |

**Table S1:** Metabolites identified in the $^1$H NMR spectra of the *Brassica Oleracea* var *italica*. In bold are evidenced the resonances chosen for metabolite quantification; s: singlet, d: doublet, t: triplet, q: quadruplet, dd: doublet of doublets, m: multiplet.