SUPPLEMENTARY INFORMATION

Metabolites

Metabolite profiling of Mexican chard (Beta vulgaris L. var. Cicla) growth under nutritional stress

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To make the Hoagland, nitrogen, potassium and calcium excess/deficit solutions that was used in the present study, Fermont analytical chemical reagent was used (Productos químicos Monterrey S.A de C.V. Monterrey N.L. México. www.pqm.com.mx). Every nutrimental solution was prepared from the following ten stock solutions in the laboratory, the preparation of which are as follows:

a) ***Stock solution A.*** Calcium nitrate tetrahydrate 1.25 M (product number: 41293, CAS: 13477-34-4, purity: 99.8%).

In a 1000 mL beaker with 400 mL of bi-distilled water was added little portions of calcium nitrate tetrahydrate with the aim of facility the solubility until complete 295.2 grams. Then it was transferred into a 1 L volumetric flask and complete the volume to get a solution 1.25M with contained of 50.1 mg/mL of calcium and 35 mg/mL of nitrogen.

b) ***Stock solution B***. Potassium nitrate 1.25 M (product number: 41843, CAS:7757-79-1, purity: 99.4%).

In a 500 mL beaker with 300 mL of bi-distilled water were slowly added 126.4 grams of potassium nitrate. When the salt was completely dissolved, it was transferred into a 1 L volumetric flask and complete the volume to get a solution 1.25 M containing 48.8 mg/mL of potassium and 17.5 mg/mL of nitrogen.

c) ***Stock solution C.*** Magnesium sulfate heptahydrate 0.5 M (product number: 63623, CAS: 10034-99-8, purity: 99.9%).

It was dissolved 123.3 grams of magnesium sulfate heptahydrate into a 500 mL beaker with 400 mL of bi-distilled water, then it was transferred into a 1 L volumetric flask and calibrated to this volume to get a 0.5 M solution and a content of 12.1 mg/mL of magnesium and 16 mg/mL of sulfur.

d) ***Stock solution D***. Potassium phosphate monobasic 0.25 M (product code: 35862, CAS: 7778-77-0, purity: min 99%).

In a 500 mL beaker with 300 mL of bi-distilled water was dissolved slowly 34.02 grams of Potassium phosphate monobasic, then It was transferred into a 1 L volumetric flask and complete the volume to get a 0.25 M solution and a content of 9.7 mg/mL of Potassium and 7.75 mg/mL of phosphorus.

e) ***Stock solution E.*** Ammonium nitrate 0.2 M (product code: 41053, CAS: 6484-52-2, purity: 99.9%).

16 grams of ammonium nitrate was dissolved on 200 mL of bi-distilled water and then it was transferred into a 1 L volumetric flask and calibrated to this volume to get a solution 0.2 M and 5.6 mg/mL of nitrogen contained.

f) ***Stock solution F***. Oligoelements.

To prepare 1L of trace element nutrient solution, 2.9 grams of boric acid (product code: 05102, CAS: 10043-35-3, purity: 99.7%) was dissolved in 100 mL of double-distilled water in five separate 200 mL beakers with 1.80 grams of manganese chloride heptahydrate (1.80 grams of manganese chloride heptahydrate). (product code: 24652, CAS: 13446-34-9, purity: 99.9%), 0.22 grams of zinc sulfate heptahydrate (product code: 63962, CAS: 7446-20-0, purity: 99.9%), 0.1 grams of copper sulfate pentahydrate (product code: 63362, CAS: 7758-99-8, purity: 99.3%) and 0.12 grams of sodium molybdate dihydrate (product code : 40902, CAS: 10102-40-6, purity: 99.99%). When all the salts were completely solubilized, they were transferred into a 1 L volumetric flask and calibrated to this volume to get a solution with 0.5 mg/mL of Boron, 0.5 mg/mL of manganese , 0.64 mg/mL of chloride, 0.05 mg/mL of zing, 0.036 of sulfur, 0.025 mg/mL of copper, 0.047 mg/mL of molybdenum and 0.022 mg/mL de sodium.

g) ***Stock solution G.*** EDTA-Fe.

To prepare 1 L of EDTA-Fe stock solution, 10.4 grams of EDTA disodium (product code: 05801, CAS: 6381-92-6, purity: 99.9%) were dissolved in 200 mL of water, then 14 grams of ferrous sulfate heptahydrate (product code: 63591, CAS: 7782-63-0, purity: 99.9) were slowly added. The resulting solution was transfer into a 1 L volumetric flask and calibrated to this volume to obtain a solution containing 2.81 mg/mL of iron, 1.28 mg/mL of sodium and 1.61 mg/mL de sulfur.

h) ***Stock solution H.*** Acetic acid 1M (product code: 03015, CAS: 64-19-7, purity: 99.7%).

57.2 mL of glacial acetic acid was slowly added into a 1 L volumetric flask with 200 mL of bi-distilled water and then calibrated to this volume to obtain a solution 1 M containing 59 mg/mL of acetate.

i) ***Stock solution I.*** Calcium acetate hydrate 0.625 M (product code: 11252, CAS: 5743-26-0, purity: min 99%).

This solution was prepared at the time of use. 55.05 grams of calcium acetate hydrate was slowly dissolved in a beaker with 300 mL of double distilled water, then transferred to a 1 L volumetric flask and calibrated to this volume to obtain a 0.625 M solution containing 25 mg/mL calcium and 73.8 mg/mL acetate.

j) ***Stock solution J.*** Potassium acetate 1M (product code: 11842, CAS: 127-09-3, purity: min 99%).

In a 500 mL beaker with 300 mL of bi-distilled water 98.15 grams of potassium acetate was slowly dissolved, then it was transferred to a 1 L volumetric flask and calibrated to this volume to obtain a 1 M solution and a content of 39 mg/mL calcium and 59 mg/mL acetate.

Preparation of the nutrient solutions from the above stock solutions used in this study

To make 1L of each nutrient solution used in this article, Table S.I.1. shows the mL needed of the stock solutions described above, and Table S.I.2 shows the mineral concentration (ppm) of any nutrient solution.

Table S1. Required volume (mL) of each stock solution necessary for the preparation of the different nutrient solutions.

Nutrimental condition/ Reactive (mL)	**$Ca(NO_3)_2$ $4H_20$ (A)**	**KNO_3 (B)**	**$MgSO_4$ $7H_20$ (C)**	**KH_2PO_4 (D)**	**NH_4 NO_2 (E)**	**Oligoelements (F)**	**EDTA-Fe (G)**	**Acetic acid (H)**	**Calcium acetate (I)**	**Potassium acetate (J)**
Hoagland solution	4	4	4	4	1	1	1	7.5	0	0
Nitrogen deficiency	1	4	4	4	0.5	1	1	0	1	0
Nitrogen excess	4	4	4	4	20	1	1	7.5	0	0
Potassium deficiency	4	1.6	4	4	8.5	1	1	7.5	0	0
Potassium excess	4	4	4	4	1	1	1	4.5	0	3
Calcium deficiency	2	4	4	4	13.5	1	1	7.5	0	0
Calcium excess	4	4	4	4	1	1	1	2.5	4	0

Table S2. Macro and microelements concentration (ppm) of each different nutrient solution. Bold numbers refer to deficiency/excess of N, K, and Ca.

Element	**HS**	**ND**	**NE**	**KD**	**KE**	**CaD**	**CaE**
N	215.64	**107.82**	**322.07**	215.65	215.65	215.65	215.65
K	234.57	234.57	234.57	**117.28**	**351.85**	234.57	234.57
Ca	200.37	200.37	200.37	200.37	200.37	**100.18**	**300.56**
P	30.97						
S	65.78						
Cl	0.64						
Na	1.30						
Mg	48.61						
B	0.49						
Fe	2.81						
Mn	0.50						
Zn	0.05						
Cu	0.02						
Mo	0.04						
Acetate	442.5						

HS = Hoagland solution; NE = Nitrogen excess, ND = Nitrogen deficiency; KE = Potassium excess, KD = Potassium deficiency; CaE = Calcium excess, CaD = Calcium deficiency.

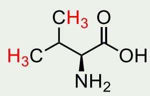
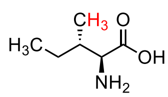
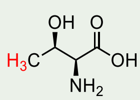
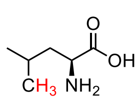
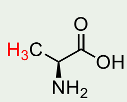
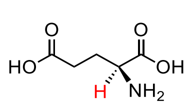
Table S3. Ingredients needed to prepare one liter of stock solution.

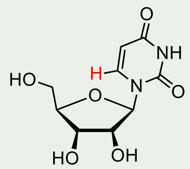
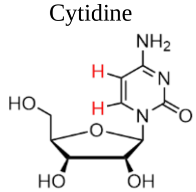
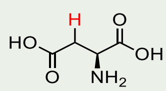
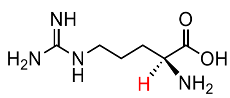
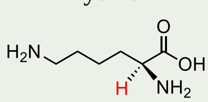
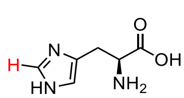
Stock solution	**Macronutrients**	**Concentration (g L^{-1})**
A	$Ca(NO_3)_2$ $4H_20$	295.18
B	KNO_3	126.36
C	$MgSO_4$ $7H_2O$	123.34
D	KH_2PO_4	34.02
E	NH_4NO_2	16.01
F	**Oligoelements**	**Concentration (g L^{-1})**
a)	H_3BO_3	2.86
b)	$MnCl_2$ $4H_20$	1.80
c)	$ZnSO_4$ $7H_2O$	0.21
d)	$CuSO_4$ $5H_20$	0.1
e)	Na_2MoO_4 $2H_20$	0.12
G	**Iron chelate**	**Concentration (g L^{-1})**
a)	EDTA 2Na	10.4
b)	$FeSO_4$ $7H_20$	14.0

Table S4. Concentration (ppm) of macro and micro elements to prepare the standard Hoagland solution.

Element	Concentration (ppm)
N	215.64
K	234.57
Ca	200.37
P	30.97
S	65.78
Cl	0.64
Na	1.30
Mg	48.61
B	0.49
Fe	2.81
Mn	0.50
Zn	0.05
Cu	0.02
Mo	0.04

Table S5. Assigned ^{1}H and ^{13}C NMR resonances (red colored atoms) of identified metabolites of *Beta vulgaris* var. cicla showing their chemical shifts (ppm), homonuclear scalar coupling (*J*, Hz), signal multiplicity and PLS-DA loading scores.

Metabolite (Assigned ^{1}H in red)	^{1}H δ (ppm)	^{13}C δ (ppm)	*J* (Hz)	Multiplicity	PLS-DA loading (See Figure 4) (a.u.)
L-Valine 	0.98 1.04	19.7 20.8	6.96 7.02	d d	-0.087 (NE), -0.031 (ND), -0.061 (CaE), -0.061 (CaD), -0.080 (KE), -0.079 (KD), -0.091 (HS)
L-Isoleucine 	1.01	17.4	7.05	d	-0.089 (NE), -0.039 (ND), -0.045 (CaE), -0.055 (CaD), -0.077 (KE), -0.050 (KD), -0.104 (HS)
L-Threonine 	1.33	22.3	6.58	d	-0.079 (NE), -0.056 (ND), -0.039 (CaE), -0.016 (CaD), -0.084 (KE), -0.074 (KD), -0.065 (HS)
L-Leucine 	0.96	24.9	5.95	td	-0.089 (NE), -0.026 (ND), 0.019 (CaE), 0.048 (CaD), -0.066 (KE), -0.060 (KD), -0.077 (HS)
L-Alanine 	1.48	19.0	7.32	d	-0.035 (NE), -0.050 (ND), -0.037 (CaE), -0.058 (CaD), -0.046 (KE), -0.098 (KD), 0.041 (HS)
L-Glutamic acid 	3.78	57.5	6.95	dd	0.072 (NE), 0.095 (ND), 0.060 (CaE), 0.026 (CaD), 0.084 (KE), 0.099 (KD), 0.099 (HS)

Uridine 	7.88	144.2	8.08	d	ND
Cytidine 	6.07 7.84	100.8 143.1	7.63 7.76	d d	ND
L-Aspartic acid 	2.82	39.3	17.51, 3.85	dd	-0.031 (NE), 0.002 (ND), -0.072 (CaE), -0.014 (CaD), -0.074 (KE), -0.070 (KD), 0.028 (HS)
L-Arginine 	3.77	57.4	6.18	m	0.078 (NE), 0.089 (ND), -0.024 (CaE), 0.098 (CaD), 0.082 (KE), 0.118 (KD), 0.026 (HS)
L-Lysine 	3.76	57.5	6.2	m	-0.003 (NE), 0.050 (ND), -0.033 (CaE), 0.063 (CaD), -0.035 (KE), 0.079 (KD), 0.069 (HS)
L-Histidine 	8.02	138.3	-	m	-0.089 (NE), -0.051 (ND), -0.074 (CaE), -0.093 (CaD), -0.103 (KE), -0.091 (KD), 0.066 (HS)

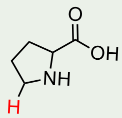
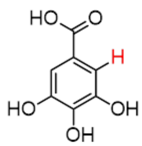
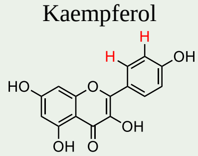
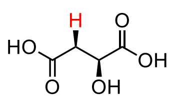
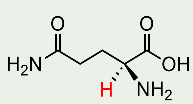
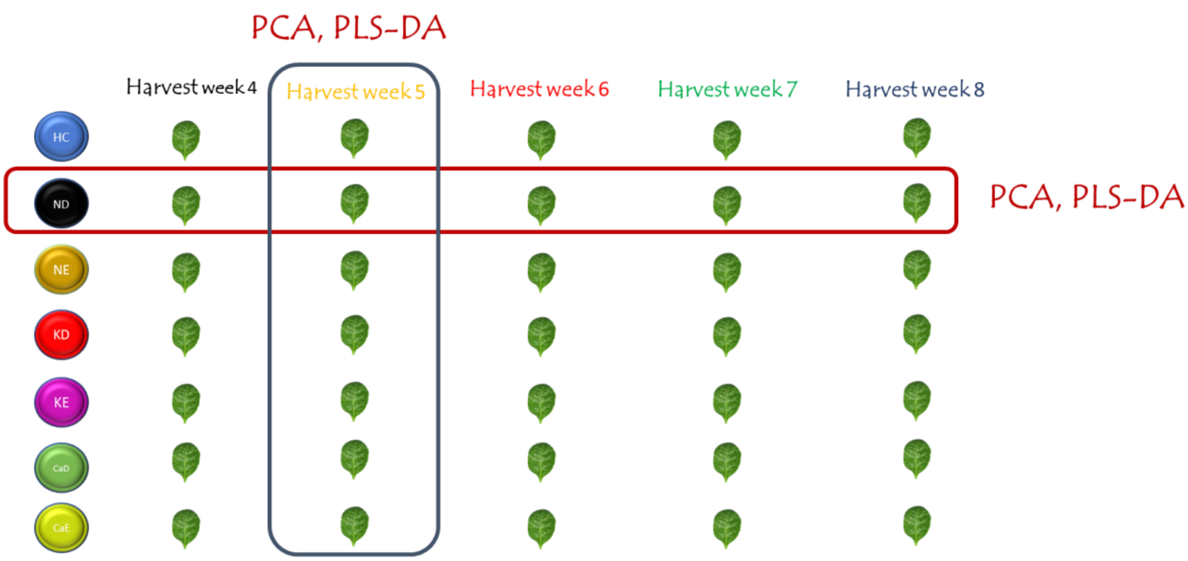
L-Proline 	3.48	48.2	11.59	m	0.099 (NE), 0.095 (ND), -0.005 (CaE), 0.094 (CaD), 0.097 (KE), 0.119 (KD), 0.073 (HS)
Gallic acid 	7.06	112.1	-	m	-0.091 (NE), -0.083 (ND), -0.089 (CaE), -0.080 (CaD), -0.098 (KE), -0.112 (KD), -0.106 (HS)
Kaempferol 	7.11 7.95	116.3 128.6	9.10 9.12	dd dd	-0.090 (NE), -0.068 (ND), -0.090 (CaE), -0.077 (CaD), -0.096 (KE), -0.103 (KD), -0.132 (HS)
Malic acid 	2.68	45.7	14.95, 3.01	dd	-0.068 (NE), -0.021 (ND), -0.094 (CaE), 0.066 (CaD), -0.058 (KE), -0.077 (KD), 0.077 (HS)
L-Glutamine 	3.83	57.5	6.29	m	0.097 (NE), 0.096 (ND), 0.065 (CaE), 0.103 (CaD), 0.100 (KE), 0.120 (KD), 0.36 (HS)

Figure S1. *Beta Vulgaris* var. cicla seedling after 28 days of growth.



Each harvest week was analyzed by nutritional condition (n=7), and each of these was analyzed by harvest week (n=5)

Figure S2. Sampling of *Beta Vulgaris* var. cicla by nutritional status (red box) and week of harvest (blue box).

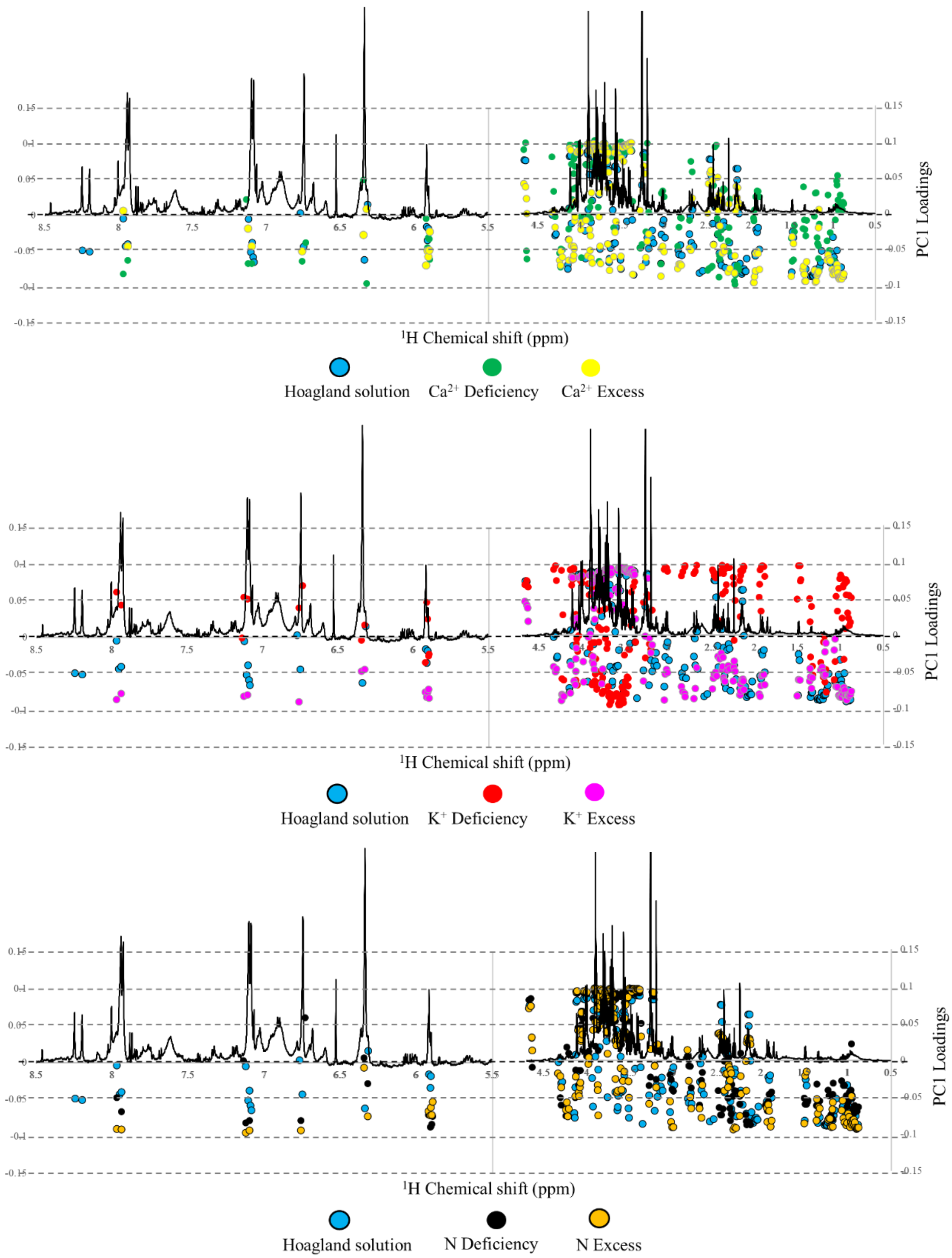


Figure S3. Non-supervised Principal Component Analysis (PCA) loading plots of *Beta vulgaris var. cicla* four-weeks plant growth cycle with standard Hoagland solution (blue spots), in comparison with excess/deficiency of Ca^{2+} (top), K^{+} (middle) and N (bottom) supported with each PC1 loading as a function of chemical shift to identify discriminating metabolites.

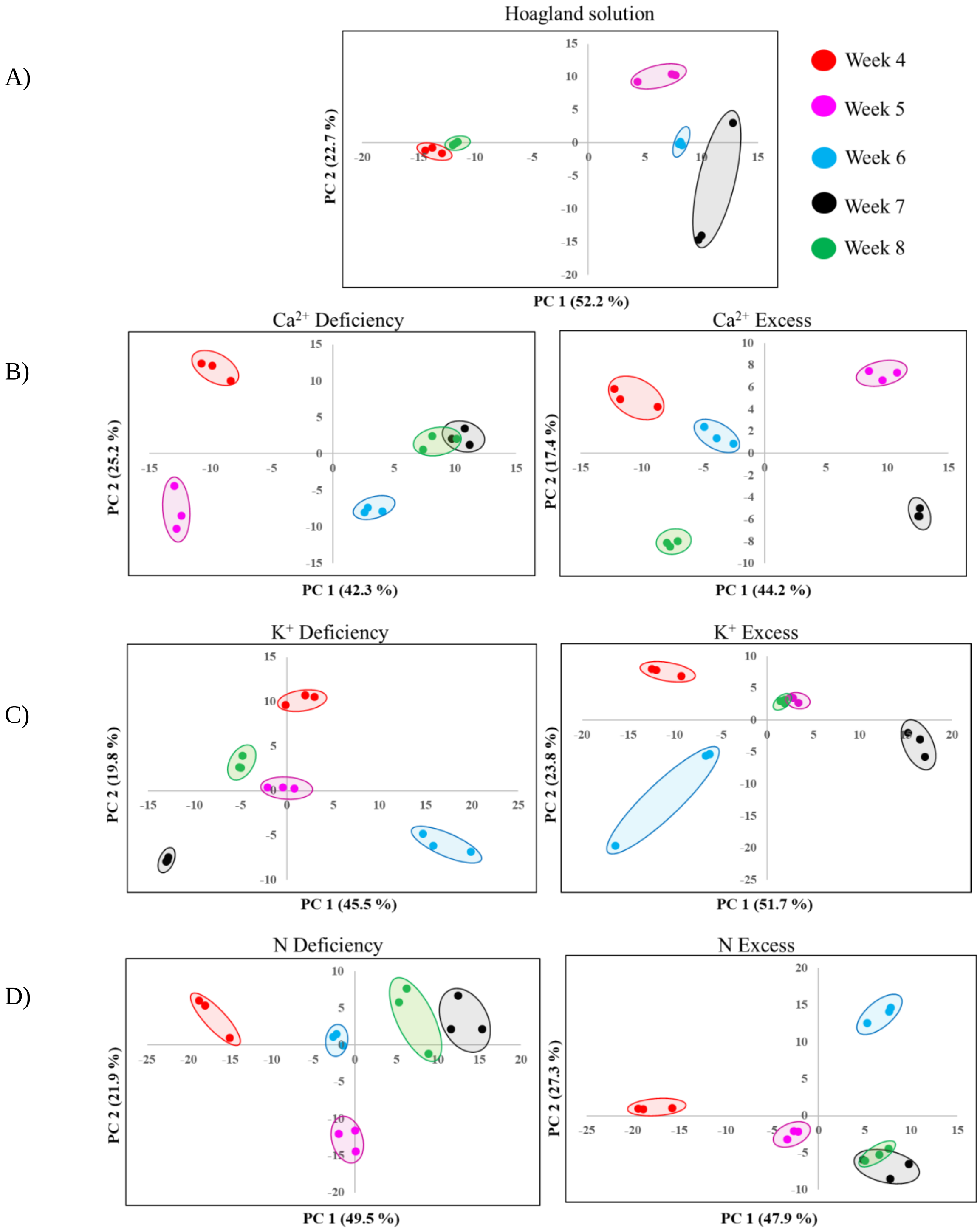


Figure S4. Non-supervised Principal Component Analysis (PCA) score plots of *Beta vulgaris var. cicla* four-weeks plant growth cycle with A) standard Hoagland solution, deficiency/excess of B) Ca^{2+}, C) K^{+}, and D) N. The temporal discriminant factor defined as weeks of plant growth (Week 4: red ellipses; week 5: magenta ellipses; week 6: blue ellipses; week 7: black ellipses; and week 8: green ellipses) is defined with T2 Hotelling ellipses at a 95% confidence interval.

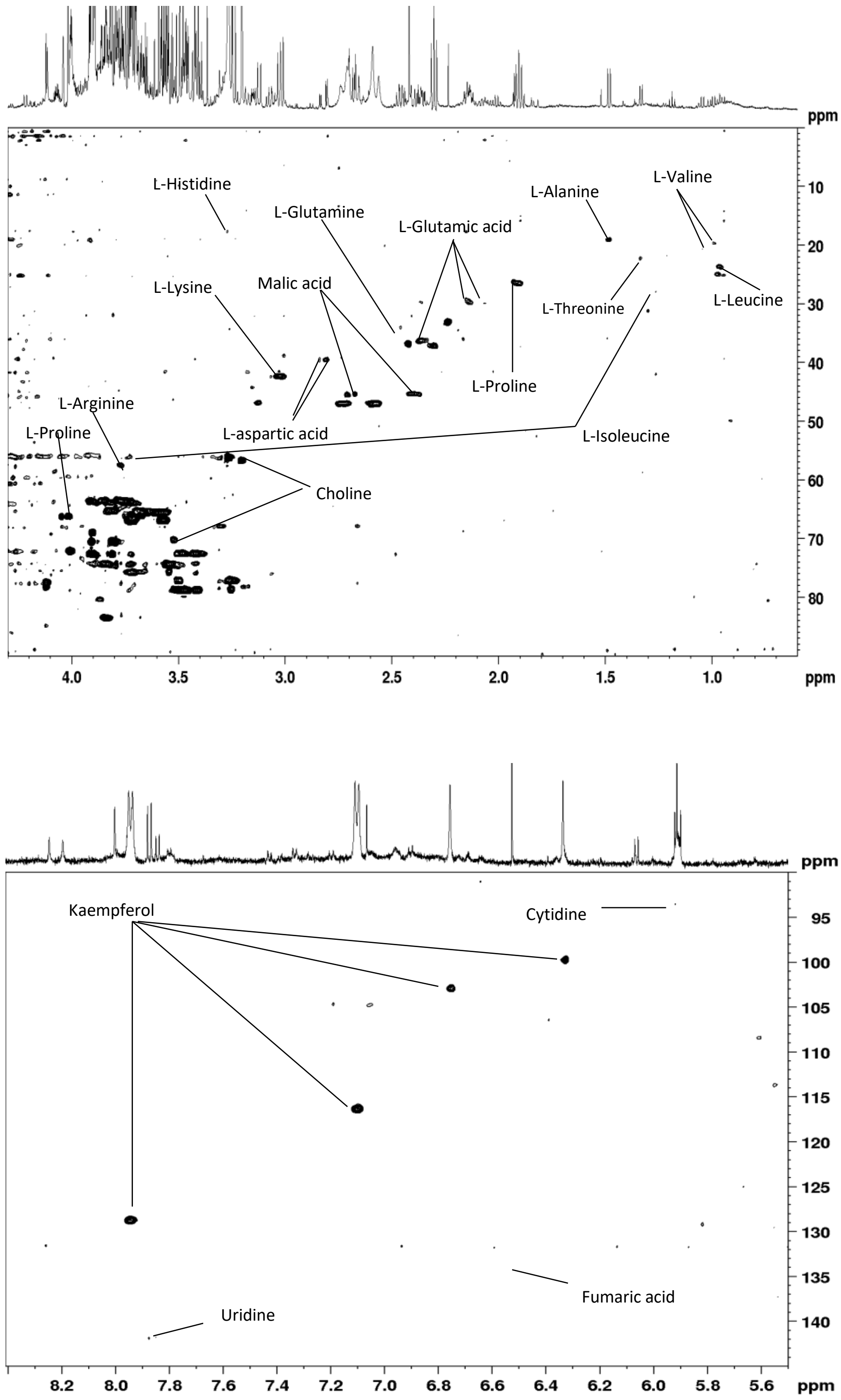


Figure S5. 2D ^{1}H-^{13}C heteronuclear correlation (HSQC) NMR experiment used to corroborate metabolites identification.

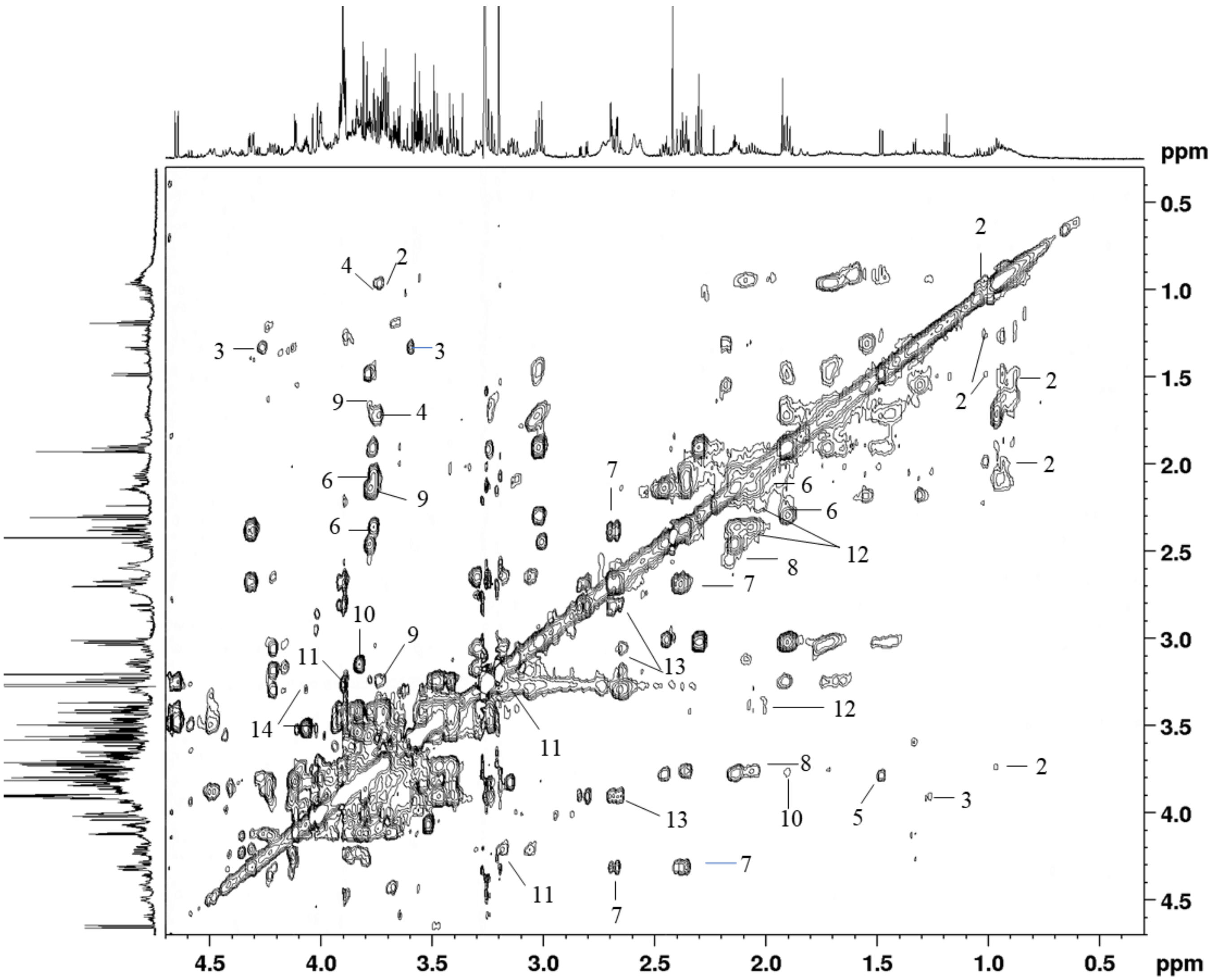


Figure S6. 2D NMR [1]H-1H homonuclear total correlation (TOCSY) experiment. 2. L-Isoleucine; 3. L-Threonine; 4. L-Leucine; 5. L-Alanina; 6. L-Glutamic acid; 7. Malic acid; 8. L-Glutamine; 9. L-Arginine; 10. L-Lysine; 11. L-Histidine; 12. L-Proline; 13. L-Aspartic acid; 14. Choline. Figure S5: 15. Fumaric acid; 16. Cytidine; 17. Uridine; 18. Gallic acid: 19. Kaempferol.

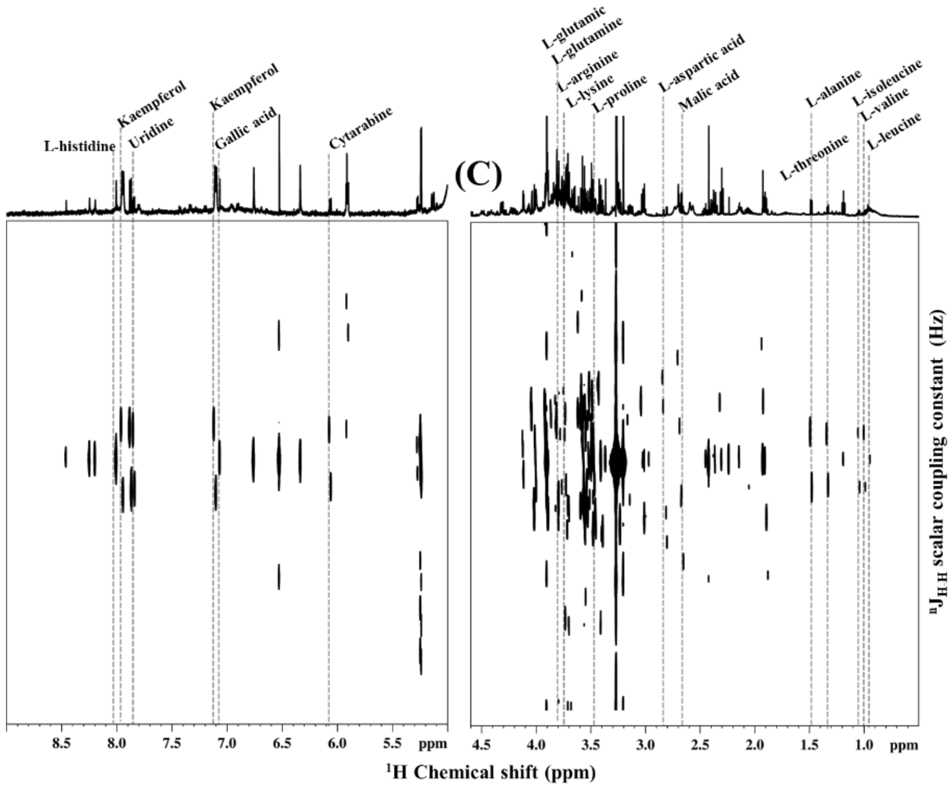


Figure S7. 2D J-resolved NMR experiment used to corroborate metabolite identification in *Beta vulgaris* var. cicla extracts.