Supplementary methods

Irrigation schedule

The net irrigation volume (I_{net}) was first calculated for each individual substrate based on the substrate's percentage available water in the container substrate (AW_{cont}), the substrate volume in L m^{-2} (V_{cont}) and the crop-specific irrigation coefficient (f) being a dimensionless value based on the irrigation system and the uniformity of the substrate. These values can be seen in Table 1. I_{net} was calculated by equation 1.

Table1: Parameters used for calculating the irrigation time.

Substrate	AW_{cont} (%)	V_{cont} (L m^{-2})	f	I_{net} (L m^{-2})	I_{gros} (L m^{-2})	I_{time} (min)
Rockwool	88	11.25	0.05	0.50	0.74	3.71
Perlite	33	16.50	0.05	0.27	0.40	2.04
Coco peat	45	15.00	0.08	0.54	0.81	4.05

[a] For each substrate the available water (AW_{cont}), the substrate volume in L m^{-2} (V_{cont}), and the crop-specific irrigation coefficient (f) parameters, and the calculated net irrigation volume (I_{net}), gross irrigation volume (I_{gros}) and irrigation time in minutes (I_{time}) are listed.

$$I_{net} = AW_{cont}/100 \cdot V_{cont} \cdot f \quad (1)$$

The gross irrigation volume (I_{gros}) was calculated according to equation 2. The I_{gros} is simply I_{net} multiplied with a safety coefficient (K_s). K_s is dependent on the risk of substrate salination and the irrigation uniformity amongst the substrate. A value of 1.15 is generally used for a salt tolerant crop, with good irrigation uniformity in the substrate and good quality water. In this case a K_s value of 1.5 was chosen for all substrates.

$$I_{gros} = I_{net} \cdot K_s \quad (2)$$

The duration of each irrigation in minutes (I_{time}) was then calculated based upon the I_{gros} and the number of dripping points per metre squared (d; m^{-2}) and the discharge rate of each dripping point in litres per hour (r, L h^{-1}). In this experiment 6 dripping points, with a discharge rate of 2.0 L h^{-1} were used. I_{time} was then calculated according to equation 3.

$$I_{time} = (I_{gross} \cdot 60)/(d \cdot r) \quad (3)$$

The irrigation frequency was then calculated based upon the estimated daily evapotranspiration (ET in L m^{-2}). Since a irrigation controller was used in this experiment, the number of irrigations per day (N_I) were calculated by dividing ET with I_{net} (Equation 4).

$$N_I = ET/I_{net} \quad (4)$$

Three estimated daily ET were used throughout the length of the experiment. At the start of the experiment from 13th of May 2017 to 31st of May 2017 an estimated ET of 2 L m^{-2} was used. From 31st of May 2017 onwards an estimated ET of either 4 L m^{-2} or 6 L m^{-2} was used. An estimated ET of 4 L m^{-2} was used under cloudy weather conditions, whilst an estimated ET of 6 L m^{-2} was used under hot and sunny conditions. The irrigation controller (Rain Bird ESP-Me) was set with a specific set of irrigation starting times throughout the day and with the irrigation duration for each specific substrate. Since the irrigation controller was only able to set the irrigation duration in full minutes the I_{time} values were rounded to the nearest minute.

Table 2: The number of irrigations based on estimated ET rate.

Substrate[a]	Estimated ET		
	2 L m^{-2}	4 L m^{-2}	6 L m^{-2}
Rockwool	4	8	12
Perlite	7	15	22
Coco peat	4	7	11

[a] For each substrate type the number of irrigations per day were calculated based on the estimated *ET* rates of 2 L m^{-2}, 4 L m^{-2} and 6 L m^{-2}.

Quantification of amino acids in tomato fruit sap by high performance liquid chromatography

To measure the amino acid content of tomato fruit, an HPLC method was developed. The quantification of amino acids in tomato fruit involves pre-column derivatisation with 2,4-dinitro-1-fluorobenzene (DNFB) and further separation of the derivatives on a C18 column. 5-Aminovaleric acid was added as an internal standard. The DNFB-derivatised amino acids were detected at 363 nm using a UV detector. Separation of the derivatives was performed on a Shimadzu (Nakagyo-ku, Japan) LC-10vp system consisting of a SCL-10Avp system controller, two LC-10ADvp pumps each equipped with a DGU-14A degasser and a FCV-10AL low pressure valve for eluent selection and connected for high pressure gradient elution, a SIL-10A autosampler, a CTO-10Avp column oven set to 27°C and a SPD-10A UV detector set to 363 nm. For separation of the amino acids derivatives a Nucleodur 100-5 C18ec 250 x 4 mm column (Macherey Nagel, Düren, Germany) preceded by a Nucleodur 100-5 C18ec 4x3 mm precolumn (Macherey Nagel) was used. The eluents consisted of 50 mM *N*-methylmorpholine set with acetic acid to pH 5.68 in 10% acetonitrile (eluent A) and 100% acetonitrile (eluent B). A flow rate of 0.8 ml min^{-1} was used, which allowed separation of the derivatised amino acids in 63 min using the following gradient: initial conditions consisted of 95% eluent A, 5% eluent B and were kept constant till 17 min. From 17 min to 30 min the gradient was changed linearly to 80% eluent A and 20% Eluent B. Between 30 min and 48 min the gradient was changed to 75% eluent A and 25% eluent B. From 48 min to 54 min eluent B was further increased linearly to 70%. From 54 min to 55 min the gradient was changed linearly to 20% eluent A, 80% eluent B and kept isocratic till 57 min. Between 57 min to 58 min the gradient was returned linearly to the initial conditions (95% eluent A, 5% eluent B) and kept isocratic till 63 min. An injection volume of 15 µl was used. The Clarity software package (DataApex, Prague, Czech Republic) was used to evaluate the resulting chromatograms. A detailed step by step protocol is provided below.

Reagents

Derivatisation reagent: DNFB 30 mM (mix 377 µl 2,4-dinitro-1-fluorobenzene with 100 ml ACN 100%; the solution can be kept at room temperature for up to 1 month)

Borate buffer 200 mM, pH 9.5 (dissolve 6.18 g boric acid in approximately 400 ml water and adjusted to pH to 9.5 with sodium hydroxide 4 M; add water to 500 ml)

Eluent A: 50 mM *N*-methylmorpholine set with acetic acid to pH 5.68 in 10 % acetonitrile (Place a 3000 ml beaker on a balance and tare. Weigh 10.12 g *N*-methylmorpholine, add approximately 1600 ml water, transfer 200 ml of 100% acetonitrile using a volumetric pipette into the beaker and set the pH of the solution to 5.68 using glacial acetic acid. Transfer the solution to a 2000 ml volumetric flask and top with water to the mark. Filter the solution through a 0.22 µm nylon filter)

Eluent B: acetonitrile, 100%

Internal standard: 5-aminovaleric acid 1000 mg L^{-1} (dissolve 55.0 mg of 2-aminovaleric acid hydrochloride in 10% ACN to a total volume of 50 ml. The solution can be kept at 4°C for up to 1 month or for leats 2 years at -20°C)

Amino acids stock for tomato samples (The amino acid stock solution was prepared according to Table 3. The solution can be kept at 4°C for up to 1 month or for at leats 2 years at -20°C)

Table 1: Concentration of amino acids in amino acid stock solution, and the amount to be weighed

Amino acid or compound	Concentration of stock solution [a] (mg L^{-1})	Compound	Amount to be weighed [b] (mg)
Asp	1500.0	L-Aspartic acid	150.0
Glu	6000.0	Monosodium glutamate monohydrate	763.2
Asn	500.0	L-Asparagine	50.0
His	100.0	L-Histidine	10.0
Ser	100.0	L-Serine	10.0
Gln	2000.0	L-Glutamine	200.0
Arg	200.0	L-Arginine	20.0
Gly	200.0	L-Glycine	20.0
Thr	150.0	L-Threonine	15.0
Pro	500.0	L-Proline	50.0
Ala	200.0	L-Alanine	20.0
GABA	1500.0	γ-Aminobutyric acid	150.0
Val	100.0	L-Valine	10.0
Met	100.0	L-Methionine	10.0
Cys	200.0	L-Cysteine	20.0
Ile	100.0	L-Isoleucine	10.0
Leu	100.0	L-Leucine	10.0
NH_3	100.0	Ammonium sulfate	38.8
Trp	100.0	L-Tryptophan	10.0
Phe	500.0	L-Phenylalanine	50.0
Orn	100.0	L-Ornithine	10.0
Lys	500.0	L-Lysine	50.0
Tyr	100.0	L-Tyrosine	10.0

[a] The concentration of amino acids in the stock solution used for preparation standards.

[b] The amount of compound to be weighed for preparation of 100 ml stock solution.

Sample preparation

1. Homogenise approximately 100 g tomato fruits with an Ultra-Turrax or a Warring Blender homogeniser.
2. Transfer the homogenate into 50 ml tubes and centrifuge at 4000 g for 5 min.

3. Filter approximately 2 ml of the supernatant through a 0.22 µm membrane syringe filter (PP, nylon or hydrophilic PTFE membrane) and use the filtrate for SPE. *Note: the filtrate can be stored at -20° until analysis.*
4. Using a volumetric pipet, transfer exactly 25 ml of the clear supernatant into a beaker placed on an analytical balance and weigh the supernatant. Calculate the density of the supernatant by dividing the weight by the volume. *Note: the density is required for conversion of the concentration of amino acids from mg L^{-1} to the content in mg kg^{-1}.*

Derivatisation

1. Transfer 30 µl tomato extract into an autosampler vial and add 100 µl internal standard and 670 µl borate buffer.
2. Prepare the standards by mixing the following solutions in autosampler vials (Table 4):
3. Add 400 µl derivatisation reagent to each standard and sample, close the vial and shake vigorously.
4. Place the samples at 60°C for 1 h.
5. Add 20 µl of glacial acetic acid to each sample, close the vial and shake vigorously.
6. The derivatised samples are analysed directly by HPLC. It is also possible to store them at 4°C for up to one week prior analysis.

Table 4: Preparation of standards.

Standard No.	Amino acid stock solution µl	Internal standard µl	Borate buffer acid pH 9.5 µl
St 1	0	100	700
St 2	2	100	698
St 3	5	100	695
St 4	10	100	690
St 5	20	100	680
St 6	30	100	670

HPLC

Analyse the samples by HPLC using the following conditions. An HPLC system with a high pressure gradient must be used to allow application of the gradient indicated in Table 5. Typical chromatograms of a standard and a tomato sample are shown in Figure 1.

Column: Nucleodur 100- 5 C18ec 250x4.0 mm
Pre-column: Nucleodur 100-5 C18ec 4x3 mm
Injection volume: 15 µl
Column oven: 27°C
Detector: UV, 363 nm
Eluent A: 50 mM *N*-methylmorpholine set with acetic acid to pH 5.68 in 10 % ACN
Eluent B: ACN 100%
Flow rate: 0.8 ml min^{-1}

Table 5: Elution program

Time in min	Eluent A in %	Eluent B in %
0.0	95	5
17.0	95	5
30.0	80	20
48.0	75	25
54.0	30	70
55.0	20	80
57.0	20	80
58.0	95	5
63.0 (STOP)	95	5

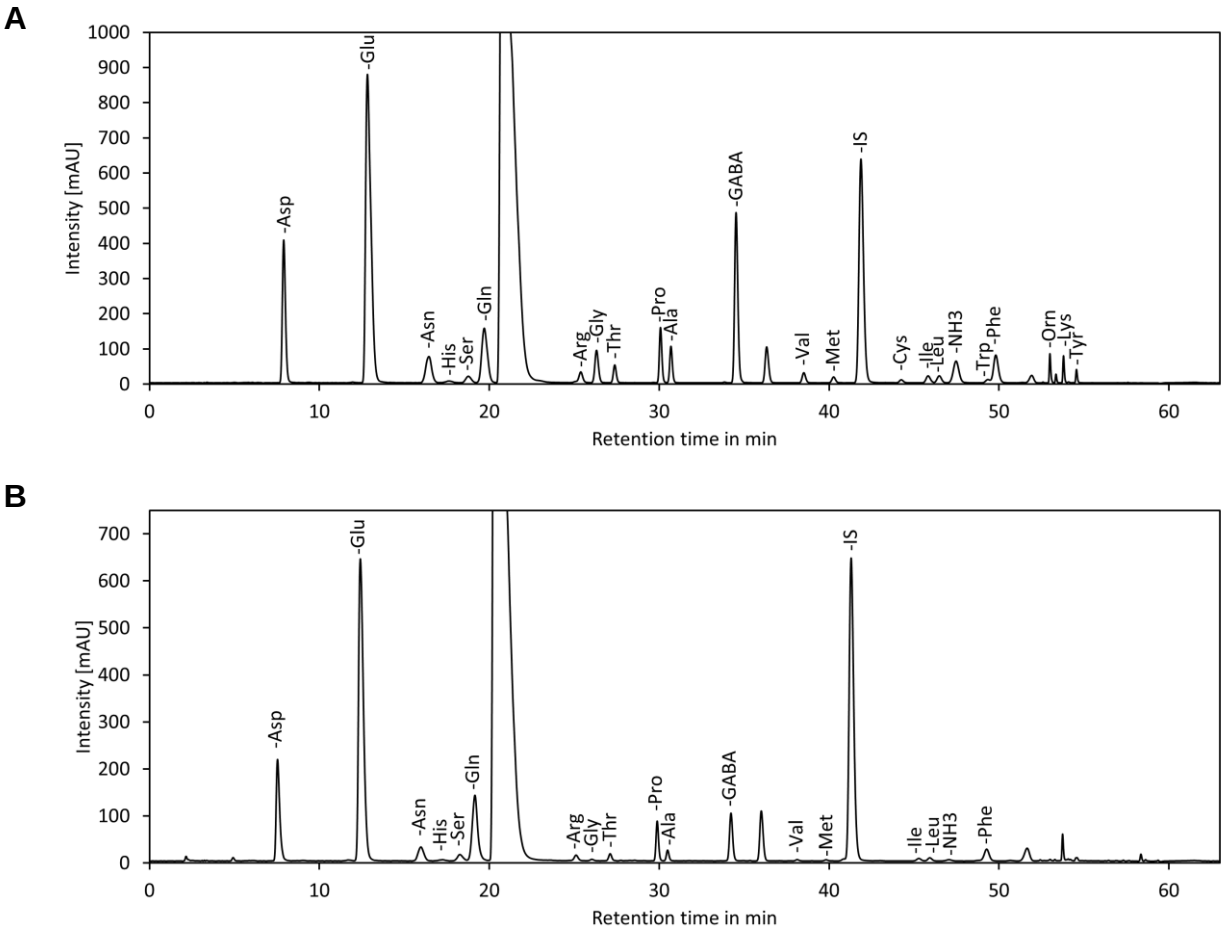

Figure 1: (**A**) HPLC chromatogram of a standard used for the quantification of tomato samples. (**B**) A typical HPLC chromatogram of a tomato sample.