

SUPPLEMENTARY DATA

MATERIALS AND METHODS FOR FIG. 3

Plant material

The plants of *Nepenthes truncata* used in our experiments were cultivated in greenhouse with irradiance up to $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR at Department of Plant Physiology, Comenius University in Bratislava, Slovakia. They were grown in plastic pots with *Sphagnum* / perlite / bark / moss mixture substrate and had possibility to catch animal prey.

Simultaneous measurements of gas exchange and chlorophyll a fluorescence (Fig. 3B,C,D,E)

Rates of net photosynthesis (A_N), stomatal conductance (g_s) and chlorophyll *a* fluorescence were measured simultaneously with a CIRAS-2 (PP-Systems, Hitchin, UK) and a Fluorcam FC1000-LC (Photon Systems Instruments, Brno, Czech Republic) attached to an PLC6 cuvette (PP-Systems, Hitchin, UK) connected to an infrared gas analyser as described previously (Pavlovič *et al.*, 2009, 2011b). The middle part of the lamina (or lid in the case of pitcher as the only flat portion of the pitcher) was enclosed in the automatic leaf cuvette PLC6 (PP-Systems, Hitchin, UK). The light intensity was set up to $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Then the light intensity was decreased stepwise with irradiation periods of 3.5 min and subsequent saturation pulses were applied until $70 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR was reached, for estimation of maximum chlorophyll fluorescence in the light-adapted state (F'_m). The light was provided by red ($\lambda = 620 \text{ nm}$) LED diodes. Simultaneously, the rate of net photosynthesis (A_N) was recorded at CO_2 concentration $380 \mu\text{mol mol}^{-1}$, leaf temperature $23 \pm 1 \text{ }^\circ\text{C}$ and relative air humidity 65–70%. Effective photochemical quantum yield of photosystem II (ϕ_{PSII}) was calculated according to Maxwell and Johnson (2000). The A/Ci response curve of photosynthesis was measured with the same infrared gas analyser starting with air CO_2 concentration $0 \mu\text{mol mol}^{-1}$ at light intensity $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Then the CO_2 concentration was increased stepwise with periods 7 minutes until concentration $1500 \mu\text{mol mol}^{-1}$ was reached. The A_N was plotted against intercellular CO_2 concentration (C_i). The apparent quantum yield of CO_2 fixation (ϕ_{CO_2}) was calculated as the initial slope of light response curve and apparent Rubisco carboxylation efficiency (ϵ) as the initial slope of A/Ci response curve (Pavlovič *et al.*, 2009).

Elemental analysis (Fig. 3G)

Plant samples were dried at $70 \text{ }^\circ\text{C}$ and the aboveground plant biomass was weighed and ground to a fine powder. Samples (1 mg) were packed into tin capsules and the nitrogen content was determined in a Vario MICRO Cube Elemental Analyzer (Elementar, Hanau, Germany) as described previously (Pavlovič *et al.*, 2014). For P analyses, 1.8–2.4 mg dry weight was mineralized with HClO_4 and 6–7 mg with HNO_3 for metallic cation analyses. Analyses of P were performed colorimetrically with an automatic FIAstar 5010 Analyzer (Tecator, Sweden), while metallic cation concentrations were estimated by atomic absorption flame spectrometry using a Varian AA240FS (Agilent, Santa Clara, CA, USA) atomic absorption flame spectrometer. Five samples were used for each foliar material.

Chlorophyll, carotenoid and anthocyanin extraction and quantification (Fig. 3H)

The half of the leaf or pitcher lid was dried at $70 \text{ }^\circ\text{C}$ to determine the dry weight percentage. Remaining portions of the tissue were ground in a mortar and pestle with a small amount of sand and extracted with 80% (v/v) chilled acetone with MgCO_3 to avoid acidification and pheophytinization of the pigments. The samples were centrifuged at 8000 g at $4 \text{ }^\circ\text{C}$ for 5 min. Assimilation pigment concentration in the supernatant was determined spectrophotometrically (Jenway 6705 UV/Vis, Bibby Scientific, Essex, UK) at 663 nm for chl *a*, 647 nm for chl *b*, 470 nm

for carotenoids and 537 nm for anthocyanins. Assimilation pigment concentrations were calculated and corrected for the presence of anthocyanins according to Sims and Gamon (2002) and expressed in mg pigments g⁻¹ dry weight.

Isolation of proteins and Western blot analysis (Fig. 3F)

Because the A_N of pitchers was very low, we investigated the presence of some photosynthesis-related proteins. Fifty mg of the assimilation leaf and different parts of trap were homogenized in liquid nitrogen and extracted into extraction buffer containing 28 mM dithiothreitol, 28 mM Na₂CO₃, 175 mM sucrose, 5% SDS and 10 mM EDTA and protease inhibitor (Protease inhibitor cocktail set VI, Calbiochem, USA). Concentration of total soluble proteins in samples was determined using the Bicinchoninic Acid Kit for Protein Determination (Sigma-Aldrich, St. Louis, MO, USA) and absorbance measured at 562 nm (Jenway 6705 UV/Vis, Bibby Scientific, Essex, UK). The same amount of proteins was electrophoresed in 12 % (v/v) SDS-polyacrylamide gel followed by transfer to the Hybond-C membranes (Amersham, Freiburg, Germany) by Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Biorad, Hercules, CA, USA). To check the correct protein transfer, the membranes were stained with Ponceau Red. Antibodies against proteins of photosystem II (D1), photosystem I (LHCI), large and small subunits of Rubisco (RbcL, RbcS) and ATP synthase were purchased from Agrisera (Vännäs, Sweden). As secondary antibody, the goat antirabbit IgG (H + L)-HRP conjugate (Bio-Rad, Hercules, CA, USA) was used. Blots were visualized using Immobilon Western chemiluminiscent kit (Millipore, Billerica, MA, USA) and medical X-ray film (FOMA BIOCHEMIA, Hradec Králové, Czech Republic) according to Pavlovič *et al.* (2011b).

MATERIALS AND METHODS FOR FIG. 4

Plant material

The plants of *Dionaea muscipula* used in our experiments were grown under greenhouse condition in well drained peat moss in plastic pots with irradiance up to 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR irrigated with distilled water at Department of Plant Physiology, Comenius University in Bratislava.

Simultaneous measurements of gas exchange and chlorophyll a fluorescence (Fig. 4A,B,C).

Measurements of chlorophyll *a* fluorescence were performed with a Fluorcam FC-1000 LC (Photon Systems Instruments, Brno, Czech Republic) attached to the PLC6 cuvette connected with a CIRAS-2 infrared gas analyser (PP-system, Hitchin, UK) as described previously (Pavlovič *et al.*, 2011a). Before each measurement the plant was dark adapted for 15 min. One leaf was placed inside a PLC6 cuvette. Because the diameter of the cuvette window was 18 mm, the traps used in the experiments did not exceed this size. After 2 min, the minimal fluorescence (F_0) at a light intensity $< 0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR was measured. Thereafter, the maximal fluorescence (F_m) was measured using a saturation pulse (light intensity 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, duration 800 ms). Then an actinic light was switched on (80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) and, after stabilization of the net photosynthetic rate (A_N), the saturation pulses were given every 168 s (3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, 800 ms duration) for determination of the maximal fluorescence in the light-adapted state (F'_m). The trigger hairs of the trap were mechanically stimulated by manipulation of the wire protruding outside the cuvette for 10 s at time 660-670 seconds. Photosynthetic parameters (F_v/F_m , ϕ_{PSII} , NPQ, and qP) were calculated according to Maxwell and Johnson (2000). Simultaneously, the infrared gas analyser monitored CO₂ and H₂O exchange every 2 s at a leaf temperature of $22 \pm 1^\circ\text{C}$, ambient CO₂ concentration of 380 $\mu\text{mol mol}^{-1}$, a relative air humidity of 60–70%, and a light intensity of 80

$\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (red emitting LEDs, $\lambda = 620 \text{ nm}$). Finally, the whole experiment was performed in the dark to measure dark mitochondrial respiration (R_D) and to calculate maximum quantum yield of PSII (F_v/F_m). Changes in the CO_2 concentration in the measuring chamber were recorded with a constant delay (the time taken for gas to pass from the cuvette to the infrared gas analyser). This delay was taken into account in figures presented here. After measurements, the leaves were dried at 70°C for 5 d, weighed, and the A_N was calculated in $\text{nmol CO}_2 \text{ g}^{-1} \text{ DW s}^{-1}$.

Measurement of electrical signals (Fig. 4D)

The extracellular electrical potential was measured with an intracellular electrometer (mod. 3100, A. M. Systems, Inc., Carlsborg, WA, USA) placed inside a Faraday cage according to Pavlovič *et al.* (2011a). Each cut leaf was fixed inside a small measuring chamber so that the lamina was dipped in a mild saline solution (0.1 mM CaCl_2 , 0.5 mM KCl), while the electrodes were placed on the abaxial surface of the closed trap. A glass micropipette containing an Ag/AgCl wire and filled with 3 M KCl was mounted with a half cell holder and connected to the headstage of the probe. An identical electrode was placed in the measuring chamber to serve as a reference electrode. The electrodes were connected to the amplifier and the signal was recorded continuously during trap stimulation at a 1 kHz rate of sampling frequency with home-made lab-view software. The trap was stimulated for 10 s and the electrical signals were collected. The action potentials were measured in the light ($80 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) at ambient CO_2 concentration.

Light response curve of photosynthesis (Fig. 4E)

The open trap of Venus flytrap was placed inside the leaf PLC6 cuvette connected with a CIRAS-2 infrared gas analyser (PP-system, Hitchin, UK) and the light response curve was measured as mentioned above. After the measurements, the digestive cycle in the trap was induced by strip of filter paper soaked with 50 mM NH_4Cl (for details see Libiaková *et al.*, 2014). Then after 36 hours the same trap in narrowed phase was placed inside the leaf PLC6 cuvette and the light response curve was measured again. After measurements, the leaves were dried at 70°C for 5 d, weighed, and the A_N was calculated in $\text{nmol CO}_2 \text{ g}^{-1} \text{ DW s}^{-1}$.

Quantification of jasmonates (Fig. 4F)

Five control and five induced traps (36 hours after induction as mentioned above) were collected for hormone analysis. Because it is known that wounding plant tissue caused by leaf cuts can increase the level of jasmonates, we used a fast method of sample collection. The samples were cut and immediately (up to 10 seconds) frozen in liquid nitrogen and stored at -80°C until extractions. Frozen plant material (15–20 mg) was homogenized using an MM 301 vibration mill (Retsch GmbH & Co. KG, Haan, Germany) at a frequency of 27 Hz for 3 min and extracted with 1 ml of ice cold 10% $\text{MeOH}/\text{H}_2\text{O}$ (v/v) extraction solution in the presence of stable isotopically labelled internal standards ($[\text{}^2\text{H}_6]\text{-}(\pm)\text{-JA}$, $[\text{}^2\text{H}_2]\text{-}(-)\text{-JA-Ile}$, $[\text{}^2\text{H}_5]\text{-IAA}$, $[\text{}^2\text{H}_4]\text{-SA}$, $[\text{}^2\text{H}_6]\text{-}(\text{+})\text{-cis,trans-ABA}$), provided by Olchemim (Olomouc, Czech Republic). The samples were incubated 20 min at 4°C by shaking using a laboratory rotator and centrifuged (3 min, 20.000 rpm, 4°C). Collected supernatants were further purified by solid-phase extraction (SPE). After sample application onto equilibrated 30 mg polymer-based solid-phase sorbent (Oasis® HLB, Waters), columns were washed with 1 ml of extraction solution and analytes were eluted by 3 ml of 80% $\text{MeOH}/\text{H}_2\text{O}$ (v/v). Samples were evaporated up to dryness under the stream of nitrogen and dissolved in 30 μl of mobile phase (15% acetonitrile: 85% 10 mM HCOOH , v/v). Final analysis of selected phytohormones was performed by an Acquity UPLC® System (Waters, Milford, MA, USA) coupled to quadrupole mass spectrometer Xevo™ TQ MS (Waters MS Technologies, Manchester, UK) in the following conditions. Reversed-phase separation was performed using Acquity UPLC CSH® C18 (100 x 2.1 mm; 1.7 μm ; Waters) column and analytes were eluted with linear (0 to 5

min, 15% A; 5 to 15 min; 45% A), logarithmic (15 to 28 min, 48.6% A) and linear (28 to 29 min; 100% A) gradient of acetonitrile (A) and 10 mM formic acid (B), as mobile phases, at a flow rate 0.4 ml min⁻¹. Phytohormones were determined in multiple reaction monitoring mode using mass transitions: 215.2 > 58.8, 326.2 > 151.1, 181.2 > 134.1, 141.1 > 96.8, 269.2 > 159.1, 209.2 > 58.8, 324.3 > 151.2, 293.3 > 275.3, 176.3 > 130.2, 137.1 > 92.8, 263.2 > 153.1 for [2H₆](±)-JA, [²H₂]-(-)-JA-Ile, [²H₅]-IAA, [²H₄]-SA, [²H₆]-(+)-*cis,trans*-ABA, (-)-JA, (-)-JA-Ile, *cis*-(+)-OPDA, IAA, SA and ABA, respectively. Optimized parameters of MS/MS measurements were following: capillary/cone voltage, 3 kV/ 23–30 V; source/desolvation temperature, 120/550 °C; cone/desolvation gas flow, 70/650 L h⁻¹; collision energy, 12–23 eV; collision gas flow, 0.21 mL min⁻¹. Quantification was carried out by using isotope dilution method and a calibration curves with a good correlation coefficients (0.9963–0.9999) were obtained (Floková *et al.*, 2014). Three independent technical measurements were performed for five biological replicates.

Statistical analysis

Statistical differences among different tissues were evaluated by one way analysis of variance (ANOVA) followed by Tukey's test (Origin 8.5.1., Origin Lab corporation, Northampton, MA, USA).

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