Mineral nutrition in hydroponically-grown *Pinguicula*

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Introduction

Hydroponic cultivation consists of growing plants on inert substrates with liquid mineral nutrient solutions. It has recently become popular for home-growing plants in limited spaces and for large-scale greenhouse production of crop plants. It is also a valuable tool for the scientific investigations of plant mineral nutrition. Despite the importance of mineral nutrition in the carnivorous syndrome (Juniper *et al.* 1989; Adamec 1997, 2002; Ellison 2006), there is no recent report on the potential usefulness of hydroponics as an investigation tool for studying mineral nutrition of carnivorous plants. The goal of this study was to evaluate the growth response of carnivorous plants of the genus *Pinguicula* on a peat-based and a rockwool-based hydroponic set-ups under various conditions of mineral deficiencies.

Materials and Methods

Plant material

In total, 21 *Pinguicula* species and hybrids were used in the study. Several Mexican species used in this study were derived from seed stocks originally collected from field sites in Mexico and later grown among hobbyists in Europe: *P. agnata* from El Chico, *P. gigantea* from either Ayautla or Synalta, *P. moranensis* from Zacapoaxtla. *P.* sp. "huahuapan" had an affinity with *P. rectifolia, P.* sp. "la vuelta" with *P. moranensis* and *P.* sp. "pachuca" had no affinity with any known species (for affinity relationships of these undescribed plants with described species, see Cieslak *et al.* 2005). Hybrids used in this study (*P. agnata* × *P.* sp. "huahuapan", *P. gigantea* × *P. moctezumae*, and *P. grandiflora* × *P. vallisneriifolia*) were all prepared by one of the authors (L. L.). Specimens of other species had a commercial origin (Nature et Paysages, France).

Hydroponic growth conditions

All plants were propagated *in vitro* according to Legendre (2011) before being immediately transferred to the hydroponic set-ups during this study. Starting stock plants had a rosette diameter of 0.5 cm unless otherwise stated.

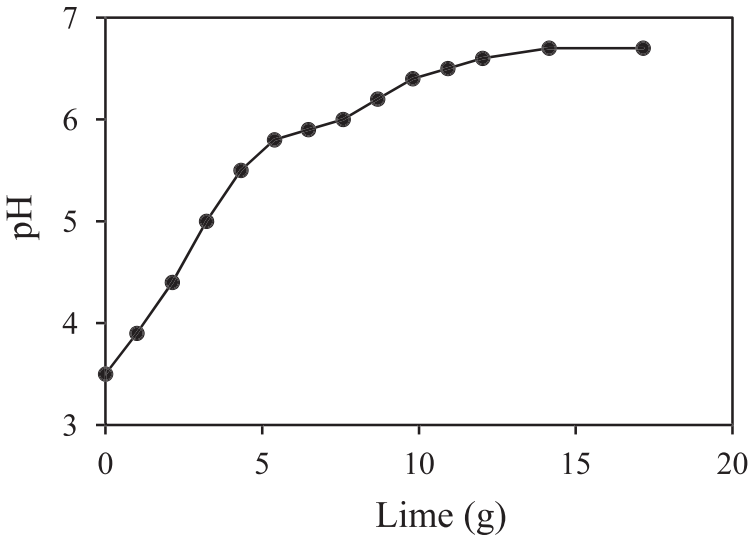

Figure 1: pH-titration curve of 100 g of Lithuanian peat with milled garden lime.

Growth conditions on peat

Plants were grown either on a mixture of peat and sand (2:1) (Lithuanian peat at pH 3.5 and 2 mm quartz sand) or on a similar mixture fertilized as originally published by Baker (1957) as the University of California (UC) basic mix (5 kg dry *Sphagnum* peat, half of the peat volume of 2 mm quartz sand, 11.34 g KNO_3, 11.34 g K_2SO_4, 113.6 g superphosphate 0-20-0, 341 g milled dolomitic lime, 113.6 g milled calcitic limestone). Both growth media were sterilized by autoclaving at 110°C for 2 h. They were laid in closed 70×45×20 cm polystyrene foam containers. Distilled water was added until it reached 2 cm above soil level. After 2 days, excess water was drained away by opening holes at the bottom of the containers. The stock plants were planted in the soil and the containers were placed in a greenhouse under regular top watering with no tray underneath (mist turned on for 15 min every other hour – deionized water was used). Temperature in the naturally lit greenhouse was 35-40°C during the days and 25°C at night. Relative humidity was kept above 90%.

Three to 6 specimens of each of the 13 tested species were planted in a random manner in the same container. Each experiment was replicated three times consecutively. Diameters of plant rosettes were measured after 4 months. Statistical analyses were made by Student t-test.

In order to assess the effect of lime addition on peat pH, a pH-titration curve was constructed by adding small increments of milled lime to a peat-water mixture/suspension. In this experiment, the pH rose markedly from 3.5 to subsequently stabilize at around 6.3-6.7 (see Fig. 1). The UC basic mix used in this study had a pH value of 6.5.

Growth conditions on rockwool

Rockwool slabs (50×44×10 cm) were irrigated with a mineral solution in a tidal table set-up. The mineral nutrient solution was pumped upward into the dish that contained the plants for 15 min at regular intervals 4 times per day. An overflow tube connected this dish

Table 1. Composition of the mineral nutrient solutions after Hoagland and Arnon (1950) modified by Jones (1997) for studying mineral deficiency effects.

Nutrient stock solutions	Concentrations in stocks (g/l)	Volume of stocks used in final solution (ml of stock per litre of final solution)					
		Complete	- N	- P	- K	- Ca	- Mg
1 M $Ca(NO_3)_2.4H_2O$	236	5	0	4	5	0	4
1 M KNO_3	101	5	0	6	0	5	6
1 M KH_2PO_4	136	1	0	0	0	1	1
1 M $MgSO_4.7H_2O$	246	2	2	2	2	2	0
50 mM FeNaEDTA	18.4	1	1	1	1	1	1
50 mM K_2SO_4	8.7	0	5	0	0	0	3
10 mM $CaSO_4.2H_2O$	1.72	0	100	0	0	0	0
50 mM $Ca(HPO_4)$	6.8	5	0	5	0	0	0
Micronutrient stock H_3BO_3 $MnSO_4.H_2O$ $ZnSO_4.7H_2O$ $CuSO_4.5H_2O$ $(NH_4)_6Mo_7O_{24}$	 2.86 1 0.22 0.08 0.02	1	1	1	1	1	1

to the nutrient solution storage container to allow the water table to stabilize 1 cm below the base of the plants. When the pump was turned off, the mineral solution flowed back to the storage tank through the pump. The formulas of the mineral nutrient solution used in this study are summarized in Table 1.

Plants (1 cm diameter) were laid on the rockwool and rooting took place in less than a week. Plants were illuminated by fluorescent tubes (industrial white) placed 40 cm above them (10 cm spacing) 16 h each day in an enclosed wooden box. Relative humidity oscillated from 45 to 55% between day and night, respectively.

All species used in an experiment were planted in the same dish (one dish per mineral nutrient solution – minimum of 6 plants per species per dish planted in a random manner). Each experiment was replicated at least 3 times in three independent set-ups. Significance of growth characteristics were estimated with the Student t-test ($p<0.001$).

Mineral content analyses

After 40 d of cultivation on the rockwool set-up, entire plants were collected. Their roots were removed and the leaves dried (at 45°C in a ventilated oven for a week) and powdered for later mineral nutrient analyses. The analyses were conducted on single plants and a minimum of three parallel plants were sampled individually per growth dish for each experimental condition.

The powdered leaf mass was digested and mineralized with concentrated acids and ana-

lysed for N, P, K, Na, Ca, and Mg content as described in Adamec (2002). Results of the nutrient analyses were expressed as % of dry weight (DW) for each nutrient.

Results and Discussion

A comparison of the growth increase of *Pinguicula* plants on fertilized (UC basic mix) and non-fertilized peat-sand revealed that most of the tested plants performed much better on the fertilized mixture (Table 2). The exceptions were *P. primuliflora, P. filifolia*, *P. moranensis*, *P.* sp. "la vuelta", and *P. lusitanica* (similar size plants on both media) and *P. sharpii* (died on the UC basic mix). Even though *P. moranensis* and *P.* sp. "la vuelta" did not attain a larger size, they produced more leaves (more active growth) on the fertilized medium. Typically, all plants had short-lived leaves that died (and rotted away) upon contact with the substrate when grown on the non-fertilized medium so that two layers of leaves were never seen stacked above each other. On the contrary, all Mexican *Pinguicula* species (except for *P. sharpii*) developed 2-3 layers of leaves stacked above each other in the fertilized medium (UC basic mix). In a later experiment, temperate European species (*P. vulgaris*, *P. grandiflora*, *P. dertosiensis*) also developed larger rosettes with more active growth on the UC basic mix (data not shown). The use of the UC basic mix generated some unusual phenotypes in some species, such as the appearance of red veins in *P. agnata* and the production of plantlets at the base of *P. lusitanica* and *P. crystallina* (Table 2). These

Table 2. Comparison of the rosette diameter *Pinguicula* species grown on pure peat/sand or on fertilized peat/sand (UC basic mix) after being watered with distilled water during 4 months (starting plant diameter was 0.5 cm). # Statistically-significant differences (t-test) at $p<0.05$ (*ns*, $p>0.05$).

Species name	Plant diameter (mean ± SD) on	
	non-fertilized mixture	fertilized mixture
P. agnata	2.5 ± 0.1	8.2 ± 3.3#
P. crystallina	2.0 ± 1.1	3.7 ± 0.5#
P. emarginata	2.3 ± 0.6	4.0 ± 0.1#
P. filifolia	4.3 ± 0.6	5.3 ± 0.6ns
P. gigantea "ayautla"	2.5 ± 0.2	24 ± 0.1#
P. gigantea "synalta"	9.0 ± 0.1	21 ± 1.0#
P. lusitanica	1.7 ± 0.3	1.7 ± 0.3ns
P. moranensis	5.1 ± 0.1	4.0 ± 0.5ns
P. primuliflora	1.7 ± 0.9	2.2 ± 1.6ns
P. sharpii	3.0 ± 0.2	Dead
P. sp. "huahuapan"	1.5 ± 0.8	2.3 ± 0.6#
P. sp. "la vuelta"	4.0 ± 1.0	4.2 ± 0.8ns
P. sp. "pachuca"	4.0 ± 0.1	5.8 ± 1.3#
P. agnata × *P.* sp. "huahuapan"	0.5 ± 0.1	4.0 ± 1.4#

could be detached and used to multiply the plants. All in all, these results show that *Pinguicula* species can be divided into two subgroups that differ in their growing response to a fertilised substrate. Coincidently, with the exception of *P. sharpii*, this correlates with a major evolutionary event within this genus (Cieslak *et al*. 2005), separating species of the subgenus *Isoloba* from those of the subgenera *Pinguicula* and *Temnoceras*. It also corresponds to a separation of species with opposite growth patterns: tropical growth-form *vs*. temperate growth-form.

Similar observations were made after growing a set of *Pinguicula* species on rockwool with the full-strength Hoagland and Arnon's (1950) hydroponic mineral nutrient solution. Temperate European (*P. vulgaris*, *P. longifolia*, *P. dertosiensis*, *P. nevadensis*, *P. grandiflora*, *P. vallisneriifolia*) as well as most Mexican *Pinguicula* species (*P. moctezumae*, *P. laueana*, *P. moranensis*, *P. rectifolia*, *P. emarginata*, *P. gypsicola*, *P. agnata*, *P. gigantea*) grew very well under these conditions, while the Mexican *P. sharpii*, Caribbean *P. filifolia*, and south-east USA species *P. lutea* died within weeks (data not shown). As shown in Table 3, the plants that fared well grew up to giant sizes after only 3 months of cultivation on the Hoagland and Arnon's (1950) complete nutrient solution (started to bloom shortly after the measurements were made, *ca* 100 days after planting).

Plants grown on a N-deficient solution were markedly smaller. They were pale green to yellow while plants were dark green on the complete solution. Very reproducibly, the leaves of some species became deep reddish-purple on their whole lamina (*P. laueana*, *P.* sp. "la vuelta"), or just along the veins (*P. grandiflora* × *P. vallisneriifolia*), a pigmentation that was at best faint on the complete (N-supplemented) Hoagland and Arnon's (1950) nutrient solution. At the end of the experiment, *P. laueana*, *P. vulgaris*, and *P.* sp. "la vuelta" were starting to enter dormancy on the N-deficient solution. This experiment has been replicated 3 times with similar results.

On a couple of occasions, it was observed that some of the plants on N-deficient nutrient solution (especially those with no reddish pigmentation) spontaneously caught large

Table 3. Effect of nitrogen deficiency on *Pinguicula* growth. Plants were grown in the rockwool hydroponic setup. Measurements were made on 2-4 parallel plants, 92 days after planting 1 cm diameter plantlets and irrigating with Hoagland and Arnon's (1950) full-strength or N-deficient nutrient solution. Differences in growth were significant for all species ($p<0.05$, t-test).

Species name	Rosette diameter (cm ± SD) on	
	complete solution	N-deficient solution
P. laueana	13.6 ± 0.4	3.8 ± 0.5
P. moctezumae	18.5 ± 0.7	7.5 ± 0.7
P. vulgaris	5.4 ± 0.9	1.1 ± 0.1
P. grandiflora × *P. vallisneriifolia*	24.7 ± 0.7	14.3 ± 5.2
P. gigantea × *P. moctezumae*	43.1 ± 1.2	7.5 ± 1.5
P. sp. "huahuapan"	10.5 ± 0.7	6.5 ± 0.5
P. sp. "la vuelta"	15.8 ± 0.5	5.1 ± 0.2
P. sp. "pachuca"	14.0 ± 1.9	7.7 ± 1.5

numbers of insects (10-100 times more root gnats than plants grown on the complete solution). This was very surprising because the trays with both mineral variant solutions were grown side-by-side in the same growth box (closed except during door opening). Unfortunately, insect captures were rare and all attempts to artificially introduce insects as prey failed so that this observation could not be repeated. Though the capture of insects is expected to provide the plants with some external source of nitrogen (and eventually additional minerals not provided by the nutrient solutions), the presence of captured insects on a plant never correlated with an increase in size or a greening of the leaves when compared to plants of the same tray that had not caught any insect. It is likely that the high nutrient concentrations in the nutrient solutions far prevailed over the effects of nutrients originating from the tiny preys.

Between day 17 and 30 after planting, plants of *P.* sp. "la vuelta" and *P.* sp. "pachuca" grown with distilled water (on rockwool or peat) and on N- or K-deficient nutrient solutions increased their rosette diameter by merely 11%, while those grown on the complete solution, Ca-, Mg-, and P-deficient solutions increased by 30%. The differences were significant using Bonferroni test ($p<0.05$). Plants grown on distilled water and on N- or K-deficient nutrient solutions had lighter green leaves (*P.* sp. "pachuca") or red-purple leaves (*P.* sp. "la vuelta") that were always sticky to the touch. Plants grown on the complete solution or on Ca-, Mg-, or P-deficient solutions had dark green leaves and were not sticky (mucilage or glands were not visible to the naked eye, either).

Table 4. Mineral nutrient content of *P.* sp. "la vuelta" and *P.* sp. "pachuca" plants grown under different fertilization regimes. Analyses were made after 40 days of cultivation either on rockwool with variants of the Hoagland and Arnon's (1950) solution (complete: original formula; none: no minerals; -Ca: Ca deficiency; -Mg: Mg deficiency; -K: K deficiency; -N: N deficiency; Dd water: distilled water) or on peat/sand mixture with distilled water. At this stage, the fastest growing plants had not yet doubled in size. Values represent the mean ± SD, n=6 (4 *P.* sp. "la vuelta" and 2 *P.* sp. "pachuca" plants, both species yielding similar results). For the complete solution, n=1.

Substrate	Nutrient solution	Plant mineral content (% DW)					
		N	P	K	Ca	Mg	Na
Peat	Dd water	0.71 ± 0.27	0.044 ± 0.003	0.85 ± 0.37	0.74 ± 0.18	0.34 ± 0.12	0.043 ± 0.012
Rockwool	Dd water	1.14 ± 0.25	0.055 ± 0.013	0.96 ± 0.46	1.06 ± 0.37	0.34 ± 0.11	0.028 ± 0.013
Rockwool	-Ca	2.25 ± 0.24	0.74 ± 0.15	4.56 ± 1.66	0.26 ± 0.18	1.04 ± 0.48	0.066 ± 0.017
Rockwool	-Mg	2.89 ± 0.24	0.50 ± 0.17	4.23 ± 1.28	1.90 ± 0.34	0.13 ± 0.024	0.054 ± 0.018
Rockwool	-K	1.76 ± 0.37	0.058 ± 0.009	1.10 ± 0.40	1.58 ± 0.53	0.52 ± 0.17	0.061 ± 0.047
Rockwool	-N	0.65 ± 0.15	0.051 ± 0.011	1.38 ± 0.40	0.79 ± 0.40	0.60 ± 0.25	0.044 ± 0.016
Rockwool	Complete	3.21	0.73	3.40	1.08	0.41	0.078

Plants grown on the complete solution had much higher tissue nitrogen, potassium, and phosphorus contents than those grown with distilled water (Table 4; the results were similar during distilled water watering on both rockwool and peat). Calcium and magnesium contents were not raised markedly by the use of the complete solution. Nitrogen deficiency appeared to have pleiotropic effects and led to decreased N, K, and P accumulation, while Ca and Mg remained almost unaffected. In agreement with the previous observation that plants grown on K-deficient media had similar phenotypes as those grown on N-deficient media, K-deficient media had the same effects on mineral accumulation in the plants as did N-deficient ones. Accumulations of N, P, and K were therefore found to be linked to each other, and all of these minerals were required for fast growth. On the other hand, Ca and Mg deficiencies did not affect P and N uptake but raised P uptake. The absence of Ca in the solution increased Mg accumulation, and *vice versa*. Thus, the absence of one of these two divalent cations led to a greater accumulation of the other three cations (K, Na, and the other divalent cation) in the plant. Even though tissue Ca content in the plant did not relate to plant growth, high Ca contents have been suggested to enhance plant tolerance to fungal attack (Legendre & Kibellis 2005). On the other hand, high foliar Ca content in carnivorous plants can indicate that the plants were grown under greenhouse conditions at low air humidity, *i.e.*, at high transpiration rates (see Adamec 2002).

In conclusion, the tissue nutrient content, but Ca and Mg, found in two Mexican *Pinguicula* species grown in nutrient-poor substrates (Table 4) was very similar to that reported for circumboreal *P. vulgaris* grown experimentally in slightly enriched peaty substrates (Aldenius *et al.* 1983; Karlsson & Carlsson 1984). Tissue Ca and Mg contents in these Mexican calcicole species remained surprisingly high, even when watered with distilled water (*cf.* Adamec 1997). However, when the two Mexican species were grown in the complete, rather concentrated, nutrient solution (Table 1) their tissue N, P, and K contents roughly tripled and were comparable with those reported for many species of rapidly growing wetland plants (*cf.* Dykyjová 1979). Studies conducted on temperate European *Pinguicula* species (*P. vulgaris, P. alpina, P. villosa*) revealed that they have the capacity to take up mineral nutrients for plant growth both by roots and leaves (Aldenius *et al.* 1983; Karlsson & Carlsson 1984; Hanslin & Karlsson 1996; for a review see Adamec 1997). In this context, this study proved a very great capacity of a large set of *Pinguicula* species (though not all) for nutrient uptake by roots (Table 2, 3) and unveiled a complex network of accumulation interrelationships among them (Table 4).

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