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Soilless Propagation of Haberlea rhodopensis Friv. Using Different Hydroponic Systems and Substrata

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Abstract. *Haberlea rhodopensis* (Gesneriaceae) is a tertiary relict with high conservation value, endemic to the Balkan Peninsula. The interest to this species is due mainly to its resurrection ability and multiple pharmacological activities, although it is valued also as an ornamental plant for use in rock gardens. *H. rhodopensis* plants are very slow-growing and no efficient method for their mass propagation has been set up until now. The present study reports the first trials on soilless cultivation of the species. Two aero-hydroponic systems with vertical and horizontal arrangements were used, studying the impact of different inert substrata, together with either leaf treatment with Indole-3-butyric acid (IBA) or leaf age and status, on rosette formation. In addition, plant propagation from seeds was tested on a small hydroponic system. Leaf rooting and survival were relatively high, up to 86.7%; however variants differed by root quality and time for root formation. Best results were obtained when IBA-treated leaves were either immediately put in perlite/agrolava substrate on the vertical system (46.7% leaves with rosettes, 2.9 well-shaped rosettes per leaf) or rooted in wet perlite prior to cultivation on the horizontal system in agrolava fractions (85.0% leaves with rosettes, 2.2 well-shaped rosettes per leaf). Rosettes were transferred to soil mixture and acclimatized in a greenhouse, and the largest 47 of them reached 10 cm in diameter for one year. Possible procedure improvements are discussed aiming at enhancement of *H. rhodopensis* soilless propagation.

Key words: endemics, Orpheus flower, resurrection plants, soilless cultivation, plant reproduction.

Introduction

Haberlea rhodopensis Friv. (Gesneriaceae) is a tertiary relict and endemic species to the Balkan Peninsula (Szeląg & Somlyay, 2009; Petrova & Vladimirov, 2010). In the tertiary period it was widely distributed in Europe and Asia; however, after the last glaciations its area shrank only to the territories of Bulgaria and Greece. This perennial herbaceous plant with leaf rosette and blue-violet flowers is growing on shady humid places, in crevices of limestone and silicate rocks in the zone of beech and pine forests at altitude from 150 to 1500 m. It is called "resurrection plant" as its vegetative parts are able to withstand up to 30 months of almost complete water loss and to recover fast under normal conditions (Gantschev, 1950). Known also as "Flower of Orpheus", *H. rhodopensis* was used by Thracians as a

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medicinal plant for treatment of many diseases. The species is protected in Bulgaria by the Biodiversity Act (2002) and the Medicinal Plants Act (2000), and is listed with the category "Least concern" in the Red List of Vascular Plants of Bulgaria (Petrova & Vladimirov, 2009). In the Red Data Book of Greece, it is listed under category "Vulnerable" (Theodoropulos et al., 2009) and is protected under the Greek Law Presidential Degree 67/81. Its natural localities fall in five Natura 2000 sites (Bazos & Petrova, 2013).

Currently *H. rhodopensis* is used as an ornamental plant at very small scale (Bazos & Petrova, 2013) because of its limited distribution. The increasing interest to the species is due mainly to its ability for resurrection (Georgieva et al., 2012) and its multiple pharmacological activities and potential rejuvenation effect (Popov et al., 2011; Berkov et al., 2011; Radev et al., 2012). However, the gathering of *H. rhodopensis* plants is forbidden because of its limited resources. The habitat is not threatened and the overall population trend is stable being mostly in inaccessible areas. Cultivation of the species is recommended for *ex situ* conservation in botanic gardens (Bazos & Petrova, 2013). Along with the phytochemical analyses there are some trials oriented to plant propagation. In this relation, the age structure of the populations has been studied as well as the seed germination under laboratory conditions and the propagation peculiarities (Bogacheva-Milkoteva et al., 2013). Experiments on *in vitro* propagation of *H. rhodopensis* were carried out as well (Djilianov et al., 2005). Monitoring of the species in Bulgaria determined nine populations with 67 localities, and a representative gene bank of *in vitro* plants originating from different populations has been established aiming at further reinforcement of some localities. However, authors reported many difficulties related to the low percentage of seedling survival and their very slow growth (Daskalova et al., 2011a, 2011b).

An alternative propagation of *H. rhodopensis* could be by hydroponic techniques, i.e. by using water solutions of mineral nutrients instead of soil, with or without artificial medium consisting of some inert material such as perlite, gravel, clay pebbles for mechanical support of the plants. Soilless cultivation has many advantages, among them most important being the crop yields, which increase significantly as plants are cultivated in greenhouse-type facilities under controlled ambient conditions and receive balanced nutrients according to their specific needs (Texier, 2013). Thus, crop productivity does not depend on the seasons, weather, precipitations, pest infestations, neither on the soil type, fertility, salinity, pH, and soil-related weeds, diseases and pests. Soilless technologies are environmentally friendly, as the use of water is minimized owing to the surplus solution recycling. There are different types of hydroponic systems; among them aeroponic ones are newer and of higher technology, where roots are not submerged in the solution and their humidification is ensured by mist; they are appropriate also for commercial level of plant growing (Mugundhan et al., 2011). In the aero-hydroponic systems no mist is used, and the roots are in meshy pots constantly or periodically sprinkled with nutrient solution by means of a pump. Until now, hydroponics has been applied mainly for vegetables; however, it could be very effective for rare species with conservation importance and medicinal and aromatic plants with resource deficiency, and there are some examples such as *Mentha, Stevia, Arnica, Ocimum* (Giurgiu et al., 2014). Smaller plants like *H. rhodopensis* could be grown in two or more shelfs, thus allowing establishment of vertical farming and additional increase of the cultivation efficiency.

The main goal of the present study was to stimulate the rooting of *H. rhodopensis* separate leaves and the formation of rosettes by applying different aero-hydroponic systems and substrata, as a first step of its successful propagation. Another challenge was to test the opportunity to obtain plants via hydroponically germinated seeds.

Materials and methods

Plant material. Leaves and seed capsules were collected from randomly chosen *H. rhodopensis* plants of the population in the Rhodope Mountains, near the village of Sitovo, Plovdiv district, in October 2017.

Leaf rooting and rosette formation. Leaf rooting was studied by two aero-hydroponic systems: vertical Green Diamond (GHE), and horizontal Aeroflo-20 (GHE) with 120 and 20 meshy pots (all 8 cm in diameter), respectively. As a control, 36 fresh leaves were put into a flower box; stalks dipped in wet perlite, covered with a glace to keep air humidity high, and left in the greenhouse.

Two factors with possible influence on the rooting: substrate and indole-3-butyric acid (IBA) were examined on the vertical system. The substrate consisted of fine material (peat cubes, mineral wool, or perlite, in a semi-permeable tissue), surrounded by larger particles (agrolava pebbles or keramzite pellets, medium fractions). The treatment with the auxin was done by dipping the stalks of the leaves into 25% IBA powder (Rhizopon BV, The Netherlands) prior to their embedding in the substrate. The combination of substrata and IBA-treated or control non-treated leaves resulted in eight parallel variants distributed in four pairs: peat cubes surrounded by agrolava pebbles, with IBA-treated leaves (variant PC-AP-IBA) or with control leaves (variant PC-AP-C); mineral wool surrounded by agrolava pebbles, with IBA-treated leaves (variant MW-AP-IBA) or with control leaves (variant MW-AP-C); perlite surrounded by agrolava pebbles, with IBA-treated leaves (variant P-AP-IBA) or with control leaves (variant P-AP-C); and perlite surrounded by keramzite pellets, with IBA-treated leaves (variant P-KP-IBA) or with control leaves (variant P-KP-C). Total 15 leaves freshly gathered from the native plants were used per variant.

On the Aeroflo-20 horizontal system three variants were tested consecutively, with 20 leaves per variant, thus studying the impact of the substrate and the leaf age and status. Two different substrata were used: perlite in a semi-permeable tissue surrounded by agrolava pebbles, and agrolava pebbles alone. All leaves were treated with IBA powder as described for the vertical system. The first trial was with substrate perlite surrounded by agrolava pebbles and leaves freshly gathered from the native plants, (variant P-AP-FL). Leaves of the second trial were stored in wet perlite during the 6 months of the first trial, and they formed meantime short roots and some nucleus of rosettes. The substrate used was agrolava pebbles (variant AP-6m-L). Leaves for the last trial were taken from entire *H. rhodopensis* plants after their wintering in the greenhouse, and put in wet perlite for one month prior to the beginning of the experiment. The substrate was agrolava pebbles (variant AP-1m-L).

The nutrient solution comprised distilled and tap water in proportion 3:1, supplemented with Flora Micro, Flora Grow, and Flora Bloom (GHE), and with the bacterium *Trichoderma harzianum* (0.1 g/L). This bacterium was added preventively as a biocontrol agent against some fungal pathogens and for enhancement of the root growth (Harman, 2000). The pH was maintained between 5.5 and 6.5, and electrical conductivity (EC) between 0.40 and 0.98 $mS.cm^{-1}$. The substrate wetting on the two systems was constant but the solution was running in a different way: strong streams of the horizontal system were laterally sprayed directly on the substrate, whereas the substrata in the vertical system were gently sprinkled by drops falling obliquely from about 50 cm of distance. The experiments were conducted in a room phytotron with mixed daylight and artificial light (Metal Halide Superveg lamps, 250 W) 16/8 h photoperiod, between 50 and 54 $\mu mol\ m^{-2}\ s^{-1}$, and variations around-the-clock of the temperature (23 ± 4 ºC) and air humidity (32 to 68%).

Criteria for selection of the best variant were: leaf rooting and survival rate, leaf capacity to form rosettes, and number of well-shaped large rosettes at least 3 cm in diameter.

Propagation by seeds. Seeds were germinated on Cutting Board hydroponic system (GHE) with 27 meshy pots, 6 cm in diameter. Fresh dust-like seeds were stratified at 6 °C for 2 weeks before sowing on the pots which were filled in with peat cubes surrounded by agrolava pebbles and wetted with standard nutrient solution (pH 5.5-6.5; EC 0.4-0.6 $mS.cm^{-1}$) through bubbles formed during the permanent pump stream of the solution. Seeds were darkened during the first month, and after the beginning of germination they were exposed to mixed daily and artificial light between 22 and 27 $\mu mol\ m^{-2}\ s^{-1}$, with light/dark photoperiod 16/8 h. Seedlings with 5-10 mm diameter of the rosettes were transferred to a tray with soil mixture and perlite in proportion 1:1 (v/v), and grown in the phytotron, until they grew enough to be transferred in pots. In addition, other seeds were put on 10 water damped coco-fiber cubes, in a tray with transparent cover, periodically removed after seed germination, when condense appeared, in order to control air humidity.

Rosettes acclimation in a greenhouse. All rosettes obtained in different ways were transferred in pots (9 cm in diameter) with soil mixture (Biobizz worldwide) containing peat moss, sphagnum peat and perlite (NPK:14-16-18), and sand in proportion 2:1 (v/v), the larger ones alone, and the rosette clusters together with the initial leaf, partially exhausted during the soilless cultivation. They were additionally grown in a room phytotron under controlled conditions (day-and-night temperature and air humidity fluctuations: 24 ± 4 °C, 55 ± 15%) and finally in an unheated greenhouse. Final number of survival rosettes was counted 13 months after the beginning of the first experiments. Well-shaped rosettes with diameter over 3 cm were defined as large, while the minor ones, often forming clusters of individuals not well distinguished from one another were called small.

Statistical analysis. Statistical analyses were done using Excel "Data analysis tool – Anova: Two Factor Without Replication". All percentage data were transformed using Excel formula: ASIN(SQRT()) to normalize error distribution prior variance analysis.

Results and Discussion

Leaf rooting and formation of rosettes took about 6 months on both vertical and horizontal aero-hydroponic systems (Fig. 1a, 1b); however, some differences were noticed.

Leaf rooting and rosette formation on the vertical aero-hydroponic system

Both substrate and IBA influenced the success of the overall process of rosette formation on Green Diamond vertical system (Table 1). In most variants the percentages of leaf rooting and survival were relatively high: 60.0 to 86.7%. Non-rooted leaves turned brown and died.

Concerning the percentage of leaf rooting, no significant differences were noticed neither between variants differing by their substrate, nor between IBA-treated and control non-treated leaves (Table 1-A). However, some variants differed regarding the root quality and the time of their formation. Thus, best results were obtained in variant P-AP-IBA where all survived leaves developed vigorous root system for 12 weeks (Fig. 1c). Rooting in the other variants took 8 to 12 weeks more. Only the organic peat substrate proved inappropriate and roots in variants PC-AP-C and PC-AP-IBA remained short and weak, many leaves dropped out and only few rosettes formed; therefore, these variants were further ignored.

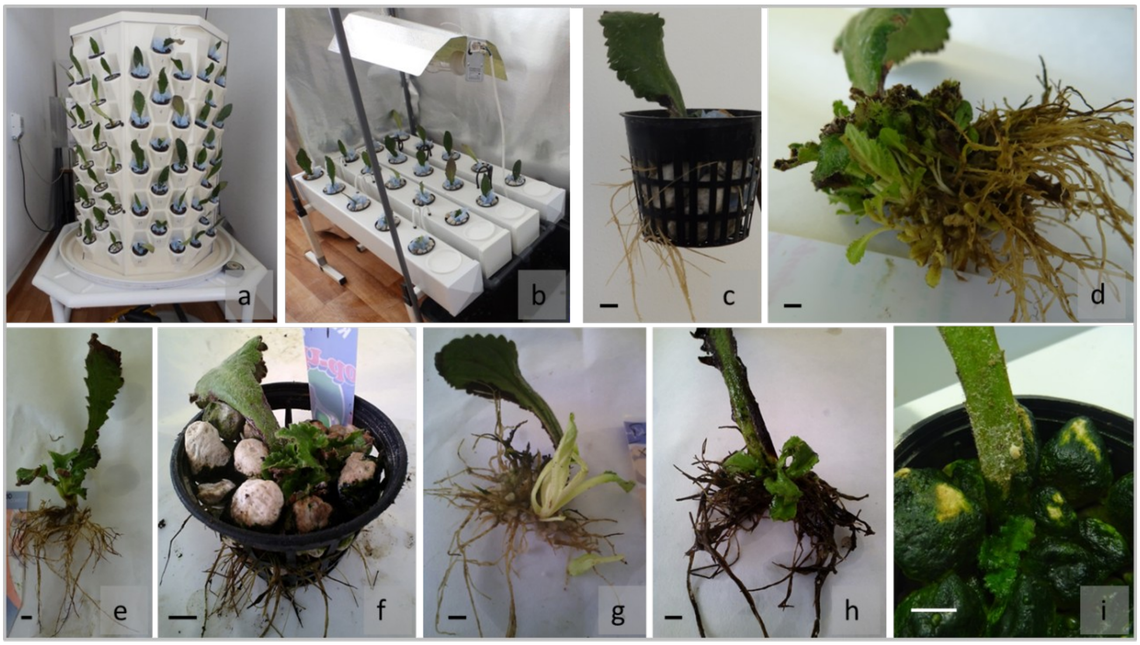

Fig. 1. *H. rhodopensis* rosettes formation on aero-hydroponic systems: a) Vertical Green Diamond system; b) Horizontal Aeroflo-20 system; c) Roots on variant P-AP-IBA, 12 weeks old; d) Numerous rosettes formed at the leaf base in variant MW-AP-IBA; e) A single rosette formed in variant MW-AP-C; f) Rosettes formed on Aeroflo-20 in variant AP-6m-L; g) Etiolated rosette formed under the agrolava pebbles; h) Partial putrefaction of the root system; i) Algae development on the substrata. Scale bars = 10 mm.

Table 1. Leaf rooting and rosettes formation on the vertical aero-hydroponic system, for 6 months. Effect of substrate and IBA-treatment on leaf rooting (A), rosette formation (B), and number of large rosettes (C), estimated with ANOVA Two-factor without replication. Legend: * - plus additional small rosettes; PC – peat cubes; AP – agrolava pebbles; IBA – IBA-treated leaves; C – control non-treated leaves; MW – mineral wool; P – perlite; KP – keramzite pellets.

Variant	Rooted leaves (%)	Leaves with rosettes (%)	Number of large rosettes	Large rosettes per leaf
PC-AP-C	53.3	13.3	2	1.0
PC-AP-IBA	46.7	6.7	1	1.0
MW-AP-C	73.3	33.3	6	1.2
MW-AP-IBA	66.7	46.7	17*	2.4
P-AP-C	60.0	40.0	8	1.3
P-AP-IBA	86.7	46.7	20*	2.9
P-KP-C	66.7	26.7	10	2.5
P-KP-IBA	53.3	40.0	7*	1.2

(A)

Source of variation	Df	MS	F	p-value
Substrate type	3	0.026007676	1.247809922	0.42995825
IBA Treatment	1	0.000162102	0.007777413	0.935283122
Error	3	0.020842658		

(B)

Source of variation	Df	MS	F	p-value
Substrate type	2	0.005684031	6.592599167	0.131707203
IBA Treatment	1	0.020022653	23.22319022	0.040464754
Error	2	0.000862184		

(C)

Source of variation	Df	MS	F	p-value
Substrate type	1	6.25	25.0	0.126
IBA Treatment	1	132.25	529.0	0.028
Error	1	0.25		

The choice of the relevant substrate is considered as very important for the success of the soilless cultivation. It should depend on the plant species requirements as well as on the way the constant wetting is realized (Giurgiu et al., 2014). Optimal conditions are determined mostly for vegetables usually grown on mineral wool that keeps 80% of the nutrient solution and contains 15% air while its fibers represent only 5% of the substrate. The proportion between the solution and the air inside is important for the root growth and over-wetting could cause their putrefaction. Giurgiu et al. (2014) recommended fraction size between 2 and 7 mm of diameter like perlite. Among the substrata used in the present experiment perlite has better perviousness compared to mineral wool and peat cubes, and both agrolava and keramzite were of larger fraction sizes than perlite. In nature, *H. rhodopensis* plants are adapted to very specific conditions presenting combination of humid ground and steep slopes and escarps allowing water drainage; therefore, we tested several substrata differing in fraction size and capacity to retain water.

Single rosettes of *H. rhodopensis* began to appear after 3 months of leaf cultivation. For the 6-month duration of the experiment, half of the survived leaves gave rise of rosettes (38 from 76 rooted leaves). Unexpectedly, the formation of rosettes was significantly enhanced by IBA-treatment ($p < 0.05$, Table 1-B). No significant difference was noticed between the three substrata regarding the percentage of the leaves forming rosettes (Table 1-B).

The total number of well-shaped rosettes with 4-6 leaves each, and diameter between 4 and 8 cm obtained in all variants was 71, and 63.4% of them formed in the variants stimulated with IBA (Table 1). In these variants additional numerous small rosettes were observed whose number was impossible to count (Fig. 1d). The highest numbers of large rosettes were noticed in variants P-AP-IBA and MW-AP-IBA: 20 and 17, respectively, while in the corresponding control variants P-AP-C and MW-AP-C their numbers were much lower (Fig. 1e) ($p < 0.05$, Table 1-C). The average numbers of rosettes per leaf in these variants were twice higher when leaves were IBA-treated, the highest one being 2.9, recorded in variant P-AP-IBA (Table 1). All these parameters, along with the fastest leaf rooting, distinguished variant P-AP-IBA as the best one on the vertical system.

Leaf rooting and rosette formation on the horizontal aero-hydroponic system

On the Aeroflo-20 horizontal system the trials took different time due to the differences in the leaf age and status which proved to be of crucial importance (Table 2). Thus, the previous leaf rooting in variant AP-6m-L facilitated the formation of rosettes: they were numerous and well-shaped, grew faster and reached the size suitable for potting in soil mixture for only one and a half month (Fig. 1f). Eighty-five percent of the leaves in this variant formed rosettes. In variant AP-1m-L, with the same substrate, leaf stalks had callus tissue at the beginning of the treatment, and most of them developed roots, but only 25% of the leaves formed rosettes and half of them represented clusters of very small rosettes. The worst variant was that with freshly gathered leaves put in perlite surrounded by agrolava pebbles (P-AP-FL) due to the rooting difficulties.

Comparison between the two aero-hydroponic systems showed important differences related to the way of the substrate wetting. On the horizontal system the excessive solution could not strain off from the dense perlite particles thus causing over-wetting, whereas the drainage on the vertical system was easy. The larger agrolava fraction applied alone was most appropriate for the horizontal system, avoiding the substrate over-wetting. However, the space between agrolava pebbles allowed formation of some rosettes below the substrate surface, which caused their etiolation (Fig. 1g). Young roots appearing on agrolava pebbles in variant AP-1m-L were vulnerable to the excessive solution, which caused partial root putrefaction (Fig. 1h). Although 16 leaves rooted (80%) only 6 of them survived and finally 5 leaves formed either one well-shaped rosette or a cluster consisting of 4 to 7 small rosettes (Table 2).

Also, algae multiplication was observed after about 2 or 3 months from the beginning of the cultivation. Microscopic Zygnemophyta green algae that are largely spread easily contaminated the solutions used in the soilless cultivation. Their fast growing and reproduction led to formation of compact algae layer on the substrate surface and hampered the development of *H. rhodopensis* rosettes (Fig. 1i).

Acclimation of rosettes to soil and growth rate comparison

Seven months after the transfer to soil mixture, the number of rosettes formed on the vertical Green Diamond system decreased almost twice (47.9% survival rosettes) and the effect of IBA was lost in a long-term period because most of the smaller rosettes died. New rosettes formed rarely after the transfer to the soil. At this stage the survived rosettes in the greenhouse were with diameter more than 10 cm and height of about 7 cm, with several well-shaped leaves (Fig. 2a). At the same, the rosettes obtained during the second trial on the Aeroflo-20 horizontal system (variant AP-6m-L) and transferred to soil mixture 2 months later, were about 7 cm in diameter (Fig. 2b). Young rosettes in the natural populations have also 4-6 leaves, and numerous mature plants are growing close to each other, the length of the leaves depending on the environmental conditions (Fig. 2c). A total of 47 rosettes (34 obtained on the vertical and 13 on the horizontal aero-hydroponic systems) were further grown in the greenhouse.

The control leaves under glace cover in the flower box with wet perlite needed about 2 months to develop roots. Their survival was high as 86.1% of the leaves rooted and were transferred to pots with light soil mixture and agrolava pebbles in proportion 2:1. At the end of the 13th month, 13.9% of the leaves had rosettes and the total number of rosettes was 11, but they were much smaller compared to those obtained on the aero-hydroponic systems (Fig. 2d). Even the 5-month old rosettes from the last test on the horizontal system were larger (Fig. 2e).

Formation of daughter rosettes on *H. rhodopensis* leaves under *ex situ* conditions was previously reported as possible but not effective, even if only ten leaves were used for the test (Bogacheva-Milkoteva et al., 2013). Authors used light soil for leaf rooting and observed rosettes on three of the leaves but they were extremely small and unable to grow and develop. Taking in consideration the slow rate of *H. rhodopensis* plant growth in nature, our results with soilless production of rosettes on leaves are promising as the largest rosettes reached autonomy for only one year.

Trials for propagation by seeds

Seeds were abundant and too small (Fig. 3a), therefore they were sown on the peat cubes without counting, most probably several hundred on each cube. First seeds germinated two weeks after their setting on the Cutting Board hydroponic system. At the end of the first month, numerous seeds germinated and formed small green cotyledons, on 23 of all 27 pots, running into several dozens of seeds per peat cube (Fig. 3b, 3c). The growth of the seedlings was very low; rosettes with diameter about 5 mm, consisting of 4 to 6 leaves, formed at the end of the third month. Several rosettes at the periphery of the pots grew faster, reaching 10 mm for the same time, probably because they had more space (Fig. 3d). Meantime, mosses developed on the peat cube surface and the substrate was infested by insects and fungi, causing putrefaction of part of the rosettes (Fig. 3e). Plantlets were treated with bio fungicide and bio insecticide but the infection was heavy and the effect of the treatments was temporary. At the end of the 5th month, the largest 30 rosettes were transferred to a tray with soil mixture and perlite in proportion 1:1, but 2 months later only one rosette survived. After 6 more months it reached diameter 4 cm (Fig. 3f) but its growth was extremely slow.

Table 2. Rosette formation on the Aeroflo-20 horizontal aero-hydroponic system. Legend: P – perlite; AP – agrolava pebbles; FL – fresh leaves; L – leaves stored in wet perlite: 6m – for 6 months, 1m – for one month.

Variant	Time	Leaves with rosettes (%)	Number of well-shaped rosettes	Number of clusters with small rosettes
P-AP-FL	6 months	10.0	1	1
AP-6m L	1.5 months	85.0	38	0
AP-1m L	2 months	25.0	2	3

Fig. 2. Potted rosettes and native plants: a) Rosettes formed on the vertical aero-hydroponic system, 7 months after the transfer to soil mixture; b) Rosette formed on the horizontal aero-hydroponic system, 5 months after the transfer to soil mixture; c) Native mature plants in their habitat; d) Rosettes formed at the base of a control leaf in wet perlite in flower box, 13 months after the start; e) Rosette cluster at the base of a leaf from the last trial on the horizontal system, 5 months after the experiment start. Scale bars = 10 mm.

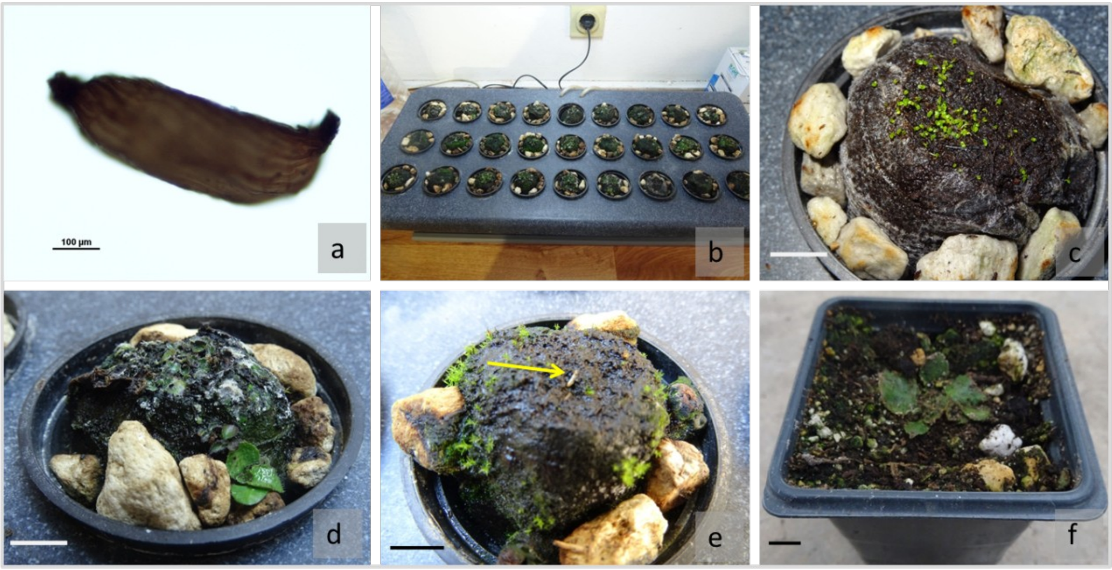

Fig. 3. Seeds derived rosettes: a) Seed; b) Seed germination on Cutting Board hydroponic system; c) One month old seedlings; d) Rosette with diameter 10 mm; e) Infected substrate with insects (the arrow indicates insect larvae); f) Rosette with diameter 3 cm in the greenhouse, 13 months after seed germination. Scale bars = 10 mm.

Coco-fiber cubes seemed less appropriate as few seeds germinated on this substrate, and some of them necrotized 2 weeks later, while the rest remained several months at cotyledon phase and finally died.

Obviously, there was no problem with seed fertility and germination, which corresponds to the results reported about the high rate of pollination of the native plants from several populations (Bogacheva-Milkoteva et al. 2013). The same authors reported easy seed germination in laboratory conditions on wet filter paper in petri dishes, but extremely slow growth (seedlings at cotyledon stage with length less than 4 mm for 5 months), and only 1% of survival rosettes.

Seeds of *H. rhodopensis* were germinated also *in vitro* after a drastic surface disinfection procedure to eliminate microbial contamination (Djilianov et al., 2005). Authors obtained plantlets after long germination and succeeded first in finding out the appropriate medium for this species, and then in multiplying rosettes by direct organogenesis, using pieces of *in vitro* leaves as explants. They obtained clusters of very small plantlets, and finally fully developed *in vitro* plantlets, for about 7 months. *Ex vitro* adapted plantlets were designed to serve as uniform and initial plant material of high quality for further research. Other authors tried to build a strategy for conservation of *H. rhodopensis* including *in vitro* propagation as a tool for production of numerous plants intended to be used for reinforcement of the natural populations (Daskalova et al., 2011a, 2011b). Authors claimed to have created *in vitro* gene bank of the species with plants from many populations; however, their later work was focused on research such as genetic diversity and chloroplast genome, and there was no more information about plant propagation, most probably because of the difficult *ex vitro* adaptation of the plants and their very slow growth.

Conclusions

First trials on soilless propagation of *H. rhodopensis* using leaves as initial material are promising, as plant production, growth and development have been enhanced; seeds are not recommended. Among the two tested aero-hydroponic systems, the variant with perlite/agrolava substrate was the best one on the Green Diamond vertical system, while the horizontal Aeroflo was more appropriate in combination with agrolava pebbles alone as substrate. However, fraction with smaller pebbles in horizontal system should be used to avoid rosette development below the surface. Rooting of IBA-treated leaves in wet perlite for two months should be applied prior to the soilless cultivation in order to synchronize the process of rosette formation and to ensure fast growth of the rosettes, thus allowing their transfer to pots with soil mixture at the end of the second month. Use of lower concentration of IBA would be better, ensuring rooting but avoiding excessive formation of miniscule rosettes, thus increasing rosette survival. Shortening of the soilless cultivation stage will contribute to avoidance of algae contamination as well. The whole optimized procedure including rosette strengthening in greenhouse and development to 10 cm in diameter could be performed in 9 months. Plants are designed for further grown in the *ex situ* collection of IBER-BAS with consideration of their specific requirements to the environmental conditions.

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