Thesis of PhD Dissertation

POSSIBILITIES OF ORCHID GENE PRESERVATION AND PROPAGATION

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ABBREVIATIONS

Sources of acquiring (plant):

Sa.: Own material

Szka.: Propagated material from trade

Ka.: material from trade

Sources of acquiring (seed):

Mcs.: Seed exchange

Fb: Pollination in ELTE Botanical Garden

Gy.: Collection from temperate zone

Tgy.: Collection from tropical habitat

Mb.: Seed bank

Ogy.: Orchid collectors

Media:

Fa: Fa medium, Fast (1982)

Fast-Kukulczanka modified Fa medium, (Prof. Kukulczanka oral notification)

FM: modified Fa medium, R. Eszéki, Szendrák (1992)

FMB: FM medium supplement – dried potatoes (10 g/1200 ml)

KC: KnudsonC medium (Knudson, 1922)

KM: modified KC medium (R. Eszéki, Győrváry, 2000)

MS: Murashige, Skoog medium (Murashige, Skoog, 1962)

$^1/_2$ MS: half-strong Murashige, Skoog t medium (Murashige, Skoog, 1962)

M: modified half-strong Murashige, Skoog medium (R. Eszéki, 2007)

MV: M medium/ sources of vitamin instead of Yeast 250 mg l^{-1} Polivitaplex 200 mg l^{-1}

MCN: M medium, supplement - fresh Jerusalem artichoke (10 g/35 ml)

MCL: M medium, supplement - homogenized Jerusalem artichoke (100 ml l^{-1})

MCS: M medium, supplement - dried Jerusalem artichoke (1,5 g/35 ml)

MC_{25} : M medium, supplement - homogenized Jerusalem artichoke (25 ml l^{-1})

MC_{50}: M medium, supplement - homogenized Jerusalem artichoke (50 ml l^{-1})

MC_{100} : M medium, supplement - homogenized Jerusalem artichoke (100 ml l^{-1})

MC_{200} : M medium, supplement - homogenized Jerusalem artichoke (200 ml l^{-1})

DEB: Debergh medium (Van Waes és Debergh 1986)

DEB+K: Debergh medium + 20 ml coconut-water

ZAK: ZAK medium (Borris, 1969)

Yeast extract: yeast extract for laboratory use (powder like water soluble) Oxoid product

Soil mix:

Tp.: Novobalt peat and perlite

Tp.+K.: Novobalt peat and perlite + fir bark

Tp.+S.: Novobalt peat and perlite + Sphagnum moss

Tp.+Ko.: Novobalt peat and perlite + coconut fibre

Tp.+A.: Novobalt peat and perlite + clay-granulate

PRELIMINARIES AND PROPOSED AIMS OF THE STUDY

Introduction

The topic of my dissertation is connecting with the tropical and the native orchid species too. After I became a member of the staff of ELTE Botanical Garden in 1986 my duty was to provide for the orchid collection. During the years I tried to list the old botanical collection and work out a system in which the new acquired plants were classified too. This meant a started point to the sterile propagation. The orchid laboratory started to work in 1987. Our most important aim was from the outset to propagation of botanical species from seeds, because we like to maintenance and increase the collection respecting the importance of genetic variability. The orchid collection of the Botanical Garden has given an adequate background to the propagation and maintenance of endangered orchid species considering not only our plant material but for other collectors and institutions too, because here they can get assistance solving their propagation and identification problems.

Raising of the problems, aims of the experiments

1. View of the efforts of the conservation of nature the first step meant for saving orchids working up the different methods of international cooperation and accepting the legal regulation. Not to be the only response on the human damage the prohibition, there is always a curiosity in the people observing and taking photos of these speciality. The botanical gardens have an important role in this than the place for the *ex situ* conservation. The cultivation of orchids in collections is a method for gene protection and the knowledge about their needs in habitats and botanical features mean the base of this. I took part in this work in the orchid collection of ELTE Botanical Garden with my morphological observations at tropical-subtropical orchids, the examination during the adaptation of new species and with the evaluation of my results.

2. Orchids have a very special lifestyle, it's evidence also in their reproduction. In nature orchid seeds, which have no stored food reserves can become plants only the supporting of mycorrhizal associations (Molnár, 2001). It isn't possibly the germination of seeds among artificial conditions without the presence of symbiotic fungi (symbiotic seed sowing) or without the nutrients obtained from the fungus (asymbiotic seed sowing) (Domokos, 1972). The framework and the orchid collection of the ELTE Botanical Garden give a base for the propagation and maintenance of endangered orchids. During my work the most important material for asymbiotic seed sowing the different seed sources. It's an important role to clear which sources are reliable.

3. During *in vitro* propagation of orchids a possibility for optimalization of applied media the application of complex additives. My aim was on the base of literature's items to examine already successful applied additives in new orchid cultures and to find new, also efficient and easy prepared plant originating material and to work out the using method too.

4. My aim was to work out an optimal soil mix for transplantation of tropical orchids (*Paphiopedilum spp.)* originated *in vitro*. Part of my work was observing a connection of the bulb size and the leaf and flower development during the acclimatization of sterile seedlings of *Liparis loeselii* (L.) Rich., our endangered fen orchid.

The main aims of my work were the followings:

- Presentation of the maintenance of tropical orchid species in collection.
- Suggestion on some morphological terms which are missing in Hungarian.
- Examination of the possibilities for preservation of biological and genetic bases.
- The development of asymbiotic propagation methods of orchid species (*Paphiopedilum venustum* (Wall. ex Sims) Pfitzer, *Paphiopedilum sukhakulii* Schoser & Senghas, *Liparis loeselii* (L.) Rich.)

- Examinations of transplantation and acclimatization of orchid seedlings (*Paphiopedilum venustum* (Wall. ex Sims) Pfitzer, *Liparis loeselii* (L.) Rich.) originated from *in vitro.*

MATERIAL AND METHODS

1. Stock examination in the orchid collection of the ELTE Botanical Garden

1. 1. Comparison of my morphological observations with the literary data

- There are many books available in Hungarian about the cultivation and importance of orchids originating from subtropical and tropical areas (Domokos, 1972; Makara, 1982; Tátrai, 2004; Ježek, 2005), but without detailed botanical knowledge, thus several important morphological terms are missing in Hungarian, while they can be found in German and English.

I reported about my experiences related to the literature which were collected during the time I've been taking care of the orchid collection.

1. 2. Newly acquired orchid species adaptation in the orchid collection

In December 2009 The ELTE Botanical Garden got a possibility in the framework of The New Hungary Development Plan for expansion of the botanical orchid collection which was enriched with 107 new specimens which related 54 different genera. Most of the plants were botanical species I noted the data of the plants concerning the source of acquiring, their place in the greenhouse and the applied transplantation methods. I dealt with the problems of nomenclature according to the correct labels were rechecked with the present accepted database, 'The Plant List' (XXX, 2010). There were three types of origin determined of the new acquired species: Own material – Sa.; Propagated from trade – Szka.; Acquired from trade – Ka. Two states measuring of the plant material were happened on 20th December 2009 and after that on 15th May 2010. I rechecked by

the flowering plants the correct identification with the use of the available orchid literature (Makara, 1982; Griffiths, 1994, Ježek, 2005, Lavarack et al., 2006, XXX, 2010).

2. Observations about seed-germination in the laboratory of ELTE Botanical Garden

2. 1. Correspondence between germinating power of seeds and the sources of acquiring

In my dissertation I evaluated the germination results of 6 years, from 18. June 2006 to 07. June 2012. In this period I achieved 240 seed sowing. The object of the measure was the successful germination from the different sources (Mcs.: Seed exchange, Fb: Pollination in ELTE Botanical Garden, Gy: Collection from temperate zone Tgy.: Collection from tropical habitat, Mb.: Seed bank, Ogy.: Orchid collectors).

We applied for seed sowing of orchids from temperate zone and also tropical-subtropical species the FM (modified Fast medium).

2. 2. In vitro experiments with plant origin additives

The first experiment was adjusted with *Paphiopedilum venustum* (Wall. ex Sims) Pfitzer seedlings had 2-3 leaves and 1-2 roots on 19. March 2008. I applied the M basic medium (modified $^1/_2$ MS (Murashige, Skoog, 1962) medium), in 5 treatments. In the case of MV medium 200 mg l^{-1} powdered Polivitaplex tablet was added instead of 250 mg l^{-1} Yeast extract as B vitamin source. In the case of the other media I added the Jerusalem artichoke in various prepared forms: MCN medium - fresh Jerusalem artichoke 10 g/35 ml, MCL medium - homogenized Jerusalem artichoke 100 ml l^{-1}, MCS medium - dried Jerusalem artichoke 1,5 g/35 ml.

The next experiment was adjusted with *Paphiopedilum sukhakulii* Schoser

& Senghas seedlings had 2 leaves and 1-2 roots on 18. December 2009. I supplemented with increasing doses of Jerusalem artichoke the examined media (25-50-100-200 ml l^{-1}). In either case the material of the experiments were placed in the culture room on 24 ^{o}C 10/14 h photoperiod, 1000 lux light intensity.

The fen orchid (*Liparis loeselii* (L.) Rich.) is one of the most endangered orchid from our swampy areas. My aim was on the base of the seed germination experiments of Illyés and Molnár (2011) to choose a maintenance medium which enable the valuable *Liparis loeselii* (L.) Rich. stock preservation for a long period in the ELTE Botanical Garden (2007-2009). I examined various media (Fast (Fast 1982)-Kukulczanka, DEB (Van Waes és Debergh 1986), ZAK (Borris, 1969), FM (R. Eszéki és Szendrák, 1992), KM (R. Eszéki és Győrváry, 2000), FMB (R. Eszéki et al. 2009). I deposed the *Liparis loeselii* (L.) Rich. stock, following the life cycle of their natural habitat, winter in a room with 10-15 ^{o}C and summer in a room 24 ^{o}C on natural light.

3. Experiments for acclimatization

I took seedlings of *Paphiopedilum venustum* (Wall. ex Sims) Pfitzer species out in different soil mix for the purpose to examine that the various components have a negative or positive effect for the growing of seedlings or sufficient a standard mix (tiny fir bark, Novobalt peat, coconut fibre and perlite in 2:2:1:1 ratio. The basic mix contained of Novobalt peat and perlite in 5:1 ratio (Tp.). I mixed to this base the next complements in 1:1 ratio: fir bark (Tp.+K.), Sphagnum moss (Tp.+S.), coconut fibre (Tp.+Ko.), clay-granulate (Tp.+A.). I didn't added to the soil mixes fertilizer. The acclimatization of the seedlings happened in a warm part of the greenhouse, on a humid, minimum 22 °C place.

Only one part of native orchids' growing their maintenance among *in vitro*, it's important also to find out what are the possibilities of their transplanting, how these plants suitable for acclimatization. Between 1995 and 2012 spring some plantlets of *Dactylorhiza maculata* (L.) Soó, *Anacamptis morio* (L.) R.M.Bateman,

Pridgeon & M.V.Chase, *Anacamptis palustris* (Jacq.) R.M.Bateman, Pridgeon & M.W.Chase *(ssp palustris), Liparis loeselii* (L.) Rich and *Platanthera bifolia* (L.) Rich species were transferred. Our experiences of this period promoted further transplantation of *Liparis loeselii* (L.) Rich. in a greater volume. I examined in the 2012. February transplanted stock of *Liparis loeselii* (L.) Rich. the connection between the bulb size, the leaves and flower formation of the plantlets.

4. The methods of observations and evaluations

In my dissertation based on the of the international literature for the types of special root- and sprout development I provided items in Hungarian - where they are absent - with supporting examples and illustrations.

During the adaptation of new acquired species, 5 months after the first state measuring I assessed what changes happened in the state of the plants and which specimens were perished. The criteria of the state measuring were: sprout and also root development was observed, only root development, only sprout development was observed, neither sprout, nor root development was observed. I noted the bud and flower formation too. I tabulated the collected data in Excel table and represented in histograms.

During the examination of the germination results of orchid seeds, the data were summarized in Excel table on the base of seed sowing register from 18 June 2006 to 07 June 2012 (6 years) and the correspondences were represented on diagrams. The number of the germinated protocorms were defined by estimate, I counted the number of the protocorms on a 0,5 cm^2 area and after that I multiplied the result with 30,4 (the area of the medium/0,5). On the base of the pieces of the protocorms were formed I made an increasing scale.

In the course of *in vitro* examination of *Paphiopedilum* species I compared the efficiency of the various media on the base of the vivid plants' ratio, the root growing, the number and length of the leaves of the plantlets.

The measurement of chlorophyll content in *Paphiopedilum venustum* (Wall. ex

Sims) Pfitzer plantlets' leaves was achieved by spectrophotometer.

Chlorophyll (a+b) microgram/gram fresh weight = (20,2*A(644) + 8,02*A(663)) * V/w

V – volume of tissue-extract (ml)

w - fresh weight of the tissue (g)

During the *in vitro* stock maintenance of *Liparis loeselii* (L.) Rich. the examination and stereomicroscopic viewing of the flasks happened the base of the next criterion: development of the bulbs, intensity of the green colour.

During the examination of acclimatization in the case of *Paphiopedilum venustum* (Wall. ex Sims) Pfitzer the examined parameters were the followings: number of roots, length of roots, and number of leaves. The results of the measurement were summarized in Excel table and represented on diagram.

During the acclimatization of *Liparis loeselii* (L.) Rich. I divided the bulbs on the base of their sizes into two groups: I. group: 3-5 mm x 4-5 mm ø bulbs, II. group: 5-8 mm x 5-10 mm ø bulbs. Two measurements were made in both groups. I summarized the data concerned the size of the bulbs, development of leaves respectively the flower formation in tables and I represented the connections on diagrams.

RESULTS

1. Stock examination in the orchid collection of the ELTE Botanical Garden

1.1. Comparison of my morphological observations with the literary data

I created the next morphological terms in Hungarian, which connected the features of sprout and root development of tropical orchids: *homoblastic pseudobulb, heteroblastic pseudobulb, reed stemmed pseudobulb, adhesive root and root-nest.*

Out of the two main pseudobulb type the homoblastic pseudobulb is typical e.g. by

Ansellia, Arpophyllum, Brassavola and *Cattleya* genera, the heteroblastic pseudobulb is typical by *Anguloa, Aspasia* and *Bifrenaria* genera. The woody reed-stemmed pseudobulb cross-section is round that is typical e.g. by the *Epidendrum radicans,* Pav. ex Lindl., *Epidendrum pseudepidendrum* Rchb.f., *Sobralia macrantha* Lindl., *Arundina graminifolia* (D.Don) Hochr. species. At the formation of the root-nest, which collects the decaying leaves and the mould for the plant the thin branching adhesive root get into shape of a dense web by growing the base of negative geotropism such as by *Ansellia africana* Lindl. species.

On the base of some successful achieved hand pollinating during the 2011 year I reported the time of the pod harvest of some tropical and subtropical species, which varies at the observed species e.g. *Dendrobium atroviolaceum* Rolfe, *Eulophia guineensis* Lindl., *Epidendrum stamfordianum* Bateman this period is taken from 2 to 7 month. In regard to the natural pollination I found some example in our orchid collection. The *Epidendrum nocturnum* Jacq. and *Guarianthe aurantiaca* (Bateman ex Lindl.) Dressler & W.E.Higgins species are often cleistogamous. The *Liparis nakaharae* Hayata, *Prosthechea* ochracea (Lindl.) W.E.Higgins and *Oeceoclades maculata* (Lindl.) species are self-pollinated.
The presence of symbiotic fungi were proved by the germination from spontaneous seed-spreading (06. 08. 2007.) of *Peristeria elata* Hook. seedlings in the greenhouse.

1. 2. Newly acquired orchid species' adaptation in the orchid collection

After 5 months, that the new acquired orchid species were placed in the greenhouse in the case when the potting medium and the growing conditions were sufficient and the winter resting period, which is typify on the species was terminated, the root and sprout development started. At the first status measuring in the "own material" group fresh roots and sprouts were observed by 44.44% of the plants, this increased at the next status measuring on 85,18 %. At the first status measuring in the "propagated from trade" group most of the plants (58.33 %) had

fresh roots and sprouts, this increased to 75 % at the next measuring. But at the first status measuring in the "acquired from trade" group most of the plants (38 %) had fresh sprouts, but without fresh roots, this changed at the next measuring too, the root development of the plants started and the rate of the plants with fresh roots and sprouts increased to 90,4 %.

With the offset of the root and sprout development of new acquired orchid species (2010. year), then with the growth and strength of the specimens (2011. year) several orchids started the bud and flower formation. The data of flowering were summarized on 20th December 2011, accepted data was a plant with opening flower. In the acquiring year (2009.) two specimens flowered and two formed buds. The increase of flowering tendency indicates that during the 2010 year 12 specimens and during the 2010 year 14 specimens flowered. In both years (in 2010 and 2011) 30 specimens repeatedly flowered.

By the acquired orchids which are access to the flowering status I found in more cases identification problems which are deserving attention, at the level of species and genera too, e.g. one of the new *Coelogyne pandurata* Lindl. specimen is *C.* x Burfordense hybrid, which is originated from the cross breeding of *C. asperata* Lindl. and *C. pandurata* Lindl.

2. Observations about seed-germination in the laboratory of ELTE Botanical Garden

2. 1. Correspondence between germinating power of seeds and the sources of acquiring

During the six years (2006-2012) I got the next result in the case of asymbiotic germination of tropical-subtropical species (64), comparing the number of effective seed sowing (75) with the total number (159) on the base of the source of supplies. The seed sowing (56) originated from the pollination of ELTE Botanical Garden (Fb.) were successful in 60,71 %. The germination result was 50

% in the case of seed sowing (54) originated from orchid collectors (Ogy.). Although 4 seed sowing was made with seeds from natural habitats (Tgy.), these germinated in 50 %. In the case of seed bank (Mb.) 40,1 % of germination result from 22 seed sowing seems successful result too. But the 13 percentage from 23 seed sowing by seeds originated from seed exchange - Index Seminum - (Mcs.) is very low.

During the six years (2006-2012) in the case of asymbiotic germination of temperate orchids (16), when I compared the number of effective seed sowing (24) with the total number (81), on the base of the source of supplies I got the next results. I got the lowest ratio (9,3 %) in the case of seeds originated from seed exchange - Index Seminum (Mcs.), although most of the seed sowings (43) originated from this source. The germination result was 50 % in the case of 4 seed sowing (mother specimens growing in own garden) originated from orchid collectors (Ogy.). With seeds from natural habitats (Gy.) were made 27 seed sowing, these germinated in 48,17 %. In the case of seed bank (Mb.) 57,14 % of germination result from 7 seed sowing seems good too.

2. 2. In vitro experiments with plant origin additives

The growing and development of the seedlings of *Paphiopedilum venustum* (Wall. ex Sims) Pfitzer I assessed first without the disturbance of the seedlings after 12 weeks on the control medium and the media supplemented with Jerusalem artichoke. The best root development and shoot growth the plantlets reached on the MV medium (200 mg l^{-1} Polivitaplex) and here was the fewest ruined seedlings (5,5 %). The medium supplemented with dried Jerusalem artichoke (MCS) was toxic for the plants, all of them died (100 %). On the Yeast extract (250 mg l^{-1}) containing M basic medium root formation was observed, short, average under 0,5 mm roots were formed (2,58 pieces/plants). On the MV medium the average root size was between 0,5-1,5 cm. On the medium supplemented with fresh Jerusalem artichoke's cubes (MCN) I observed (1,06

pieces/plants) starting root formation, with under 0,5 cm root size.

After 4 months I evaluated the whole experiment. On the MV medium, which earlier showed the bests results, the development of the plantlets slowed up. Here was the longest root size (35,25 cm), but these roots were thin and straggling radial into the medium. Comparing with this, on the M medium shorter (10,75 cm), but better quality roots developed. I got plantlets only on the MCL and MCN medium with sufficient leaf development, which were ready for transplanting, here was typical also the formation of root-ball. These roots similarly to the roots of the full grown plants were thick and hairy. The mottled leaves, which is typical by the species and indicates intensive development the 40 % of survived plantlets showed. Where I observed the ruin of the plantlets, this wasn't wholehearted. On the MCL medium by the rootstock of the dying plantlets (36,36 %) protocorm likes bodies (PLB) were formed with 3,5 sprout/ plant.

The results of the measurement of chlorophyll content showed that the total chlorophyll concentration was the highest in the seedlings which developed on the MV medium. On the MCN and MCL medium intensive plantlet growing occured together with lower chlorophyll formation.

1. table Size-categories of *Paphiopedilum sukhakulii* Schoser & Senghas seedlings on the examined media /M, $MC_{25,}$ MC_{50} MC_{100} MC_{200} (18.10. 2010.)

Kategory	Number of roots (p.)	Length of roots (mm)	Number of leaves (p.)	Length of leaves (mm)	Length of sprout (mm)
0	0<3	0<50	0<3	0<50	0<50
I.	3,09	100-200	2,81	100-150	50-80
II.	4,14	200-300	3	150-200	80-100
III.	4	200-400	2,88	150-300	80-120
IV.	4	200-500	3	200-350	100-200

On the fortieth week, I evaluated the growing and development of the seedlings of *Paphiopedilum sukhakulii* Schoser & Senghas on the examined media on the base of five size-categories (0.-IV.) (1. table). Transplantable sizes were the III.-IV. categories. On the M basic medium most of the plantlets belonged into the

0. and I. categories, so the seedlings didn't show sufficient development. The most equable development was observed if the medium was supplemented with 25 ml l^{-1} homogenized Jerusalem artichoke (MC_{25}), here developed the highest number of seedlings belonged to the III. category. On the medium supplemented with 50 ml l^{-1} homogenized Jerusalem artichoke (MC_{50}) the II. category represented more serious rate. On the medium supplemented with 100 ml l^{-1} homogenized Jerusalem artichoke (MC_{100}) most of the seedlings belonged to the I. category and in the case of the medium was supplemented with 200 ml l^{-1} homogenized Jerusalem artichoke (MC_{200}) their rate was the highest in the 0. category, although on this medium also remarkably developed plants were found, equable growing plantlets (IV. category). The formation of mottled leaves, which is typified of the species also, was variable on the different media. On the M medium it was observed by the 20 % of the seedlings, it increased on the MC_{25} medium, but it fell back in the case of MC_{50} onto 16,6 %. Mottled leaves didn't form on MC_{100} and MC_{200} media. Formation of sprouts occurred in more cases; I experienced the highest rate, 30 % on MC_{25} medium. In the case of MC_{100} medium that was 16 % and on MC_{50} medium 6,6 %.

On achievement of seed germination experiments of Illyés and Molnár (2011), valuable plant stock of *Liparis loeseli* (L.) Rich remained in the laboratory of the ELTE Botanical Garden which were transferred every year, at their sprouting. On the base of the examination results of 2004-2007 periods I chose the FMB medium (FM + 10 g dried potatoes /1200 ml d. w.) for the stock maintenance of *Liparis loeselii* (L.) Rich. *in vitro* culture between the DEB, ZAK, FAST, FM, KM media. On FMB medium sufficient root formation, increase of bulb size, intensive leaf development occurred, so on 15th 02. 2007. (after 4 years and 10 months) the experiments for transplanting of plants and acclimatization could start.

4. Experiments for acclimatization

The changes of the growing and development of *Paphiopedilum venustum*

(Wall. ex Sims) Pfitzer seedlings among greenhouse conditions on the influence of the different soil mixes I evaluated on 21st 09. 2011. (after 11 months). In the basic soil mix (peat and perlite, Tp.) the root system grew only in the upper layer of the soil (3 pieces/plants, the longest root 3 cm), which was underdeveloped comparing to the *Sphagnum* moss containing mix (Tp.+S) (3,55 pieces/plants, the longest root 4,33 cm). In the case of the fir barks containing soil mix (Tp.+K.) the growing of roots wasn't equal, although some stronger roots also formed (2,6 pieces/plants, the longest root 1,7 cm). The coconut fibre containing mix (Tp.+Ko.) gave the most interesting result (2,71 pieces/plants, the longest root 3,57 cm). Although here was the highest mortality – in the case of the other mixes it wasn't higher than 10 % - here reached 30 %, the alive plants showed strong development. Least of all the (Tp.+A.) clay-granulate containing mix was adequate, here typical were the weak, thin roots which grew in the upper layer of the soil mix (2,2 pieces/plants, the longest root 2,15 cm).

During the transplanting of native orchids (1995 2011.) among *ex situ* circumstances, every species sprouted also in the next year. By the *Dactylorhiza maculata* (L.) Soó, *Anacamptis morio* (L.) R.M.Bateman, Pridgeon & M.V.Chase species the further ruining of the plants occured because of the problems of pest control (slug chewing). In the third year *Anacamptis palustris* (Jacq.) R.M.Bateman, Pridgeon & M.W.Chase *(ssp palustris)* didn't sprout, probably the environmental factors weren't sufficient. Here were similar problems by *Liparis loeselii* (L.) Rich. species because of the support of sufficient water supply. By *Platanthera bifolia* (L.) Rich. species, I hadn't already observations in the third year (2011. transplanting).

Evaluation of the growing results of the seedlings of fen orchid, *Liparis loeselii* (L.) Rich., among *ex situ* conditions were got round two occasions. By the first status measure, two months after the transplanting when the rate of the sprouting of the bulbs were measured, in the I. group (3-5mm x 4-5mm) 58,62 % in the II. group (5-8 mm x 5-10 mm) 54,83 % of the bulbs sprouted. The next

status measure just had happened after two weeks. By this time in the I. group 77,58 % and in the II. group 70,96 % of the bulbs sprouted. Here, in four cases flower stems developed (13 %), and after that by one specimen was formed a capsule. In the case of the sprouted bulbs in the I. group regarding the total number of transplanted bulbs in highest rate bulbs with one leaves developed (61 %) and in the II. group in highest rate, bulbs with two leaves developed (48 %).

NEW SCIENTIFIC RESULTS

1. Foremost I created special terms on the next morphological features in Hungarian vocational literature: *homoblastic pseudobulb, heteroblastic pseudobulb, reed stemmed pseudobulb, adhesive root and root-nest.*
2. I gave data in accordance with speciality of germination about some tropical-subtropical and native orchid species and with the germination of different originated seeds I determined the supply sources reliability.
3. I first practised two plant originated complex additives - *Solanum tuberosum* L. and *Helianthus tuberosum* L. – during the asymbiotic propagation of orchid species.
4. I determined the optimal compound of medium during the *in vitro* cultivation of *Liparis loselii* (L.) Rich. an endangered native orchid species.
5. I appointed that during the transplanting necessary to compile a soil mix which is suited to the claims of the tropical species.
6. I proved during the cultivation of *Liparis loeselii* (L.) Rich. among *ex situ* circumstances the effect of the bulb size on the measure of leaf and flower development.

CONCLUSIONS AND RECOMMENDATION

Knowledge of morphological features, so e.g. the separation of bulb types

is important for botanists and orchid enthusiasts too, so where these terms were missing in Hungarian, I kept important to get into shape on the base of German and English literature (Whitner et al., 1974; Fast 1980; Röth, 1982). In the case of the plants which didn't reach the flowering status yet, this lends assistance to identification to the unknown species or hybrids and so the determination of sufficient keeping conditions.

During the greenhouse adaptation of the 2009 year acquired orchid species I observed that the choose of the soil mix according to the species' requirements and beside this the adequate evolving of greenhouse conditions (moisture, humidity, light, temperature, aeration) have essential importance for the optimal root and sprout development and respectively the bud and flower formation occurred.

During the six years (2006-2012), on the base of the germination results appointed that in the case of subtropical and tropical species, the germination ability of seeds originated from seed exchange - Index Seminum (Mcs.) aren't reliable. Seed request from these sources is reasonable only for very rare species. In that case, it's very important to note the items of the sender Botanical Garden and to record the germination results before the next seed request. In all cases sowing is necessary from fresh seeds, or by sufficient storage (dryly, in refrigerator) seeds with good germination ability. It's clear that the seed exchange - Index Seminum (Mcs.) isn't sufficient by temperate orchids too, because these species loose their germination ability sooner. By native species the single method is the acquiring and sowing of seeds from permitted collection of natural habitat.

The examination of chlorophyll contents of seedlings of *Paphiopedilum venustum* (Wall. ex Sims) Pfitzer showed conflicted results on the various medium. The total chlorophyll concentration was higher in the weak developed seedlings (34,265 micrograms/gram), on the MV medium and it was lower in the strong growing seedlings on the MCL (23.02 micrograms/gram) and MCN medium (29.97 micrograms/gram). Accordance with the investigation of Yates and Curtis (1949) in the case of asymbiotic propagation of epiphytic orchids root development

increased with increasing concentration of sucrose, both in terms of length and number of roots, while the length of shoots reduced. They concluded that beside abundant carbohydrate supplies the change over to photosynthetic nutrition delayed. In seedlings of *Cymbidium* sp. sprout, root and chlorophyll development were all inhibited by high concentrations of sucrose (16 g/l) (Vanséveren-Van Espen, 1973). Probably the nutrients which are available in the media complemented with Jerusalem artichoke – not only the plus sucrose – ensured the sufficient development beside low rate of assimilation. The expanded root system, with retarded sprout growing, which was observed on MV medium is explicable that in the end the plants search with their roots the important but absent nutrients. Ichihashi (1979) wrote a similar effect during the asymbiotic propagation of *Bletilla striata* (Thunb.) Rchb.f. seedlings, by under 20 mM ion concentration. After his interpretation, in ecophysiological terms the plant is stimulated by the low concentration of nutrients to pursuit of soil minerals.

The evaluation of the effect of Jerusalem artichoke supplemented media emerged the question of high rate of mortality too. Although these are subtropical or tropical genera the problems during their propagation are similar to temperate orchids. It isn't occasion that the researchers tried to work out special medium formula for their asymbiotic propagation e.g. Thomale-Detert, N3f medium (Domokos, 1972). By the propagation of *Paphiopedilum* x *Maudiae* Fast (1980) obtained seedlings only on medium supplemented with 10 % homogenized banana, which were ready for acclimatization. Van Waes (1984) during the asymbiotic propagation of temperate orchids in the case of high ion concentration of media observed abnormal growing, thicker base of the plants and vitrification by the sprout apex. Malmgren (1989) on the media with the concentration of 3 g l^{-1} peptone observed the brown colour of the medium and the ruining of the seedlings. I observed also this effect by the ruining seedlings. In my opinion applying homogenized Jerusalem artichoke (plant origin additive) for the cultivation of *Paphiopedilum* species can give the best results after the initiation of root growth.

During the nurse culture the optimal dosage is the basic medium complemented with 25 ml^{-1} homogenized Jerusalem artichoke tuber.

I appointed that during the transplanting necessary to compile a soil mix which is suited to the claims of the tropical species. In the case of *Paphiopedilum venustum* (Wall. ex Sims) Pfitzer species increasing the quantity of *Sphagnum* moss and parallel decreasing the loosen components (fir-bark, clay granulate) has positive effect on root and sprout growth.

My experiences under artificial conditions contributed to the conservation of *Liparis loeselii* (L.) Rich, the fen orchid, these observations agreed with literature data, according to which the bulbs only after certain development can form two leaves and flower (Mrkvicka 1990). The relationships of the bulb size, thus the bulbs only after certain development (5-8 mm x 5-10 mm) can be able to develop two leaves (48 %) and flower (13 %), should promote the protocols for successful recovery. The life cycle of the species is joined the tempo of the natural habitat, this is documented by literature data (Illyés, 2005).

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