Asymbiotic propagation of hardy orchids at the ELTE Botanical Garden – review of the work during the period 1988-2012

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The laboratory of the ELTE Botanical Garden started to work in 1986 and our primary aim was the asymbiotic micropropagation of tropical orchids. We have started to focus on the propagation from seeds of hardy orchids since 1988. In the case of 19 native Hungarian species the most successful among the examined media was a modified Fast medium (Fast, 1982). During the period 1990 to 1997 we observed and monitored the germination and *in vitro* cultivation of 20 different temperate orchid species.

We examined the negative influences of the polyphenol formation factors on the shoots and roots development of *Anacamptis palustris ssp. palustris* and *Dactylorhiza maculata* species and protocorms of *Epipactis helleborine* on five different media. The browning of the medium decreased on lower temperature (15-18 ^{0}C) and under natural illumination. The positive effect of applying potato cubes as plant origin natural complex additives directed our attention to extend the studies. Experiments were carried out with *Dactylorhiza maculata* protocorms and plantlets. Results proved that potato products were suitable growth promoters on the protocorm and shoot growth of *Dactylorhiza maculata* comparing to the control Fast medium.

Based on the examinations during 2004-2007, we identified the culture medium for the *in vitro* culture of *Liparis loeseli* sterile stock. The FMB medium (FM + 10 g dried potato / 1200 ml d. w.) gave the best response.

During six years (2006-2012) we got a definite result when we compared the number of effective seed sowing (24) with the total number (81) in the case of temperate orchids' species, on the base of the source of supplies.

During these decades we have also several experiments for acclimatization. *Anacamptis morio* which was transferred in 1991 and repeatedly in 1994 and have survived for several years after the acclimatization *in vivo*. In the case of the transfers between 1995 and 2011 for *Dactylorhiza maculata, Anacamptis morio, Anacamptis palustris ssp palustris, Liparis loeselii* and *Platanthera bifolia* species, the plantlets have survived two years after the acclimatization.

Successful cultivation has gone under *ex situ* circumstances of *Liparis loeselii* seedlings derived from *in vitro* since 2012 February. 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.