Lankesteriana International Journal on Orchidology
ISSN: 1409-3871
lankesteriana@ucr.ac.cr
Universidad de Costa Rica
Costa Rica

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Lankesteriana International Journal on Orchidology, vol. 7, núm. 1-2, marzo, 2007, pp. 129-133
Universidad de Costa Rica
Cartago, Costa Rica

LANKESTERIANA 7(1-2): 129-133. 2007.

EX SITU CONSERVATION OF TROPICAL ORCHIDS IN UKRAINE

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KEY WORDS: Orchidaceae, *ex situ* conservation, living collection, *in vitro* propagation, orchid exhibition greenhouse

The problem of biodiversity conservation of tropical floras having the global ecological, economical and social importance, has already exceeded the boundaries of individual countries. Successful conservation actions require joint efforts of scientists on the local, national, regional, and international levels (Wyse Jackson & Sutherland, 2000, Grodzynsky *et al.*, 2001).

The National Botanical Garden (NBG) of the National Academy of Sciences of Ukraine plays the leading role in *ex situ* conservation and propagation of tropical plants in Ukraine. Diverse studies aimed at protection of rare species of various families of angiosperms, particularly tropical orchids, were carried out at this institution for more than 30 years.

Since many representatives of the orchid family are under threat of extinction in their native habitats in the tropics, these investigations are viewed among the highest priorities of our research agenda. The main factors threatening the natural populations of tropical orchids include (1) large-scale collecting of these plants for commercial purposes as ornamental or medicinal plants, and (2) changes of ecological conditions in natural habitats. These changes are caused by human activities, such as agricultural development of territories for crop cultures, industrial development, road construction, mining, recreation, timber harvesting (especially clear-cutting of forests). These activities result in to deforestation, habitat fragmentation, forest fires, invasions of alien species, and other adverse processes (Averyanov *et al.*, 2000, Cribb *et al.*, 2005, Dunkan *et al.*, 2005).

Taking into account the disastrous rate of degradation of virgin or even semi-natural tropical forests (which are natural habitats of many species) as well as biological peculiarities of orchids (mycosymbiotrophic interactions, prolonged and complicated life cycles, pollination limitation), it often happens that the measures taken to protect orchids in their natural habitats, such as monitoring of population, establishment and management of protected areas, are not sufficient for ensuring their survival in nature. Because of that, *ex situ* conservation of tropical plants shall be viewed, along with *in situ* protection, as an efficient alternative way of biodiversity conservation. It is especially true for tropical orchids, especially those species which are threatened with extinction within their native ranges.

The main activities of NBG in the field of conservation of tropical orchid species *ex situ* include maintenance of living collections, propagation of these plants through *in vitro* asymbiotic seed germination and tissue culture techniques, and creation of the orchid exhibition greenhouse, *Orchidarium* (fig. 1).

FIGURE 1. Fragment of the orchid display Orchidarium.

At present the NBG living collection of tropical orchids contains about 4500 plants representing approximately 170 genera and 450 natural species (not counting artificial hybrids and cultivars). Most of species of the collection (more than 70%) are native to South-East Asia, whereas the remaining 30 % are orchids from South America and Central America, with a few genera from Africa (including Madagascar). Since 1999 the whole NBG collection of tropical plants (including orchids) has the status of a National Heritage Collection of Ukraine. The list of National Heritage units, including the most important science and arts collections, is designated by relevant ministries and agencies and approved by the Cabinet of Ministers of Ukraine.

The NBG collection is taxonomically representative; it includes the taxa belonging to four out of five currently recognized subfamilies (Chase 2005, Pridgeon *et al*. 2005) of Orchidaceae (Cypripedioideae, Vanilloideae, Orchidoideae, and Epidendroideae).

The major part of the plant collection was accumulated by NBG researchers during the 1980-1990s through field trips and collecting activities in the wild in various floristic regions of Paleotropis and Neotropis, usually in close cooperation with local botanists. In addition to that, samples of plants were received as gifts from botanical gardens throughout the world (Caracas, Venezuela; Beijing, China; Warsaw, Poland; Moscow & St. Petersburg, Russia), donated by private persons, as well as purchased from well-known floricultural commercial companies (Mandai Orchids, Singapore; Vacherot & Lecoufle, France; Floriana, Brazil; Winkler's Orchids, Argentine; Saigon Orchids, Vietnam, and others).

The most valuable part of the orchid collection is represented by orchid species of the flora of Vietnam. This collection, comprising about 1/5 of the total number of species in the orchid flora of Vietnam, was developed due to scientific collaboration between NBG and the Center for Biodiversity and Development of the Institute of Tropical Biology of Vietnam. Within the framework of partnership between these institutions, five expeditions have been carried out, which resulted in new accessions to the living orchid collection.

During the recent years the orchid collection is replenished and managed taking into consideration the international priorities in the Garden's policy of collection development, as outlined in the International Agenda for Botanic Garden in Conservation (Wyse Jackson & Sutherland, 2000), CITES Orchid Checklists (Roberts *et al.*, 1995, 1997, 2001) and Global Strategy for Plant Conservation (2002). The NBG collection of tropical orchids was registered at the Administrative Organ of CITES in Ukraine (Ministry of Environment, registration No. 6939/19/1-10 of 23 June 2004).

While creating the collection, the strategic goal was to represent most widely the floristic, ecological and morphological diversity of Orchidaceae, with an emphasis on rare and vulnerable orchid species.

Botanical gardens maintaining the collections of tropical plants are responsible for their long-term persistence and sustainability, which is extremely topical at present, when there are strict limitations of CITES concerning sampling plants from natural habitats. To achieve successful acclimatization of orchid plants collected in the wild (commonly on fallen trunks and branches of dead trees), ecological requirements of each species must be met under glasshouse conditions.

Though the orchid family as a whole occupies a huge range of diverse habitats, individual species commonly demonstrate restricted distribution patterns with specific habitat preferences (Cribb, 1998, Vu Ngoc Long, 2002, Averyanov & Averyanova, 2003, Averyanov *et al.*, 2003, Pridgeon *et al.*, 2005).

Precise data on ecological requirements of many tropical orchid species remain surprisingly poorly known, and it is especially alarming if we consider an incredible rate of degradation of primary tropical forests. Thus, the best way to fill the gap in information on ecological requirements of tropical orchids is field observations in the wild.

Long-term maintenance and reproduction of live plants under glasshouse conditions with the aim of *ex situ* biodiversity conservation and subsequent repatriation are possible only on the basis of investigations of their ecological requirements, developmental biology both *in situ* and under glasshouse conditions, vegetative architecture, and anatomical and ecophysiological peculiarities.

Understanding of these peculiarities of orchids determines the adaptability of orchid plants of different ecological groups (terrestrials, epiphytes, and lithophytes)

under glasshouse conditions. On the other hand, studying of this subject is the prerequisite for development of techniques and procedures for propagation and cultivation.

The collection of orchids at the National Botanical Gardens consists of more than 450 species, all of which cannot be studied in detail, considering available resources and reasonable time limits. Because of that, for in-depth studies we identified priority groups, including such genera as *Angraecum* Bory, *Calanthe* R.Br., *Cattleya* Lindl., *Coelogyne* Lindl., *Cymbidium* Sw., *Dendrobium* Sw., *Laelia* Lindl., *Paphiopedilum* Pfitz.

While selecting the taxa, the following features were taken into account: frequency of occurrence in natural habitats, number of taxa per genus in the NBG collection, economic value (ornamental and medicinal plants), vegetative architecture, and conservation status. The preference was given to genera most widely represented in our collection, with special reference to rare orchid species of South-East Asia and South America.

Mass-propagation of orchid plants is a critical component of any long-term *ex situ* conservation program. Application of *in vitro* propagation techniques to rare tropical orchid species is, undoubtedly, a powerful tool for *ex situ* biodiversity conservation. Until recently, many tropical native orchids species from South America (*Cattleya* spp., *Laelia* spp., *Oncidium* spp.), South-East Asia (*Calanthe* spp., *Coelogyne* spp., *Dendrobium* spp., *Paphiopedilum* spp.), Africa and Madagascar (*Angraecum eburneum* Bory, *A. sesquipedale* Thouars) were propagated at the NBG through a range of asymbiotic seed germination techniques and tissue culture procedures aimed at preserving a number of individuals under artificial conditions in glasshouses of the temperate zone, and in protecting in such way these species from extinction (Cherevchenko, 1984, Buyun *et al.*, 2004, Lavrentyeva *et al.*, 2005).

Development of propagation methods for numerous species of tropical orchids in the NBG was preceded by long-term observations and dedicated studies in their reproductive biology (duration of anthesis, terms of pollination of flowers, and duration of fruit maturation).

Under glasshouse conditions, where specific pollinators are absent, artificial pollination of flowers is the only way to obtain fruits with viable seeds. For this reason, hundreds combinations of pollinations of flowers belonging to different species have been carried out in NBG greenhouses, depending on the breeding systems and quantity of samples of the species studied. As a result of these experiments, we obtained seeds of more than 100 species.

The list of natural species of *Paphiopedilum* propagated in NBG in *in vitro* culture includes up to 10 species (*P. appletonianum* (Gower) Rolfe, *P. callosum* (Rchb.f.) Stein, *P. delenatii* Guillaum., *P. insigne* (Wall. ex Lindl.) Pfitz., *P. lawrenceanum* (Rchb. f) Pfitz., *P. villosum* (Lindl.) Stein, *P. wardii* Summerh.). The urgency of protection of these species both *in situ* and *ex situ* is dictated not only by increasing demand for these plants in the world but also by their developmental biology and habitat preferences and peculiarities. Many *Paphiopedilum* species are obligate lithophytes; therefore, after the fires their populations are not restored at all (Averyanov, *et al.*, 2000). The plants propagated *in vitro* meet the huge demand for these plants, thus reducing the pressure on natural populations. *Paphiopedilum delenatii* is an excellent object to illustrate how a rare plant can be saved and propagated in cultivation for a long time (Cribb, 1998).

Undoubtedly, the use of glasshouse collections of living plants grown under artificial climate conditions in the temperate zone cannot ensure conservation of the whole genetic diversity of orchids. This way of conservation of tropical plants can be viewed rather as urgent measures because when rare species are poorly sampled only a miserable portion of their actual genetic diversity is preserved. In addition, the plant samples are often borrowed from living collections of other botanical gardens after long-term cultivation under glasshouse conditions.

Taking into account all mentioned above, the main trends of the use of fund collections for conservation of orchid diversity is to satisfy the increasing demand for plants of the natural orchid species through using seedlings and plantlets propagated *in vitro*, as well as creation of orchid exhibits.

At present the protection of biodiversity of rare tropical orchid species *ex situ* should not be limited by listing the specimens of rare and vulnerable species main-

tained in greenhouses of botanic gardens of the temperate zone climate. It requires fundamental understanding of factors controlling orchid plant development and acclimatization/adaptation in artificially created conditions using different experimental methods. These methods should be specifically designed for solving numerous theoretical and practical issues of adaptation of orchid species cultivated under glasshouse conditions. The availability of the collections of orchid species belonging to different ecological types opens wide prospects for investigating different types of life strategies, which provide survival in a wide range of ecological conditions, both in nature and in glasshouse. Finally, this will contribute to development of the most appropriate propagation methods and cultivation techniques under glasshouse conditions.

The original results of studies of seed coat patterns is an example of such investigations undertaken in NBG for elucidating structural morphological adaptations of orchids to different ecological niches. Seed testa sculpture patterns of more than 140 tropical orchid species belonging to 70 genera were investigated in NBG using scanning electron microscope (SEM) (Buyun, 2006). Actuality of this investigation can be explained by the fact that seed coat sculpture, as well as external surface sculpture of any plant organ directly exposed to the environment, can bring important information reflecting the pathways of morphological adaptation of orchid plants to specific environmental conditions (Kurzweil, 1993, Thompson *et al.*, 2001). Thus, ecological considerations of differences between seed coat sculpture of studied orchids (epiphytes, terrestrials, and lithophytes) highlighted by SEM are useful both for enhancing *in vitro* seed culture as a means of promoting conservation of tropical orchids *ex situ* and for elaborating appropriate techniques for their cultivation under glasshouse conditions.

To summarize, the main aspects of tropical orchid investigations in the NBG cover the following fields: (1) studies of developmental biology of orchids under glasshouse conditions (with special reference to reproductive biology of epiphytes and lithophytes as the most vulnerable groups); (2) investigation of structural adaptations of orchids to survival under a wide range of different habitats; (3) development of *in vitro* orchid propagation methods and cultivation techniques.

Beside this, the exhibition greenhouse *Orchidarium*, which was opened for public in 2005, can be considered as a source of material for scientific investigations, a wide range of educational programs, as well as an efficient tool in raising public awareness in issues related to conservation of rare tropical orchid species suffering from over-collecting and continuous loss of their natural habitats.

Two international conferences on biology and conservation of tropical and native orchids were held at NBG in 1983 and 1999, which emphasize the role of NBG as a leading Ukrainian center of *ex situ* conservation of tropical orchids.

ACKNOWLEDGMENT. The authors are grateful to Sergei L. Mosyakin for providing comments on the manuscript and improving the style of the English text.

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