Selbyana 26(1,2): 229–235. 2005.

ORCHID CONSERVATION THROUGH SEED BANKING: INS AND OUTS

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ABSTRACT. Some conservation strategies set aside large areas for nature reserves. Native plants, including orchids, are collected from these reserves for scientific study by public and private foundations, and seeds are collected and stored. This last strategy, seed banking, appears to be the simplest to execute and the most economical. Problems arise, however, regarding the most efficient methods for storing orchid seed. Such problems involve temperature, seed moisture, and seed survivability in each type of storage. One advantage is that several pods of half sibling plants may be stored and variability preserved in a very small package. On the other hand, orchid seeds, especially seeds of tropical orchids, appear to have short-lived seeds and thus do not survive to long-term storage.

Key words: seed storage, orthodox seed, recalcitrant, variability, storage conditions, cryopreservation

INTRODUCTION

Native plants, including orchids, are collected from forests (subjected to deforestation or not) for scientific study by public and private foundations, and sometimes seeds are collected and stored. Infrequently, new species are appearing in private collections without being known by scientists. When seeds are collected, rarely are they sowed or stored. The recent strategy of seed storage should be the easiest and the simplest way to preserve a large amount of variability of this group of plants.

Seed banks can be established as two types—short-term storage and long-term storage. For orchid conservation, the rapid degradation of natural habitats around the world makes long-term storage—known as seed gene banks—essential. Seeds are kept under −20°C and 5%MC (moisture content) or equilibrated up to 30%RH (relative humidity). This prevents pathogens that would be active in MC above 10%; it also prevents metabolic processes that in upper MC would age the seeds too fast, leading to rapid viability loss.

The parameters of orchid seed storage are not well studied. Orchid seeds are small, with only a few storage cells and a rudimentary embryo, and little knowledge exists relating to their physiological and anatomical aspects (Koopowitz 2001). Thus problems arise regarding the most efficient methods for seed orchid storing. Such problems involve temperature, seed moisture, and seed survivability in each type of storage. First, the seed type must be known, before selecting the best-suited storage. Seeds have been divided in two categories: a) Orthodox seeds that tolerate desiccation (<10% MC) and low temperatures during storage and that have a variable longevity determined by the species; and b) Recalcitrant (or unorthodox) seeds that do not tolerate water loss and/or low storage temperatures (Bewley & Black 1994). A third category, intermediate seeds, was defined by Ellis et al. (1990) as including seeds that tolerate some drying and cold storage but do not stay viable for long periods.

Pritchard and Seaton (1993) suggested a seed classification with two categories: "orthodox" and "intermediate" (or recalcitrant). Orthodox seeds tolerate desiccation and seed moisture contents in equilibrium with about 30%RH, or even lower; these seeds increase in longevity with reduction of moisture contents (20–5%) and/or storage temperatures (62–0°C). They also are able to maintain ca. 50% of viability after 8–14 years, when stored dry at 5–8°C. Pritchard and Seaton, however, recognized two sub-groups of orthodox seed storage behavior among orchid species: "truly orthodox" and "essentially orthodox." The truly orthodox species are capable of long-term preservation under conventional seed bank conditions (i.e., 5%MC and −20°C) without problems. *Orchis morio* seeds, for example, equilibrated to 15%RH at 15°C subsequently are maintained for 6–7 years in hermetic storage at −20°C (TABLE 1). In contrast, essentially orthodox seeds of *Cattleya aurantiaca* and possibly many other tropical orchids show reduced longevity at cool (below 5°C) and subzero temperatures with drier seeds (below 5%MC or in equilibrium with <31%RH). In dealing with essentially orthodox species, long-term conservation under conventional seed bank conditions is problematic. For the essentially orthodox sub-group, Pritchard and Seaton used the

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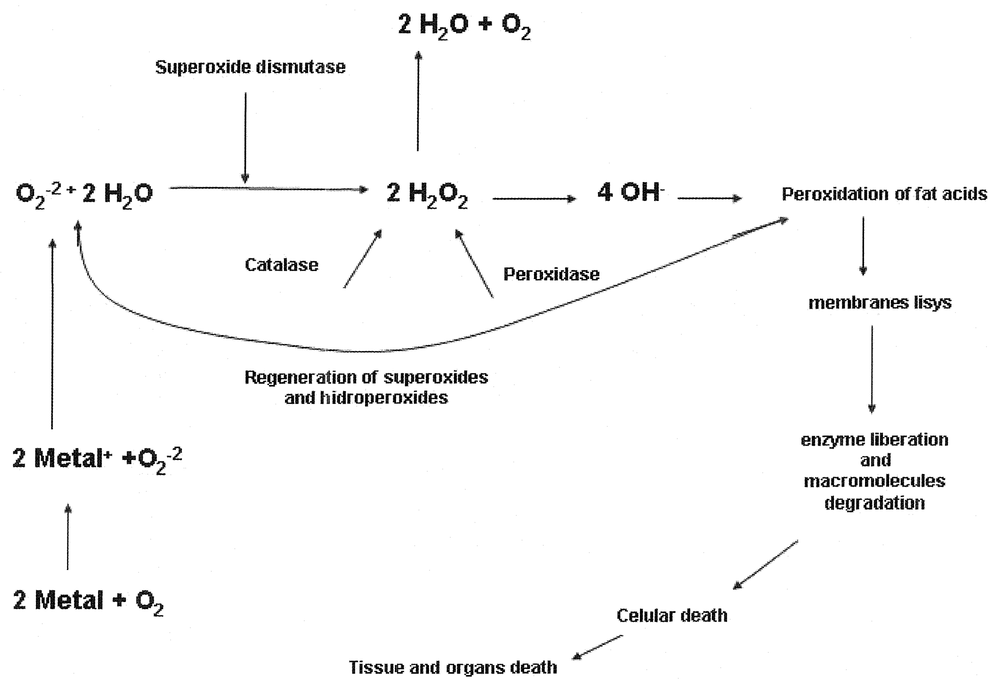

FIGURE 1. Scheme of fatty acid deterioration in seeds.

classification of intermediate seed storage behavior.

MATERIALS AND METHODS

Problems arise with ageing processes because the orchid seed reserve is constituted of mostly fatty acids (Hew 1987, Stancato et al. 1998). Such compounds are subject to peroxidation, a process based on active oxygen molecules generated by respiration (FIGURE 1), one of the most deleterious events, simply because peroxidation is very harmful to membranes and other macromolecules. Some reports relate seed vigor and free radical scavenger enzymes (Bailly et al. 1996, 1998, 2002), which lead readily to a massive loss of cell viability and finally to seed death, if stored under inappropriate conditions.

Contradictory data on orchid seed conservation may result in conflict among seed physiologists, as reflected in TABLE 1. *Cattleya aurantiaca* seeds, for example, had 25% of germination after 17 years of storage at 4°C over $CaCl_2$ (Shoushtari et al. 1994), but germination of the same species ranged 36–70% after 6 years of storage with 3.7% or 10.4%MC, respectively (Pritchard & Seaton 1993). Most orchid seeds were dead after several years of storage at 4°C over $CaCl_2$ (Pritchard & Seaton 1993, Shoushtari et al. 1994), and some species lost viability after just a few months of storage (Bowling & Thompson 1972; TABLE 1). Several species of *Sophronitis* sensu van den Berg, especially the rupiculous ones, did not tolerate more than 6 months of cold storage (4°C), neither over $CaCl_2$ nor over Silica Gel Blue (TABLE 2). *Sophronitis purpurata* and *S. tenebrosa,* two lowland species, were stored, however, in those conditions for 4 years without loosing germinability, but seed vigor declined readily, and after another year of storage, they were reaching zero germination. The same pattern could be found in the bifoliate *Cattleya,* where some species, such as *C. amethystoglossa* and *C. nobilior* that stayed vigorous for 4 years, dropped to zero at the end of the fifth year (TABLE 2).

Some orchids, from Southeast Asia, such as *Ascocentrum, Vanda,* and *Phalaenopsis,* did not allow storage for more than 6 months, and their vigor declined monthly (TABLE 2).

In addition to the seed type (orthodox and intermediate or recalcitrant), the final phase of the maturation period determines whether initially high vigorous seeds are obtained. This final period is recognized as one of the most important factors influencing the storage potential of a species. Seeds that are subject to high humidity, temperature >28°C, or cold <15°C have an inferior storage potential. High drying temperatures, even if they occur naturally, may reduce

TABLE 1. Longevity of orchid seed during storage, according to the literature.

Species	Storage conditions & time	Final germination	Seed classification	Author(s)
Tropical Epiphytic				
Acampe papillosa	Hermetic storage at −10°C/2 mon	>75%	Orthodox	*
Catasetum dilectum	Hermetic storage at −10°C/2 mon	<20%	Intermediate	*
Cattleya aurantiaca	6 yrs hermetic storage at 5°C with moisture contents 3.7–10.4%	36–70%	Intermediate	**
Cattleya aurantiaca	4°C over $CaCl_2$/17 yrs	25%	Orthodox	***
Cattleya elongata	4°C over $CaCl_2$/14 yrs	30%	Orthodox	***
Cattleya intermedia	4°C over $CaCl_2$/15 yrs	90%	Orthodox	***
Cattleya trianae	4°C over $CaCl_2$/19 yrs	1%	Intermediate?	***
Cattleya trianae	3 yrs storage at room temperature	40%	Orthodox	**
Cattleya loddigesii	Hermetic storage at −10°C/2 mon	20–50%	Intermediate	*
Cattleya luteola	Hermetic storage at −10°C/7 mon	>75%	Orthodox	*
Cuitlauzina pendida	4°C over $CaCl_2$/11 yrs	1%	Orthodox	***
Dendrohium crumenatum	4°C over $CaCl_2$/14 yrs	100%	Orthodox	***
Epidendrum nocturnum	Hermetic storage at −10°C/6 mon	20–50%	Intermediate	*
Epidendrum palludiflorum	Hermetic storage at −10°C/3 mon	20–50%	Intermediate	*
Epidendrum patens	Hermetic storage at −10°C/7 mon	>75%	Orthodox	*
Epidendrum scepterum	Hermetic storage at −10°C/3 mon	20–50%	Intermediate	*
Grammatophyllum speciosum	4°C over $CaCl_2$/13 yrs	10%	Orthodox	***
Laelia fiava	4°C over $CaCl_2$/14 yrs	None	?	***
Laelia lobata	4°C over $CaCl_2$/11 yrs	25%	Orthodox	***
Laelia purpurata var. *Alba*	4°C over $CaCl_2$/12 yrs	90%	Orthodox	***
Schomburgkia tibicinis	4°C over $CaCl_2$/15 yrs	100	Orthodox	***
Sophronitis coccinea	Hermetic storage at −10°C/3 mon	20–50%	Intermediate	*
Stanhopea sp.	4°C over $CaCl_2$/15 yrs	25%	Orthodox	***
Tropical Terrestrial				
Cyrtopodium punctatum	4°C over $CaCl_2$/15 yrs	None	?	***
Arundina graminifolia	Hermetic storage at −10°C/2 mon	>75%	Orthodox	*
Oeceoclades maculata	Hermetic storage at −10°C/7 mon	>75%	Orthodox	*
Phaius maculatus	4°C over $CaCl_2$/15 yrs	20%	Orthodox	***
Spatoglottis plicata	Hermetic storage at −10°C/7 mon	>75%	Orthodox	*
Zvgopetalum mackayi	4°C over $CaCl_2$/15 yrs	25%	Orthodox	***
Temperate Terrestrial				
Anacamptis piramidalis	4°C over silica	10%	Intermediate	**
Calanthe discolorxseiboldii	Hermetic air dry storage/−15–17°C/yr	20%	Intermediate	**
Calanthe discolor	Hermetic air dry storage/−15–17°C/yr	5%	Intermediate	**
Calanthe sp.	−4°C	7%	Intermediate	**
Cymbidium madidum	4°C over $CaCl_2$/12 yrs	40%	Orthodox	***
Cymbidium hybrids	8°C over $CaCl_2$/8 yrs	2–5%	Orthodox	**
Cypripedium hybrids	10 yrs storage over $CaCl_2$ at 8°C	50%	Orthodox	**
Dactylorhyza fuchsii	6.8 yrs hermetic storage at −20°C	42%	Orthodox	**
Dactylorhyza fuchsii	Liquid nitrogen	58%	Orthodox	**
Dactylorhyza maculata	1 yr storage at room	11%	Intermediate	**
Dactylorhyza maculata	1 yr over silica gel at 4°C	28%	Intermediate	**
Dactylorhyza maculata	0.5 yrs at 50–70% RH and 4°C	95%	Intermediate	**
Dactylorhyza sambucina	1 yr storage at room temperature	22%	Orthodox	**

TABLE 1. Continued.

Species	Storage conditions & time	Final germination	Seed classification	Author(s)
Dactylorhyza sambucina	Silica gel at 4°C	36%	Orthodox	**
Disa uniflora	15 min in liquid nitrogen	Survive	Orthodox	†
Disa uniflora	"Drierite" desiccant at 6°C	Good	Orthodox	**
Ophrys sphegodes	15 min in liquid nitrogen	Survive	Orthodox	††
Orchis morio	1 hr in liquid nitrogen	Survive	Orthodox	††
Orchis morio	1 yr hermetic air-dry storage at −10°C	Viability maintained	Orthodox	**
Orchis morio	6 yrs hermetic storage at −20°C	Viability maintained	Orthodox	**

* Bowling & Thompson 1972, ** Pritchard & Seaton 1993, *** Shoushtari et al. 1994, † Pritchard 1984, †† Pritchard 1985.

the initial seed quality (Bewley & Black 1994). Another factor to be considered is the nutritional state of the mother plant and the maturation degree at the pod harvest time (Carvalho & Nakagawa 2000). These factors could explain the behavior of different samples of the same species (see TABLE 1).

Fungi Conservation

Orchids have a significant symbiosis with fungi, and the need for fungi conservation must be recognized, if posterior recuperation through symbiotic or non-symbiotic germination is to occur. Seeds of *Caladenia, Diuris, Pterostylis,* and *Thelmytra,* dried over silica for 24 hours and stored for a year at 4, 18, or 22°C, germinated better than freshly collected seeds (4 weeks after dehiscence). Germination, however, was highest overall after storage of dried seed in liquid nitrogen (−196°C). Mycorrhizal fungi also were successfully preserved in the same conditions (Batty et al. 2001).

Epiphytes vs. Terrestrials

In comparing an epiphytic species with a terrestrial one, the terrestrial showed lower water loss rates and smaller activation energies for water loss, and it absorbed water from lower relative humidity. In contrast, the epiphytic lacked the enhanced water retention capacity of the terrestrial, implying that epiphytic orchids are able to germinate quickly when an adequately moist substrate is given (Yoder et al. 2000). Such differences could influence orchid seed conservation, because terrestrial temperate orchid seeds appeared to be more dependent on associated fungi to germinate and took water from lower RH than did epiphytic ones. On the other hand, seeds of *Sobralia dichotoma* showed adaptations to water retention, such as pectin sheets and cellulose capillars that cover the seeds, which allow the seed to get and maintain water from the atmosphere close to the embryo, preventing desiccation (Prustch et al. 2000). These adaptations, found in seeds of both terrestrials and epiphytes, make seed banking even more laborious. Comprehending the relationship of water uptake and desiccation tolerance may help in establishing adequate forms of storage for the different kinds of orchid seeds, in these processes that are the key to correct management of seeds prior to storing.

Cryopreservation

Cryopreservation of seeds, or embryos, is a real possibility and a good alternative for long-term storage. According to Stanwood (1985), cryopreservation virtually would be an "eternal" conservation. Cryoconservation (i.e., conservation under very low temperatures, commonly under liquid nitrogen) has been reported for plant embryos, either somatic or sexual (Tessereau et al. 1994, Gonzalez-Benito et al. 1998, Bomal & Tremblay 2000). Others have reported successful storage of orchid seeds (Pritchard et al. 1999, Wood et al. 2000, Batty et al. 2001). Batty et al. (2001) reported on cryopreservation of orchid seeds and their symbiotic fungi in seeds dried over silica gel for 24 hours, and Wood et al. (2000) reported on pre-treating seeds in alginate beads. Cryopreservation may be the best alternative for orchid seeds, because this method can be applied to embryos, even those that originated from recalcitrant seeds (Chin 1995, Pesce 1995).

For stable botanical varieties, at least 4000 viable seeds per variety need to be stored; and for the more variable varieties, 12,000 viable seeds per variety are needed. Such large amounts of seeds are necessary to preserve the variability of living collections of these orchid populations.

TABLE 2. Longevity of orchid seeds during storage and their classification, Presidente Prudente, Brazil, 2004.

Species	Storage conditions & time to no germination	Seed classification
Ascocenda	4°C over silica/3 mon	Recalcitrant
Ascocentrum curvifolium	4°C over silica/6 mon	Intermediate
Cattleya forbesii	4°C over silica/6 mon	Intermediate
Cattleya amethystoglossa	4°C over $CaCl_2$/4 yrs	Orthodox
Cattleya nobilior	4°C over silica/4 yrs	Orthodox
Cattleya tigrina	4°C over silica/6 mon	Intermediate
Constatia cristinae	4°C over silica/4 mon	Recalcitrant
Oncidium pumilum	4°C over $CaCl_2$/12 mon	Orthodox
Oncidium ithmithi	4°C over silica/3 yrs	Orthodox
Oncidium hastatum	4°C over silica/3 yrs	Orthodox
Phalaenopsis	4°C over silica/1 mon	Recalcitrant
Sophronitis crispata	4°C over silica/12 mon	Orthodox
Sophronitis brevicaulis	4°C over silica/6 mon	Intermediate
Sophronitis briegerii	4°C over silica/12 mon	Orthodox
Sophronitis duvenii	4°C over silica/4 mon	Recalcitrant
Sophronitis endsfeldzii	4°C over silica/6 mon	Orthodox
Sophronitis milleri	4°C over silica/4 mon	Recalcitrant
Sophronitis purpurata	4°C over $CaCl_2$/5 yrs	Orthodox
Sophronitis rupestris	4°C over silica/6 mon	Intermediate
Sophronitis sanguiloba	4°C over silica/6 mon	Intermediate
Sophronitis tenebrosa	4°C over $CaCl_2$/5 yrs	Orthodox
Sophronitis sp. 1	4°C over silica/6 mon	Intermediate
Sophronitis sp. 2	4°C over silica/6 mon	Intermediate
Vanda tesselata	4°C over silica/6 mon	Intermediate
Vanda testacea	4°C over silica/6 mon	Intermediate

For most orchids, however, the amount of seeds needing to be sowed or stored is much less; for example, adequate sowing or storing of *Cattleya* may require only 40 mg of seeds (Shoushtari et al. 1994). *Oncidium pumilum* or *Campylocentrum pubirrachis* may only require the collection of several pods, and this amount of seed is easily stored in micro assay tubes under appropriate conditions. Even if it is possible to obtain sufficient amounts of seed from one pod, it still will be necessary to have many pods to store much of the variability, with repetitions, around the world.

The mortality factor is a concept that needs to be considered in attempting to conserve orchid seeds. Not all the seeds in one pod (around 10 million in a *Cattleya* pod) are equal. Two factors must be considered: a) Genetic factors based on each seed being a different combination of parent genes, and b) Position in the pod, as some seeds are liberated in different maturation points. Normally, natural selection acts to determine that the majority of the genotypes will not survive. Under artificial conditions, the rigorous conditions faced by the seeds during germination are relaxed and a great number of offspring seedlings can reach maturity. This emphasizes why it is so difficult to re-establish a species in the wild, after it has been under artificial cultivation for a while. A surviving artificially propagated population is but a pale image of what the whole species was. Some conservationists counter that something is better than nothing (Koopowitz 2001).

RESULTS

Advantages of Seed Banking

Seed banking of orchid species offers numerous advantages. Seeds with correct, controlled conditions of storage may stay viable for long periods and ready to germinate. Such seeds may deteriorate slowly, either because of pathogens or from biochemical degradation (ageing). The amount of variability among the response of species to storage may be high because of the sample sizes and areas used for storage. Seeds and their symbiotic fungi may be stored together. Seed banks are less expensive when compared to living collections. Short-term storage banks, such as the Orchid Seedbank Project in Chandler, Arizona, USA, sponsored by Aaron Hicks, may merit support. Seeds are being exchanged easily, without bureaucratic oversight, by mail, except for restrictions by the U.N. Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES); and plants are being propagated by several orchid growers.

Disadvantages of Seed Banking

Renovation of orchid seed stocks may be problematic, especially if the mother plants have disappeared, as the time between sowing of the seed and flowering may be lengthy, as much as 12–15 years in *Euanthe sanderiana,* or 5–7 years for several *Cattleya* species. Seed banks, although less expensive to operate than living collections, are still very expensive to maintain over the long run. Seed banks are subject to failures because of energy breakdowns or fires, which could damage irreversibly any collection, and replicates must be stored in different places. Access to seed samples, which requires precise rules, regulations, and controls, may be a problem. Seeds, as any other part of the plant, are subjected to CITES restrictions, which in turn, may make movement of seeds across international borders difficult.

Conclusions

The main idea of banking orchid seed is to preserve variability, especially in those species highly threatened in nature and/or not well known. Environments, such as rain forests, are being destroyed around the world, as they are exploited for wood, crops, or grazing. Some environments that contain endemic orchid species, such as the rupiculous *Sophronitis* or *Constantia,* are much more vulnerable to human impact. Direct and/or indirect impacts, such as those from pollution or hydroelectric reservoir construction, are causing massive alterations in microclimate, with dangerous consequences for orchid species. For example, *Pleurothallis,* which is not a preferred genus for the vast majority of orchid growers, may vanish without being known. Therefore, if possible, those threatened species need to be collected and seeds sowed or stored.

Short-term storage banks allow maintenance of seed collections for use by researchers, hobbyists, and growers with less bureaucratic oversight but with restrictions set by CITES. Long-term storage banks will become a reality when knowledge about correct storage conditions for orchid seeds reaches consensus. Currently, very little is known about conserving the seed of this huge botanical family.

Seed banking, by itself, is not a definitive solution. It may be possible, however, for seed samples to be divided among several banks, with at least one living collection maintained cooperatively by seed bank managers.

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