Hardy Orchids and Peonies From In Vitro Culture©

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HARDY ORCHIDS

Some believe the orchid family to be the most evolved because orchids have adapted to attract specific pollinators and/or to marginal conditions where other plants can't grow. This specificity to pollinators and habitat has also made orchids vulnerable to extinction when their habitat or that of their pollinators is disturbed. All orchids have been placed under protection of CITES, the Convention on the International Trade of Endangered Species. This document regulates the international shipping of all orchids. Orchids that have been micropropagated or hybrids between species are issued a CITES certificate along with a phytosanitary certificate with no additional difficulty.

Orchids that are successfully pollinated develop a capsule that may contain thousands to millions of seeds. These tiny seeds are easily carried by wind or float on water and thus orchids are found on all continents except Antarctica. With an estimated 25,000 to 30,000 species worldwide, orchids are the largest flowering plant family. Adding to this species diversity, are the even larger number of hybrids that have been made between species. To produce a hybrid, it is often enough to take the pollen from one species and transfer it to the stigma of a flower from another species of the same or closely related genus. The ease of interspecies hybridization may be due to the seed structure of orchids. An orchid seed consists of an embryo and a seed coat, but contains no endosperm to nourish the embryo. The lack of an endosperm may be the reason that interspecies embryos are not aborted.

Tropical orchids may be epiphytes or terrestrial, but temperate climate or "hardy" orchids are all terrestrial. Terrestrial orchids possess fleshy roots which may be rhizomatous or tuberous. Hardy terrestrial orchids differ from tropical orchids in the development of overwintering buds at the crown of the plant. A cold period is required before vegetative growth can continue. Orchids are relatively slow growing and many have adapted to grow in marginal conditions. As a result, orchids are most often killed by overwatering causing root rot, or by too much fertilizer. Location is a critical success factor for tropical orchids growing in the house or hardy orchids growing in the garden. Many of the orchids of horticultural interest grow on the edge of the forest with dappled sunlight. We grow all our orchids in pots under 50% shade. The substrate or soil must be well drained. We use additional perlite in our peatmoss-based mix but coarse sand may also be used if the soil is not porous enough. Fertilizer should only be applied at reduced concentrations of 12.5% to 25% that used for other plants. Most of the hardy orchids grow at pH 6–7, with *C. acaule* the most notable exception.

The stemless or pink lady's slipper, *Cypripedium acaule*, is probably the best known native orchid in North America. Unfortunately it requires a very acidic pH 4–4.5. Many have tried to transplant *C. acaule* from the wild into their gardens to find that it flowered the first year, came back smaller the second, and was gone

by the third year. This has led to the myth that orchids can't be transplanted. It is thought that the mycorrhizae associated with *C. acaule* require an acid pH. *Cypripedium acaule* is tolerant of a low pH and is protected by it. When *C. acaule* is transplanted it is very difficult to maintain an acid pH, the mycorrhizae die off and *C. acaule* soon follows. This problem does not occur with other hardy orchids that grow at pH 6–7, though transplanting is discouraged and in many cases illegal. The better option is the purchase of tissue-cultured plants.

In 1922, American plant physiologist Lewis Knudson found orchids could be germinated on agar without mycorrhizae, but with the sugars and minerals that would have been provided by the fungus. As mentioned earlier, orchid seed doesn't contain an endosperm which serves to nourish the embryo until it becomes autotrophic. In nature, orchid seed must associate with mycorrhizae to break open the seed coat and nourish the embryo. This doesn't come without a cost. The balance between nourishing and digesting the embryo completely is a delicate one. Only a minute number of seeds will germinate and develop into a mature plant. Germination in vitro may be accomplished either symbiotically, in the presence of mycorrhizae, or asymbiotically, replacing the mycorrhizae with an entirely artificial medium.

Planteck Biotechnologies uses asymbiotic germination for all of the species they are working with. Capsules are harvested in the fall and the seed is dried for storage. The seed is placed in culture by surface sterilization of the seed and plating on an artificial medium in a sterile flow hood. The first stage of germination is called a protocorm. Full development into a seedling may take up to 1 year. Seedlings require a cold period, often a minimum of 3 months, before vegetative growth can continue. Planting out may be done at this stage or plants may be held in vitro for 1 or 2 more years to develop larger buds and stronger root systems. First flowering depends on the species and may occur in 2 years, or 5 to 6 years for many *Cypripedium* sp.

In the 1960s, the apical meristem was used for cloning and mass propagation of some orchid species and hybrids. This led to a revolution in the orchid market, with plants available in large numbers, true-to-type and more quickly than by germination. *Phalaenopsis* are now found in many grocery stores and hardware chains with garden centers, at prices that almost anyone can afford. Unfortunately mericloning has not been successful with either the tropical or hardy slipper orchids. As a result, these plants are not as widely available. This will likely change as labs develop the technology to mericlone the slipper orchid genera.

In landscaping, most of the hardy orchids do well under dappled shade with sun either in the early morning or late afternoon. Hostas and ferns make good companion plants if they are not planted too close. Orchids are poor competitors and more aggressive plants growing in their vicinity must be controlled. In fact, in the wild, orchids are often seen growing singly or in small clumps. In pots and in the garden, much larger clumps with up to 50 stems may be achieved.

PEONIES

Peonies, in contrast to orchids, are considered more primitive on the evolutionary scale and were originally grouped with the *Ranunculaceae*, the buttercup family. They now have their own plant family designation: the *Paeoniaceae* comprised of one genus: *Paeonia*. The genus is made up of three sections: *Moutan*, which are known as tree peonies; *Onaepia*, which includes only two species of little commer-

cial interest, and *Paeon*, which are known as herbaceous peonies. *Paeonia lactiflora*, which is in the *Paeon* section, is the most widely cultivated species. The Chinese have been cultivating this species for almost 2000 years, both for medicinal purposes and as an ornamental flower. However it is not as highly regarded as the tree peony in China, which is found throughout Chinese history and art, and is a prized New Year's gift symbolizing prosperity. Peonies were introduced to Europe in the 18th century. In the late 1800s and early 1900s, France had the most active peony breeding programs. Today this has shifted to the U.S.A., where it remains in the hands of amateurs and specialized nurseries.

I mention peony taxonomy because it is important to the breeding and grouping of peonies. Peony varieties are sold as being of *P. lactiflora* origin, as hybrid herbaceous (crosses within the *Paeon* section), as Chinese or Japanese tree peonies (referring to their origin and not botanical differences), or as hybrid tree peonies (crosses within the *Moutan* section). To this we can now also add another category, the Itoh or intersectional peonies. These are crosses between the *Paeon* and *Moutan* sections, more specifically, between *P. lactiflora* (herbaceous) and *P. lutea* hybrids (tree peony). This cross was thought impossible until a Japanese nurseryman, Toichi Itoh, succeeded in 1948 by crossing 'Kakoden' (*P. lactiflora*) with 'Alice Harding' (*P.* ×*lemoinei* hybrid). Unfortunately he did not live to see his plants flower. An American, Louis Smirnow, purchased four selections from Itoh's widow and introduced them to North America as 'Yellow Crown', 'Yellow Emperor', 'Yellow Heaven', and 'Yellow Dream'. These first hybrids are inferior to the new Itohs now available, but they inspired a number of American breeders to attempt similar crosses, in particular Roger Anderson, Don Hollingsworth, and Don Smith.

The Itohs have been available on the collector's market for about 20 years, but prices of US$200 – $1000 for a 3–5 eye division kept them well out of reach of all but the wealthiest collectors. High prices and lack of availability have made these varieties particularly attractive for micropropagation, however there were reasons that they were not being commercially micropropagated. Peonies have a determinant herbaceous growth habit, meaning that next season's growth is determined by bud and storage root development in the fall. This determinant growth habit makes peonies recalcitrant to in vitro propagation. Despite this, Planteck Biotechnologies has developed an economically feasible method for the micropropagation of peonies.

Planteck's micropropagation program has been successful with all three major groups of peonies: herbaceous, tree, and Itohs. Genotype still has an important role to play and some cultivars from each of these groups respond better than others. The tree peonies generally multiply very well, but rooting is more difficult and the resulting young plants are not as vigorous as the herbaceous or Itoh cultivars. This is only natural given that tree peonies are usually grafted onto herbaceous nurse roots until their own roots are strong enough to take over. The advantage of micropropagated tree peonies is that they are immediately growing on their own roots. 'High Noon' was our first micropropagated tree peony to flower. Unfortunately, although there is a high demand for tree peonies in Europe and Asia, only relatively small quantities of any one cultivar are required to meet the demand. Given the infrastructure required for in vitro propagation, it is not economically feasible to produce small quantities of individual cultivars by tissue culture. Also, the European market is flooded by cheap tree peonies grown in China, and micropropagation can't compete with field production for pricing.

The same may be said for most herbaceous cultivars which were developed in the first half of the 20th century and are readily available from traditional field propagation. However, new herbaceous varieties with stronger stems are being bred and micropropagation has a definite advantage to bringing new varieties to the market more quickly and in sufficient number to meet the demand (and therefore at a more accessible price). Once again, new varieties must first be tested for their response to in vitro propagation. Itoh peonies, with limited supplies and resulting high prices, are perfect candidates for in vitro propagation and have been the focus of Planteck's research program.

Peony breeding has remained the domain of amateurs and specialized nurseries. Perhaps the possibility of micropropagation will encourage a more organized effort to improve the overall quality of the peony. So far peony breeding has focused primarily on flower colour and more recently, stem strength for herbaceous peonies. With the hundreds of peonies registered and already on the market, demand for any one variety will be small. If a new variety had an additional quality, such as cold resistance or increased vigour in tree peonies, the increase in demand could be substantial. It should also be remembered that peonies are in demand for the cut flower market, especially in Europe, and there is room for the development of new varieties for the cut flower market as well. In both these cases a large demand for a new variety will make them ideal candidates for in vitro propagation.

Peony micropropagation as developed by Planteck Biotechnologies follows the standard procedure of culture establishment, shoot multiplication, and rooting. Peonies have two types of roots: large tuberous storage roots and fibrous feeder roots. Acclimatization of peony microplants is not particularly difficult but peonies are slow growing so attention must be paid to fungal diseases. *Botrytis* in particular can be a problem for the first month in the greenhouse before plants are fully established. Overwatering must be avoided and preventative fungicide treatments are recommended. In the first growing season, peony microplants develop two to three leaves. Repotting is recommended after 6–8 weeks to allow the storage roots to develop without excessive twisting. At dormancy, at least one larger bud and several smaller ones have formed at the crown of the plant. First flowering occurs in the third or fourth growing season. By this time, herbaceous and Itoh cultivars should be planted in the field or garden. To date, all of our micropropagated peonies have flowered true-to-type. Our first micropropagated peony flowered in 2003. Since then hundreds have reached flowering size in our demonstration garden.

ADDITIONAL READING

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