

## Polyploidy in *Phalaenopsis* orchid improvement

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**ABSTRACT:** *The use of naturally occurring unreduced gametes and of artificially induced polyploidy has increased the fertility of triploid Phalaenopsis orchids. The fertile hexaploids may be used in a breeding program to improve flower quality.*

POLYPLOIDY has played an important role in the improvement of many plant species and hybrids. The field of floriculture has probably benefited the most because 1) polyploidy can increase genetic variability, and 2) polyploidy tends to increase the size and substance and improve the form of flowers. Because of these characteristics, breeders have unconsciously selected and used polyploid forms as parents. As a result of autopolyploidy, near perfection has been achieved in size, substance, and form in the white, cut-flower, *Phalaenopsis* orchids (*Phal. amabilis*).

In the 1960s a number of *Phalaenopsis* species with very brightly colored and patterned flowers were reintroduced into cultivation. Many of these novelty species, however, produce inflorescences with only a very few, tiny, starry-shaped flowers. These diploid species have been crossed with the modern autopolyploid white types to improve their size, form, and floriferousness. In many instances, these characteristics were improved but flower color was substantially diluted. In addition, many of these hybrids were triploid and were almost completely sterile.

### Materials and Methods

Orchid protocorms were obtained either through seed germination or tissue culture as previously described<sup>[1]</sup>. Chromosome doubling was accomplished by placing protocorms on medium containing half-strength Murashige and Skoog's salts and vitamins, 2 gm/L bacto-peptone, 2 ml/L 1-methyl-2-pyrrolidinone (a polyphenol inhibitor), 30 gm/L sucrose, 8 gm/L agar, and 0.5 mg/L filter sterilized (0.45 µm Nalgene) colchicines<sup>[1]</sup>. After 10 days in the dark at 26°C, the protocorms were transferred to the same medium but lacking the colchicine. After several months, the small plantlets were removed from culture and established in the greenhouse.

### Results and Discussion

Most commercially valuable orchids are hybrids. In some instances, their hybridity can be quite complex involving up to four genera (e.g., *Potinara* = *Cattleya* X *Laelia* X *Sophranitis* X *Brassavola*). Thus, both allo- and autopolyploidy could play a role in increasing fertility. There are several intergeneric *Phalaenopsis* hybrids that are only fertile as allopolyploids (e.g., *Asconopsis* = *Ascocenda* X *Phalaenopsis*). In most instances, however, the major limitation in using orchid hybrids in a breeding program is not due to their hybridity but to the result of triploidy.

Not all triploid plants are sterile, especially in orchids. Due to the enormous number of seed within a typical orchid seed capsule (e.g.,  $1 \times 10^6$ ) a 0.01 percent fertility can result in 10 seedlings per cross. In other non-orchid plants, thousands of

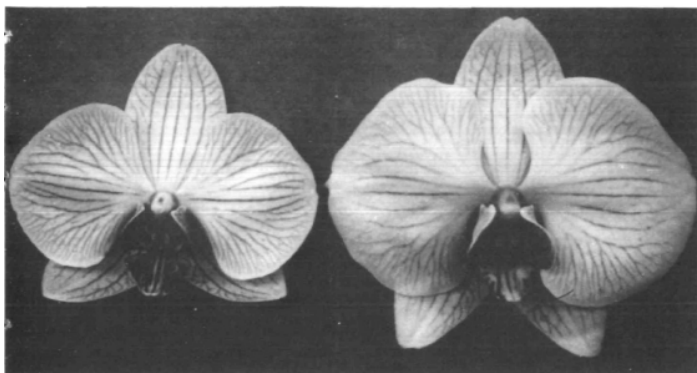
capsules would be needed to yield the same number of progeny.

The fertility of triploid orchids does, however, vary considerably. For example, each of the triploid clones of the grex *Sic. Jewel Box* differs in its fertility. The clone *Beverly* is the most fertile with greater than 1000 viable seed/capsule; the clone *Sheherazade* is intermediate producing hundreds of viable seed/capsule; and the clone *Dark Waters* is essentially sterile and has never produced viable seed.

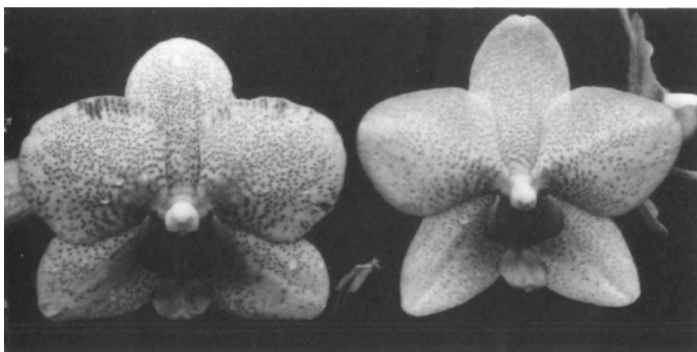
The fertility of most orchid triploids can be attributed to production of unreduced gametes. Storey<sup>[8]</sup> found that the progeny of *Cattleya Rembrandt* ( $2n = 3x = 60$ ) X *Laeliocattleya Pasadena* ( $2n = 4x = ca 80$ ) were pentaploid with approximately 100 chromosomes. Similarly, Niimoto and Randolph<sup>7</sup> found a large number of the progeny of *Cattleya Bow Bells* ( $2n = 3x = ca 60$ ) X *Cattleya Helen P. Dane* ( $2n = 4x = 80$ ) to be hypopentaploid ( $2n = 5x = ca 90$ ).

McCoy<sup>[3]</sup> has found that there is a genetic basis for the production of unreduced gametes. In alfalfa, there is a single gene recessive (*rprp*) that when homozygous leads to the production of up to 50 percent unreduced gametes. McCoy also has shown that the environment can alter the frequency of unreduced gametes; however, no one specific condition could be singled out that would either increase or decrease their production. One would expect that the fertility of a specific triploid should vary depending upon the environment. This seems to be the case. In general,





**FIGURE 1** Polyploidy in the improvement of "striped" *Phalaenopsis*: diploid form,  $2n = 2x = 38$  (left), and tetraploid form,  $2n = 4x = 76$  (right).



**FIGURE 2** Polyploidy in *Phalaenopsis* Golden Sands 'Canary': hexaploid form,  $2n = 6x = 114$  (left); and triploid form,  $2n = 3x = 57$  (right).

it was found that triploid *Phalaenopsis* are more fertile when they are fully mature and at least 4 to 5 years old. Freed (pers. comm.) of Freed's Orchids, Inc. of Malibu, California also has found that triploid orchids are more fertile when fully mature and not at their first or second flowering. Large mature plants that are grown well are buffered against short term environmental stresses that would alter the frequency of reduced gametes. Stress can lead to a decrease the frequency of unreduced gametes. McHale<sup>[4]</sup> and that high temperature stress reduced the frequency of unreduced gametes almost two-fold in *Solatum* species. Unreduced gametes can be used to improve the "Peppermint-striped" *Phalaenopsis* types (Figure 1). The triploid progeny from crosses between the diploid veined types and the tetraploid white types had much improved size, form, and substance, but lacked the peppermint striping.

These tetraploids were backcrossed to the diploid parent yield several hundred progeny raised. Almost all the progeny were tetraploid ( $2n = 4x = ca 76$ ). The intensity of striping varied considerably. Two to three percent were as intensely marked as the polyloid progenitor, but were much larger, fuller, and heavier (Figure 1). One cannot always rely upon naturally processed, unreduced gametes to restore fertility. For example, very few seedlings are obtained from crosses involving the triploid *Phal.* Golden Sands. In addition, most of the seedlings that are raised are aneuploid and produce deformed flowers. Through colchicine doubling a hexaploid form of *Phal.* Golden Sands was produced (Figure 2). Approximately 50 percent of the treated protocorms developed in polyploid plants. The hexaploid's flowers, besides being heavier and fuller, were much darker than the triploid's.

The induced tetraploids of *Phal. equestris* and *Phal.* Coral Isle also were much more intense than the untreated diploids. Morrison<sup>[5]</sup> has reported a similar effect in marigold where the tetraploid forms were more intensely colored than the diploid forms. Polyploidy also can change the qualitative expression of genes involved in flower color. In *Briza media*, Murray and Williams<sup>[6]</sup> have shown that the naturally occurring tetraploids contain c-glycosyl derivatives of luteolin while the diploids contain apigenin derivatives, which have one less OH group. It appears that polyploidy can both derepress and repress flavonoid genes. Levy<sup>[2]</sup> has found that in phlox the tetraploid forms expressed a different set of glycoflavones than did diploid types. Polyploidy by changing gene dosages can have a dramatic effect on gene expression. The hexaploid form of *Phal.* Golden Sands was backcrossed to the tetraploid white parent.

Approximately 5 percent of the seedlings produced clear yellow/green flowers without spotting. The other 95 percent of the progeny had various degrees of spotting. The form, size, and substance of all the offspring was much improved. The best of this cross, which was registered as *Phal.* Meadowlark ( $2n = 5x = ca 95$ ), was then backcrossed to the highly colored diploid parent. The pentaploid *Phal.* Meadowlark, behaves as a tetraploid in that most of the viable seedlings when crossed with a diploid are triploid. In *Cattleyas*, pentaploids behave somewhat differently. Vajrabhaya and Randolph<sup>[9]</sup> found that most of the offspring from diploid X pentaploid crosses had chromosome numbers between the triploid and tetraploid levels. In *Cattleyas*, aneuploidy is tolerated more than in *Phalaenopsis*.



In our crosses involving pentaploids, the aneuploid progeny do not develop past the protocorm stage, while in cattleyas the aneuploid plants could develop into somewhat normal seedlings. In the *Phalaenopsis* pentaploid crosses about 50 percent of the viable seed did not mature or develop into normal seedlings. These individuals were highly aneuploid. The seedlings from the backcross of *Phal.* Meadowlark to the diploid species retained both the intense coloration of the species and the size, form, and floriferousness of the white-flowered types.

Both unreduced gametes and colchicine-induced polyploidy has lead to major advances in orchid improvement in a relatively short period of time. It appears that polyploidy could play a very important role in the improvement of *Phalaenopsis* orchids.

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