Trends of *Phalaenopsis* orchid breeding research

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Abstract

Phalaenopsis is one of the most popular and important orchids produced at commercial scale. New cultivars are produced by selection of seedling plants during their flowering stage. After selection of orchid plants with desirable traits, tissue culture and mass production of young plantlets were achieved through flower stalk node culture in vitro. Cross hybridization by artificial pollination among different parental hybrids usually can lead to capsule set and seed formation. However, breeding barriers are present in Phalaenopsis and its alliance genera with the mechanisms largely remained unknown. Meiotic chromosome behavior of the pollen mother cells (PMC) was investigated to examine whether it is correlated with seed set in different breeding parents of both Doritaenopsis and Phalaenopsis orchids. The results showed that high percentage of normal tetrad, in addition to regular bivalent chromosome paring, generally suggests high fertility. Unreduced gametes such as dyads were observed during the PMC meiotic process and the number of the dyads could be increased by treating young flower buds with mitotic inhibitors such as colchicines and trifluralin. Through cross pollination of chemically conditioned pollinia, chance of obtaining polyploidy seedlings is expected to be increased.

Through these meiotic analysis and chemical treatment, breeding efficiency of phalaenopsis orchids will be increased.

Introduction

More than 50 species of *Phalaenopsis* genus have been found in the South-East Asia regions, including Taiwan, Philippine, Indonesia, Malaysia, Hainan Island, New Guinea, Thailand, and China (Christenson, 2001; Sweet, 1980). Its alliance genera such as *Doritis* and *Kingidium*, also have been used to produce the integeneric hybrids (http://www.phals.net/Definitions_e.html), especially *Doritaenopsis*. Many new *Phalaenopsis* and *Doritaenopsis* hybrids every year are registered through RHS (http://apps.rhs.org.uk/horticulturaldatabase/orchidregister/#). Potted Phalaenopsis orchids have become the most important flowering potted plants in the global markets (Griesbach, 2002). Therefore, breeding and selection of novel hybrids are the utmost to explore the Phalaenopsis market. To this point, how we can increase breeding efficiency by overcoming hybridization barriers, which occur very often during pollination and fertilization, is very helpful to the orchid industry.

Hybridization barriers in higher plants

The first step of fertilization in plants is pollination. Pollen tubes are allowed to grow into the stylar tissues in the campatible pollination. Increase in detectable levels of calcium ion was observed in the stylar tissues of lily after successful pollination and in the synergid cells within embryo sac of *Brassica napus* two days after fertilization (Zhao et al., 2004; Yu et al., 1998), which suggest an important role played by calcium in the process of reproduction (Ge et al., 2007). By monitoring Ca^{2+} dynamics during pollination in the transgenic *Arabidopsis* expressing yellow cameleon, a Ca^{2+} indicator based on green fluorescent protein, it was shown that

calcium was increased during pollen hydration and pollen tube penetrated into the papilla cell wall (Iwano et al., 2004). Some chemical guidance molecules, such as pectin and stigma/stylar cysteine-rich adhesin (SCA) have also been reported in the compatible pollination (Lord, 2003). Other molecules such as sugars and peptides may also help to guide pollen tube growth (Weterings and Russell, 2004). High levels of free auxin in stigmas and styles of *Nicotiana tabacum* during pollen germination and pollen-tube growth were detected by immunolocalization, which suggest the dependent role of auxin in pollination and subsequent fertilization (Chen and Zhao, 2008; Sundberg and Ostergaard, 2009). Certain compounds, including lipids, in the stigmatic exudates may help successful pollen tube germination (Sanchez et al., 2004).

In higher plants, two aspects of interspecific hybridization barriers are observed, including prezygotic and postzygotic barriers which may occur during or after syngamy (Stebbins, 1958; Rieseberg and Carney, 1998; Tiffin et al., 2001; Bushell et al., 2003). Imprinting and epigenetic regulation in interspecific hybrid failure have been suggested to operate in Arabidopsis, in addition to ploidy differences (Bomblies and Weigel, 2010).

One solution to the interspecific crossing barrier is to rescue the immature embryo by in vitro culture (Ondřej et al., 2001), or through in vitro fertilization (Wang et al., 2006). Alternatively, doubling the chromosome number by colchicines treatment of in vitro explants may help homeologous pairing in the doubled plants and thus improve the fertility (Hermsen et al., 1981; Lu and Bridgen, 1997).

Meiotic chromosome behavior and fertility in Doritaenopsis and Phalaenopsis orchids

Modern Phalaenopsis hybrids are usually derived from the cross hybridization between two different species or between a hybrid and a species. According to pedigree analysis by using the 'Wildcatt' software, one hybrid might be contributed by as many as 13-14 species via stepwise introgression. In addition, many hybrids have been evolved with higher ploidy level, especially tetraploid (Kuo et al., 2005). Most interspecific hybrids or intergeneric hybrid may carry diploidy. In dendrobium, the species from different sections may be represented as different genome constitution, therefore intersectional crossing might have abnormal meiotic pairing due to their nonhomologous chromosomes (Kamemoto et al., 1999). The chromosome set can be doubled by in vitro induction of mitotic inhibitors such as colchicines, oryzaline and some herbicides. With doubled chromosomes, the fertility of a hybrid may be restored completely or partially (van Tuyl et al., 1992; Lu and Bridgen, 1997).

Only limited information on breeding barrier of Phalaenopsis is available. In our experience, the chance of failure in capsule development in many crossing for either Doritaenopsis or Phalaenopsis orchids was high in practical experience. Crossing of diploid species *P*. *equestris* as female parent to tetraploid hybrids usually failed to set seeds. However, if the diploid species was used as pollen donor, 50-57% of the crosses produced viable seeds (Tang and Chen, 2007). When the triploid *P*. Taipei Gold 'STM' plant was crossed reciprocally to a tetraploid *P*. Sogo Yukidian 'V3', with regular meiosis (Hsu et al., 2010), no capsule or seed set was obtained. The cause for this crossing failure was attributed to the nature of low fertility in triploid as well as problem in stigmatic cavity clousure (Chuang et al., 2008). Despite auxins (IBA and NAA) were applied on to the stigma after pollination and cavity closed, no pollen germination was observed in the same cross combination (Chuang et al., 2008).

As the mechanisms of hybridization barriers in Phalaenopsis orchids are largely unknown, an alternative to look for the clue was by investigating the meiotic chromosome behavior of pollen mother cells (Bolaños-Villegas and Chen, 2007; Bolaños-Villegas et al., 2008), which may help to reveal suitable cultivars with good chromosome pairing and high pollen viability for use in the breeding program. The reason of using PMC as target material is their easy accessibility, because if we examine female meiosis of Phalaenopsis orchids, the fertilization event would occur only 60 days or 11 weeks after pollination and not very convenient for study (Nadeau et al., 1996; Lee et al., 2008).

Several Doritaenopsis hybrids were investigated for cross pollination, pollen viability, and chromosomal behavior during meiosis. A positive relation was found among high seed set, high frequency of viable tetrads, high degree of chromosome pairing, and low frequency of chromosomal aberrations such as inversions and translocations (Bolaños-Villegas and Chen, 2007; Bolaños-Villegas et al., 2008). In other words, those hybrids with lower percentage of tetrads, higher numbers of micronuclei or multivalent chromosome pairing tends to have low fertility, especially for the early generation *Doritaenopsis* hybrids such as *Dtps*. I-Hsin Purple Jewel, *Dtps*. Taihort Gem and *Dtps*. Queen Beer (Bolaños-Villegas and Chen, 2007; Bolaños-Villegas et al., 2008). The former two are also triploid with low or no fertility possibly due to abnormal meiotic behavior and low rate of normal tetrads. Generally, even numbered ploidy hybrids tend to have higher rate of tetrads and thus possessing certain degree of fertility. Triploids are generally sterile and only in some cases partially fertile which were supposed to be involved in polyploidy evolution (Bretagnolle and Thompson, 1995).

We further investigated some harlequin-type and standard pink Doritaenopsis for their meiosis, a high percentage of tetrads was observed in the PMCs of most hybrids

and thus their high fertility. One exception is with Dtps. Sogo Sofei, which had low fertility in several reciprocal crossing despite of its high tetrad percentage (Hsu et al., 2010).

Possibility of inducing unreduced gametes in Phalaenosis orchids

As mentioned above, meiotic pairing might be abnormal for *Dendrobium* species of different sections. In some cases, chromosome set were doubled between two different genomes and resulted with allopolyploid or amphidiploid. In many plants, such as Dendrobium orchid and a cactus, unreduced male or female gametes, occurring at very low rate, may contribute to produce polyploidy progeny (Kamemoto et al., 1999; Tel-Zur et al., 2003). Occasionally we could observe a tetraploid Phalaenopsis seedling derived from diploid plant crossed with another diploid, such as a tetraploid *P. equestris* was obtained and its ploidy level confirmed by root tip chromosome squash (Kuo et al., 2005). We suggest natural unreduced gamete as a good source for producing polyploidy progeny after cross pollination, despite with very low frequency.

To increase the number of unreduced gametes, mitotic inhibitors such as colchicine, oryzalin, and trifluralin have been used to prior to meiosis. Crossing plants with more unreduced gametes will increase the chance of polyploidy progenies (Bretagnolle and Thompson, 1995). We have used two tetraploid hybrids, namely *P.* Sogo Yukidian ‘V3’ and *P.* Tai Lin Redangel ‘V31’, as model phalaenopsis orchids to induce unreduced gametes. The reason for choosing both hybrids is their large flowers and pollinia for easy chemical treatment. Young flower buds were surface smeared with lanolin paste containing either colchicine or trifluralin. Both mitotic inhibitors increased the frequency of dyads in V3, while colchicines could induce higher number of unreduced dyads in V31 (Hsu et al., 2010).

Conclusion and perspectives

Meiotic chromosome behaviors of PMCs could be used as criteria for evaluation of parental fertility. They also revealed the state of unreduced gametes (monad, dyads and triads) and thus offer the opportunity to be induced at higher frequency by mitotic inhibitor treatments. Doubling the chromosome sets also may improve fertility. As yet we are too far from understating in depth of the hybridization barriers, those chemicals or metabolites which may help increase the pollination success and capsule setting so to overcome the barriers deserve further exploitation. Understanding hybridization barriers at molecular level may also shed light on the signals regulating the successful pollination and subsequent fertilization.

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