Tools to Develop Genetic Model Plants in the Orchidaceae Family

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Introduction

The Orchidaceae family is estimated to contain about 28,000 species and over 100,000 hybrids, making it one of the largest taxonomic groups among flowering plants [1]. The variations in flower colours, floral organ morphology and scents make orchids highly sought-after in ornamental horticulture. In addition, orchids are found in nearly all regions of the world with diverse adaptations, making them an invaluable resource to study plant evolution and speciation.

Despite orchids' great economic values and potential in basic research, currently there are no model plants from the Orchidaceae family. Unlike the model plant Arabidopsis (*Arabidopsis thaliana*), the orchid genomes are often large, heterogeneous and complex, making genetic analyses difficult [2]. Currently transformation techniques have been developed for several orchid species [3-5], and Virus-Induced Gene Silencing (VIGS) has been used to examine loss-of-function phenotypes [6]. *The wind orchid (Neofinetia falcate) is also being advocated as a model plant for its short stature, small genome and large mutant collection [7].* Nevertheless, many barriers still prevent orchids from becoming accessible for genetic analyses. Here we summarize two findings that may help increase the utility of orchids as a genetic model organism.

Firstly, orchid transposons may be a useful tool in mutation mapping. Currently there are no efficient mutagenesis methods for orchids, and the existing mutants are largely isolated and maintained by hobbyists [7]. Transposon may be able to quickly and methodically isolate orchid mutants. Undesirable phenotypic defects can often appear in cell culture clones during commercial breeding, likely due to random transposon excision and re-insertion events. A *Phalaenopsis* mutant with the floral column partially transformed into petals has previously been identified and characterized [8]. This floral organ conversion resembles the Arabidopsis *agamous (ag)* mutant, suggesting this mutant is likely affected in a C class floral organ identity gene [9,10]. Homologues of floral identity genes have been identified in *Phalaenopsis*, suggesting orchids may utilize floral development mechanism similar to Arabidopsis [11,12]. Furthermore, wild type-like revertants were isolated from the progenies of this *Phalaenopsis* mutant, indicating the mutation is likely caused by a transposon insertion event, and the phenotype reversed when the transposon is excised [8]. Currently the sequence and identity of this transposon remains unknown, but if this transposon can be identified it may help to develop *Phalaenopsis* as a genetic model plant. Snapdragon (*Antirrhinum majus*) is a successful model plant partially due to its genome contains transposons with known sequences. This allows the establishment of transposon tagging lines to easily identify genes affected by transposon insertion [13]. In addition, the function of the gene in question can easily be tested by isolating revertants resulted from the excision of the transposon.

A second challenge in establishing orchid as a model plant is its long generation time. Ornamental orchids typically takes two to three years to propagate one generation, making standard genetic analyses unfeasibly long in orchids. In vitro flowering system has been developed for *Dendrobium* to accelerate flowering, although its culturing conditions are heavily species-dependent [14]. Recently an Artificial Cultivation System (ACS) was developed to propagate two obligate mycoheterotrophic species, *Gastrodia* pubilabiata and G. confuse [15]. The ACS set-up consists a cedar log as growth medium, along with humus and cedar cones to promote fungal growth contained within a plastic box [15]. Interestingly, G. pubilabiata was not only viable in the ACS, but was able to set seeds up to three times a year, compare to in the natural habitat where it may take at least one year to set seed [15]. These results suggest that orchid generation time can potentially be shortened under artificial conditions, such that the timeframes of genetic analyses become feasible.

In summary, advancements in the Orchidaceae family research highlight the orchids' potential to be used as a genetic tool for basic research. One direction is to utilize orchid transposon as a mutation mapping tool. Phenotypic instability is a significant problem in the orchid breeding industry. However if these mutants are indeed caused by transposon insertions, then these mutant populations can be a useful resource to identify novel genes in development. Another advancement is to shorten the orchid generation time. Using the ACS, *Gastrodia* species can be propagated faster compared to under natural conditions, and currently the *Gastrodia* inbred line can be maintained up to the F3 generation. In conjunction with the current established molecular genetic techniques and genome annotation efforts, it is conceivable in the near future that genetic analyses in the Orchidaceae family become common practices

References

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