Phalaenopsis aphrodite (moth orchid): Functional genomics and biotechnology

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Abstract

Phalaenopsis aphrodite **(moth orchid) is one of the most important economic ornamental crops in the world. It has many special biological characteristics that are worth of studying. However, the complex size of the genome and the lack of molecular tool kits hamper the research and development of this plant species. Fortunately, the latest developments in sequencing technology and molecular tool kits such as gene transformation systems, VIGS technology, and gene editing platform etc. make it possible for functional gene discovery and precise genetic engineering. Undoubtedly, it will accelerate the application of basic research, orchid biotechnology application, and speed up the development of** ***Phalaenopsis*** **orchid industry**

Keywords: *Phalaenopsis aphrodite*, functional gene, horticultural traits, biotechnology, molecular breeding, genetic engineer.

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Introduction

Phalaenopsis orchid is one of the main commodities in international trade and global ornamental market *Phal.* orchids have distinctive biological and physiological characteristics. *Phal aphrodite* performs typical Crassulacean acid metabolism (CAM) photosynthesis and it has thick and succulent leaves.

Biological features of orchid

Phal. Orchids evolved to maximize CO2 uptake and reduce water loss by opening stomata at night under drought environments; therefore, they acquire high water use efficiency. CAM orchid uptakes very low rate of CO2 (<6 µmol•m–2•s–1) at night [1] and that causes slow growth rate in orchids. Orchids also have other features such as unique flower shape and have a special ABCDE model for flower development [2]. In the mature capsules orchid seeds are immature and without endosperm [3], therefore, symbiosis with mycorrhizae is essential for orchid seed germination in nature [4]. However, numerous *in vitro* seed germination media containing appropriate nutrients have been developed and are beneficial for orchid seedling development. Besides, several characteristics for example polyploidy, huge and complexity of orchid genome size and lack of molecular toolkits still hinder basic research of this orchid.

Advance research platforms/tool kits for orchids

The convenience of next-generation sequencing technologies significantly increases the opportunity to exam sequence information of non-model crop species. There are two very useful orchid transcriptomic databases in Taiwan, named OrchidBase 2.0 (http://orchidbase.itps.ncku.edu.tw) [1-5] and Orchidstra 2.0 (http://orchidstra2.abrc.sinica.edu.tw/orchidstra2/index.php) that are easily accessible to the public. Notably, Orchidstra 2.0 database provides a large number of transcriptome resources of orchid species with high economic value, and detail gene expression patterns in various organs. The genome sequence of *Phal. equestris* (a close relative of moth orchid) was published in year 2015 [6]. To be noted, in April 2018, Academia Sinica published the genome sequence of moth orchid (*Phal. aphrodite*) with high quality chromosome scale assembly [7]. These valuable bioinformatics databases actively support basic functional genomics research, accelerate the discovery of novel genes, and achieve precise molecular breeding in the near future.

Phalaenopsis orchid protoplast isolation method and transient gene expression system were established and enable detail molecular study such as: protein subcellular localization, protein-protein interaction, and promoter transient assays etc. [8]. Virus-induced gene silencing (VIGS) technology was developed and it provides transient, rapid, and enable high throughput screening and validation of orchid genes [9]. In additions, scientists will use knock out or overexpression approaches to validate the target gene function. Therefore, a promising and stable transformation system is critical. Our laboratory has developed an efficient Agrobacterium-mediated transformation of orchid, which is an efficient, stable, and transgene is heritable to the next generation [10]. This useful gene transformation technique significantly contributes to gene discovery, genetic engineering beneficial traits (such as blue color and scent) to commercial orchids. This high throughput orchid transformation system has enabled proof-of-concept and establishment of CRISPR/Cas9 technology in *Phalaenopsis* [11].

Orchid functional genomics studies

Several flowering marker genes of *Phalaenopsis* have been studied [12-14]. Multiple floral stalks and flowers increase the value of pot and cut flower *Phalaenopsis*. SPK1, a bHLH TF, controls *Phalaenopsis* spike initiation and affects multiple spiking [15]. *Phalaenopsis phototropin 1* and *2* have been isolated and function in chloroplast movements (accumulation and avoidance) to reduce photoinihbition and increase photosynthesis [16].

Floral scent is one of the most attractive horticulture traits of ornamental flowers [17]. It is believed that geranyl diphosphate synthase (GDPS) and terpene synthase (TPS) are the two key enzymes for monoterpene biosynthesis. Promoter of *GDPS* with a tandem repeat is crucial for scent activity [18]. Attainment of a blue orchid is the dream of some orchid enthusiasts. It has been reported that several MYB transcription factors play an important role in orchid flower pigmentation [19]. Researchers infiltrated Agrobacteria carrying *Delphinium grandiflorum DgF3'5'H* and *Phal. equestris PeMYB2* to V3 white orchid and produced purple blue color pigment formation [20]. In addition, a report about transgenic Arabidopsis and tobacco with autoluminescence was published recently [21]. The autoluminescence target gene driven by a flower specific promoter which is then overexpressed in orchid will have a huge and attractive market. Heterologous overexpression of rice *gibberellin 2-oxidase 6 (GA2ox6)* generated a miniature *Phalaenopsis* [22]. Moth orchids are severely infected by Odontoglossum ringspot tobamovirus (ORSV) and Cymbidium mosaic potexvirus (CymMV) etc. Researchers showed heterologous overexpressing dual resistance genes of ORSV and CymMV in tobacco transgenic plants and significantly enhanced tobacco plant resistant to the viruses. This provides an exciting scenario where similar strategies can be used to generate transgenic orchids and generate host plants resistance orchids against viruses in the near future [23].

Conclusion and Future Prospective

Discovery of more useful and functional genes will facilitate precise molecular breeding. Moreover, identification and utilization of tissue-specific promoters are essential for precise crop design. Genetic modify non-edible ornamental plants such as orchids are more easily accepted by consumers compare to those GM foods such as rice, soybean, maize etc. However, viable pollen might cause gene flow between GMO and wild relatives and its effect on biodiversity are major concerns. Therefore, male sterile orchids that have inactive pollinia might be a solution and need more study. In short, integration of research platforms and updated tool kits enable fundamental R&D of orchid biotechnology and accelerate development of the orchid industry.

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