ISSN 1817-3721, E-ISSN 1818-8745
- Review paper

Plant Tissue Cult. & Biotech. **29**(2): 133-149, 2019 (June)

PTC&B

Recent Advances in *Phalaenopsis* Orchid Improvement using Omics Approaches

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Key words: *Phalaenopsis*, Orchid, Genomics, Omics approaches

Abstract

With recent advances in high-throughput sequencing (HTS) technologies to improve plants, there is a need to release orchid specific genomic resources and platforms that are crucial for managing omics elements in systematic manner. Authors provide details about the recent developments in biotechnological techniques, genomics, transcriptomics, proteomics, metabolomics and their applications for the industrial production, propagation, conservation and manipulation of *Phalaenopsis* orchid.

Introduction

Phalaenopsis belongs to most diverse, second largest and widespread family Orchidaceae, which comprises more than 25,000 species, prominently monopodial epiphytes (Averyanov and Averyanov 2006, Sheelavanthmath et al. 2005). Orchid flower is complex and typically zygomorphic with attractive odor components for pollinators like bees, moths, flies and birds (Cozzolino and Widmer 2005).

In recent years, there is a rise in market value of orchid flowers in international business (Tsai 2011). Orchids are the second most economically valued in USA (USDA 2006). To date, *Phalaenopsis* has been identified as the most popular potted orchids in the world (Minh et al. 2017). Several countries such as Thailand, Malaysia, Singapore, South Korea, and Sri Lanka cultivate orchids as a cash crop and Taiwan tops in the world production (Khoddamzadeh et al. 2011).

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DOI: https://doi.org/10.3329/ptcb.v29i1.41986

Unregulated flower collection and destruction of plant habitat are resulting in reduction of orchid's diversification (Vij and Pathak 2012). Microscopic seed size, lack of endosperm with less than 5% germination rates and requirement of species-specific mycorrhizal fungi during germination (Shefferson et al. 2005, Bonnardeaux et al. 2007) and production of heterozygous plants are major limitations of seed propagation. To overcome the limitations seed propagation, *in vitro* clonal propagation protocols of *Phalaenopsis* hybrids has been developed using various vegetative parts of the plants (Teixeira da Silva et al. 2014, Vendrame and Khoddamzadeh 2016, Yeung 2017). Remarkable amount of research in micropropagation of *Phalaenopsis* played an important role in ex *situ* conservation (Khoddamzadeh et al. 2011, Vendrame and Khoddamzadeh 2016). Besides symbiotic germination approaches on *Phalaenopsis,* non-symbiotic germination technologies are also being used for mass propagation of orchids (Griesbach 2002). However, browning of culture owing to exudation of phenolics during micropropagation, fungi and bacterial contamination of explants and somaclonal variation are some of the challenges for successful *in vitro* culture (Khoddamzadeh et al. 2010).

Phalaenopsis species is a diploid plant with 38 chromosomes (2n = 2x) and the genome size of *P. equestris* is the smallest among other species of *Orchidacea*e (Leitch et al. 2009). Several studies on transcriptome sequencing have provided new insights into the structural and functional organization of the *Phalaenopsis* genome (Hsiao et al. 2006). It also aided in understanding and identification of putative genes involved in recent biosynthesis pathway. Manipulation of the biosynthesis pathway of fragrance can be used to produce high levels of aroma into monoterpene biosynthesis whereas this character will increase economic value of *Phalaenopsis* hybrid flowers. There are only a few species of *Phalaenopsis* which have a distinctive aroma (Yeh et al. 2014). Although recent efforts have been focused on genes identification focusing on scent, color, shape of flora (Hsu et al. 2015), there are still some missing genes in these pathways. Hsiao et al. (2006) elucidate the aroma biosynthetic pathway. A total of 31 volatile compounds were identified from *Phalaenopsis* 'Nobby's Pacific Sunset' orchids (Yeh et al. 2014).

Biologically active compounds such as phenolics and flavonoids have been identified in *Phalaenopsis* spp. (Minh et al. 2016). Root extract could play a role as antioxidant components. Manako et al. (2001) reported two phenanthropyran derivatives from *P. equestri*. Presence of pyrrolizidine alkaloid from root tips and young flower buds of *Phalaenopsis* orchids was reported by Anke et al. (2008).

This review provides details about the recent developments in biotechnological techniques, genomics and their applications for the industrial production, propagation, conservation and manipulation of *Phalaenopsis* orchid.

Plant tissue culture approaches: The improvement of biotechnological approaches aid in improving floricultural species for commercial production of orchid (Hossain et al. 2013). In the recent years, several reports on micropropagation of *Phalaenopsis* orchids show that selection of suitable explants are the critical factors for the success of plant tissue culture (Vasil et al. 2008, Vendrame and Khoddamzadeh et al. 2016). Composition of media also significantly affect the induction, number and plant regeneration efficiency in *Phalaenopsis*. (Kosiret et al. (2004) has been reported six medium compositions for direct shoot regeneration of *Phalaenopsis*. Plant regeneration in many genera has been achieved through flower stalks bud, stem nodes (Balilashaki et al. 2014), leaf tissues (Mayer et al. 2010, Niknejad et al. 2011, Vendrame and Khoddamzadeh 2016), shoot tips (Pant and Thapa et al. 2012). Sinha et al. (2010) studied the vegetative propagation of *Phalaenopsis* using young leaf segments on gelrite-gelled half strength MS supplemented with 2% sucrose, 2.0 mg/l BA, 0.5 mg/l NAA, 10% coconut water (CW), 2 g/l peptone and 1 g/l activated charcoal, the protocorm like bodies (PLBs) were induced within 12 weeks of culture. PLB formation is considered either direct or indirect embryogenesis (Martin and Madassery et al. 2006, Hong et al. 2008). The clonal propagation on a large scale of *Phalaenopsis* via the culture of protocorms has been reported by Paek et al. (2011). They reported that genotype, seed maturity and media composition influence seed germination rate and protocorm formation. The *in vitro* regeneration of *Phalaenopsis* orchid also dependent on activated charcoal (absorbs ethylene and phenolic inhibitors) supplement in culture medium. Cytokinins in combination with auxins also have shown to induce the PLB formation from leaf sections (Niknejad et al. 2011). A successful method for mass propagation of PLBs of *Phalaenopsis* elucidates using bioreactor system where leaves emerging from nodes (Young et al. 2000). Kuo et al. (2005) reported factors affecting direct somatic embryogenesis in the orchid *Phalaenopsis* 'Little Steve'. Direct somatic embryogenesis was reported from leaf explants of *Phalaenopsis amabilis* (Chen and Chang et al. 2006). Feng and Chen (2014) developed an efficient protocol for regeneration of *Phalaenopsis aphrodite* subsp. *Formosana* via inducing direct somatic embryogenesis. The important economic targets for *in vitro* propagation of epiphytic orchids includes the creation of variation in leaf types, flower color, fragrance and plant shape. Raynalta et al. (2018) studied the clonal fidelity of micropropagated *Phalaenopsis* plantlets by using of SNAP markers, they showed that TDZ and polyvinylpyrrolidone (PVP) induced PLB from leaf explants. 11.8% possible variants out of 34 evaluated plantlets were seen based on the assessment SNAP markers. Reports showed *in vitro* technologies can improve the *ex situ* conservation of orchid genetic resources (Aktar et al. 2008, An et al. 2011, Hossain et al. 2013).

Asymbiotic seed germination and the use of microbes has been reported in *Phalaenopsis* Blume orchids (Lesar et al. 2012). The influence of pollination season and maturity of capsule have been investigated under asymbiotic seed germination in three

Phalaenopsis orchid hybrids, namely, 'Athens', 'Moscow' and 'Lusaka' flowers (Balilashaki et al. 2014). Winter season was the suitable seasons of pollination and the highest germination percentages observed in 5-month-old winter-pollinated capsules.

Genomics: Genomics study is difficult for *Phalaenopsis* orchid because of its larger genome size and long life cycles. The chromosome sizes of *Phalaenopsis* species ranging from 1.5 to 3.5 μm are grouped into low, medium and high nuclear DNA content (Chen et al. 2010a). Lin et al. (2001) used flow-cytometry and estimated DNA contents of the nuclei in 18 *Phalaenopsis* Blume and *Doritis pulcherrima* Lindl. species. They observed a 6.07-fold variation in genome size within 18 *Phalaenopsis* species, ranging from 2.74 pg/ diploid nuclear DNA content (2C) for *P. sanderiana* to 16.61 pg/2C for *P. parishii* which suggested that the 2C-values of the *Phalaenopsis* sp. correlate with their chromosome sizes and also observed highest degree of endoreduplication in *P. equestris* leaves. Through chromosomal doubling new commercial hybrids were produced in *Phalaenopsis* (Chen et al. 2010b) and these hybrids can be used for comparative analyses of DNA content on evolution of *Phalaenopsis* or help to the orchid breeders and molecular geneticists for the selection of parental varieties for hybridization. Kao et al. (2001) analyzed karyotypes of nine representative *Phalaenopsis* species and *D. pulcherrima* and reported genome size and the amount of constitutive heterochromatin (CH) among the species. Molecular markers such as RFLP, RAPD and DAF used in diversity studies of *Phalaenopsis*. Goh et al. (2005) used RAPD markers for genetic distance and relationship investigation of 149 accessions in *Phalaenopsis,* they were useful for separation of the genus into seven clusters. RAPD analysis of 20 species of *Phalaenopsis*, was useful for producing genetic maps and marker-assisted selection in crop plants (Niknejad et al. 2009). Sequence-based microsatellite markers used for the study of molecular characterization and relationships (Fattmah and Sukma 2011) in orchids. Twenty-eight polymorphic microsatellite markers were screened for delimiting species within genus *Phalaenopsis* by Ko et al. (2017). cpDNA markers was used to compare *P. equestris* and *P. aphrodite*, they showed similar AT content, genome size, gene order and codon usage (Chang et al. 2017).

Genomic *in situ* hybridization (GISH) and RFLP analysis were conducted to identify the intergeneric hybridization status of putative hybrids. Both GISH and RFLP analyses were effective for F_1 hybrids detection (Liu et al. 2016a). The modified drop method has been improved by Kuo et al. (2016), it could be used for fluorescence *in situ* hybridization (FISH) mapping of DNA fragments in cytogenetic studies in *Phalaenopsis* orchids. FISH technique was used for chalcone synthase (CHS) localization on *Phalaenopsis* orchid chromosomes (Kuo et al. 2018) and also was used for detection of the accuracy of genome assembly of *Phalaenopsis aphrodite* (Chao et al. 2018).

The genome sequence of *Phalaenopsis equestris* (Cai et al. 2015) suggest that, gene duplication might have contributed to the CAM photosynthesis process in *P. equestris* and found MADS-box C/D-class, B-class *AP3* and *AGL6*-class genes, play role in

morphology of orchid flowers. A draft genome for *Phalaenopsis pulcherrima* 'B8802' and *Phalaenopsis* 'KHM190' cultivars have been generated by Huang et al. (2016). The differences between two orchids allowed the identification of 691,532 single-nucleotide polymorphisms. They also discovered the gibberellins synthesis pathway that regulates the expression of flowering time genes during the reproductive phase in orchids.

Transcriptomics: Transcriptome approach is used to study of the total mRNA molecules, gene fusions, and allele specific expression patterns with a clear, complete view from the molecular mechanisms of floral transcription in orchids. There are several reports on gene expression of orchid flower. Chalcone synthase (CHS), is the key gene in flavonoid biosynthesis pathway was isolated from *Phalaenopsis* hybrid flowers (Han et al. 2006), highly expressed *Pchs1* in petals and lips concomitant with the accumulation pattern of the anthocyanin in its flowers. Floral pigmentation patterning was studied in *Phalaenopsis* spp., and three R2R3-MYB transcription factors *PeMYB2, PeMYB11*, and *PeMYB12* were detected concomitant with red color formation in different varieties of tissues such as sepals/petals and lip. *PeMYB2*, *PeMYB11* and *PeMYB12* were responsive to the anthocyanin production in the sepals/petals (Hsu et al. 2015).

Real-time RT-PCR analysis on selected ESTs (Expres sed Sequence Tags) showed that auxin-regulated protein kinase, cyclophilin, and *TCP*-like genes are upregulated in mutant flower buds (Chen et al. 2005). A total of 5593 ESTs obtained from the flower buds of *Phalaenopsis equestris* (diploid species of *Phalaenopsis*) whereas a unigene set of 3688 sequences were identified via cluster analysis (Tsai et al. 2006).

Hsiao et al. (2006) successfully compared the transcripts in *Phalaenopsis bellina* and *Phalaenopsis equestris* flowers. Enzymes in the monoterpenoids biosynthetic pathway were recognized through data mining of the *P. bellina* floral EST database (dbEST). Systematic computational approaches were used to characterize the microRNA (miRNA) in *Phalaenopsis aphrodite.* A sum of 23 novel miRNAs expressed in the flower, their targets was predicted by miRBase in *P. aphrodite* (Chao et al. 2014). Huang et al. (2016) reported draft sequence and assembly of the genome of *Phalaenopsis* 'KHM190' cultivar generated 89.5 Gb RNA-seq and 113 million sRNA-seq reads for identifying 188 miRNA families.

There are several reports available on the orchid MADS-Box genes encoding transcriptional factors which are employed for the important roles on orchid floral development and evolutionary studies. Based on the exon/intron and domain structures, this ABCDE gene family is divided into two lineages, type I and type II in orchids (Smaczniak et al. 2012). Previous analysis of the patterns of expression in the floral organs of *Phalaenopsis equestris* orchid MADS-box genes identified and characterized four B-class *Phalaenopsis DEF*-like MADS-box genes, including *PeMADS2*, *PeMADS3*, *PeMADS4* and *PeMADS5* that they may play distinctive morphogenetic roles in the flowers (Tsai et al. 2006). Miranda and Palomino (2014) tested eight MADS-box candidate SEP-, FUL-, AG-, and STK-like genes in wild-type and peloric *Phalaenopsis* flowers. Their

result represented that *SEPALLATA-like* genes cleaved in two major clades, SEP1, 2, 4-like genes by four sub-clade *OsMADS1, OsMADS5*, und RMADS217-like genes (*OsMADS34*) and SEP3-like gene (divided in three major groups) which expressed in all flower organs.

Advances in sequencing technologies and a functional genomic study in orchids reported by Su et al. (2011), where they employed two strategies, high-throughput sequencing platform technologies, Roche 454 and Illumina/Solexato maximize assembly output. C- and a D-class gene, *PeMADS1* and *PeMADS7* in *Phalaenopsis equestris* are involved in evolution, orchid gynostemium and ovule developmental processes (Chen et al. 2012). The first transcriptome analysis based on deep sequencing was reported by Tsai et al. (2015) for developing EST-SSR loci in *P. aphrodite* subsp. *formosana.* They obtained a total of 1,439 EST-SSR loci, including di-, tri-, tetra-, penta- and hexanucleotide motifs from *Phalaenopsis* species whereas di- and tri-nucleotide detected as two most frequent motifs in this orchid species. Transcriptome and expression profile analysis during *Phalaenopsis* explant browning *in vitro* culture assayed by Xu et al. (2015), functional annotation led to the discovery of different expressed gene (DEGs) mainly involved in phenylpropanoid pathway and flavonoid biosynthesis. Previous studies reported significant changes in those two pathway (Jones and Saxena 2013). Transcriptome sequencing using Illumina platform from floral organ tissues (sepal, petal, labellum and gynostemium) of the *Phalaenopsis* wild-type and peloric mutant has revealed the critical regulators of the MADS-box TFs in *Phalaenopsis* labellum formation, and also identified five MADS genes, *PhAGL6a* (CUFF.17763), *PhAGL6b* (CUFF.17763.1), *PhMADS1* (CUFF.36625.1), *PhMADS4* (CUFF.25909) and *PhMADS5* (CUFF.39479.1) genes with differential expression in floral-organ development in *Phalaenopsis*. An increased *PhMADS4*, *PhAGL6a* and *PhAGL6b* transcript levels observed in lip-like petals and lip-like sepals of peloric mutant flowers whereas *PhMADS1* transcript was expressed strongly in the gynostemium of both wild types and peloric mutants and the PhMADS5 transcript level showed a positive regulator of petal and sepal development (Huang et al. 2015) and identified the four isoforms of PhAGL6b on the C-terminus region with the MADS-box genes as potential regulatory components of labellum organ development which involved alternative splicing in the big lip mutant (Huang et al. 2016). They showed the expression of flowering time genes control by the gibberellin synthesis pathway. Genome and transcriptome information help the genetic improvement and breeding of the *Phalaenopsis* orchids. *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for 11 diverse *P. equestris* tissue performed by Niu et al. (2016), and obtained transcriptomes from root, stem, seed and floral organs, and found 24, 21, 22 and 7 disease resistance (R) genes in the flower bud, root, stem and in the 7-day-seeds, respectively and did not observe the YABBY gene family (use in determining leaf polarity) in roots and seeds. Eight transcripts have been identified

during the comparative transcriptome analysis between scented and scentless orchids, among them PbbHLH4 regulates floral monoterpene biosynthesis in *Phalaenopsis* orchid (Chuang et al. 2018). *Phalaenopsis* flowering locus *VE* (*PaFVE*) gene has been characterized by Koh et al. (2018) via spatial and temporal expression studies. It regulates floral organ maturation and flowering time.

Proteomics: Systematic study of the orchid genome has generated a lot of information in the past. Genome annotation has discovered a number of new orchid genes not previously known in production and evolutionary biology (Cai et al. 2015). Several research efforts have been undertaken targeting on enhancement of orchid properties using proteomics-based methods including two-dimensional electrophoresis (2-DE) and mass spectrometry. Proteomics-based applications have been applied in orchid for mass production, mycorrhizal fungi interaction, drought stress and cell cycle regulation (Hsiao et al. 2011, Hossain et al. 2013). Liao et al. 2004, made a proteomic effort for CymMV capsid protein gene known for its role to silence the *P. amabilis* and enhances its resistance to CymMV. It has been reported that expression of exogenous lipid transfers protein-encoding gene responsible for improving the plants frost resistance (Qin et al. 2011). Lai et al. (2013) identified 27 novel differentially expressed proteins by using two-dimensional electrophoresis and further examined them by mass spectrometry. Functional annotation of these proteins revealed that they play a critical role in wide range of biological processes including disease resistance, stress response, transcriptional regulation, energy metabolism, and protein modification. Identified proteins may provide new insights towards understanding of the interactive responses in protein expression of *P. amabilis* during infection with CymMV and/or ORSV. Chen et al. (2018) reported a combined proteomic approach with ultrastructural observation and physiological-biochemical analysis during pollination-induced petal senescence in *Phalaenopsis* and yielded 42 differentially regulated proteins. Out of 42 proteins, 17 were found upregulated, while 25 were down regulated. Identification and functional characterization of differentially regulated proteins can be utilized as putative markers of senescence in *Phalaenopsis*.

The two-dimensional electrophoresis and LC/MS/MS have been used to show the differential expressions of *PsbP* and *PsbO* between the green and yellow leaf sectors of a variegated mutant of *Phalaenopsis aphrodite* subsp. *Formosana* (Tsi et al. 2017). Proteomic changes via matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/TOF-MS) has been examined by Chen et al. (2018) to reveal the mechanism regulation of petal senescence in *Phalaenopsis*.

In spite of various bioinformatics based algorithms and tools available for functional annotation and protein structure modeling, extensive analysis was limited to a few selected protein families. For instance, UniProt hosts mere 1,011 protein entries for *Phalaenopsis*, of which only 74 reviewed; leaving a huge scope for both bioinformatics and

in vitro studies. Currently, there is a demand for solved crystal structure or modeled 3D structures that can accelerate the Computer-Added Drug Design (CADD) to simulate drug-receptor interactions. Furthermore, 12 crystal structures for orchids are available in protein data bank (PDB) (http://www.rcsb.org/pdb/results/results.do? tabtoshow = Current and qrid = 1DCCD321) (Fig. 1), which could bridge that demand in finding insights into the above-mentioned mechanisms.

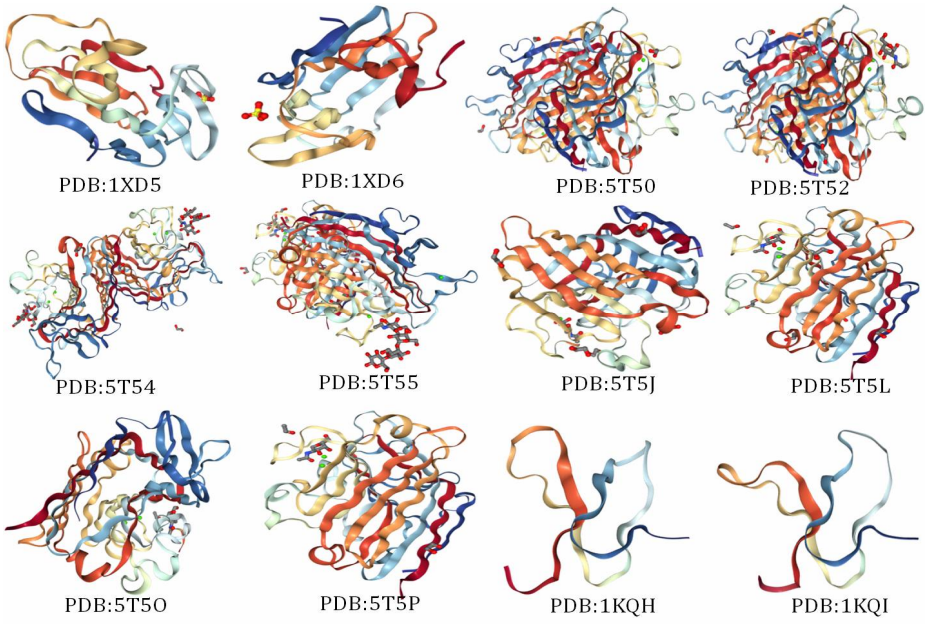

Fig. 1. 3D view of orchid protein structures available in protein data bank (PDB).

Metabolomics: 3% of these total orchid plant-derived compounds are known (Gutierrez 2010, Qasem and Foy 2001) and there are a very few reports available on phytochemical and biochemical aspects of orchids as a potential source of medicinal property. Recent advances in elucidating the biological properties of orchid species and its potential role in health-care suggest they can be used for treatment of various diseases such as anti-rheumatic, anti-carcinogenic, antivirus, antimicrobials, anticonvulsive, neuroprotective, and hypoglycemic activities (Gutierrez 2010, Yonzone et al. 2012; Marasini and Joshi 2013, Avasthi et al. 2013). Various studies on chemical components of orchids and suggest they possess phytoconstituents like phenols, alkaloids, glycosides, triterpenoids, flavonoids and stilbenoids (Gutierrez 2010, Kalaiarasan et al. 2011, Teoh 2016). According to Manako et al. (2001) study, spectroscopic (NMR, MS and so on) analysis identified 3-methoxy-2,7-dihydroxy-5H-phenanthro [4,5-*bcd*] pyran and 2,3,7-trihydroxy-5H-phenanthro [4,5-*bcd*] pyran from *Phalaenopsis equestris.* The accumulation

of different phenolic compounds examined by Andreotti et al. (2006) reported that amount of the phenols being synthesized in different parts of plant are affected by environmental conditions such as stress, UV- light and and so on. Ling and Subramaniam (2007) examined anthocyanins, anthocyanidins, chlorophylls, phenolics, proteins and sugar contents of 12 different samples of *Phalaenopsis violacea* and the reported cyaniding at a concentration of 11.53 ± µg/ml, delphinidin (12.73 ± 0.08 µg/ml), malvidin (7.65 ± 0.05 µg/ml), pelargonidin (8.98 ± 0.06 µg/ml), peonidin (21.24 ± 0.13 µg/ml) and petunidin (117.12 ± 0.69 µg/ml). The protein and total phenol concentration obtained for *Phalaenopsis violacea* were 1.78 ± 0 and 55.00 ± 4.15 µg, respectively. Frölich et al. (2006) suggested that pathways of typical compounds for plant secondary metabolism, orchidaceae alkaloids like T-phalaenopsine (necine base trachelanthamidine) more than 90% of total alkaloid and its stereoisomer Is-phalaenopsine (necine base isoretronecanol) as two 1,2-saturated pyrrolizidine monoesters identified by GC–MS. Analysis of phalaenopsine biosynthesis with ^{14}C-labeled putrecine indicated, the aerial roots of rosette plants were the sites of phalaenopsine biosynthesis. The tissue distribution of pyrrolizidine alkaloids in *Phalaenopsis* suggests in young and developing tissues (e.g., root tips and young leaves), peripheral tissues (e.g., of flower stalks) and reproductive organs (flower buds and flowers), has highest accumulation (Anke et al. 2008). *Phalaenopsis* orchids produce pyrrolizidine alkaloids of the phalaenopsine type as a defense factor that the first enzyme of pyrrolizidine alkaloid biosynthesis is homospermidine synthase (HSS) (Nurhayati et al. 2009). Anke et al. (2008) suggested no linkage was observed between plant development and HSS expression pattern (in the tips of aerial roots as the first site) and both of them independently happen during angiosperm evolution as expressed in a variety of tissues (Ober and Kaltenegger 2009).

Minh et al. (2016) investigated the leaves and roots extracts of six different hybrids of *Phalaenopsis* spp. For phenolic compounds and antiradical properties. They reported that the roots containing ferulic acid, *p*-coumaric acid, and sinapic acid and has extensive amount of natural antioxidants compared to leaves. Extracts from *Dactylorhiza hatagirea* (*Orchidaceae*) plant have been reported for its antibacterial activity (Dutta and Karn 2007). The medicinal potential of *Dactylorhiza hatagirea* was studied by Pant and Rinchen (2012) in traditional and modern medicine system. Kuo et al. (2010) detected the compositions of flavones and anthocyanin at 375 and 530 nm via high-performance liquid chromatography/ultraviolet detector (HPLC/UV) in various *Phalaenopsis* hybrids with red flower color, eventually using the scavenging of the α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical assayed the antioxidant properties and also reported the anti-tyrosinase activities of the pigment constituents. Compounds of (3',7-di-*O*-sinapylglucosyl)-3-glucosyl cyaniding, saponarin and apigenin 6-*C*-ribosido-7-*O*- glucoside

were observed with the IC_{50} values of 27.3, 307.1 and 41.6 μM, respectively, and strong anti-tyrosinase activities was apperceived only by apigenin 6-*C*-ribosido-7-*O*-glucoside.

Modification of the metabolic rate and the growth *Phalaenopsis* by genetic engineering has been studied by Chen et al. (2010), and selected *Phalaenopsis* transformed with the *Vitreoscilla* hemoglobin gene via injection of DNA solution into immature capsules and analyzed by Western blotting, eventually indicated line B47 has increased the growth in the vegetative and reproductive stage. The antimicrobial activity of *Dendrobium nobile* and *Phalaenopsis* flower extracts compared to five different antibiotics pyogenic skin infections isolates which the promising result were observed in case of flower extracts of *Dendrobium nobile* and *Phalaenopsis* that have been rich in key metabolites (Ashraf et al. 2013). Based on data obtained from metabolites of *Phalaenopsis* species can be used in the development of novel pharmaceutical sciences. The sucrose concentration in the stem significantly was increased at dawn and dusk of *Phalaenopsis aphroide* after warm-night treatment, so sucrose in the stem might be playing an important role in to sustain the viability of the dormant spike bud. All leaves at dawn of *Phalaenopsis aphroide* contained the highest citrate concentration under the warm-night treatment. Citrate accumulation helps to protect the leaves from warm-night stress (Liu et al. 2013). The three light treatments; (1) 40% blue 60% red, (2) 100% red, and (3) 100% white (control) were employed (Ouzounis et al. 2014) in greenhouse systems of *Phalaenopsis* production, where they observed that leaf area and total fresh weight were highest in the 40% B/60% R and 100% red for *Phalaenopsis* 'Vivien' and *Phalaenopsis* 'Purple star', respectively. They also studied quantitation of secondary metabolites by HPLC and their results indicated the additional blue light increased amount of flavonoids and carotenoids in *Phalaenopsis*. LC-MS technique based metabolomics has been performed on the effects of light qualities on *Phalaenopsis,* obtained results were showed the different light environments affected on the compounds of *Phalaenopsis,* so the environment conditions plays an important role in chemical instability of *Phalaenopsis* orchid, and also observed the significant differences between the molecular weights that involved biosynthesis of alkaloids derived from shikimate pathway. This study would be helpful to produce commercial *Phalaenopsis* orchid (Liu et al. 2016 b) exploring the existing possibilities as highlighted above.

Bioinformatics data bases for orchid: Recent advances in high-throughput sequencing (HTS) technologies, coincident with dramatic declines in cost**,** have enabled the scientific community to screen the whole genome and generate hypothesis leading to improving plants. Further, recently released orchid specific genomic resources and platforms are crucial for managing omics elements in systematic manners and extraction of desired genomic information. Details of important orchid databases have been shown in Table 1.

In this review, authors have summarized recent developments in genetics, genomics and their applications for the industrial production of *Phalaenopsis* orchid. The new hybrids produced through somaclonal variants are used for the production of important metabolites. These could be better explored through the genome databases and

important networks can be constructed which will further improve this value of the orchids.

Table 1. List of available orchid specific databases.

Database	No. of species	Description	Gene ontology	BLAST option	Statistics	URL	Reference
Orchidstra	6	Integrated orchid functional genomics database	Browse and search	Yes	114,933 (Coding genes)	http://orchidstra.abrc.sinica.edu.tw/	Su *et al.* (2013a)
Orchidstra 2.0	18	Database of transcriptome information for the Orchidaceae	Browse and search	Yes	510,947 (Coding genes)	http://orchidstra2.abrc.sinica.edu.tw	Chao *et al.* (2017)
OrchidBase 2.0	10	Orchidaceae floral transcriptomes data base	Browse only	No	1,562,071 (Unigenes)	http://orchidbase.itps.ncku.edu.tw	Tsai *et al.* (2013)
OOGB	1	Oncidium orchid Genome base	No	Yes	50,908 (Contig sequences)	http://predictor.nchu.edu.tw/oogb/	Chang *et al.* (2011)
Monocots PLAZA 4.0	1	Plant-oriented online resource for comparative, evolutionary and functional genomics	Browse only	No	29,431 (Coding genes)	https://bioinformatics.psb.ugent.be/plaza/versions/plaza_v4_monocots/	Van Bel *et al.* (2018)

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(Manuscript received on 13 February, 2019; revised on 3 April, 2019)