The genome and transcriptome of the *Phalaenopsis* yield insights into floral organ development and flowering regulation

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Phalaenopsis orchid is an important potted flower with high economic value around the world. We report the 3.1 Gb draft genome assembly of an important winter flowering *Phalaenopsis* 'KHM190' cultivar. We generated 89.5 Gb RNA-seq and 113 million sRNA-seq reads to use these data to identify 41,153 protein-coding genes and 188 miRNA families. We also generated a draft genome for *Phalaenopsis pulcherrima* 'B8802', a summer flowering species, via resequencing. Comparison of genome data between the two *Phalaenopsis* cultivars allowed the identification of 691,532 single-nucleotide polymorphisms. In this study, we reveal the key role of *PhAGL6b* in the regulation of labellum organ development involves alternative splicing in big lip mutant. Petal or sepal overexpressing *PhAGL6b* lead to the conversion into lip-like structure. We also evidenced the gibberellin pathway that regulates the expression of flowering time genes during the reproductive phase change induced by cool temperature. Our work thus depicted a valuable resource for the flowering control, flower architecture development, and breeding of the *Phalaenopsis* orchids.

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Abstract

Phalaenopsis **orchid is an important potted flower with high economic value around the world. We report the 3.1 Gb draft genome assembly of an important winter flowering** ***Phalaenopsis*** **'KHM190' cultivar. We generated 89.5 Gb RNA-seq and 113 million sRNA-seq reads to use these data to identify 41,153 protein-coding genes and 188 miRNA families. We also generated a draft genome for** ***Phalaenopsis pulcherrima*** **'B8802', a summer flowering species, via resequencing. Comparison of genome data between the two** ***Phalaenopsis*** **cultivars allowed the identification of 691,532 single-nucleotide polymorphisms. In this study, we reveal the key role of** ***PhAGL6b*** **in the regulation of labellum organ development involves alternative splicing in big lip mutant. Petal or sepal overexpressing** ***PhAGL6b*** **lead to the conversion into lip-like structure. We also evidenced the gibberellin pathway that regulates the expression of flowering time genes during the reproductive phase change induced by cool temperature. Our work thus depicted a valuable resource for the flowering control, flower architecture development, and breeding of the** ***Phalaenopsis*** **orchids.**

1. Introduction

Phalaenopsis is a genus within the family Orchidaceae and comprises approximately 66 species distributed throughout tropical Asia (Christenson 2002). The predicted *Phalaenopsis* genome size is approximately 1.5 gigabases (Gb), which is distributed across 19 chromosomes (Lin et al. 2001). *Phalaenopsis* flowers have a zygomorphic floral structure, including three sepals (in the first floral whorl), two petals and the third petal develops into a labellum in early stage of development, which is a distinctive feature of a highly modified floral part in second floral whorl unique to orchids. The gynostemium contains the male and female reproductive organs in the center (Rudall & Bateman 2002). In the ABCDE model, B-class genes play important role to perianth development in orchid species (Chang et al. 2010; Mondragon-Palomino & Theissen 2011; Tsai et al. 2004). In addition, *PhAGL6a* and *PhAGL6b*, expressed specifically in the *Phalaenopsis* labellum, were implied to play as a positive regulator of labellum formation (Huang et al. 2015; Su et al. 2013). However, the relationship between the function of genes involved in floral-organ development and morphological features remains poorly understood.

Phalaenopsis orchids are produced in large quantity annually and are traded as the most important potted plants worldwide. During greenhouse production of young plants, the high temperature >28°C was routinely used to promote vegetative growth and inhibit spike initiation (Blanchard & Runkle 2006). Conversely, a lower ambient temperature (24/18°C day/night) is used to induce spiking (Chen et al. 2008) to produce flowering plants. Spike induction in *Phalaenopsis* orchid by this cool temperature is the key to precisely control its flowering date. Several studies have indicated that cool temperature during the night are necessary for *Phalaenopsis* orchids to flower (Blanchard & Runkle 2006; Chen et al. 1994; Chen et al. 2008; Wang 1995). Despite a number of expressed sequence tags (ESTs), RNA-seqs and sRNA-seqs from several tisues of *Phalaenopsis* have been reported and deposited in GenBank or OrchidBase (An & Chan 2012; An et al. 2011; Hsiao et al. 2011; Su et al. 2011), only a few flowering related genes or miRNAs have been identified and characterized. Besides, the clues to the spike initiation during reproductive phase change in the shorten stem, which may produce signals related to flowering during cool temperature induction, have not been dealt with. So far, the molecular mechanisms leading to spiking of *Phalaenopsis* has yet to be elucidated.

Here we report a high-quality genome and transcriptomes (mRNAs and small RNAs) of *Phalaenopsis* Brother Spring Dancer ‘KHM190’, a winter flowering hybrid with spike formation in response to low temperature. We also provide resequencing data for summer flowering species *P. pulcherrima* ‘P8802’. Our comprehensive genomic and transcriptome analyses provide valuable insights into the molecular mechanisms of important biological processes such as floral organ development and flowering time regulation.

2. METHODS SUMMARY

The genome of the *Phalaenopsis* Brother Spring Dancer 'KHM190' cultivar was sequenced on the Illumina HiSeq 2000 platform. The obtained data were used to assemble a draft genome sequence using the Velvet software (Zerbino & Birney 2008). RNA-Seq and sRNA-Seq data were generated on the same platform for genome annotation and transcriptome and small RNA analyses. Repetitive elements were identified by combining information on sequence similarity at the nucleotide and protein levels and by using de novo approaches. Gene models were predicted by combining publically available *Phalaenopsis* RNA-Seq data and RNA-Seq data generated in this project. RNA-Seq data were mapped to the repeat masked genome with Tophat (Trapnell et al. 2009) and CuffLinks (Trapnell et al. 2012). The detailed methodology and associated references are available in the SI Appendix.

3. Results and Discussion

3.1 Genome sequencing and assembly. We sequenced the genome of the *Phalaenopsis* orchid cultivar 'KHM190' (SI Appendix, Fig. S1a) using the Illumina HiSeq 2000 platform and assembled the genome with the Velvet assembler, using 300.5 Gb (90-fold coverage) of filtered high-quality sequence data (SI Appendix, Table S1). This cultivar has an estimated genome size of 3.45 Gb on the basis of a 17-mer depth distribution analysis of the sequenced reads (SI Appendix, Fig. S2 and S3 and Table S2 and S3). *De novo* assembly of the Illumina reads resulted in a sequence of 3.1 Gb, representing 89.9% of the *Phalaenopsis* orchid genome. Following gap closure, the assembly consisted of 149,151 scaffolds (≥1000 bp), with N50 lengths of 100 kb and 1.5 kb for the contigs. Approximately 90% of the total sequence was covered by 6,804 scaffolds of >100 kb, with the largest scaffold spanning 1.4 Mb (SI Appendix, Table S3-S5 and Dataset S17). The sequencing depth of 92.5% of the assembly was more than 20 reads (SI Appendix, Fig. S3), ensuring high accuracy at the nucleotide level. The GC content distribution in the *Phalaenopsis* genome was comparable with that in the genomes of *Arabidopsis* (2000), *Oryza* (2005) and *Vitis* (Jaillon et al. 2007) (SI Appendix, Fig. S4).

3.2 Gene prediction and annotation. Approximately 59.74% of the *Phalaenopsis* genome assembly was identified as repetitive elements, including long terminal repeat retrotransposons (33.44%), DNA transposons (2.91%) and unclassified repeats (21.99%) (SI Appendix, Fig. S5 and Table S6). To facilitate gene annotation, we identified 41,153 high-confidence and medium-confidence protein-coding regions with complete gene structures in the *Phalaenopsis* genome using RNA-Seq (114.1 Gb for a 157.6 Mb transcriptome assembly), based on 15 libraries representing four tissues (young floral organs, leaves, shortened stems and protocorm-like bodies (PLBs)) (SI Appendix, Table S7 and Dataset S18), and we used transcript assemblies of these regions in combination with publically available expressed sequence tags (Su et al. 2011; Tsai et

al. 2013) for gene model prediction and validation (Dataset S1-S2). We predicted 41,153 genes with an average mRNA length of 1,014 bp and a mean number of 3.83 exons per gene (Table 1 and Dataset S3). In addition to protein coding genes, we identified a total of 562 ribosomal RNAs, 655 transfer RNAs, 290 small nucleolar RNAs and 263 small nuclear RNAs in the *Phalaenopsis* genome (SI Appendix, Table S8). We also obtained 92,811,417 small RNA (sRNA) reads (18-27 bp), representing 6,976,375 unique sRNA tags (SI Appendix, Fig. S6 and Dataset S6-S7). A total of 650 miRNAs distributed in 188 families were identified (Dataset S8), and a total of 1,644 miRNA-targeted genes were predicted through the alignment of conserved miRNAs to our gene models (SI Appendix, Fig. S7 and Dataset S9-S10).

The *Phalaenopsis* gene families were compared with those of *Arabidopsis* (2000), *Oryza* (2005), and *Vitis* (Jaillon et al. 2007) using OrthoMCL (Li et al. 2003). We identified 41,153 *Phalaenopsis* genes in 15,855 families, with 8,532 gene families being shared with *Arabidopsis*, *Oryza* and *Vitis*. Another 5,143 families, containing 12,520 genes, were unique to *Phalaenopsis* (Figure 1). In comparison with the 29,431 protein-coding genes estimated for the *Phalaenopsis equestris* genome (Cai et al. 2015), our gene set for *Phalaenopsis* 'KHM190' contained 11,722 more members, suggesting a more wider representation of genes in this work. This difference in gene number may be due to different approaches between *Phalaenopsis* 'KHM190' and *Phalaenopsis equestris*. Besides, *Phalaenopsis* 'KHM190' is a hybrid while *P. equestris* species, which may show gene number difference due to different genetic background. To better annotate the *Phalaenopsis* genome for protein-coding genes, we generated RNA-seq reads obtained from four tissues as well as publically available expressed sequence tags for cross reference. We defined the function of members of these families using Gene ontology (2008), the Kyoto Encyclopedia of Genes and Genomes (Kanehisa et al. 2012) and Pfam protein motifs (Finn et al. 2014) (Figure. 2 and Dataset S3-S5 and Dataset S19).

The genes in the HC (High confidence) and MC (Medium Confidence) gene sets were functionally annotated based on homology to annotated genes from the NCBI non-redundant database (Dataset S3). The functional domains of *Phalaenopsis* genes were identified by comparing their sequences against protein databases, including the Gene Ontology (GO) (2008), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al. 2012) and Pfam (Finn et al. 2014; Finn et al. 2011) databases. GO terms were obtained using the Blast2GO program (Conesa & Gotz 2008). In the GO annotations, 16,034, 27,294, and 16,360 genes were assigned to the biological process, cellular compound, and molecular function categories, respectively (Figure. 2A). Based on KEGG pathway mapping, we were able to assign a significant proportion of the *Phalaenopsis* gene sets to KEGG functional or biological pathway categories (11,452 sequences; 140 KEGG orthologous terms) (Dataset S4). To investigate protein families, we compared the Pfam domains of *Phalaenopsis* genome. A total of 1,842 Pfam domains were detected among the *Phalaenopsis* sequences. The most abundant protein domains in *Phalaenopsis* genome were

pentatricopeptide repeats (PPRs, pfam01535), followed by the WD40 (pfam00400), EF hand (pfam00036) and ERM (Ezrin/radixin/moesin, pfam00769) domains (Figure. 2B and Dataset S5). Furthermore, conserved domains could be identified in 50.17% of the predicted protein sequences based on comparison against Pfam databases. In addition, we identified 2,610 transcription factors (6.34% of the total genes) and transcriptional regulators in 55 gene families (SI Appendix, Fig. S8-S10 and Dataset S11-S12).

3.3 Regulation of *Phalaenopsis* floral organ development. The relative expression of all *Phalaenopsis* genes was compared through RNA-Seq analysis of shoot tip tissues from shortened stems, leaf, floral organs and PLB samples, in addition to vegetative tissues, reproductive tissues, and germinating seeds from *P. aphrodite* (Su et al. 2011; Tsai et al. 2013) (SI Appendix, Fig. S12 and Dataset S1). *Phalaenopsis* orchids exhibit a unique flower morphology involving outer tepals, lateral inner tepals and a particularly conspicuous labellum (lip) (Rudall & Bateman 2002). However, our understanding of the regulation of the floral organ development of the genus is still in its infancy. To comprehensively characterize the genes involved in the development of *Phalaenopsis* floral organs, we obtained RNA-Seq data for the sepals, petals and labellum of both the wild-type and peloric mutant of *Phalaenopsis* 'KHM190' at the 0.2-cm floral bud stage, at which shows early sign of labellum differentiation. This cultivar presented an early peloric fate in its lateral inner tepals. In a peloric flower, the lateral inner tepals are converted into a lip-like morphology at this young bud stage (SI Appendix, Fig. S12a and 11b). We identified 3,743 genes that were differentially expressed in the floral organs of the wild-type and peloric mutant plants. Gene Ontology analysis of the differentially expressed genes in *Phalaenopsis* floral organs revealed functions related to biological regulation, developmental processes and nucleotide binding, which were significantly altered in both genotypes (Huang et al. 2015). Transcription factors (TFs) seem to play a role in floral organ development. Of the 3,309 putative TF genes identified in the *Phalaenopsis* genome showed differences in expression between the wild-type and peloric mutant plants (Dataset S11).

MADS-box genes are of ancient origin and are found in plants, yeasts and animals (Trobner et al. 1992). This gene family can be divided into two main lineages, referred to as type I and type II. Type I genes only share sequence similarity with type II genes in the MADS domain (Alvarez-Buylla et al. 2000). Most of the well-studied plant genes are type II genes and contain three domains that are not present in type I genes: an intervening (I) domain, a keratin-like coiled-coil (K) domain, and a C-terminal (C) domain (Munster et al. 1997). These genes are best known for their roles in the specification of floral organ development, the regulation of flowering time and other aspects of reproductive development (Dornelas et al. 2011). In addition, MADS-box genes are also widely expressed in vegetative tissues (Messenguy & Dubois 2003; Parenicova et al. 2003). The ABCDE model comprises five major classes of homeotic selector

genes: A, B, C, D and E, most of which are MADS-box genes (Theissen 2001). However, research on the ABCDE model was mainly focused on herbaceous plants and has not fully explained how diverse angiosperms evolved. The function of many other genes expressed during floral development remains obscure. *Phalaenopsis* exhibits unique flower morphology involving three types of perianth organs: outer tepals, lateral inner tepals, and a labellum (Rudall & Bateman 2002). Despite its unique floral morphological features, the molecular mechanism of floral development in *Phalaenopsis* orchid remains largely unclear, and further research is needed to identify genes involved in floral differentiation. Recently, several remarkable research studies on *Phalaenopsis* MADS-box genes have revealed important roles of some of these genes in floral development, such as four B-class *DEF*-like MADS-box genes that are differentially expressed between wild-type plants and peloric mutants with lip-like petals (Tsai et al. 2004) and a *PI-like* gene, *PeMADS6*, that is ubiquitously expressed in petaloid sepals, petals, columns and ovaries (Tsai et al. 2005).

In the *Phalaenopsis* genome sequence assembly, a total of 122 genes were predicted to encode MADS-box family proteins (SI Appendix, Fig. S8, Dataset S12). To obtain a more accurate classification, phylogenetic trees were constructed via the neighbour-joining method, with 1000 bootstraps using MEGA5 (Tamura et al. 2011).. The differentially expressed genes (DEGs) among 122 *Phalaenopsis* MADS-box genes were obtained from our *Phalaenopsis* RNA-Seq data (Dataset S11). The expression profile indicated that most MADS-box genes are widely expressed in diverse tissues. These results will be helpful to elucidate the regulatory roles of these genes in *Phalaenopsis* floral organ development.

Notably, we previously reported one of the MADS-box genes, *PhAGL6b*, upregulated in the peloric lateral inner tepals (lip-like petals) and lip organs (Huang et al. 2015). To understand the expression mode, we therefore cloned the full-length sequence of *PhAGL6b* from lip organ cDNA libraries for the wild-type, peloric mutant and big lip mutant. The big lip mutant developed a petaloid labellum instead of the regular lip observed in the wild-type flower (Figure 3B). Interestingly, we identified firstly four alternatively spliced forms of *PhAGL6b* that were specifically expressed only in the petaloid labellum of the big lip mutant (Figure 3C and 3D and SI Appendix, Fig. S11. To determine whether the alternatively spliced forms of *PhAGL6b* affect the conversion of the labellum to a petal-like organ in the big lip mutant, we performed RT-PCR of total RNA extracted from the labellum organs of plants with different big lip mutant phenotypes and wild-type plants (SI Appendix, Table S11, Figure 4A) to amplify the *PhAGL6b* transcripts. Interestingly, among all of the big lip mutant phenotypes, 500~700 bp bands were detected, corresponding to *PhAGL6b* alternatively spliced forms, which were not found in any of the other orchid plants (Figure 4A). We further examined the expression of *PhAGL6b* and its alternatively spliced forms in the labellum organs of *Phalaenopsis* plants with different big lip phenotypes and wild-type plants via real-time PCR (SI Appendix, Table S11). In the big lip

mutants, the expression of native *PhAGL6b* was reduced by 42~70%, whereas all of the alternatively spliced forms were expressed more strongly compared with the wild-type plants (figure 4B). In summary, the RT-PCR and real-time PCR experiments corroborated the specific expression of the alternatively spliced forms of *PhAGL6b* in the petal-like lip of big lip mutants. Thus, *PhAGL6b* might play crucial role in the development of the labellum in *Phalaenopsis*.

The four isoforms of the encoded PhAGL6b products differ only in the length of their C-terminus region (Figure 3D). C-domain is important for the activation of transcription of target genes (Honma & Goto 2001) and may affect the nature of the interactions with other MADS-box proteins in multimeric complexes (Geuten et al. 2006; Gramzow & Theissen 2010). In *Oncidium*, L (lip) complex (OAP3-2/OAGL6-2/OAGL6-2/OPI) is required for lip formation (Hsu et al. 2015). The *Phalaenopsis PhAGL6b* is an orthologue of *OAGL6-2*. In our study, the PhAGL6b and its different spliced forms may each other compete the *Phalaenopsis* L-like complex to affect labellum development as reported in *Oncidium* (Hsu et al. 2015). This provides a novel clue further supporting the notion that *PhAGL6b* may function as a key floral organ regulator in *Phalaenopsis* orchids, with broad impacts on petal, sepal and labellum development (Figure 3E).

3.4 Control of flowering time in *Phalaenopsis*. The flowering of *Phalaenopsis* orchids is a response to cues related to seasonal changes in light (Wang 1995), temperature (Blanchard & Runkle 2006) and other external influences (Chen et al. 1994). A cool night temperature of 18-20°C for approximately 4 weeks will generally induce spiking in most *Phalaenopsis* hybrids, while high temperature inhibits it. To compare gene expression between a constant high-temperature (30/27°C; day/night) and inducing cool temperature (22/18°C), we collected shoot tip tissues from shortened stems of mature *P. aphrodite* plants after treatment at a constant high temperature (BH) and a cool temperature (BL) (1 to 4 weeks) for RNA-Seq data analysis (SI Appendix, Fig. S12g-i). More than 7,500 *Phalaenopsis* genes were found to be highly expressed in the floral meristems during the 4 successive cool temperature periods (showing at least a 2-fold difference in the expression level in the BL condition relative to BH) (Dataset S13). The identified flowering-related genes correspond to transcription factors and genes involved in signal transduction, development and metabolism (Figure 3 and Dataset S14). The classification of these genes includes the following categories: photoperiod, gibberellins (GAs), ambient temperature, light-quality pathways, autonomous pathways and floral pathway integrators (Fornara et al. 2010; Mouradov et al. 2002). However, the genes involved in the photoperiod, ambient temperature, light quality and autonomous pathways did not show significant changes in the floral meristems during the cool temperature treatments (SI Appendix, Fig. S13 and Dataset S14). By contrast, the expression patterns of genes involved in pathways that regulate flowering, comprising a total of 22 GA pathway-related genes, were related to biosynthesis, signal transduction and responsiveness. The GA pathway-related genes and the floral pathway integrator

genes have been revealed as representative key players in the link between flowering promotion pathways and the floral transition regulation network in several plant species (Mutasa-Gottgens & Hedden 2009). In contrast to the expression patterns observed in BL and BH, the GA biosynthetic pathway and positively acting regulator genes showed high expression levels in BL. Furthermore, the expression level of negatively acting regulators, like DELLA genes identified, was suppressed by the cool temperature which allowing the activation of flowering related genes . The genes included in the flowering promotion pathways and floral pathway integrators were generally upregulated in BL (Figure 5 and Figure 6 and Dataset S11). These findings suggest that the GA pathway may play a crucial role in the regulation of flowering time in *Phalaenopsis* orchid during cool temperature.

3.5 Genetic polymorphisms for *Phalaenopsis* orchids. The *Phalaenopsis* genome assembly also provides the basis for the development of molecular marker-assisted breeding. Analysis of the *Phalaenopsis* genome revealed a total of 532,285 simple sequence repeats (SSRs) (SI Appendix, Fig. S14 and Table S9 and Dataset S15). To enable the identification of single nucleotide polymorphisms (SNPs), we re-sequenced the genome of a summer flowering species, *P. pulcherrima* 'B8802', with about tenfolds coverage. Comparison of the genome data from the two *Phalaenopsis* accessions (KHM190 and B8802) allowed the discovery of 691,532 SNPs, which should be valuable for future development of SNP markers for *Phalaenopsis* marker-assisted selection. (SI Appendix, Fig. S15 and Table S10 and Dataset S16). *P. pulcherrima* is an important parent for small flower and summer-flowering cultivars in breeding program. These SNP markers may contribute valuable tools for varietal identification, genetic linkage map development, genetic diversity analysis, and marker-assisted selection breeding in *Phalaenopsis* orchid.

4. Conclusion

In this study, we sequenced, de novo assembled, and extensively annotated the genome of one of the most important *Phalaenopsis* hybrid. We also annotated the genome with a wealth of RNA-seq and sRNA-seq from different tissues, and many genes and miRNAs related to floral organ development, flowering time and protocorm (embryo) development were identified. Importantly, this RNA-Seq and sRNA-seq data allowed us to further improve the genome annotation quality. In addition, mining of SSR and SNP molecular markers from the genome and transcriptomes is currently being adopted in advanced breeding programs and comparative genetic studies, which should contribute to efficient *Phalaenopsis* cultivar development. Despite the *P. equestris* genome has been reported recently (Cai et al. 2015), focus on floral organ development and flowering time regulation has not been dealt with. In our study, we obtained transcriptomes from shortened stems, which initiate spikes in response to low ambient temperature, and floral organs

and generated valuable data of potentially regulate flowering time key genes and floral organ development. The genome and transcriptome information of our work should provide a constructive reference resource to upgrade the efficiency of cultivation and genetic improvement of *Phalaenopsis* orchids.

Data deposition:

The *Phalaenopsis* genome assembly, transcriptomic and sRNA-seq data were deposited in Genbank with BioProject ID PRJNA271641. The version described in this paper is the first version, JXCR00000000. All short-read data are available via Sequence Read Archive: SRR1747138, SRR1753943, SRR1753944, SRR1753945, SRR1753946, SRR1753947, SRR1753948, SRR1753949, SRR1753950, SRR1752971, SRR1753106, SRR1753165, SRR1753166 (*Phalaenopsis* 'KHM190' genomic DNA); SRR1762751, SRR1762752, SRR1762753 (*Phalaenopsis* 'B8802' genomic DNA); SRR1760428, SRR1760429, SRR1760430, SRR1760432, SRR1760433, SRR1760435, SRR1760436, SRR1760438, SRR1760439, SRX396172, SRX396784, SRX396785, SRX396786, SRX396787, SRX396788 (RNA-seq); SRR1760091, SRR1760211, SRR1760212, SRR1760213, SRR1760270, SRR1760271, SRR1760523, SRR1760524, SRR1760525, SRR1760526, SRR1760527, SRR1760528, SRR1760530, SRR1760531, SRR1760532 (small RNA)

Figure Legends

Figure 1. Venn diagram showing unique and shared gene families between and among *Phalaenopsis*, *Oryza*, *Arabidopsis* and *Vitis*.

Figure 2. GO (A) and Pfam (B) annotation of Phalaenopsis protein-coding genes.

Figure 3. Possible evolutionary relationship of *PhAGL6b* in the regulation of lip formation and floral symmetry in *Phalaenopsis* orchid.

(A) Wild-type flower. (B) A big lip mutant of *Phalaenopsis* World Class 'Big Foot'. (C) Representative RT-PCR result showing the mRNA splicing pattern of *PhAGL6b* in wild-type (W) and big lip mutant (M). (D) Alignment of the amino acid sequences of alternatively spliced forms of *PhAGL6b*. (E) Model of *PhAGL6b* spatial expression for controlling *Phalaenopsis* floral symmetry. Ectopic expression of *PhAGL6b* in the distal domain (petal; pink), petal converts into a lip-like structure that leads to radial symmetry. Ectopic expression in proximal domain, (sepal; blue) sepal converts into a lip-like structure that leads to bilateral symmetry[15]. The alternative processing of *PhAGL6b* transcripts produced in proximal domain (labellum; pink), labellum converts into a petal-like structure that leads to radial symmetry. *PhAGL6b* expression patterns in *Phalaenopsis* floral organs are either an expansion or a reduction across labellum. This implies that *PhAGL6b* may be a key regulator to the bilateral or radially symmetrical evolvements. Pink color: 2nd whorl of the flower; blue color: 1st whorl of the flower; fan-shaped symbol: petal or petal-like structure; triangle symbol: labellum or lip-like structure; Curved symbol: sepal.

Figure 4. Different labellum types of wild-type and big lip mutant *Phalaenopsis* flowers. RT-PCR analysis of the mRNA splicing pattern of *PhAGL6b* in wild-type plants (98201-WT1 and 98201-WT2) and different big lip mutant types (A). Splicing variants of *PhAGL6b*, as detected via qRT-PCR in the labellum organ of different big lip mutant types (B).

Figure 5. Expression profiles of genes of flowering time regulation pathway with high temperature and cool temperature treatment. Only the genes with twofold change in expression during cool temperature treatments are revealed

Figure 6. Predicted pathway in the regulation of spike induction in *Phalaenopsis*.

Red color indicates that the involved genes are more highly expressed in the GA biosynthesis pathway; whereas pink color of gene names indicates their differential expression in the GA response pathway. Blue colors of gene names represent the activation of flower architecture genes. Red arrows show the steps of the GA signaling stage; Pink arrows direct the steps of inflorescence evocation stage; Blue arrows reveal the steps of flower stalk initiation stage. Black arrows indicate the genes downregulated 2X over. *GA20ox*, *GA3ox*, *GAMYB*, *FT*, *SOC1*, *LFY* and *AP1* are upregulated 2X over.

Supplementary files

SUPPLEMENTARY INFORMATION APPENDIX

Dataset 1-14

Dataset 13

Dataset 15

Dataset 16

Dataset 17:https://drive.google.com/open?id=0B_TRDroXHRivc1MwYjJwT1ZlZVU

Dataset 18

Dataset 19

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Figure 1(on next page)

Figure 1

Figure 1. Venn diagram showing unique and shared gene families between and among *Phalaenopsis*, *Oryza*, *Arabidopsis* and *Vitis*.

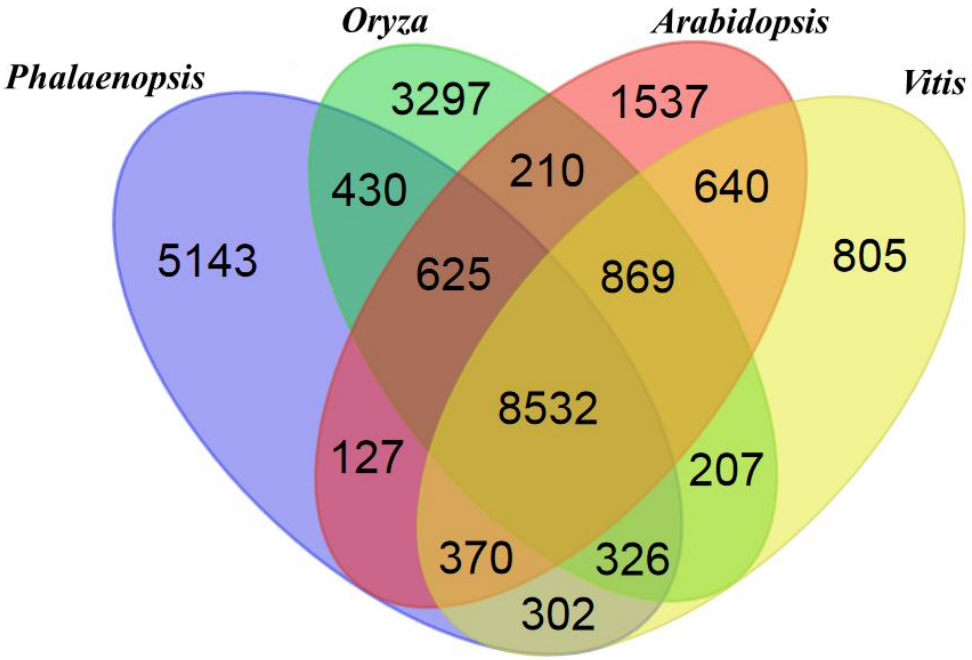
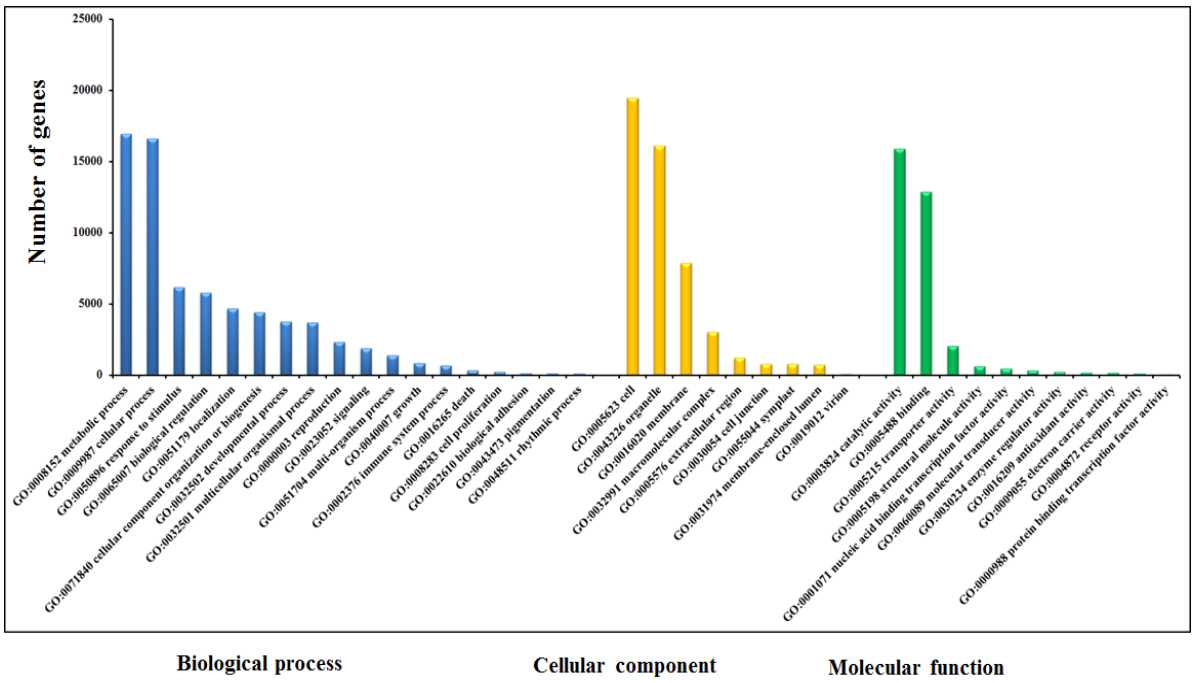

Oryza
Arabidopsis
Phalaenopsis
Vitis
3297
1537
430
210
640
5143
625
869
805
8532
127
207
370
326
302

Figure 2(on next page)

Figure 2

Figure 2. GO (A) and Pfam (B) annotation of Phalaenopsis protein-coding genes

A


Number of genes
25000
20000
15000
10000
5000
0
GO:0008152 metabolic process
GO:0009987 cellular process
GO:0050896 response to stimulus
GO:0065007 biological regulation
GO:0051179 localization
GO:0071840 cellular component organization or biogenesis
GO:0032502 developmental process
GO:0032501 multicellular organismal process
GO:0000003 reproduction
GO:0023052 signaling
GO:0040007 growth
GO:0002376 immune system process
GO:0008283 cell proliferation
GO:0022610 biological adhesion
GO:0043473 pigmentation
GO:0048511 rhythmic process
GO:0005623 cell
GO:0043226 organelle
GO:0016020 membrane
GO:0032991 macromolecular complex
GO:0005576 extracellular region
GO:0030054 cell junction
GO:0055044 symplast
GO:0031974 membrane-enclosed lumen
GO:0019012 virion
GO:0003824 catalytic activity
GO:0005488 binding
GO:0005215 transporter activity
GO:0005198 structural molecule activity
GO:0001071 nucleic acid binding transcription factor activity
GO:0060089 molecular transducer activity
GO:0030234 enzyme regulator activity
GO:0016209 antioxidant activity
GO:0009055 electron carrier activity
GO:0004872 receptor activity
GO:0000988 protein binding transcription factor activity
Biological process
Cellular component
Molecular function

B

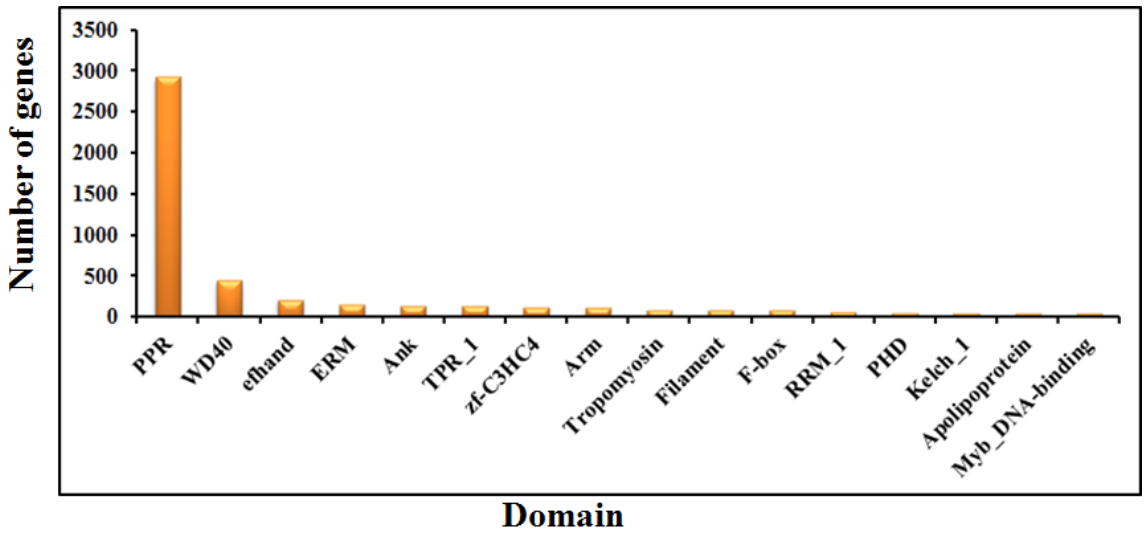

Number of genes
3500
3000
2500
2000
1500
1000
500
0
PPR
WD40
efhand
ERM
Ank
TPR_1
zf-C3HC4
Arm
Tropomyosin
Filament
F-box
RRM_1
PHD
Kelch_1
Apolipoprotein
Myb_DNA-binding
Domain

Figure 3(on next page)

Figure 3

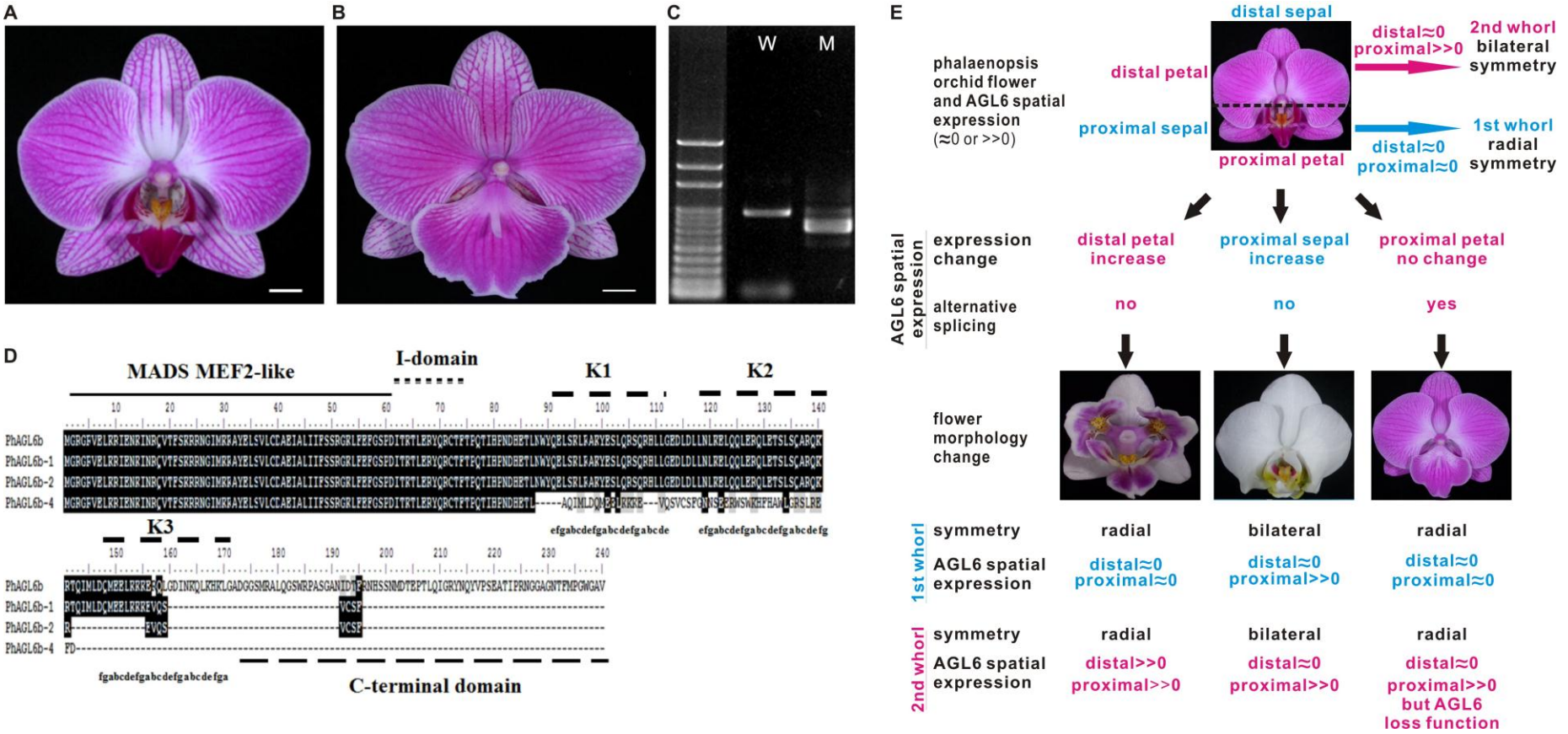
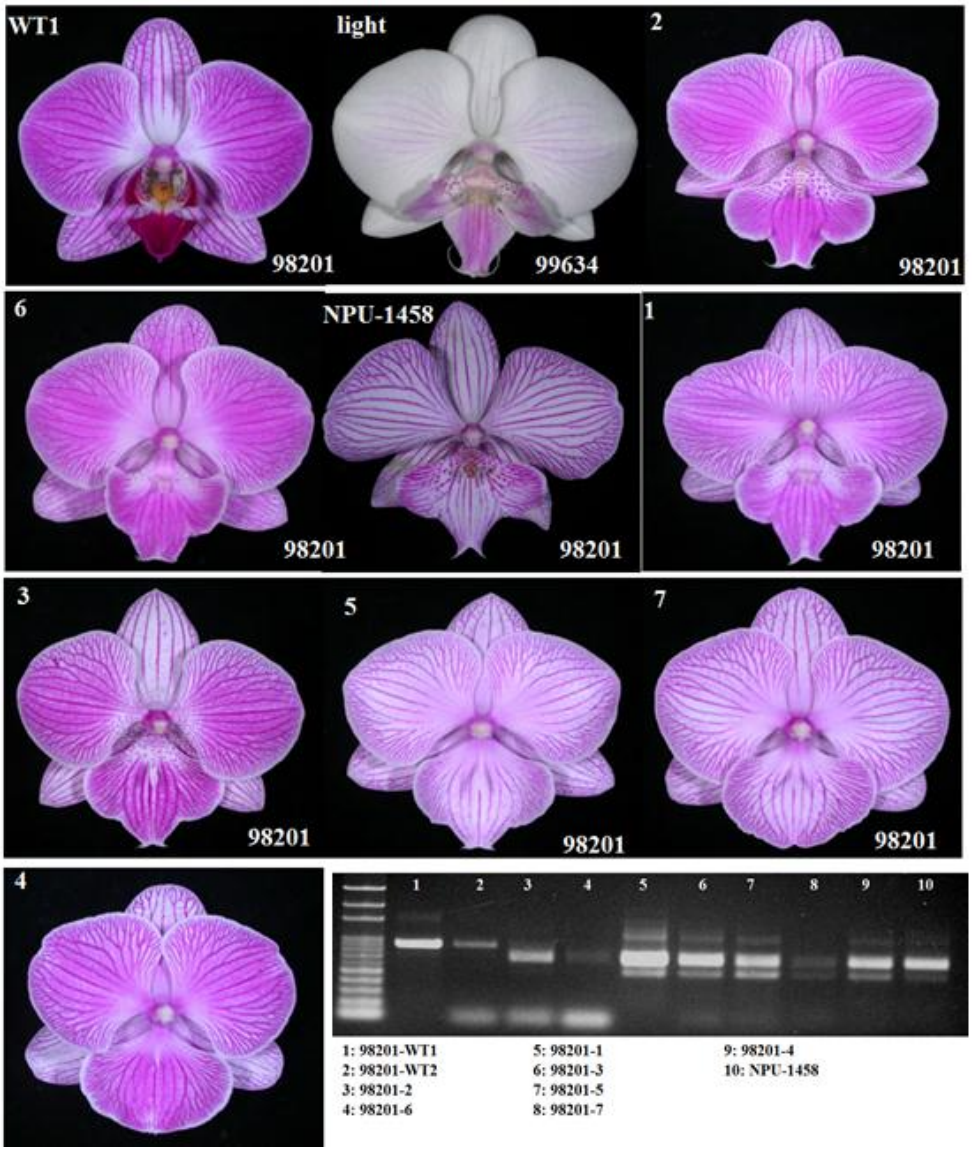

A
B
C
W
M
D
MADS MEF2-like
I-domain
K1
K2
K3
C-terminal domain
PhAGL6b
PhAGL6b-1
PhAGL6b-2
PhAGL6b-4
E
phalaenopsis orchid flower and AGL6 spatial expression (≈0 or >>0)
distal sepal
distal petal
proximal sepal
proximal petal
distal≈0 proximal>>0
2nd whorl bilateral symmetry
distal≈0 proximal≈0
1st whorl radial symmetry
AGL6 spatial expression
expression change
distal petal increase
proximal sepal increase
proximal petal no change
alternative splicing
no
no
yes
flower morphology change
1st whorl
symmetry
radial
bilateral
radial
AGL6 spatial expression
distal≈0 proximal≈0
distal≈0 proximal>>0
distal≈0 proximal≈0
2nd whorl
symmetry
radial
bilateral
radial
AGL6 spatial expression
distal>>0 proximal>>0
distal≈0 proximal>>0
distal≈0 proximal>>0 but AGL6 loss function

Figure 4(on next page)

Figure 4

Figure 4. Different labellum types of wild-type and big lip mutant *Phalaenopsis* flowers. RT-PCR analysis of the mRNA splicing pattern of *PhAGL6b* in wild-type plants (98201-WT1 and 98201-WT2) and different big lip mutant types (A). Splicing variants of *PhAGL6b*, as detected via qRT-PCR in the labellum organ of different big lip mutant types (B).

A


WT1
light
2
98201
99634
98201
6
NPU-1458
1
98201
98201
98201
3
5
7
98201
98201
98201
4
1 2 3 4 5 6 7 8 9 10
1: 98201-WT1
2: 98201-WT2
3: 98201-2
4: 98201-6
5: 98201-1
6: 98201-3
7: 98201-5
8: 98201-7
9: 98201-4
10: NPU-1458

B

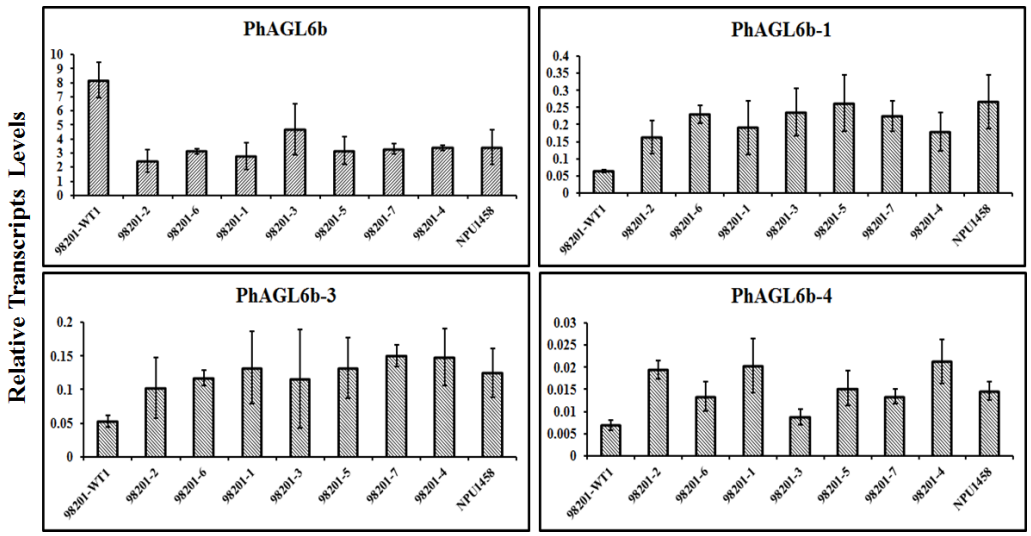

Relative Transcripts Levels
PhAGL6b
PhAGL6b-1
PhAGL6b-3
PhAGL6b-4
98201-WT1
98201-2
98201-6
98201-1
98201-3
98201-5
98201-7
98201-4
NPU1458

Figure 5(on next page)

Figure 5

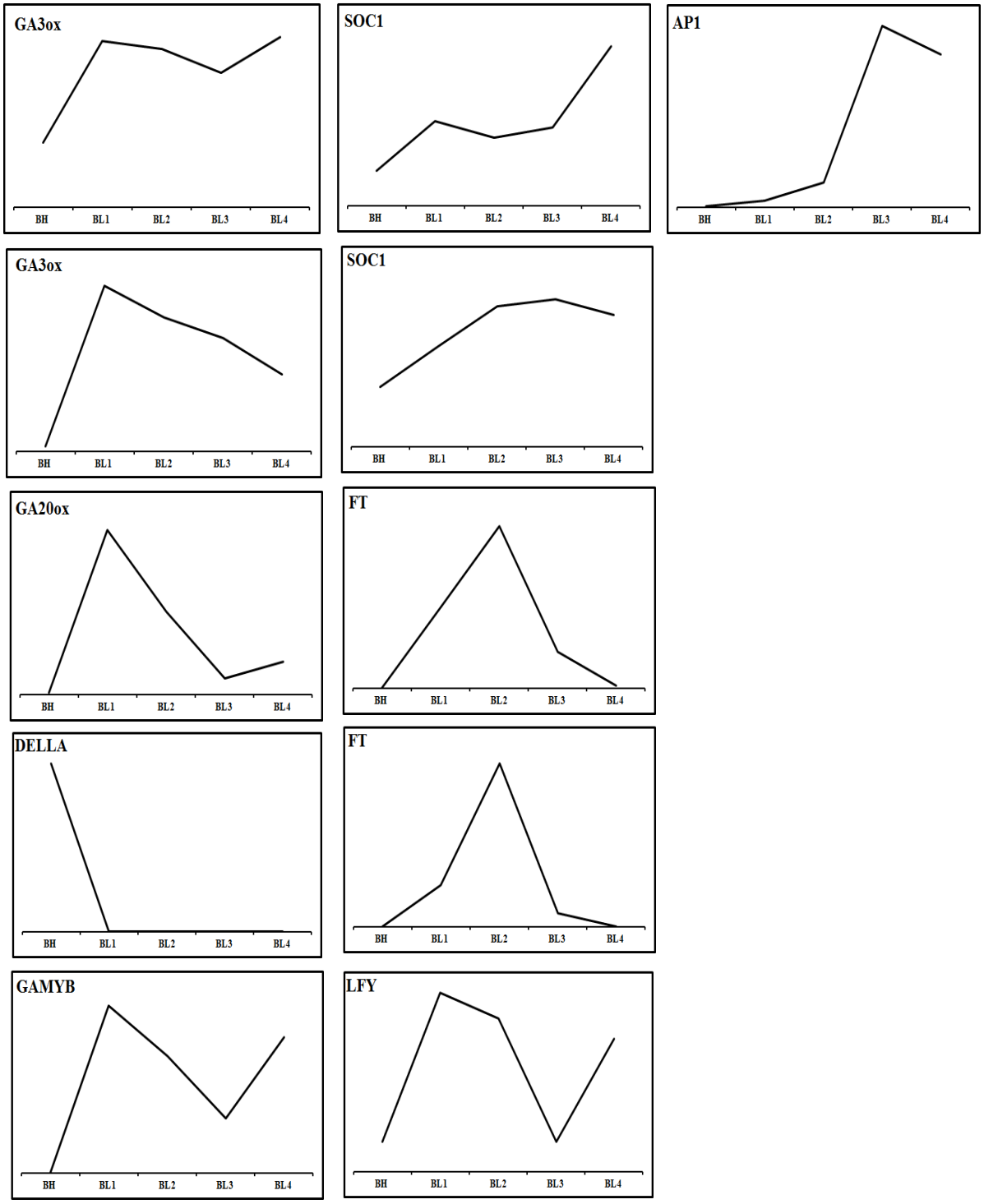

GA3ox
SOC1
AP1
GA3ox
SOC1
GA20ox
FT
DELLA
FT
GAMYB
LFY
BH
BL1
BL2
BL3
BL4

Figure 6(on next page)

Figure 6

Figure 6. Predicted pathway in the regulation of spikeinduction in *Phalaenopsis*.

Red color indicates that the involved genes are more highly expressed in the GA biosynthesis pathway; whereas pink color of gene names indicates their differential expression in the GA response pathway. Blue colors of gene names represent the activation of flower architecturegenes. Red arrows show the steps ofthe GA signaling stage; Pink arrows direct the steps of inflorescence evocation stage; Blue arrowsrevealthe steps offlower stalk initiation stage. Black arrows indicate the genes downregulated 2X over.*GA20ox*,*GA3ox*,*GAMYB*,*FT*,*SOC1*, *LFY*and*AP1*are upregulated 2X over.

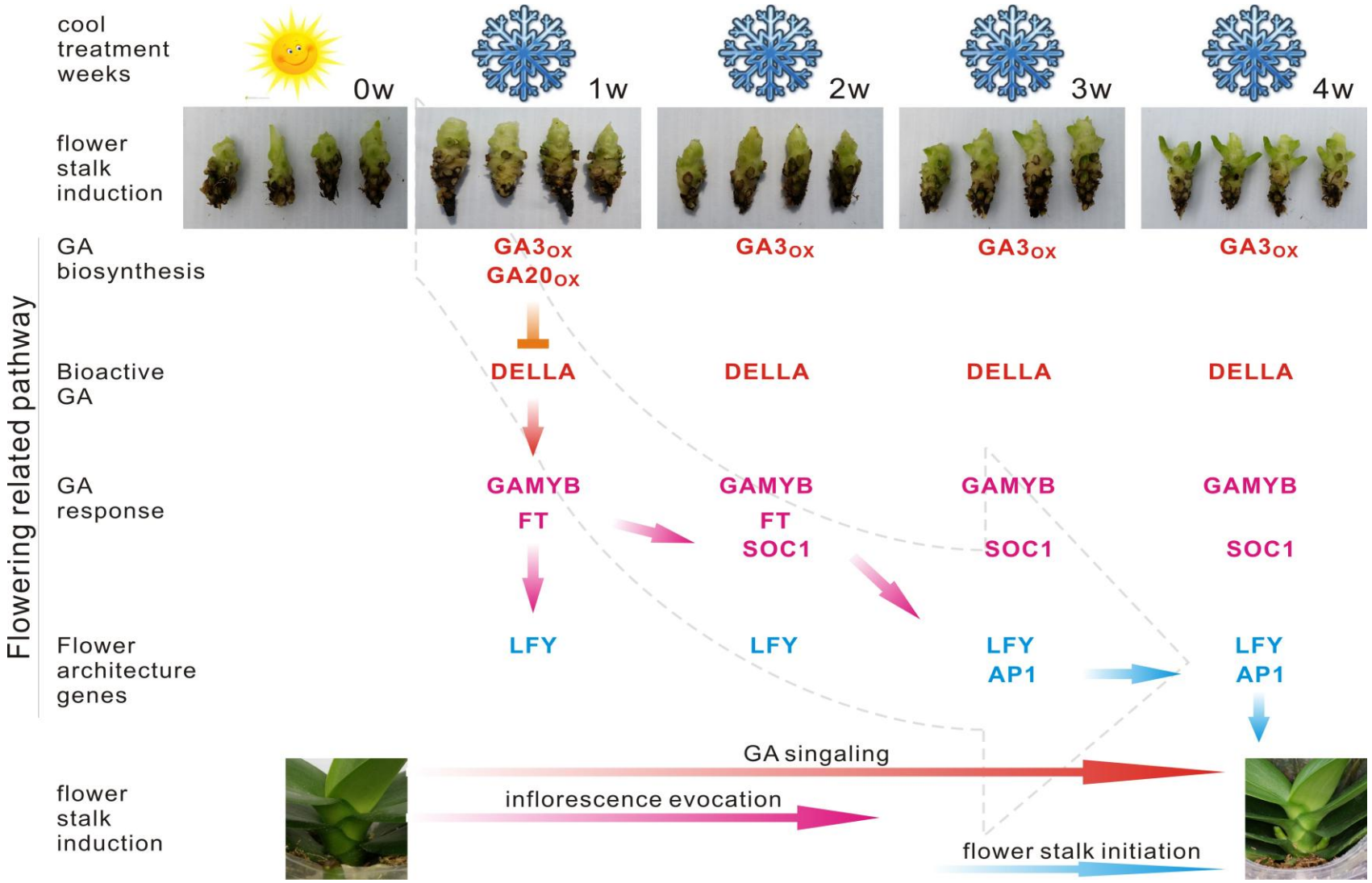

cool treatment weeks
0w
1w
2w
3w
4w
flower stalk induction
GA biosynthesis
GA3ox
GA20ox
GA3ox
GA3ox
GA3ox
Bioactive GA
DELLA
DELLA
DELLA
DELLA
GA response
GAMYB
FT
GAMYB
FT
SOC1
GAMYB
SOC1
GAMYB
SOC1
Flower architecture genes
LFY
LFY
LFY
AP1
LFY
AP1
Flowering related pathway
flower stalk induction
GA singaling
inflorescence evocation
flower stalk initiation

Table 1(on next page)

Table 1

Table 1 Statistics of the *Phalaenopsis* draft genome

Estimate of genome size	**3.45 Gb**
Chromosome number (2n)	**38**
Total size of assembled contigs	**3.1 Gb**
Number of contigs (≥1kp)	**630,316**
Largest contig	**50,944**
N50 length (contig)	**1,489**
Number of scaffolds (≥1kp)	**149,151**
Total size of assembled scaffolds	**3,104,268,398**
N50 length (scaffolds)	**100,943**
Longest scaffold	**1,402,447**
GC content	**30.7**
Number of gene models	**41,153**
Mean coding sequence length	**1,014 bp**
Mean exon length/ number	**264 bp / 3.83**
Mean intron length/ number	**3,099 bp / 2.83**
Exon GC (%)	**41.9**
Intron GC (%)	**16.1**
Number of predicted miRNA genes	**650**
Total size of transposable elements	**1,598,926,178**