Gene 518 (2013) 91–100

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Methods Paper

Transcriptomic analysis of floral organs from *Phalaenopsis* orchid by using oligonucleotide microarray

Yu-Yun Hsiao [a,b,1], Tian-Hsiang Huang [b,c,1], Chih-Hsiung Fu [d], Shi-Ching Huang [a], Yi-Jun Chen [c], Yueh-Min Huang [d], Wen-Huei Chen [b], Wen-Chieh Tsai [b,c,*], Hong-Hwa Chen [a,b,c,*]

[a] *Department of Life Sciences, National Cheng Kung University, Tainan 701, Taiwan*
[b] *Orchid Research Center, National Cheng Kung University, Tainan 701, Taiwan*
[c] *Institute of Tropical Plant Sciences, National Cheng Kung University, Tainan 701, Taiwan*
[d] *Department of Engineering Science, National Cheng Kung University, Tainan 701, Taiwan*

ARTICLE INFO

Article history:
Accepted 27 November 2012
Available online 20 December 2012

Keywords:
Labellum
Oligomicroarray
Orchids
Phalaenopsis
Transcriptome

ABSTRACT

Orchids are one of the most species rich of all angiosperm families. Their extraordinary floral diversity, especially conspicuous labellum morphology, makes them the successful species during evolution process. Because of the fine and delicate development of the perianth, orchid provides a rich subject for studying developmental biology. However, study on molecular mechanism underling orchid floral development is still in its infancy. In this study, we developed an oligomicroarray containing 14,732 unigenes based on the information of expressed sequence tags derived from *Phalaenopsis* orchids. We applied the oligomicroarray to compare transcriptome among different types of floral organs including sepal, petal and labellum. We discovered that 173, 11, and 285 unigenes were highly differentially expressed in sepal, petal, and labellum, respectively. These unigenes were annotated with Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, and transcription factor family. Unigenes involved in energy metabolism, lipid metabolism, and terpenoid metabolism are significantly differentially distributed between labellum and two types of tepal (sepal and petal). Labellum-dominant unigenes encoding MADS-box and sepal-dominant unigenes encoding WRKY transcription factors were also identified. Further studies are required but data suggest that it will be possible to identify genes better adapted to sepal, petal and labellum function. The developed functional genomic tool will narrow the gap between approaches based on model organisms with plenty genomic resources and species that are important for developmental and evolutionary studies.

1. Introduction

The family of Orchidaceae is one of the largest families of angiosperms with enormously diversified and specialized floral morphology. The number of species may exceed 24,500 in this family (Dressler, 2005). Like all other living organisms, present-day orchids have evolved from ancestral forms as a result of selection pressure and adaptation. The family has developed elaborate mechanisms for radial adaptation. These factors include specific interaction between the orchid flower and pollinator (Cozzolino and Widmer, 2005), sequential and rapid interplay between drift and natural selection (Tremblay et al., 2005), obligate interaction with mycorrhiza (Otero and Flanagan, 2006), and Crassulacean acid metabolism and epiphytism (Silvera et al., 2009). Thus, new species of orchids still be discovered incessantly (Chase et al., 2009; Liu et al., 2011).

According to the classical view, the orchid flower is composed of five whorls of three segments, each including two perianth whorls, two staminal whorls and one carpel whorl (Fig. 1). This structure also conforms to the general flower structure of many other monocotyledonous families. The outermost whorl of orchid flower consists of three sepals

Abbreviations: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; *MADS-box*, an acronym designating a conserved DNA sequence encoding a DNA-binding protein domain, with *MADS* being derived from genes *MCM1*, *AGAMOUS*, *DEFICIENS* and *SRF*; WRKY, The WRKY transcription factor is defined by the conserved amino acid sequence WRKYGQK at its N-terminal end, together with a novel zinc-finger-like motif; MIAME, Minimum Information About a Microarray Experiment; BLAST, basic local alignment search tool; TAIR, The Arabidopsis Information Resource; PTFDB, Plant Transcription Factor Database; bp, base pair(s); Cy3(5), cyanine 3(5); *CERn*, *ECERIFERUM* gene NO. n; *CYPn*, *Cytochrome P450* gene NO. n; ARF, auxin response factor; C3H, encodes a protein containing a Cys3His zinc finger domain; HB, homeobox; ZF-HD, Zinc-finger-homeodomain; bZIP, basic region/leucine zipper motif; CO, CONSTANS; NAC, NAM (no apical meristem), ATAF, and CUC (cup-shaped cotyledon) transcription factor; TIFA, encodes a protein containing a domain TIF[F/Y]XG domain; AGL, AGAMOUS-like.

* Corresponding authors at: Institute of Tropical Plant Sciences, National Cheng Kung University, Tainan 701, Taiwan. Tel.: +886 6 5050635x3050; fax: +886 6 2356211.
E-mail addresses: yunhsiao@gmail.com (Y.-Y. Hsiao), huangtx@gmail.com (T.-H. Huang), grid.net@gmail.com (C.-H. Fu), z9408128@email.ncku.edu.tw (S.-C. Huang), ycc0111@gmail.com (Y.-J. Chen), huang@mail.ncku.edu.tw (Y.-M. Huang), a08539@gmail.com (W.-H. Chen), tsaiwc@mail.ncku.edu.tw (W.-C. Tsai), hhchen@mail.ncku.edu.tw (H.-H. Chen).
[1] These authors contributed equally to this work.

0378-1119/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2012.11.069

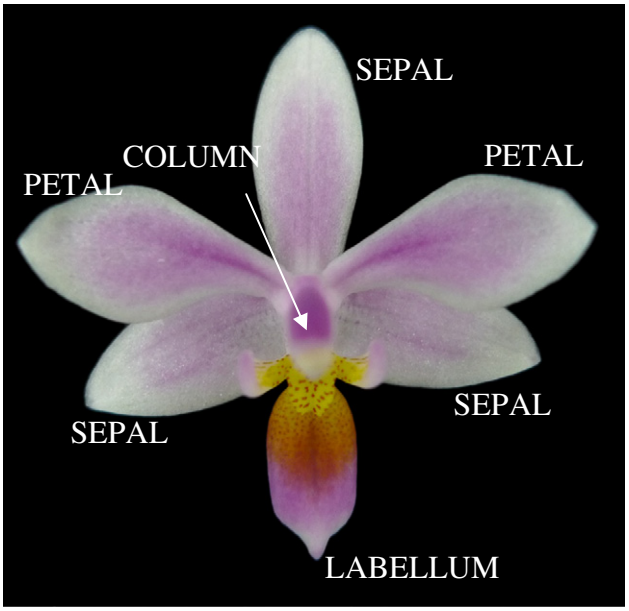

Fig. 1. Diagram of flower of *Phalaenopsis equestris*. *Phalaenopsis* orchid flowers have three sepals and three petals. One of the petals is morphologically different in structure and is known as the labellum or lip. The male and female reproductive parts are fused in a structure, the gynostemium or column, in the center of the flower.

with similar shape, size and color. Three petals make up the second whorl. The adaxial petal is called lip or labellum which is highly evolved and exhibits a distinctive shape and color pattern and can be decorated with calli, spurs and glands. The male and female reproductive organs are fused to form gynostemium in the center of flower. Within the monocots, only well-known crop species such as rice and maize have been studied thoroughly. However, their highly reduced flowers make them unsuitable for general floral development studies. All expected whorls in the flowers are present in orchids, and their highly sophisticated flower organization offers an opportunity to discover new variant genes and different levels of complexity within morphogenetic networks. However, although orchids have unique floral morphology and adaptive reproduction strategies, the relationship between the function of genes and morphological structures remains poorly understood.

Recently, several researches are focused on the roles of B-class MADS-box genes played on the orchid tepal development (Chang et al., 2010; Hsiao et al., 2011b; Kim et al., 2007; Tsai et al., 2004, 2005; Xu et al., 2006). Duplicated four-clade B-class genes with differential expression patterns in orchid floral organs, as well as divergent protein behaviors of the encoded B-class genes, support the unique evolutionary routes of B-class genes associated with unique labellum innovation in orchid (Mondragon-Palomino and Theissen, 2008; Pan et al., 2011; Tsai et al., 2008b). Very recently, the C/D-class MADS-box genes were paid attention to study their functions in gynostemium and ovule development (Chen et al., 2012; Skipper et al., 2006; Song et al., 2006; Wang et al., 2011; Xu et al., 2006). However, the regulation network of orchid flower development still not be investigated.

Microarray technology has become a usefully systematic biological research tool to provide a rapid and relatively inexpensive way to monitor in parallel the expression of thousands of transcripts (Meyers et al., 2004). Due to the use of standard platforms, laboratory protocols and procedures for processing of primary data, the results of microarrays analyses are well suited for database management and meta-analysis across multiple experiments, whilst data mining is based on powerful statistical procedures with support from functional and structural annotations of genes. The genomics resources of orchids are being rapidly expanded through a few large-scale genomics programs (Fu et al., 2011; Hsiao et al., 2006, 2011a; Hsu et al., 2011; Tsai et al., 2006). The availability of these resources represents a unique opportunity to perform comparative and genome-wide analyses of development in parallel at the gene expression level.

Here, we analyze spatial gene expression changes at sepal, petal and labellum using microarrays. We monitor more than 12,000 unique transcripts from the recently sequenced *Phalaenopsis* transcriptome (Fu et al., 2011; Hsiao et al., 2006, 2011a; Tsai et al., 2006). By correlating relative gene expression levels with changes in different forms of perianth, we provide novel insights into the floral development of orchids. This is the first time that such an analysis was performed at the present scale in any orchid species. This study, therefore, provides the foundation for future research on mechanisms underlying floral development in *Phalaenopsis* and other orchids.

2. Materials and methods

2.1. Plant materials

Phalaenopsis equestris with a red petal orange lip (Fig. 1) were grown in greenhouses at National Cheng Kung University under natural light and controlled temperature ranging from 23 °C to 27 °C. For RNA extraction, various organs of stage 3 flower buds (Tsai et al., 2004) including sepals, petals and lips were collected, immersed in liquid nitrogen, and stored at −80 °C until the RNA was extracted.

2.2. Microarray construction

Phalaenopsis microarray was customized using Agilent eArray 5.0 program according to the manufacturer's recommendations. The customized microarray (4×44 K) contained spots in two to three duplicates with 14,732 gene-specific oligonucleotides (45–60 mers in length) derived from 84,614 unigenes in OrchidBase (Fu et al., 2011).

2.3. RNA isolation, labeling, hybridization, and scanning

Total RNA was isolated from sepals, petals and lips of stage 3 floral buds following the methods described by using Tsai et al. (2004). The total RNA yield was quantified by nanodrop UV spectroscopy (Ocean Optics) and the quantity was verified by gel electrophoresis and analyzed on a RNA 6000 Nano LabChip (Agilent Technologies) using a 2100 bioanalyzer (Agilent Technologies). cDNA was synthesized from 1 mg of enriched mRNA with Cyscribe 1st-strand cDNA labeling kit, (GE Healthcare) and labeled with Cy3 or Cy5 (CyDye, PerkinElmer). The fluorescently labeled cDNA was purified with QIAGEN RNeasy cleanup kit and fragmented in fragmentation buffer (Agilent). The correspondingly labeled cDNA was mixed in GEx Hybridization Buffer HI-RPM (Agilent). Hybridization was performed in an Agilent microarray Hybridization Chamber for 17 h at 60uC. After hybridization, the slides were washed in Gene Expression Wash Buffer (Agilent) and dried by nitrogen gun blowing. Microarrays were scanned using an Agilent microarray scanner at 535 nm for Cy3 and 625 nm for Cy5. Feature Extraction 9.5.3 and image analysis software (Agilent Technologies) were used to locate and delineate every spot in the array, to integrate each spot's intensity, and to normalize data using the rank-consistency-filtering Lowess method. The data points which had flag value of non-zero or a signal-to-noise ratio smaller than 2.6 were masked. The remaining data were log2 transformed and averaged for each gene. All microarray data reported in the study is described in accordance with MIAME guidelines. For selection of genes which were significantly at each floral organ, a 2-fold change and *p*ValueLogRatio<0.05 were used as thresholds.

2.4. Sequence analysis and GO classification

All sequences were searched for their similarities to known sequences through the use of a BLASTX algorithm against the "nr" protein database. Sequence similarity was considered significant at an *E*-value