Orchid Biotechnology in Production and Improvement

Rasika G. Mudalige and Adelheid R. Kuehne
Department of Tropical Plant and Soil Sciences, University of Hawaii, 3190 Maile Way, Honolulu, HI 96822

The Orchidaceae is one of the largest families of flowering plants, with several genera being used in cut flower and potted plant production. The molecular biology of orchids, last reviewed in Kuehne (1997), covered research in phylogeny and systematics, floral physiology, and plant breeding. Only 10 orchid genes were cloned at that time. Since then, the total number of genes cloned from orchids has expanded considerably. The objective of this paper is to: 1) provide an overview of the function of some recently cloned genes; and 2) review advances made in other applications of biotechnology in orchid production and improvement.

Functions of some cloned orchid genes

About 70 genes have been cloned from seven orchid genera, namely Dendrobium, Phalaenopsis, Doritaenopsis, Aranda, Bromheadia, Vanilla, and Cymbidium. These genes can be divided into seven categories based on their presumptive functions (Table 1). Here we review genes affecting flower induction, flower color, flower senescence, and disease resistance. Other genes have been cloned that affect primary metabolism, ovule development, cell division, and cell structure (Table 1). At this time, the latter categories have had less direct impact on commercial orchid production and improvement.

Flower induction. The ability to time flowering for holidays and to hasten flowering in otherwise slow-maturing orchids is of keen interest to growers of commercially cropped orchids. Several research groups are examining the genetic aspects of flower induction and the transition of the apical meristem from a vegetative shoot apical meristem (VSAM) through a transitional shoot apical meristem (TSAM) to a reproductive meristem. Specifically, several orchid genes have been identified to be so-called homeobox and MADS box genes, which encode transcription factors and are preferentially expressed in vegetative or transitional stages (Table 1).

Homeobox genes. Five clones were identified by mRNA differential display as transcription factors involved in floral induction (Yu and Goh, 2000a). Of these, the partial cDNA clone ogv2 showed significant similarity to homeobox genes, which are a universal group of transcription factors important in development. The full-length single copy clone, DOH1, which was subsequently isolated from a Dendrobium cDNA library of VSAM, shared considerable similarity to a class of homeobox genes known as class 1 knox (knotted-like) genes (Yu et al., 2000). Northern analysis and in situ hybridization revealed that DOH1/ogv2 mRNA accumulated in all meristem-rich tissues and its expression was down-regulated at the beginning of floral transition (Yu and Goh, 2000a; Yu et al., 2000). A Dendrobium hybrid transformed with sense constructs of DOH1 gene driven by the cauliflower mosaic virus (CaMV) 35S promoter (Yu et al., 2000) revealed that the overexpression of DOH1 completely suppresses shoot organization and development. Anti-sense plants formed multiple shoot meristems and precociously flowered. With these results, the authors concluded that DOH1 is involved in controlling the formation and identity of the shoot apical meristem, and thereby involved in maintaining the basic plant architecture.

Another transcription factor, ogv27, a homologue of a Drosophila shuttle-craft gene, was isolated from the VSAM of Dendrobium and appears to be important in maintaining the vegetative state of shoot apical meristem in plants (Yu and Goh, 2000a). Also, a cDNA clone, ogv16, expressed only in the TSAM has shown significant similarity to an Arabidopsis casein kinase gene involved in protein phosphorylation and signal transduction (Yu and Goh, 2000a). The complete signal transduction pathway in floral induction of orchids is yet to be discovered.

Flower color. The biosynthesis of anthocyanins is well characterized in many ornamental plants. The synthesis of flavonoids, including anthocyanins, occurs through the phenylpropanoid pathway, which starts with the stepwise condensation of three acetate units from malonyl-coA with 4-coumaroyl CoA to yield tetrahydroxychalcone (Heller and Forkmann, 1988). This reaction is catalyzed by the enzyme chalcone synthase (CHS), which is attractive to researchers for flower color manipulation. The magnificent spectrum of colors found in orchids is mainly due to the accumulation of anthocyanins in their flowers (Arndt and Fisch, 1977).Although carotenoids and chlorophylls contribute to flower color in many orchids, there are no detailed reports of cloned orchid genes associated with the biosynthesis of these compounds except for a brief mention of an amplified restriction fragment of Phalaenopsis, AM4-1, which showed similarity to the geranyl-geranyl pyrophosphate synthase gene (Liu and Chen, 1999). Genes for other important enzymes cloned in orchids are flavanone 3-hydroxylase (F3H) and dihydroflavonol 4-reductase (DFR).

Chalcone synthase genes. The earliest report of the isolation of flower color genes in orchids appeared as an abstract only by Yong and Chu (1990), in which four cDNA clones encoding CHS were isolated from a cDNA library of a Dendrobium hybrid. These DNA sequences are absent in all the searchable databases.

Received for publication 31 Jan. 2003. Accepted for publication 13 Feb. 2003. Published in HORTSCIENCE on 14 Feb. 2004.

To whom reprint requests should be addressed.
E-mail address: heidi@hawaii.edu.

HORTSCIENCE, VOL. 39(1), FEBRUARY 2004
hybridized with sis. It is dif
KD. CHS in (Liew et al., 1998a), were shown to contain a single
pOCHS01 cDNA clone suggested to be a CHS homolog, (Yong and Chua 1990). Another
leaves with pigmented edges and roots (Liew et al., 1995). It encodes
flavonoid biosynthesis, (Liew et al., 1999) and only a few pelargonidin ac-
trol of pollination, many orchid
Flower senescence. In the absence of
databases. Later, three CHS cDNA clones, OCHS3, OCHS4, and OCHS8, isolated and
characterized from the cDNA library of Bromheadia finlaysoniana flowers (Liew et al., 1998a), were shown to contain a single
orf of 1185 bp encoding a protein of 429 KD. CHS in Dendrobium and Bromheadia are encoded by a small multigene family and is expressed in high levels in flower buds and other tissues containing anthocyanin such as leaves with pigmented edges and roots (Liew et al., 1998a; Yong and Chua 1990). Another
cDNA clone suggested to be a CHS homolog, pOCHS01, was isolated from a Phalaenopsis hybrid (Hsu et al., 1997). However, a recent BLAST search revealed that this cDNA clone is in fact 98% similar to bibenzyl synthase of Phalaenopsis and Bromheadia. Authors noted that there are at least 10 other sequences that hybridized with pOCHS01 in southern analy-
sis. It is difficult to distinguish genes encoding CHS, bibenzyl synthase and stilbene synthase due to their close similarity at the nucleotide and amino acid sequence levels (Liew et al., 1998a).

Flavonone 3-hydroxylase genes. Another key enzyme in flavonoid biosynthesis, flav-
one 3-hydroxylase, catalyzes the formation of dihydrolavonolavonol from (2S)-flavanones in plants. A full-length cDNA clone of 1393 bp encoding F3H was isolated from Bromheadia finlaysoniana (Liew et al., 1995). It encodes a protein of 415 KD with 464 amino acid residues and shared 52% to 59% and 70% to 76% homology with F3H of other plants at the nucleotide and amino acid levels, respectively.

Dihydroflavonol 4-reductase (DFR) genes. The conversion of dihydrolavonols such as dihydrolavone to dihydroflavonol (DHF), dihydroquercetin (DHQ), and dihydromyricetin (DHM) to the corresponding leucoanthocyanidins is the first committed step in anthocyanin biosynthesis and is catalyzed by the enzyme, DFR. The color of the anthocyanin produced depends on the dihydrolavonol substrate that is reduced by DFR. The substrate specificity of DFR explains the absence of certain colors from some ornamental plants, e.g., Petunia hybrida which does not catalyze the conversion of DHK to orange-colored pelargonidin (Meyer et al., 1987). In orchids, Cymbidium are noticeably devoid of orange colored flowers (Johnson et al., 1999) and only a few pelargonidin ac-
trolling of ethylene starts with the synthesis of ethylene (Johnson et al., 1995; Meyer et al., 1998). The production of ethylene is regulated by a key enzyme, DFR, which catalyzes the reduction of DHK to DHQ. The conversion of DHK to DHQ is important for the production of pink cyanidin instead of orange pelargonidin. Introduction of a DFR from a plant that efficiently reduces DHK to pelargonidin (e.g., Antheridina majus, Rosa hybrida, Gerbera hybrida, and Zea mays) might be an attractive alternative to introduce orange color into orchids such as Cymbidium and Dendrobium.

Flower senescence. In the absence of polli-
nation, many orchid flowers are known to have a very long life, some lasting up to 6 months (Goh and Arditti, 1985). This long life span may increase the chance of pollination by a highly specific insect pollinator. Pollina-
tion triggers a series of physiological and developmental changes, known collectively as “post-pollination syndrome”, which mobilizes nutrients out of nonessential parts (petioles) into essential parts, the developing ovule and ovary of a flower. The hormone ethylene plays a key role in the transduction of the pollination signal and the coordination of post-pollina-
tion development in many flowers including orchids (Porat et al., 1994a and b). The pro-
duction of ethylene starts with the synthesis of S-adenosyl methionine (SAM) catalyzed

Table 1. Isolated orchid genes or cDNA clones and their presumptive function.

| Gene or cDNA designation | Orchid genus | Presumptive function | Reference and GeneBank accession numbers |
|--------------------------|-------------|----------------------|-----------------------------------------|
| org2/DOH1                | Dendrobium  | Flower Induction     | Yu and Goh, 2000a; Yu et al., 2000 (AF100326, AF176S89) |
| om1                     | ×Aranda     | Flower specific MADS box gene | Lu et al., 1993; (X69107) |
| otg7                     | Dendrobium  | MADS-box gene        | Yu and Goh, 2000a. (AF107588) |
| DOMADS1, 2 and 3         | Dendrobium  | MADS-box genes in AP1/LAG1 subfamily | Yu and Goh, 2000b. (AF198174-b) |
| ovg27                    | Dendrobium  | Transcriptional repressor | Yu and Goh, 2000a. (AF100331) |
| otg16                    | Dendrobium  | Protein kinase        | Yu and Goh, 2000a. (AF107592) |
| Ovg14                    | Dendrobium  | Transcriptional regulator of cell cycle regulators. | Yu and Goh, 2000a. (AF100328) |
| otg4                     | Dendrobium  | Cell division control protein, a homolog of yeast NDA4 | Yu and Goh, 2000a. (AF107586) |
| Ovg30                    | Dendrobium  | DNA binding protein, regulate cell cycle progression | Yu and Goh, 2000a. (AF100333) |
| Ovg29                    | Dendrobium  | Putative 2I7 protein. Degradation of cell cycle regulatory proteins | Yu and Goh, 2000a. (AF100332) |
| otg2                     | Dendrobium  | Myosin heavy chain | Yu and Goh 2000a. (AF107585) |
| P-ACT1                   | Phalaenopsis| Actin partial clone | Nadeau et al., 1996, (U18102) |
| ACT2                     | Phalaenopsis| Actin-like protein | Huang et al., 2000, (AF246715) |
| ---                      | Phalaenopsis| Profilin, an actin binding protein | Lee et al., 1999b. (AF126263) |
| Ds-ACS1, Ds-ACS2          | ×Doritaenopsis| Flower Senescence | O’Neill et al., 1993 (L07882, L07883) |
| ACS2, ACS3                |                     | ACC synthase         | Bui and O’Neill, 1998 (AF007213, AF00721) |
| pOACS30                  | Phalaenopsis| ACC synthase         | Do and Huang, 1998. (Z77854) |
| ---                      | ×Doritaenopsis| ACC oxidase          | Nadeau et al., 1993, (L077912) |
| ---                      | ×Doritaenopsis| ACC oxidase          | Nadeau and O’Neill, 1995 (L37103) |
| pPEFEA                   | Phalaenopsis| ACC oxidase          | Lee and Huang, 1995 |
| Per1                     | Phalaenopsis| Homologue of ethylene receptor | Do et al., 1999, (AF055894) |
| Per1                     | Phalaenopsis| Ethylene response sensor | Chai et al., 1999. (AF113541) |
| POAC031/DACO1            | Phalaenopsis| Homologue of human Acyl-CoA oxidase | Do and Huang, 1996. (U66299) |

Table 1 continues on next page.