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Genetic and Molecular Aspects of *Gypsophila*

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ABSTRACT

The genus *Gypsophila* is a member of the family Caryophyllaceae, which also includes carnation (*Dianthus caryophyllus*). Phylogenetic analyses of the Caryophyllaceae have been performed using DNA markers, chloroplast DNA, and rDNA sequences. *Gypsophila* includes more than 100 species, which are distributed mainly in Eurasia. The long-day perennial *G. paniculata* and the annual *G. elegans* are popular in floriculture. In particular, *G. paniculata* is produced in large quantities for use in flower arrangements. The molecular mechanism of flowering has been extensively studied in *Arabidopsis*, a qualitative long-day plant. Many of the genes that regulate flowering time on long-day induction have been characterized. Among them, *CONSTANS* (*CO*) is a key genetic component of the long-day-dependent flowering pathway. Recent studies have suggested that at least four *CO* homologs (*GpCOLs*) are expressed in *G. paniculata*. Each GpCOL contains a CCT (CO, CO-like, TOC1) domain near the carboxyl terminus. Phylogenetic analysis of the CCT domain primary sequence indicates that the four GpCOLs are Group I CO-like proteins. The expression of two of the *GpCOLs* oscillates daily, suggesting a relationship between the *GpCOLs* and flowering in *G. paniculata*. Only a few sequences from *Gypsophila* are available in DNA databases for phylogenetic analyses, including sequences from chloroplast DNA and rDNA, as well as genes involved in anthocyanin formation. Therefore, additional studies are needed at the molecular and genetic levels.

Keywords: CONSTANS, flowering, FLOWERING LOCUS T, *Gypsophila paniculata*
Abbreviations: CO, CONSTANS; **COL**, CONSTANS-like; **FT**, FLOWERING LOCUS T

CONTENTS

INTRODUCTION

The genus *Gypsophila* belongs to the family Caryophyllaceae, which also includes carnation (*Dianthus caryophyllus*), and consists of more than 100 species that are distributed mainly in Eurasia, such as *Gypsophila elegans* and *Gypsophila paniculata*. The annual *G. elegans* is used as a cut flower and for gardening. The perennial *G. paniculata*, or "baby's breath," is a highly-branched plant with numerous small white or pink flowers that are very popular in floral arrangements; therefore, it is cultivated in large quantities year-round.

G. paniculata is a long-day plant; thus, specialized lighting can be used to prevent rosette formation and to promote flowering under short-day conditions. However, suboptimal lighting can produce poor quality inflorescences due to early bolting. In addition, during summer, increased nighttime temperatures can induce flower malformation (Doi 2006); therefore, *G. paniculata* is cultivated in cool regions during summer.

Several cultivars of *G. paniculata* have been developed that vary in terms of flowering time and flower color. The most popular early-flowering cultivar is 'Bristol Fairy', which has high-quality flowers and high productivity. Among its selections are a late-flowering cultivar called 'Perfecta,' which shows variation in flower color from white to pink, and the popular cultivar 'Red Sea,' which has pink flowers. The high temperature tolerance of the 'Magic' series cultivars is useful because flower malformation is rare, even in summer (Doi 2006).

As stated above, *Gypsophila* is important in horticulture; however, information is lacking regarding its molecular genetics. In this review, the current state of molecular re-search and expectations for the future are described.

PHYLOGENITIC ANALYSIS

Gypsophila belongs to the family Caryophyllaceae, which also includes carnation. Caryophyllaceae forms the order Caryophyllales with the Chenopodiaceae, which includes the horticulturally-important plants spinach (*Spinacia oleracea*), Nyctaginaceae, and Cactaceae. Cuenoud *et al*. (2002) carried out detailed phylogenetic analyses of the Caryophyllales based on the sequences of their plastid *matK*, *rbcL*, *and atpB* genes and their nuclear 18S rDNA sequences. Most taxa of the Caryophyllales grouped into two main clades: core and noncore. The Caryophyllaceae are included in the core Caryophyllales with the Amaranthaceae, and these families form a well-supported clade. Furthermore, the family Caryophyllaceae, which includes more than 2000 species, is divided into three subfamilies based on molecular phylogenetic data (Fior *et al*. 2006). This family shows

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complex and possibly homoplasious morphological characters that make taxonomy difficult. Therefore, molecular approaches using plastid *matK* and nuclear rDNA spacer sequences were used in the phylogenetic analysis by Fior *et al*. (2006).

GENES RELATED TO PHOTOPERIODIC FLOWER INDUCTION IN ARABIDOPSIS

The genetic pathways that control flowering have been extensively studied in the model plant *Arabidopsis*. Therefore, the current state of molecular research is described here regarding photoperiodic flower induction in *Arabidopsis* to help understand molecular analysis in *Gypsophila*. In the photoperiod pathway, photoreceptor- and circadian clock-related genes help regulate key pathway components like CONSTANS (CO) and FLOWERING LOCUS T (FT) (**Fig. 1**). Phytochrome is the well-characterized photoreceptor of red and far-red light. The phytochrome gene family includes *PHYB*, which controls flowering by inhibiting *FT* expression (Lee *et al*. 2006). Blue light also induces flowering in *Arabidopsis*. Cryptochrome is known to be the photoreceptor of blue light. The cryptochrome-like protein FLAVIN-BINDING, KELCH REPEAT, F-BOX (FKF1) also regulates *CO* expression, and may function as a photoperiodic blue light receptor (Imaizumi *et al*. 2003).

CO is a key regulator of photoperiodic flowering pathways in *Arabidopsis*. CO senses the photoperiod by receiving signals from photoreceptors and the circadian clock, and it functions as the output gene for these signals. In addition to CO, there are many CO-like proteins (COL) in *Arabidopsis*. These nuclear proteins, which regulate gene expression, contain two highly-conserved regions: zinc finger B-box(es) near the amino terminus and a CCT (CO, CO-like, TOC1) domain near the carboxyl terminus (Griffiths *et al*. 2003). The expression of some *COL* genes is regulated by the circadian clock and may be related to photoperiodic flower induction. *FT* expression, which is regulated by CO and promotes flowering, is induced under long-day conditions, and its signal moves from the leaves to the shoot apex (Lee *et al*. 2006).

MOLECULAR ANALYSIS OF FLOWERING IN *GYPSOPHILA*

One of the most important factors in the year-round cultivation of *Gypsophila* is the control of flowering time. Genetic variation leading to early and late flowering is exploited in *G. paniculata* production. Lighting and heating are also important in the control of flowering under short-day conditions. To improve the control of flowering in *G. paniculata* during cultivation, research efforts should focus on the inhibition of rosette formation, the prevention of early bolting, the interaction between photoperiod and temperature, and the reduction in costs. As the molecular mechanism of flowering in *Gypsophila* is clarified, breeding and cultivation methods will be improved.

Recent work of Kanayama *et al*. (2007) has indicated that at least four *COL* (*GpCOL1 to 4*) genes are expressed in *G. paniculata* (**Fig. 1**). Each GpCOL contains a CCT domain near its carboxyl terminus. This conserved region may include a nuclear localization sequence, suggesting that GpCOL1, GpCOL2, GpCOL3, and GpCOL4 are part of the COL gene family. The COL gene family contains three groups (Griffiths *et al*. 2003). Phylogenetic analysis of the CCT domain amino acid sequence showed that the four GpCOLs are included in Group I, which contains two zinc finger B-boxes and involves CO. Notably, the expression of two of the GpCOLs shows daily oscillations, suggesting a relationship between the GpCOLs and flowering in *G. paniculata* (Kanayama *et al*. 2007).

FT plays a key role in the induction of flowering in *Arabidopsis*, and *FT* homologs have been isolated from various species, including monocots. Thus, *Gypsophila* is expected to have an *FT* homolog that functions downstream

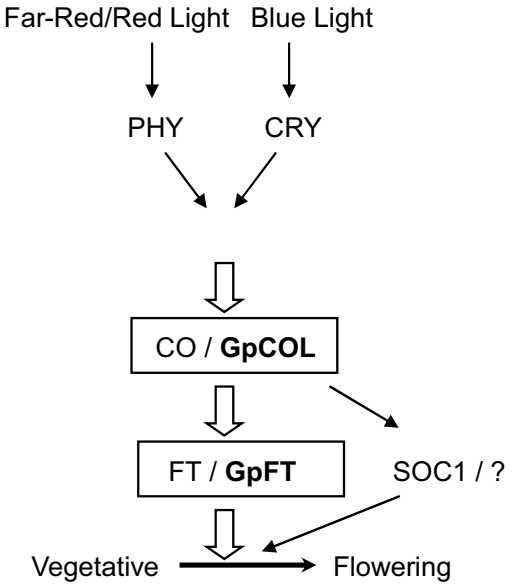

Fig. 1 Model for the function of CONSTANS (CO) and FLOWERING LOCUS T (FT) in *Arabidopsis* and *Gypsophila paniculata*. CO and GpCOL play a role in integrating circadian rhythms and light signals, which are mediated by far-red/red light receptor phytochrome (PHY) and blue light receptor cryptochrome (CRY). CO and GpCOL induce the expression of *FT* and *GpFT* that promote the transition to flowering under long-day conditions. *Gypsophila* SUPPRESSOR OF OVEREXPRESSION OF CONSTANT 1 (SOC1) homolog has yet been isolated.

of the *Gypsophila CO* homolog. By RT-PCR using degenerate primers, two cDNAs for putative *Gypsophila FT* homologs (*GpFT1* and *GpFT2*) were cloned from *G. paniculata* plant in which flowering was induced under long-day conditions (unpublished data, **Fig. 1**). The deduced amino acid sequences of *GpFT1* and *GpFT2* have high homology with *FT* and their expression seems to be induced under a long-day photoperiod.

OTHER GENES

A database search using the keyword *Gypsophila* with omission of the sequences of microorganisms such as bacterial pathogens retrieved only 13 registered sequences from the DNA Data Bank of Japan (DDBJ). Eight of those sequences, including *matK* and rDNA, were included in a phylogenetic analysis. Four of the sequences were enzymes in the anthocyanin biosynthetic pathway, and the others were related to saponin biosynthesis. Because saponin is extracted from *Gypsophila*, many biochemical studies have been reported (e.g. Acebes *et al*. 1998; Herold and Henry 2001).

In terms of flower color, cDNAs encoding flavanone 3-hydroxylase (FHT, DDBJ accession number AY515295), chalcone synthase (CHS, AY309966), dihydroflavonol 4-reductase (DFR, AY256381), and anthocyanidin synthase (ANS, AY256380) are currently included in the DDBJ database (**Fig. 2**). These genes may be important in anthocyanin biosynthesis in *Gypsophila* flowers. The most popular variety of *Gypsophila* is the white-flower cultivar 'Bristol Fairy.' Molecular analyses of anthocyanin-related genes are important for genetic engineering and the development of DNA markers for flower color. These approaches will result in the development of cultivars with diverse flower colors.

PERSPECTIVES

As indicated by the small number of registered genes in current plant databases, molecular findings in *Gypsophila* are lacking despite its importance in cut flower production. The cell fusion of *Gypsophila* with carnation or *Dianthus barbatus* was previously attempted, and interspecific hybrids were identified by nuclear rDNA analysis (Nakano and Mii 1993; Nakano *et al*. 1996); however, cell fusion is no longer a pro-

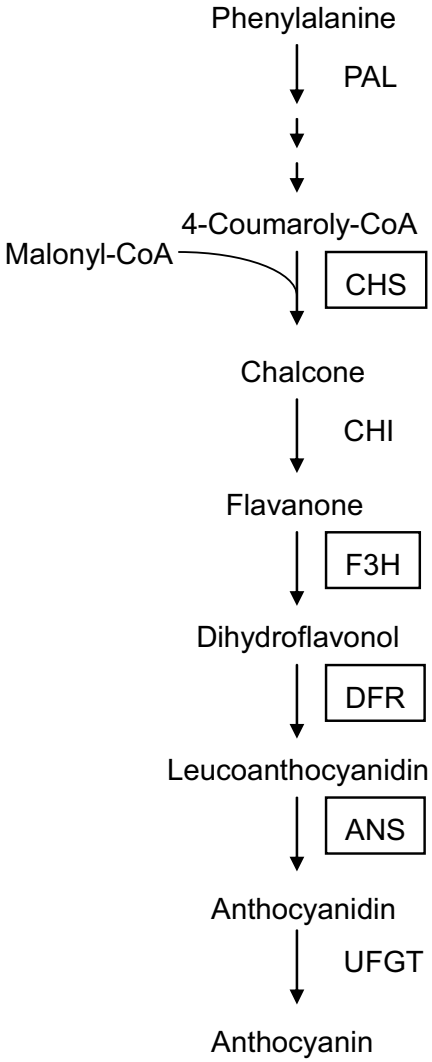

Fig. 2 Model of anthocyanin biosynthetic pathway. cDNAs for Chalcone synthase (CHS), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), and anthocyanidin synthase (ANS) have already been isolated from *Gypsophila paniculata*. Pheylalanine ammonia-lyase, PAL; chalcone isomerase, CHI; UDP-glucose;flavonoid 3-*O*-glucosyltransferase.

ductive breeding technique. Recently, a random amplified polymorphic DNA (RAPD) analysis of plants propagated by *in vitro* culture was reported (Rady 2006). By the RAPD analysis, Rady showed that low variation at the DNA level occurred during *in vitro* culture when using *Gypsophlia* shoot tips.

Future research should focus on the development of a transformation protocol for *Gypsophila*. Transformation facilitates the development of new cultivars and the identification of useful genes. To our knowledge, the transformation of *Gypsophila* has yet to be reported. Nevertheless, meristem culture for the propagation of *G. paniculata* and regeneration techniques have been established (Doi 2006). Therefore, the transformation of *Gypsophila* will be possible once the optimum conditions for *Agrobacterium* infection are determined.

Molecular studies of flower initiation and flower color, which have been preliminarily attempted as described above, are expected to continue during the next decade. In addition, flower odor should be considered for *G. panculara* production, because an unpleasant odor is emitted by its inflorescences. Although volatile compounds and their related metabolism have been analyzed in relation to this odor (Nimitkeatkai *et al*. 2005a, 2005b, 2006), molecular data for this trait are lacking. Furthermore, elucidating the mechanism of flower senescence will be important for improving the quality of *G. paniculata* cut flowers. Hoeberichts *et al*. (2005) reported apoptotic-like cell death at the early stages of *G. paniculata* petal senescence. Flower senescence in *G. paniculata* is promoted by ethylene; therefore, ethylene-related genes should be isolated from *G. paniculata* and investigated.

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