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 sequences 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.

**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 sequences 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

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**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.

**PHYLLOGENETIC 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 Caryophyllales 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...
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-BINDING1, 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 CTO (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 CTO 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 CTO 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 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 AY15295), 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-
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. paniculata 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. Hoebberichts 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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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, ANS; Leucoanthocyanidin, F3H; Flavanone, DFR; Chalcone, CHS; Anthocyanidin, ANS; Anthocyanin, UFGT; Anthocyanin biosynthetic pathway.