Induction of dwarf and early flowering phenotypes in *Tricyrtis* sp. by ectopic expression of *LEAFY* from *Arabidopsis thaliana*

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Abstract  LEAFY (LFY), which encodes a plant-specific transcription factor, plays an important role in the transition from vegetative to reproductive development. Ectopic expression of LFY has been reported to induce dwarfism and early flowering in some model plants. In order to examine the possibility of using LFY for molecular breeding of ornamental plants, we produced and characterized transgenic plants ectopically expressing LFY from *Arabidopsis thaliana* (*AtLFY*) in the liliaceous ornamental plant *Tricyrtis* sp. Nine independent transgenic plants have been obtained, and all of them exhibited dwarf phenotypes compared with the vector control. These transgenic plants could be classified into three types according to the degree of dwarfism: one showed an extremely dwarf phenotype with smaller leaves (Type I); two showed moderately dwarf phenotypes (Type II); and six showed slightly dwarf phenotypes (Type III). All of Type I, Type II and Type III transgenic plants produced flower buds 1–3 weeks earlier than the vector control. Vector control and Type III transgenic plants produced only a single apical flower bud. Type I and Type II transgenic plants often produced non-fully-opened flowers. Quantitative real-time reverse transcription-polymerase chain reaction analysis showed that the *AtLFY* expression level generally correlated with the degree of dwarfism. These results indicate that morphological alterations observed in the transgenic plants was induced by ectopic expression of *AtLFY*. Lower levels of ectopic expression of LFY may be valuable for producing dwarf and early flowering ornamental plants.

Key words:  *LEAFY* (LFY), liliaceous ornamental plant, morphological alteration, transgenic plant.

LFY, which encodes a plant-specific transcription factor, plays an important role in the transition from vegetative to reproductive development (Weigel et al. 1992). In a *lfy* mutant of *Arabidopsis thaliana*, the first flower was converted into leafy shoots and some abnormal flowers were subsequently produced (Weigel et al. 1992). On the other hand, ectopic expression of LFY-homologous genes has been reported to induce several phenotypic alterations such as dwarfism and early flowering in *A. thaliana* and *Nicotiana tabacum* (Ahearn et al. 2001; Weigel and Nilsson 1998).

Dwarfness and early-flowering are attractive traits especially for ornamental plants. To date, production of dwarf plants has been reported for several ornamental plants (Christensen et al. 2008; Godo et al. 1997; Hoshino and Mii 1998; Koike et al. 2003) by transformation with wild-type strains of *Agrobacterium rhizogenes* or rol genes from *A. rhizogenes*. Dwarf plants have also been produced in *Torenia fournieri* (Niki et al. 2006) and *Tricyrtis* sp. (Otani et al. 2013) by transformation with genes for gibberellin 2-oxidase, which catalyzes bioactive gibberellins or their immediate precursors to inactive forms. However, almost of these transgenic plants exhibited undesirable morphologies such as decreased apical dominance, wrinkled leaves, and/or only small or no flowers in addition to dwarfness. On the other hand, only a few papers have described production of early-flowering transgenic ornamental plants: transgenic *Oncidium* sp. overexpressing *Oncidium*-derived MADS-box gene (Thiruvengadam et al. 2012), transgenic *Sinningia speciosa* with suppressed miR159 (Li et al. 2013), and transgenic *Lilium longiflorum* overexpressing *FT* (Leeggangers et al. 2018). Although LFY may be an alternative gene for producing dwarfness and/or early flowering, it is still unclear whether this transformation can be useful for producing early-flowering ornamental plants.
Transformation of *Tricyrtis* with *Arabidopsis* LEAFY

flowering transgenic plants, there have been only few reports on genetic transformation of ornamental plants with *LFY*.

*Tricyrtis* spp., liliaceous geophytes native to Japan, have recently become popular as ornamental plants for pot and garden uses. We have been using *Tricyrtis* spp. as model plants in liliaceous ornamental plants for basic and applied researches for the following reasons: high transformation efficiencies, relatively small plant sizes, ease of cultivation, and taking only one year from in vitro regeneration to flowering (Nakano and Otani 2020). In the present study, we produced and characterized transgenic *Tricyrtis* sp. plants ectopically expressing *LFY* from *A. thaliana* (AtLFY). The results obtained in the present study showed a possibility of genetic transformation with *LFY* for producing dwarf and early flowering monocot ornamental plants.

Tepal-derived embryogenic callus cultures of a tetraploid somaclonal variant of *Tricyrtis* sp. ‘Shinonome’ (Nakano et al. 2006) were induced and maintained as previously described (Nakano and Otani 2020). *A. tumefaciens* strain EHA101/pIG-AtLFY was used for transformation. The T-DNA region of the binary vector pIG-AtLFY contains AtLFY (accession number NM125579 in the GenBank/EMBL/DBJ databases) under the control of the CaMV35S promoter, NPTII under the control of the NOS promoter, and HPT under the control of the CaMV35S promoter. *A. tumefaciens* strain EHA101/pIG121-Hm (accession number AB489142 in the GenBank/EMBL/DBJ databases) was also used as a vector control. Co-cultivation of embryogenic calli with *Agrobacterium*, selection of transgenic cells and tissues, and regeneration of transgenic plants were performed as previously described (Nakano and Otani 2020). Transgenic plants and the vector control plants were transplanted to pots and cultivated in a growth chamber at 25°C under a 16-h photoperiod. After two years of cultivation, morphological characterization was performed during the flowering season. Transgene transcripts in young leaves of transgenic plants were quantified by real-time RT-PCR analysis as previously described (Otani et al. 2013). The primer sets used were an AtLFY-specific primer set, AtLFY RT-F1 (5′-CCG CGT CAT TGG CTA CTC TCC-3′) and AtLFY RT-R2 (5′-TGC GTC CCA GTA ACC ACT TCC-3′); and a Thact2 (actin gene of *Tricyrtis* sp.; accession number AB196261 in the GenBank/EMBL/DBJ databases)-specific primer set, Thact1-F (5′-CCG ACT CCC TCA TGA AAA TCC-3′) and Thact2-R (5′-CTC GAG CTC CTG TTT GTA GTG A-3′). The relative amount of *AtLFY* transcripts was calculated using the comparative cycle threshold method, and the results were normalized to Thact2.

A total of nine independent transgenic lines were obtained. All of them showed dwarf phenotypes and were distinguishable from the vector control plants. Transgenic plants were classified into three types according to the degree of dwarfism (Table 1; Figure 1): an extremely dwarf Type I line (L1); moderately dwarf Type II lines (L2 and L3); and slightly dwarf Type III lines (L4, L5, L6, L7, L8 and L9). All morphological characteristics of vegetative organs of Type I plants differed significantly from the vector control plants. Type I plants had the shortest shoot length of 5.7 cm, which was only 26% of the vector control plants. Moreover, Type I plants had the smallest number of nodes per shoot, stem diameter and leaf size. Type II plants had a shoot length of 11.2 cm, which was approximately half of the vector control plants. Reduction in stem diameter and leaf size were also observed in Type II lines, but the degree of alterations was lower than Type I plants. Type III plants exhibited less dwarfism compared with Type I and Type II plants. All of Type I, Type II and Type III plants produced flowers 1–3 weeks earlier than the vector control plants (Table 1). Vector control and Type III transgenic plants produced 1–4 apical flowers, whereas Type I and Type II transgenic plants produced only a single apical flower. Non-fully-opened flowers were often observed in Type I and Type II transgenic plants. No differences in the length and diameter of fully opened flowers were observed between all three types of transgenic plants and the vector control plants.

Relative expression level of *AtLFY* in transgenic plants was determined by quantitative real-time RT-PCR analysis (Figure 2). *AtLFY* transcripts were detected in all of Type I, Type II and Type III plants, but not in the vector control plants. Among nine transgenic lines, the highest expression level of *AtLFY* was observed in Type I line, followed by Type II and Type III lines. Thus the *AtLFY* expression level generally correlated with the degree of dwarfism. These results indicate that the morphological alterations observed in transgenic *Tricyrtis* sp. plants was resulted from ectopic expression of *AtLFY*. Although transformation with wild-type strains of *A. rhizogenes*, rol genes from *A. rhizogenes* and gibberellin 2-oxidase genes frequently induced wrinkled leaves or small flowers in addition to dwarfness (Christensen et al. 2008; Godo et al. 1997; Hoshino and Mii 1998; Koike et al. 2003; Niki et al. 2006; Otani et al. 2013), no such morphological alterations were observed in the present study.

Despite the successful introduction of dwarf and early flowering traits, Type I and Type II transgenic plants with higher levels of *AtLFY* expression produced less and non-fully-opened flowers, which greatly reduce the ornamental value. On the other hand, no such unfavorable characteristics were observed in Type III transgenic plants with lower levels of *AtLFY* expression. Thus rather low levels *AtLFY* expression may be necessary for producing dwarf and early flowering
Table 1. Morphological characteristics of transgenic plants containing AtLFY and the vector control plants of Tricyrtis sp. at the flowering stage.*

<table>
<thead>
<tr>
<th>Plant line</th>
<th>Shoot length (cm)</th>
<th>Stom diameter (cm)</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
<th>Flowering date</th>
<th>No. of flowers per shoot</th>
<th>Flower length (cm)</th>
<th>Flower diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector control</td>
<td>10.0±0.6 a</td>
<td>6.0±0.5</td>
<td>7.0±0.5</td>
<td>2.0±0.5</td>
<td>Sep. 9</td>
<td>1.0±0.6 a</td>
<td>0.8±0.5 a</td>
<td>0.5±0.5 a</td>
</tr>
<tr>
<td>Type II transgenic lines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>15.2±1.1 b</td>
<td>4.5±0.5</td>
<td>1.5±0.5</td>
<td>2.0±0.5</td>
<td>Sep. 1</td>
<td>1.5±0.5 b</td>
<td>0.8±0.5 b</td>
<td>0.5±0.5 b</td>
</tr>
<tr>
<td>L2</td>
<td>15.2±1.1 b</td>
<td>4.5±0.5</td>
<td>1.5±0.5</td>
<td>2.0±0.5</td>
<td>Sep. 1</td>
<td>1.5±0.5 b</td>
<td>0.8±0.5 b</td>
<td>0.5±0.5 b</td>
</tr>
<tr>
<td>L3</td>
<td>15.2±1.1 b</td>
<td>4.5±0.5</td>
<td>1.5±0.5</td>
<td>2.0±0.5</td>
<td>Sep. 1</td>
<td>1.5±0.5 b</td>
<td>0.8±0.5 b</td>
<td>0.5±0.5 b</td>
</tr>
<tr>
<td>Type III transgenic lines</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>L4</td>
<td>15.2±1.1 b</td>
<td>4.5±0.5</td>
<td>1.5±0.5</td>
<td>2.0±0.5</td>
<td>Sep. 1</td>
<td>1.5±0.5 b</td>
<td>0.8±0.5 b</td>
<td>0.5±0.5 b</td>
</tr>
<tr>
<td>L5</td>
<td>15.2±1.1 b</td>
<td>4.5±0.5</td>
<td>1.5±0.5</td>
<td>2.0±0.5</td>
<td>Sep. 1</td>
<td>1.5±0.5 b</td>
<td>0.8±0.5 b</td>
<td>0.5±0.5 b</td>
</tr>
<tr>
<td>L6</td>
<td>15.2±1.1 b</td>
<td>4.5±0.5</td>
<td>1.5±0.5</td>
<td>2.0±0.5</td>
<td>Sep. 1</td>
<td>1.5±0.5 b</td>
<td>0.8±0.5 b</td>
<td>0.5±0.5 b</td>
</tr>
<tr>
<td>L7</td>
<td>15.2±1.1 b</td>
<td>4.5±0.5</td>
<td>1.5±0.5</td>
<td>2.0±0.5</td>
<td>Sep. 1</td>
<td>1.5±0.5 b</td>
<td>0.8±0.5 b</td>
<td>0.5±0.5 b</td>
</tr>
<tr>
<td>L8</td>
<td>15.2±1.1 b</td>
<td>4.5±0.5</td>
<td>1.5±0.5</td>
<td>2.0±0.5</td>
<td>Sep. 1</td>
<td>1.5±0.5 b</td>
<td>0.8±0.5 b</td>
<td>0.5±0.5 b</td>
</tr>
<tr>
<td>L9</td>
<td>15.2±1.1 b</td>
<td>4.5±0.5</td>
<td>1.5±0.5</td>
<td>2.0±0.5</td>
<td>Sep. 1</td>
<td>1.5±0.5 b</td>
<td>0.8±0.5 b</td>
<td>0.5±0.5 b</td>
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</table>

*Values represent the mean±standard error of triplicate. Values with different letters are significantly different at the 0.01 level with the Tukey–Kramer’s test.

transgenic plants without reduction in the flower number and non-fully-opened flowers. Higher expression levels of AtLFY in the present study might be caused by a strong activity of the CaMV35S promoter. Therefore, efficient production of dwarf transgenic Tricyrtis sp. plants with normal number of fully opened flowers may be possible by using weaker or tissue-specific promoters.

In the present study, dwarf and early flowering transgenic plants were successfully obtained in the liliaceous ornamental plant Tricyrtis sp. by ectopic expression of the LFY gene from the cruciferous plant A. thaliana. The results provide a possibility of molecular breeding for producing dwarf and early flowering monocot ornamental plants by genetic transformation with the LFY genes. We are now examining production and characterization of transgenic plants ectopically expressing AtLFY in long- and short-day ornamental plants.

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