Plant Science 185–186 (2012) 298–308

Contents lists available at SciVerse ScienceDirect

Plant Science

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

Reduced transcription of a *LEAFY*-like gene in *Alstroemeria* sp. cultivar Green Coral that cannot develop floral meristems

Masayo Hirai[a,*], Masumi Yamagishi[b], Akira Kanno[c]

[a] Graduate School of Agriculture, Hokkaido University, N9W9, Kita-ku, Sapporo 060-8589, Japan
[b] Research Faculty of Agriculture, Hokkaido University, N9W9, Kita-ku, Sapporo 060-8589, Japan
[c] Graduate School of Life Sciences, Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai 980-8577, Japan

ARTICLE INFO

Article history:
Received 30 September 2011
Received in revised form
22 December 2011
Accepted 23 December 2011
Available online 3 January 2012

Keywords:
AP1/*SQUAMOSA*
Floral meristem identity
FLORICAULA/*LEAFY*
Inflorescence
MADS-box gene
Monocots

ABSTRACT

Alstroemeria sp. cv. Green Coral has numerous bracts instead of flowers, and its cyme structures are repeated eternally. Observations of the development and morphology of inflorescence in cv. Green Coral revealed that transition from inflorescence to floral meristem was restricted. We isolated and characterized floral meristem identity genes *LEAFY*-like (*AlsLFY*) and *SQUAMOSA*-like (*AlsSQa* and *AlsSQb*) genes from *Alstroemeria ligtu*. *In situ* hybridization results indicated that *AlsSQa* and *AlsSQb* were expressed in the dome-shaped floral meristems and all floral organ primordia in *A. ligtu*. Transcripts of *AlsLFY* accumulated early in the dome-shaped floral meristems; the signals were restricted later to the outer region of the floral meristem. These results indicate that *AlsLFY*, *AlsSQa*, and *AlsSQb* function as floral meristem identity genes. Expression profiles of *AlsLFY*, *AlsSQa*, *AlsSQb*, and other MADS-box genes were compared between *A. ligtu* and cv. Green Coral. *AlsLFY*, *AlsDEFa*, and *AlsAGL6* transcripts were not detected at the shoot apices of cv. Green Coral but were detected in *A. ligtu*. The early induction and accumulation of *AlsLFY* transcripts in the inflorescence meristem of *A. ligtu* prior to development of the floral meristem suggest that downregulation of *AlsLFY* is likely to restrict the inflorescence-to-floral meristem transition in cv. Green Coral.

1. Introduction

Angiosperms show a broad diversity in when and where they produce flowers. Flowers can appear as a single bloom at the end of a branch, or arranged into a complex inflorescence composed of many flowers. Inflorescence types are classified as indeterminate or determinate, according to whether or not the shoot apices end in terminal flowers. When they do not terminate, the inflorescence is classified as indeterminate. A typical example of an indeterminate inflorescence is the raceme, such as in Arabidopsis (*Arabidopsis thaliana*) or snapdragon (*Antirrhinum majus*). In a raceme inflorescence, the apical meristem grows without limit, inducing a continuous main axis that laterally produces floral meristems. A representative determinate inflorescence is the cyme. This type of inflorescence lacks a main axis: the main shoot terminates in a flower, while growth proceeds through lateral axes formed below the terminal flower. These lateral axes again produce terminal flowers and this process is repeated several times. Cyme inflorescences include tobacco (*Nicotiana tabacum*) or white campion (*Silene latifolia*) [1].

Two types of meristem identity genes determine inflorescence structures. One type promotes transition from inflorescence meristem to floral meristem, and the other inhibits this process and maintains the inflorescence meristem state. The former are called floral meristem identity genes and the latter are antagonists. *LEAFY* (*LFY*)/*FLORICAULA* (*FLO*)-like and *APETALA 1* (*AP1*)/*SQUAMOSA* (*SQUA*)-like genes direct floral meristem identity [2–5]. In contrast, *TERMINAL FLOWER 1* (*TFL1*)/*CENTRORADIALIS* (*CEN*)-like genes maintain inflorescence meristem identity [6,7]. In Arabidopsis, *TFL1* transcription is upregulated prior to the expression of *LFY* and *AP1* in the midsection of the inflorescence meristem. *TFL1* maintains shoot development by inhibiting the transition from inflorescence meristem to floral meristem. On the periphery of the inflorescence meristem, *TFL1* transcription is inhibited by *AP1* and *LFY*, and floral meristem identity is established [8]. The interaction between *TFL1*, *LFY*, and *AP1* generates the raceme inflorescence of Arabidopsis.

Double mutation analysis has shown that floral meristem identity genes *LFY* and *AP1* are reciprocal positive regulators [9]. *LFY*-like

* Corresponding author. Present address: School of Science, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8654, Japan. Tel.: +81 11 706 4808; fax: +81 11 706 3846.
E-mail addresses: a.ligtu@gmail.com (M. Hirai), yamagisi@res.agr.hokudai.ac.jp (M. Yamagishi), kanno@ige.tohoku.ac.jp (A. Kanno).

0168-9452/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2011.12.017

genes encode a plant-specific transcription factor [10], which regulates both the transition to flowering and the subsequent patterning of young floral meristems. During vegetative growth, LFY translation increases in newly formed leaves until it is sufficiently abundant to stimulate transcription of *AP1* and *CAULIFLOWER* (*CAL*), which is a duplicated *AP1*-like gene specific to Brassicaceae [11,12], and triggers the floral meristem transition [13]. Once the floral meristem is established, *LFY* mediates the expression of the floral organ identity genes, such as *AP1*, *APETALA3* (*AP3*), *PISTILLATA* (*PI*), and *AGAMOUS* (*AG*) [14,15]. Similarly, *AP1* is an activator of *AP3* and *PI* [16].

The activated targets of floral meristem identity genes are known as the actors of the ABC model [14]. According to this model, from the outside of a flower inward, sepals are identified by A-function genes, petals are determined by A- and B-function genes, stamens by B- and C-function genes, and carpels by C-function genes. The genes that work in the ABC model (except for *APETALA 2* (*AP2*), one of the A-functional genes) belong to the MADS-box gene family. Named for Arabidopsis and snapdragon genes, class A MADS-box genes are known as *AP1*/*SQUA*-like genes; 2 lineages of class B MADS-box genes are named *AP3*/*DEFICIENS* (*DEF*)-like and *PI*/*GLOBOSA* (*GLO*)-like genes; class C MADS-box genes are known as *AG*/*PLENA* (*PLE*)-like genes. *AP1*/*SQUA*-like genes work at two different stages to determine floral meristem identity and floral organ identity.

Unlike eudicots, many non-grass monocots develop flowers having petaloid organs instead of sepals in the first whorl. To explain the flowers which have a doubled petaloid perianth (tepal), such as those in tulip and lily, the modified ABC model was proposed based on the morphological analysis of mutant tulip flowers [17]. In this model, the petaloid character of the outer tepal is explained by the expression of class B genes, not only in the second and third whorls but also in the first whorl. Genus *Alstroemeria* is included in monocot species belonging to the order Liliales and the family Alstroemeriaceae. *Alstroemeria* is occasionally called the Inca lily and is native primarily to South America [18]. It has been cultivated since the 18th century, and many cultivars are bred. *Alstroemeria* species have a compound inflorescence composed of umbel and cyme with zygomorphic flowers (Fig. 1A and C). We isolated class B genes *AlsDEFa*, *AlsDEFb*, and *AlsGLO* from *A. ligtu* and clarified their expression patterns to support the modified ABC model in *Alstroemeria* [19].

Mutants of *LFY*/*FLO*-like genes and *AP1*/*SQUA*-like genes in eudicots show defects in the transition from inflorescence meristem to floral meristem. *lfy* in Arabidopsis [5], *flo* in snapdragon [2], *aberrant leaf and flower* in petunia (*Petunia* x *hybrida*) [20], and *falsiflora* in tomato (*Solanum lycopersicum*) [21] are *lfy*/*flo*-like gene mutants. *ap1 cal fruitfull* (*ful*) triple mutant in Arabidopsis [22], *squa* in snapdragon [3], and *peam4* in pea (*Pisum sativum*) [23] are *squa*-like gene mutants. In monocots, a *lfy*-like mutant was reported as *apo2*/*rfl* in rice (*Oryza sativa*) [24] and *zfl1 zfl2* double mutant in maize (*Zea mays*) [25]. However, to the best of our knowledge, no such mutants have been identified in non-grass monocots.

Green Coral, an eccentric cultivar of *Alstroemeria*, is released by Miyake Nursery Ltd., Chiba, Japan. This cultivar has numerous bracts instead of flowers, and the cyme structure is repeated eternally (Fig. 1B and D). Its morphology suggests that the transition from inflorescence meristem to floral meristem should be aberrant in this cultivar. In this study, we evaluated the morphology and process of inflorescence formation in cv. Green Coral using light and scanning electron microscopy (SEM). We isolated and characterized floral meristem identity genes *AlsLFY*, *AlsSQa*, and *AlsSQb* from *A. ligtu* and compared the expression profiles of *AlsLFY*, *AlsSQa*, *AlsSQb*, *AlsDEFa*, *AlsDEFb*, *AlsGLO*, and *AlsAGL6* genes in *A. ligtu* and cv. Green Coral.

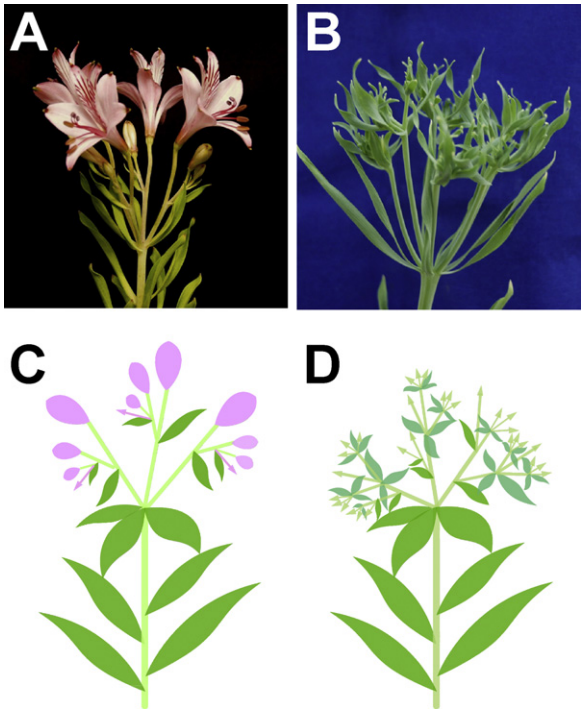

Fig. 1. Inflorescence of wild-type *Alstroemeria ligtu* (A) and cv. Green Coral (B). The schematic drawing (C and D) illustrates how secondary pedicels develop in succession on the umbel-like branches in *A. ligtu* (C), but a second umbel-like structure develops instead of pedicles in cv. Green Coral (D).

2. Materials and methods

2.1. Plant materials

Alstroemeria sp. cv. Green Coral and *Alstroemeria ligtu* ssp. *ligtu* were used in this study. Cv. Green Coral is selected from selfed-seeds of Dr. Salter's Hybrids (http://www.hinsyu.maff.go.jp/), and Dr. Salter's Hybrids are said to be derived from interspecific crosses of *A. ligtu* and its related species. Thus, we used *A. ligtu* as a control. *A. ligtu* shows the typical inflorescence and flower phenotypes of genus *Alstroemeria*. Plants were grown at the experimental farm of Hokkaido University, Japan.

2.2. Section preparation

Young inflorescence (0.5–3 cm) and vegetative shoots were fixed in 50 mM sodium phosphate (pH 7.2) containing 4% (w/v) paraformaldehyde, 0.25% (w/v) glutaraldehyde, and 0.8 mM NaOH, dehydrated in 2-methyl-2-propanol and embedded in Paraplast Plus (Sigma–Aldrich, St. Louis, MO, USA). A rotary microtome (RM2145, Leica, Solms, Germany) was used to prepare 8-μm sections of paraffin-embedded tissue, which were placed onto glass slides. Sections were washed in ethanol and xylene to remove paraffin.

2.3. Light microscopy

The sections were washed in ethanol, stained with Toluidine Blue O (Wako Pure Chemical, Osaka, Japan), and observed under a light microscope.

2.4. In situ hybridization

The sections were treated with 0.9 $\mu g\,mL^{-1}$ recombinant Proteinase K (Roche Diagnostics, Indianapolis, IN, USA) for 10 min at