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Anatomical Analysis of Inflorescence Development in *Eustoma Grandiflorum*

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Abstract

Inflorescence refers to the spatial and temporal patterns of flowers and analysis of the branching pattern would facilitate understanding of the entire structure of inflorescence. To gain an insight into the inflorescence architecture of *Eustoma*, the popularity of which has soared worldwide, we conducted a detailed characterization of inflorescence development. In particular, we focused on identifying meristem types and describing their development, both of which may affect the inflorescence structure. During reproductive development, the shoot apical meristem usually splits into two meristems, either or both of which can become inflorescence meristems and capable of producing a floral meristem. However, the inflorescence meristems ultimately abort flower production. Meanwhile, axillary meristems sometimes grow and convert into inflorescence meristems, leading to differentiation of floral meristems. Hence, different types of inflorescence result from different types of meristem, and changes in meristem activity. We elucidated the factors influencing inflorescence structure and classified them into eight groups.

Discipline: Horticulture
Additional key words: Bifurcation, floral meristem

Introduction

Eustoma grandiflorum (Raf.) Shinn (lisianthus) belongs to the Gentianaceae family and originated in North America, where it is widely grown as an ornamental flower in temperate areas. The worldwide popularity of *Eustoma* as a cut flower has soared over the last decade. In Japan, *Eustoma* ranked fifth in terms of the production value of cut flowers in 2009. Japanese seed companies have produced many new varieties over the last 60 years. Most breeding efforts have been focused on the morphology of the flower, such as flower color, double layers of petals, and early flower development. In general, the number of flowers in inflorescence is one of the key traits and their structure and temporal arrangement dictate the inflorescence structure. The practice of removing immature flower buds is recognized as important in the production of standard carnations and standard roses to obtain large and voluminous flowers. This suggests that the quality of flowers is influenced by the inflorescence structure, making it important to understand the mechanisms underlying the regulation of the inflorescence structure.

Apart from single-flower inflorescences such as gerbera, the inflorescences of most cut flowers are composed of many flowers arranged in a branching pattern. Accordingly, analysis of branching patterns should facilitate understanding of the entire inflorescence structure. In *Eustoma*, however, the developmental profile of the inflorescence remains unclear. To date, little breeding in *Eustoma* has focused on inflorescence structure; hence information concerning the development of inflorescence might be useful for the breeding and cultivation of this species.

The shoot apical meristem (SAM) is the ultimate source of all aerial structures of a plant, including the flowers. While the vegetative phase of the SAM produces leaves, the reproductive phase (i.e. inflorescence meristem) produces floral meristems that spawn floral organs, meaning each meristem can be defined by the structure(s) it produces[5]. During the life cycle of a plant, the meristem undergoes many transitions[3]. In particular, the switch from the vegetative to the inflorescence phase tends to be accompanied by changes in the spatial pattern of organ initiation; namely, changes in phyllotaxy represent a critical shift in the developmental stage.

We investigated the complex structure of the inflorescence in *Eustoma*, detailing the initial stages of inflo-

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rescence development and clarifying the types of meristem and their spatial patterns.

Materials and methods

1. Plant material and growth conditions

Eustoma grandiflorum (cv. Piccorosa Snow) seeds were sown in plug trays containing fertilized soil, and maintained at 10°C in the dark for 5 weeks in a growth cabinet to promote subsequent stem elongation. Trays were transferred to a growth chamber (Nihonika Co., Tokyo, Japan), and maintained at 28°C under a 12-h light phase with fluorescent lamps (50 μmol · m^{-2} · s^{-1}) and 18°C under a 12-h dark phase until two pairs of leaves developed. Each seedling was transplanted into a 10.5-cm plastic pot containing fertilized medium (Kureha Engeibaido, Kureha Chemical Industry Co., Ltd., Tokyo, Japan) and placed in a greenhouse.

2. Histological analysis

The plant tips were collected at the transition from the vegetative to the inflorescence phase. For paraffin sectioning, we fixed samples overnight in formalin: glacial acetic acid: 70% ethanol (1:1:18), followed by dehydration in a graded ethanol series. Following substitution with xylene, the samples were embedded in Paraplast Plus (Oxford Labware, St. Louis, MO, USA) and sectioned to a thickness of 8 μm using a rotary microtome. Sections were then stained with 0.05% toluidine blue and observed with a light microscope (AX70, Olympus, Tokyo, Japan).

For scanning electron microscopy (SEM), leaves were removed under a stereomicroscope and samples, including inflorescence meristems and floral meristems, were prepared. Samples without surface treatment were observed under a scanning electron microscope (VE-7800; Keyence, Tokyo, Japan).

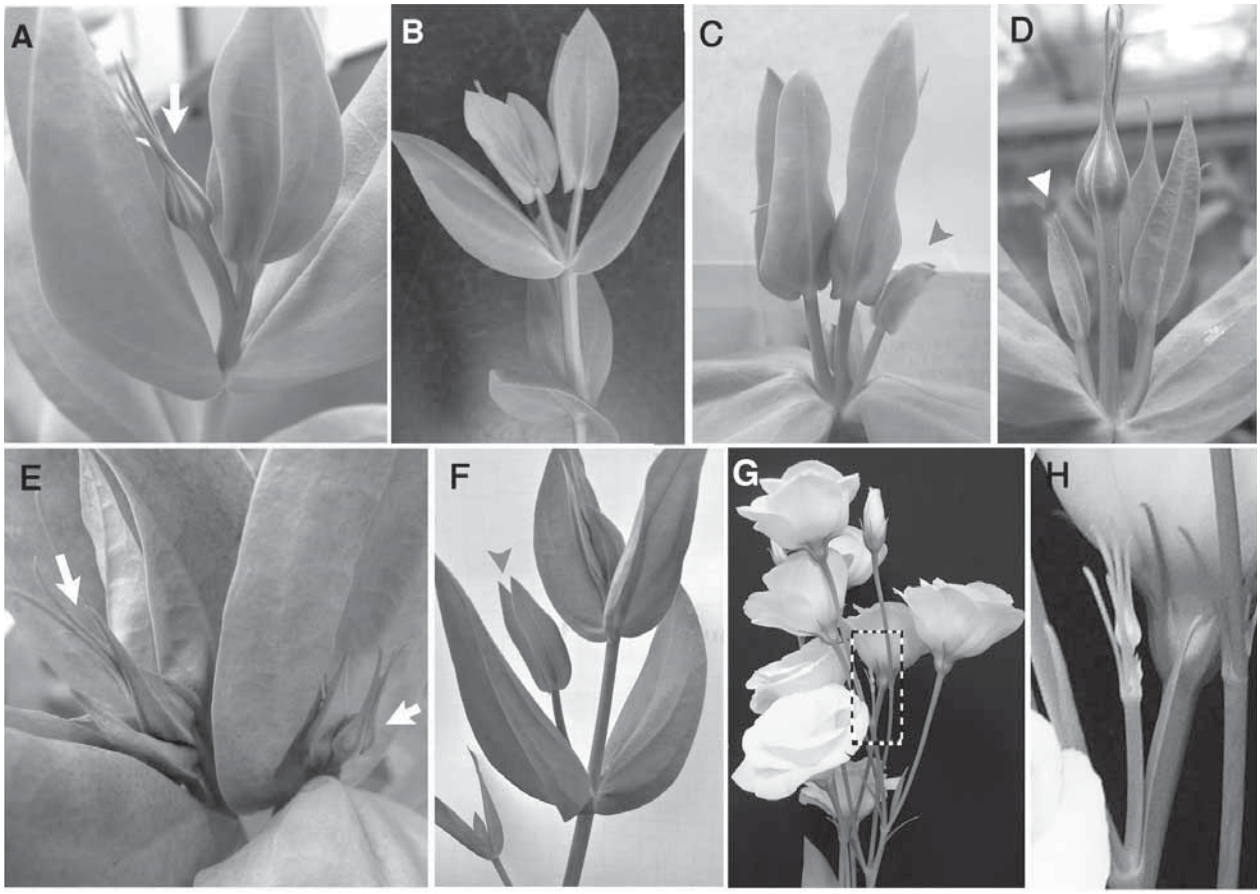

Fig. 1. Morphological features of the inflorescences in *Eustoma*

(A, B) The two major types of inflorescence: a floral bud (arrow) and inflorescence branch (A) and two inflorescence branches (B). (C, D) The two minor types of inflorescence: two main inflorescence branches and one extra branch (arrowhead) (C) and a floral bud, an inflorescence branch, and one extra branch (arrowhead)(D). (E) The two units of inflorescence branches: two pairs of bracts enclose a floral bud (arrow) and an inflorescence branch. (F) A plant with axillary inflorescence branches (arrowhead) at a node below the transition node. (G) Appearance of an inflorescence in which the tip had aborted. (H) Higher magnification of the abortion shown in (G).

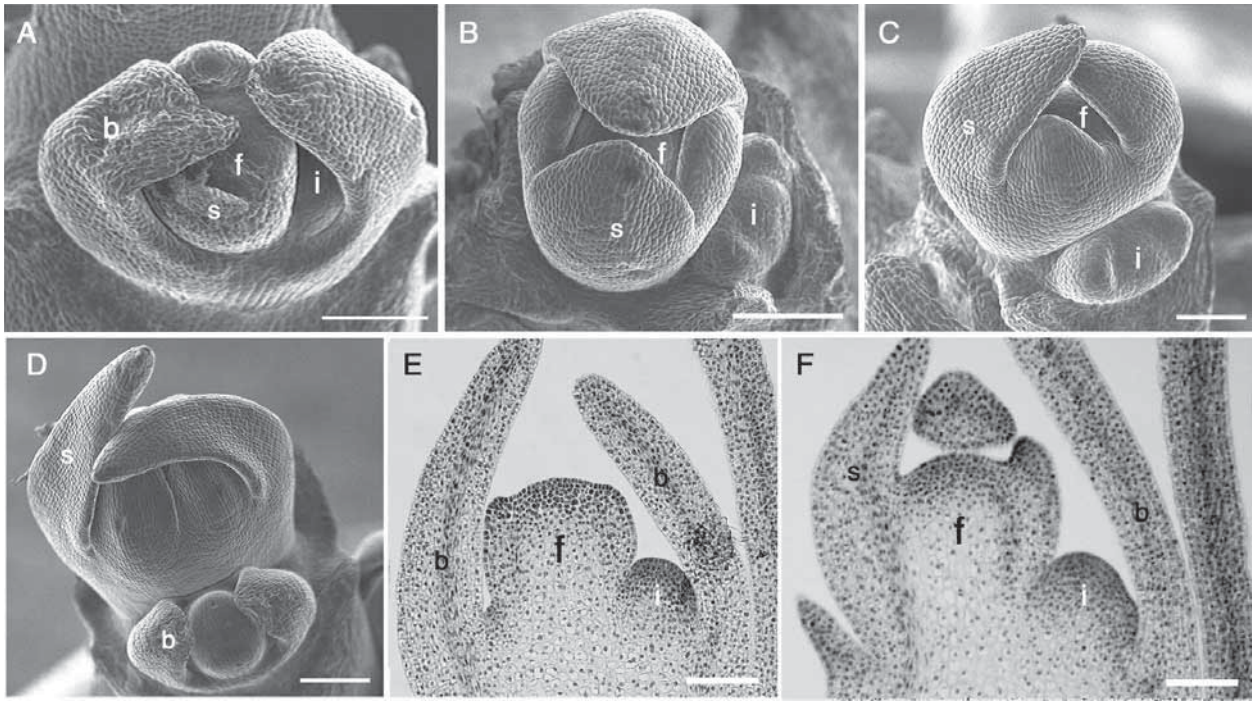

Fig. 2. Sequential developmental changes in an inflorescence meristem and a floral meristem
(A–D) Scanning electron microscope images of reproductive meristems.
(E, F) Paraffin sections of the inflorescence meristem and floral meristem producing sepals.
Abbreviations: i, inflorescence meristem; f, floral meristem; b, bract; s, sepal.
Scale bars: 200 μm (A–D), 100 μm (E, F).

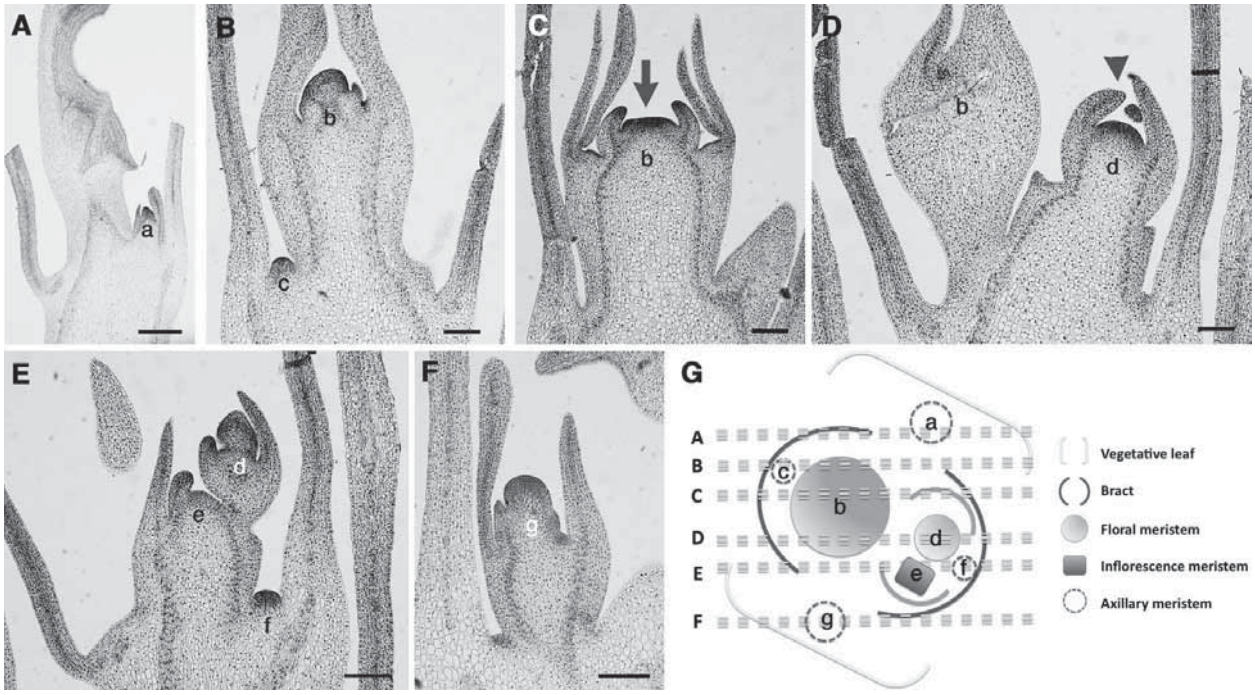

Fig. 3. Spatial patterns in meristems
(A–F) Serial sections of an inflorescence, with various small letters showing different meristems. Floral meristem producing sepals (arrowhead) and floral meristem producing petals (arrow).
Scale bars: 500 μm (A), 200 μm (B–F). (G) Schematic diagram of inflorescence. Small letters correspond to those shown in (A-F). Capital letters correspond to the sections shown in (A–F).

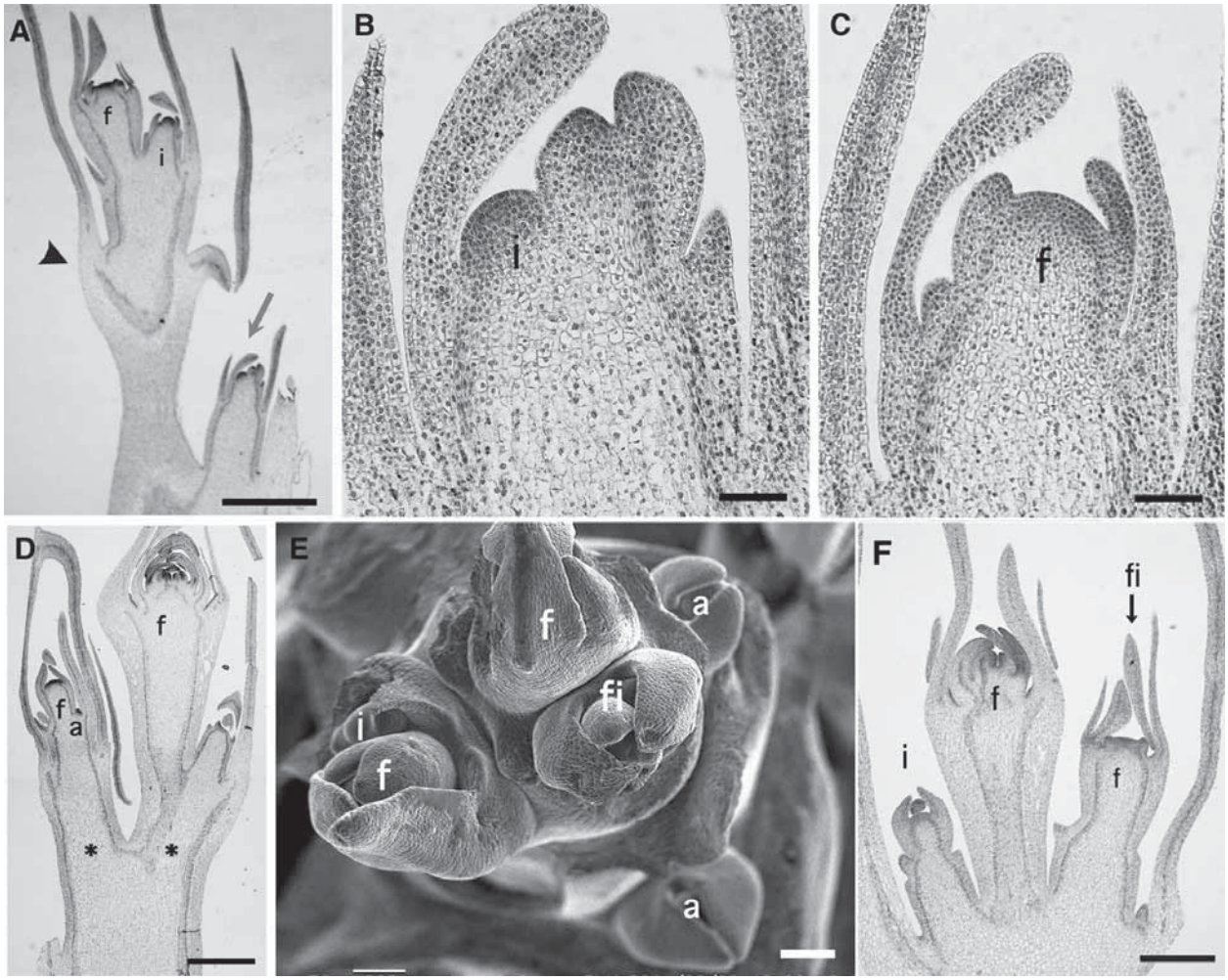

Fig. 4. Developmental profiles of an inflorescence with two inflorescence branches
(A–D) Serial sections of a young inflorescence with two main branches and an axillary branch. The axillary meristem (arrow) below the transition node (arrowhead) was converted from the vegetative to the reproductive phase. Two relatively even stems of inflorescence branches (asterisks) are also shown (D). (E) An SEM image of the initial development of an inflorescence with two branches. (F) Young inflorescence with two branches and floral buds at the transition node. Abbreviations: i, inflorescence meristem; f, floral meristem; a, axillary meristem.
Scale bars: 1 mm (A, D, F), 100 μm (B, C), 200 μm (E).

3. Characterization of cut flowers

Eighty-two days after transplanting, we harvested the plants and examined the characteristics of the inflorescence; 10 days, on average, had passed since the first flower opened.

Results

Leaves began development with an opposite and decussate phyllotaxy but their arrangement soon changed to a combination of mainly two patterns: an inflorescence branch and a flower (Fig. 1A), and two inflorescence branches (Fig. 1B). These two patterns applied to almost all specimens, although an extra inflorescence branch was rarely observed (Figs. 1C, D). Each branch was composed of a pair of bracts, a floral bud, and a new inflorescence branch (Fig. 1E), showing that cleavage of the axes may be evidence of the transition from the vegetative to the reproductive phase. In addition, axillary buds sometimes grew at a node that was lower than the transition node (Fig. 1F). Axillary buds produced several pairs of leaves as well as reproductive organs such as inflorescence branches and floral buds. Branch cleavage was reiterative until the tip of a branch ceased growth (Figs. 1G, H).

To understand inflorescence architecture, we conducted a detailed characterization of reproductive development using SEM. Furthermore, to study the spatial patterns of meristems, we observed initial reproductive organs in paraffin thin sections. The inflorescence and

	Type I	Type II	Type III	Type IV	Type V	Type VI	Type VII	Type VIII
The meristem type just after the transition	FI	FI	FI	FI	II	II	II	II
Outgrowth of axillary meristem at the node below the transition node	×	○	×	○	×	○	×	○
Formation of extra inflorescence branches	×	×	○	○	×	×	○	○
Schematics								

Fig. 5. Inflorescence types in *Eustoma* based on anatomical characterization
FI; A floral meristem and an inflorescence meristem,
II; Two inflorescence meristems.

flower meristems were adjacently and simultaneously established, and both were subtended by bracts (Fig. 2A, E). When sepal primordia differentiated from the floral meristem, the inflorescence meristem adjacent to the floral meristem produced a pair of bracts (Figs. 2B–D, F) followed immediately by a floral meristem (Fig. 2D). The remainder of the inflorescence meristem, apart from the floral meristem, became a secondary inflorescence meristem, which was large at inception (Fig. 2E). In other words, the inflorescence meristem appeared to bifurcate into two halves, either or both of which became an inflorescence or another floral meristem.

During this stage, the axillary meristems had not yet initiated in the axils of the bracts (Figs. 2E, F). Thereafter, during the petal-differentiation stage, axillary meristems were initiated between the peduncle and the bract subtending the inflorescence or floral meristem (Figs. 3B, E). Axillary meristems were also produced at the node beneath the transition node, and sometimes emerged from dormancy and became an inflorescence meristem (Figs. 3A, F, G, 4A–C). In contrast, few axillary meristems in the upper node, other than the transition node, emerged from dormancy.

When a bifurcated inflorescence meristem produced two inflorescence meristems (Figs. 4D, E, 6A), both grew almost simultaneously (Figs. 4D, E). Moreover, the inflorescence meristem sometimes produced three meristems (Fig. 4F). In summary, the floral meristem initiation pattern is mainly influenced by three factors: (1) meristem type at the transition from the vegetative to the reproductive phase, (2) outgrowth of axillary meristems at the node below the transition node, and (3) the formation of extra inflorescence branches (Fig. 5).

We also examined the number of flowers per plant. Plants that formed a single inflorescence meristem just after the transition from the vegetative to the reproductive phase had fewer flowers than those in which two inflorescence meristems formed (Fig. 6B). This result is consistent with our predictions (Fig. 6A).

Discussion

We conducted detailed analyses of inflorescence development in *Eustoma*, and found that the SAM splits during the transition from the vegetative to the reproductive phase. Although the flower meristem proliferated more vigorously than the inflorescence meristem (Fig. 2C), the establishment of both types coincided (Figs. 2A, 4B, C). Therefore, it is unlikely that inflorescence meristems form in the axils of the floral meristem. A split of the reproductive meristem has also been observed in *Petunia*, tomatoes, and peas [1, 6, 7]. Accordingly, we assume that the inflorescence type of *Eustoma* is cymose.

We elucidated some factors that influence the final architecture of the *Eustoma* inflorescence: (1) meristem type at the transition from the vegetative to the reproduc-

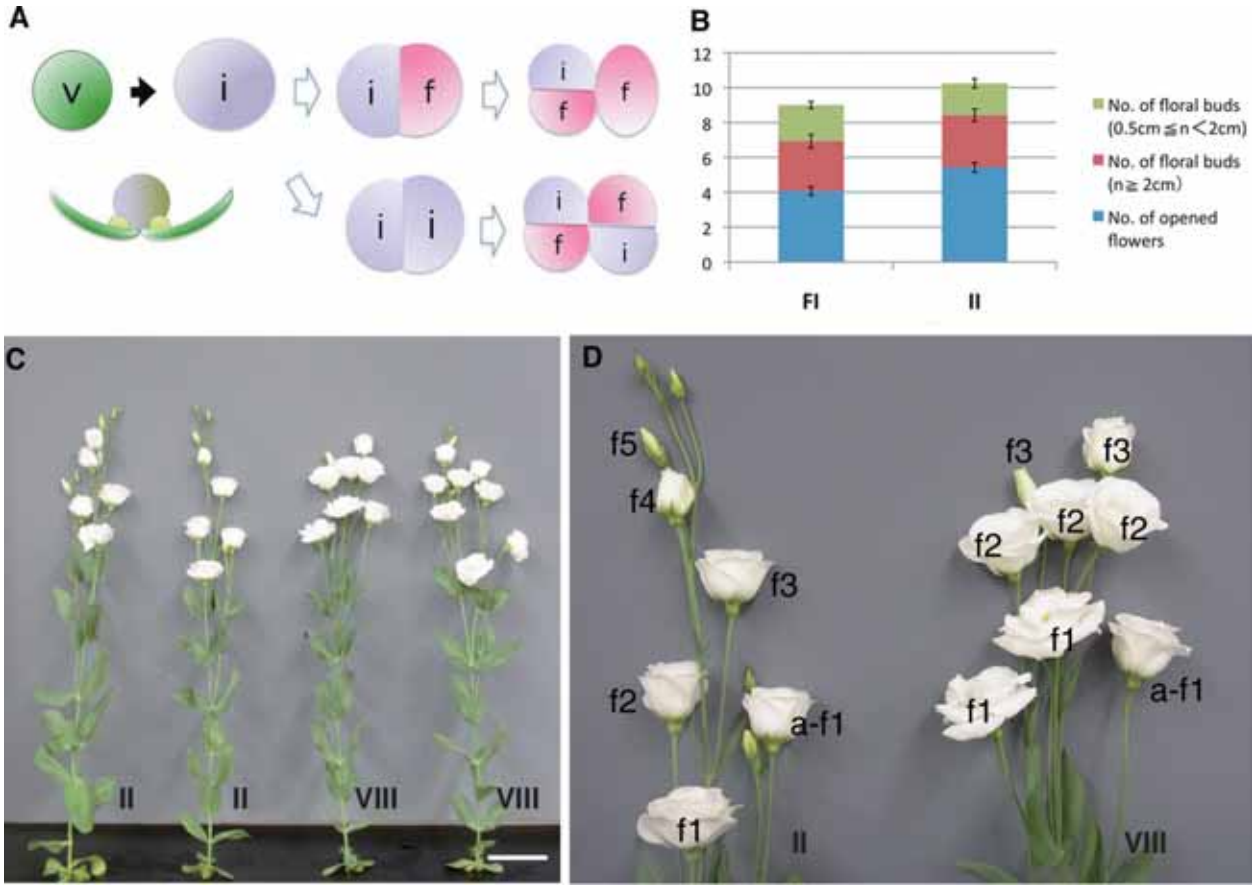

Fig. 6. Variation in inflorescence morphology

(A) Schematic diagram of the conversion of the meristem type.
(B) Number of flowers in two types of inflorescence. FI: flower meristem formed just after the transition from the vegetative to the reproductive phase; II: two inflorescence meristems formed just after the transition from the vegetative to the reproductive phase.
(C) Appearance of plants with a Type II inflorescence (left) and with a Type VIII inflorescence (right). Scale bar = 10cm.
(D) High magnification of (C).
Numbers indicate the rank from the transition node. Abbreviations: i, inflorescence meristem; f, floral meristem; a, axillary meristem;
v, vegetative meristem.

tive phase, (2) whether the axillary meristems can emerge from dormancy, (3) whether extra inflorescences can be formed from meristems, and (4) the timing of abortion of the inflorescence meristem. How each of these four factors affect inflorescence architecture is described below.

The meristem type at the phase transition affects the horizontal width of the inflorescence and the timing of the transition is thought to dictate whether or not bifurcated meristems become inflorescence meristems. Subsequently, the later transition from an inflorescence meristem to a floral meristem would elevate the node associated with that flower and increase the number of meristems with the potential to become flowers (Fig. 6A, B). In such cases, we discovered extended periods of transition. The mechanisms underlying the timing of such transitions have been investigated in several dicotyledonous plants[4], and might be genetically regulated in *Eustoma*.

The emergence of axillary meristems at and below the transition node is essential to increase the number of open flowers. In addition, when the emergence of axillary meristems from dormancy coincides with flower formation in the main axis (Figs. 4A–C), the number of open flowers tends to increase. Recent studies have shed light on the mechanisms and regulation of axillary meristem growth in other plant species[2] but mainly in relation to the outgrowth of the axillary bud in the vegetative phase. It is necessary to study the processes that result in the vigorous development of axillary inflorescence branches.

When extra inflorescence meristems form, it influences both the width of the inflorescence and synchro-

nous flower opening. For example, in Type VIII inflorescence, in which extra inflorescence meristems are formed at the secondary upper node from the transition node, there were more open flowers compared to the Type II inflorescence (Figs. 6C, D).

Finally, the timing of abortion of inflorescence meristems affects the vertical width of the inflorescence. To increase the number of flower tiers, the time between flowering to meristem abortion should be increased. One problem, however, is the significant time lag between the opening of flowers at the bottom and top of the inflorescence respectively. Because synchronous flower opening ensures commercial value as a cut flower, it is important to research this factor in more detail with the aim of increasing the number of open flowers.

The present study provides useful information on factors regulating the inflorescence architecture of *Eustoma*. Meristem type and activity influence the entire structure of the inflorescence. Moreover, each of the above-described factors appears to be influenced by environmental conditions. To better understand how each factor is regulated, further agronomic and genetic studies are needed.

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