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Fasciation in Strawberry Floral Organs and Possible Implications for Floral Transition

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Fasciation in strawberry is characterized by an enlarged and flattened receptacle, clustering of flowers, and altered inflorescence architecture. However, the developmental process of fasciated flowers remains obscure. In this study, the fasciation incidence and developmental process in the primary fruit and inflorescence architecture were evaluated and compared for the non-susceptible cultivars, 'Nyoho' and 'Sagahonoka' and one of the most susceptible cultivars, 'Ai-Berry'. The severity and frequency of flower and inflorescence fasciation was clearly greater in the vigorously growing large plants of 'Ai-Berry' compared to small plants and large plants of the other two cultivars. In 'Ai-Berry', the deformation of the large shoot apical meristem (SAM) into an oval shape was the initial symptom observed before and during floral transition. Such oval-shaped SAMs often differentiated two or more leaf primordia almost at the same time, which then developed into divided multiple vegetative SAMs before floral transition and linearly-fasciated SAMs during floral transition, respectively. The development of fasciation symptoms was observed after downregulation of *FaTFL1*. Although inflorescence or receptacle fasciation could be controlled when early and rapid floral induction was achieved by intermittent low-temperature treatment, severe fasciation was observed in late-flowered plants which were either not responsive or not subjected to this treatment. These results indicate that fasciation of floral organs may be triggered and develop during floral transition and that temperature fluctuations around boundary values between floral inhibition to induction may cause a half-finished or slowly processed floral transition and finally result in severe fasciation in vigorously growing 'Ai-Berry' plants.

Key Words: early flowering, *FaTFL1*, flower induction, propagation, transplant sizes.

Introduction

Cultivated strawberries (*Fragaria × ananassa* Duch.) were found in Europe from populations of the natural hybrid between *F. chiloensis* (L.) Mill. and *F. virginiana* Duch. in the mid-1700s. The former is a dioecious plant species, bearing large and whitish fruits, and it had been introduced from the Pacific coast of South America after the early 1700s. The latter develops hermaphrodite flowers, small, scarlet-colored attractive fruits, and was continuously introduced from North America after

Columbus' "discovery" (Darrow, 1966). After finding interspecific hybrid plants, new large-fruiting cultivars of *F. × ananassa* spread worldwide and completely replaced commercially grown *F. virginiana* and *F. chiloensis* during the 1800s. Darrow (1966) exhibited fruits of such early cultivars, including one of the oldest cultivars, 'Downton' (1820), and also newer, more famous cultivars including 'Marshall' (1893), 'Howard 17' (1909), and 'Fairfax' (1923). Photographs show wedge-shaped or severely malformed fan-shaped fruits.

Fasciation of strawberry flowers was first reported by Darrow and Borthwick (1954). Slightly affected plants can only be distinguished by broad-shaped primary flowers and by fruit with a flattened peduncle. In severely affected plants, the primary flowers develop into flattened wedge-shaped, coxcomb-shaped, or curled fruit on the flattened peduncle, and the numbers of secondary and inferior fruit greatly increase. Fruit is

much smaller than for normal plants, and the inflorescence has a witches' broom-like appearance. This disorder has not been serious except for certain large-fruiting cultivars in the Middle and South Atlantic States of the USA, with the principal cultivars not sensitive to the disorder in the region. However, the "new" large-fruiting cultivars, including 'Howard 17' and its progenies, were highly susceptible to fasciation and could not be grown in the region. The disorder was also observed more frequently in large and old plants than in young and small ones, and in short-day treated plants of certain cultivars (Darrow and Borthwick, 1954).

Modern cultivated strawberries were introduced to Japan in the early 1800s from the Netherlands. The first Japanese cultivar, 'Fukuba', was selected in 1898 from seedlings of a French cultivar, 'General Chanzy' (Kanezashi, 1997). This was not susceptible to fasciation and was a leading cultivar until the 1960s. From the 1970s to the mid-1980s, when forcing strawberry production in plastic housing became popular (Fujimoto, 1971), 'Hokowase' (released from Hyogo Prefecture in 1957) was a leading cultivar for a long time (Yoshida and Nishimoto, 2020), while a US cultivar 'Donner', one of the progenies of 'Howard 17', was most popular for semi-forcing production in the Eastern region. Large and old nursery plants of these two major cultivars usually produced sufficient early yield, but often developed large fasciated primary fruit with many small secondary or inferior fruit (Kimura, 1984).

Fruit fasciation is a fruit malformation, but moderate fasciation is not recognized as a disorder that causes economic damage. Such fruit is still valuable because of its large size. In this century, fasciated fruit is not as common because most recently released forcing cultivars were genetically improved to reduce susceptibility to the disorder, and smaller transplant propagation procedures became popular using plastic pots or trays (Yoshida and Motomura, 2011; Yoshida and Nishimoto, 2020).

However, certain recently released cultivars, including 'Fukuoka S6' (Mitsui et al., 2003) and 'Beni hoppe' (Takeuchi et al., 1999), exhibit fasciation when flowering of the primary inflorescence is unusually delayed. Such delayed flowering usually results from a delay of floral transition, often caused by unsuccessful artificial flower induction treatment before planting to bountiful soil or direct planting of runner plantlets onto substrate growing systems.

In previous studies (Yoshida, 1992; Yoshida et al., 1991), and practical production fields, we have frequently observed fasciated flowers and fruits in the cultivar 'Ai-Berry'. As described by Darrow (1966), this was severe for overwintered mother plants and common in large old plants compared to small plants among the propagated transplants for forcing production. In strawberry, it is well known that the initial symptom of floral transition is enlargement of the shoot apical meristem

(SAM) (Yoshida and Nishimoto, 2020). Aged large plants usually have a thicker crown and larger SAM, and develop larger leaves compared to young small plants or runner tips. The SAM size appears to be an important factor affecting fasciation.

The SAMs of plants are composed of stem cells that are continuously replenished and the size of SAMs is maintained through a classical feedback circuit involving the homeobox *WUSCHEL* (*WUS*) gene and the *CLAVATA* (*CLV*) gene signaling pathway (Somssich et al., 2016). In *Arabidopsis thaliana* L., *CLV* gene mutants develop fasciated inflorescences and flowers caused by enlarged SAMs (Clark et al., 1997). The *CLV* gene mutants of tomato (*Solanum lycopersicum* L.) also develop fasciated inflorescences and flowers, but the enlargement becomes more pronounced at the reproductive transition stage, unlike *Arabidopsis* (Xu et al., 2015). Both tomato and strawberry are characterized by a sympodial shoot and inflorescence architecture. After differentiating a certain number of leaf primordia, normally 8–10, the SAM of tomato is sequentially transformed into an inflorescence meristem (IM) and floral meristem (FM) generating the flower (Schmitz and Theres, 1999). Tomato is a neutral plant and does not require any environmental stimuli for floral transition; however, seasonal flowering strawberry is a facultative short-day plant and the floral transition is strictly inhibited by high temperature (Ito and Saito, 1962).

Although the fasciation of strawberries has long been recognized as a disorder, the developmental process remains obscure. Darrow and Borthwick (1954) reported that fasciation appears to initiate at the time of flower bud differentiation, but there is no evidence demonstrating a relationship between the two phenomena. Therefore, we carefully observed the developmental process of fasciation in strawberry flower buds using one of the most susceptible cultivars 'Ai-Berry', examined the effect of a floral inducing treatment on the development of fasciation, and determined the expression of *TERMINAL FLOWER 1* (*TFL1*), which is a crucial floral repressor gene in seasonal flowering *F. × ananassa*, as well as in *F. vesca* (Nakano et al., 2015), to demonstrate the implication of floral transition in the fasciation of strawberry.

Materials and Methods

Plant materials

In a greenhouse at Okayama University, runner plants were propagated from mother plants, which were grown in 36-cm bowl planters (Vantech Co., Ltd., Sakai) containing 7 L of peat moss medium and supplied with 30% OAT A solution [N 5.6, P 0.5, K 2.6, Ca 1.2, Mg 0.5, and S 0.5 (all in mM) with microelements; OAT Agrio Co., Ltd., Tokyo] three times a week. The nursery plants were propagated on pots and trays for later experiments.

In June 2018, more than 80 runner plants of 'Ai-

Berry' and a non-susceptible cultivar 'Nyoho' were rooted in 12-cm pots filled with ca. 800 mL of pre-mixed media (Ichigo ikubyou baido; Sumika Agrotech, Co., Ltd., Osaka) to obtain large aged plants and observe fasciation development in Experiment 1. Root-ed plants were detached from mother plants on July 9 and supplied with 30% OAT A solution three times a week until September 7. Such a fertigation procedure has been demonstrated to be sufficient to encourage vegetative growth, but not so effective to suppress floral transition for a long time (Kinjo et al., 2017; Yoshida and Morimoto, 2010).

In June 2019, more than 30, 70, and 90 runner plants of 'Ai-Berry' were rooted in 12-, 10.5- (ca. 560 mL), and 9-cm (ca. 370 mL) pots, respectively, and more than 70 plants of another non-susceptible cultivar 'Sagahonoka' were rooted in 10.5-cm pots. They were detached on July 13. Additionally, more than 70 plants of 'Ai-Berry' propagated from the same mother plants were rooted in 7.5-cm pots (ca. 220 mL) from late July to early August to obtain young and small plants, and these were detached on August 22. The same nutrient solution was supplied until transplanting or sampling. The 12-, 10.5-, and 7.5-cm pot-grown plants were used in Experiment 2 to determine the effect of plant size on fasciation development. The remaining 9-cm pot-grown plants were used in Experiments 3 and 4.

On July 25, 2019, runner plants of 'Ai-Berry' with more than two expanded leaves were excised from mother plants grown on the table-top substrate. Runner cuttings were rooted in three trays having 24 of 175-mL cells spaced 8 cm × 8.5 cm apart (Suku-suku tray; Marusan Sangyo Co., Ltd., Tochigi) and managed as described previously (Yoshida and Motomura, 2011). These densely grown tray plants and 50 sparsely grown plants in 9-cm pots were supplied with the same solu-tion three times a week until August 30 and used in Experiment 3 to determine the effect of artificial flower induction treatment. The remaining 40 plants in 9-cm pots were supplied with the same solution three times a week continuously and used in Experiment 4 to deter-mine *FaTFL1* expression.

Observation of fasciation development in SAM

Morphological changes in SAM were observed using a stereo microscope (SMZ-2T; Nikon Corp., Tokyo) and a digital microscope (VHX-2000; Keyence Corp., Osaka). Changes in SAM size were evaluated by mea-suring the longitudinal diameter, including the youngest leaf primordium or bract primordia, in fasciated SAMs.

Evaluation and classification of fasciation in fruit and inflorescences

As described by Darrow and Borthwick (1954), fruit fasciation originated from abnormal flowers with asym-metrical and flattened receptacles (Fig. S1) and there were variations in the severity of fasciation. Slightly

affected plants developed wedge-shaped primary fruit and 2–3 normal secondary fruit, while severely affected plants developed folding fan- or coxcomb-shaped primary fruit with flattened, fused peduncles and/or inflorescences divided into several clusters with each primary flower opening concurrently.

In this study, the degree of primary fruit fasciation was classified into five levels (Fig. 1): 0, normal conical- or spindle-shaped fruit; 1, slightly flattened wedge-shaped fruit with a round distal end; 2, wedge-shaped fruit with a flattened distal part; 3, fan-shaped fruit with a broad distal part; and 4, coxcomb-like fruit with a semicircular shape or fused partly separated receptacles. When the inflorescence of a plant was divided into multiple clusters as described later, the degree was evaluated based on the most severely affect-ed fruit.

The degree of inflorescence fasciation was classified into five levels (Fig. 2): 0 (normal), typical dichasial cyme bearing a normal or slightly fasciated primary fruit (level 1) with a couple of secondary fruits; 1 (slightly fasciated), inflorescence bearing a fasciated primary and several secondary fruits with an oval-shaped cross-section of the main peduncle; 2 (fasciat-ed), inflorescence bearing a severely fasciated primary (level 3 or 4) and many secondary fruits with a flattened or partly fused main peduncle; 3 (severely fasciated), inflorescence divided into multiple clusters in which each primary flower open concurrently or with a severely fused main peduncle; and 4 (multiple apical shoots), branched shoots in a crown formed without sympodial branching caused by SAM division before floral transition (Fig. S4).
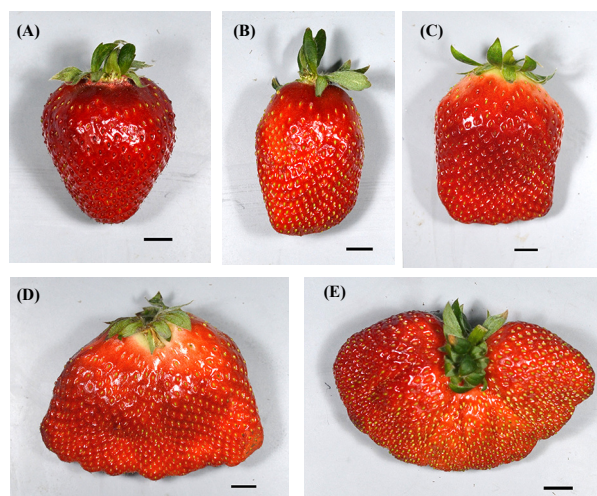

Fig. 1. Classification of fasciated primary fruit of a cluster, based on levels from 0 to 4. (A) Level 0, Normal fruits; (B) Level 1, Slightly flattened wedge-shaped fruit with a round distal end; (C) Level 2, Wedge-shaped fruit with a flattened distal part; (D) Level 3, Fan-shaped fruit with a broad distal part; (E) Level 4, Coxcomb-like fruit with a semicircular shape or fused partly separated receptacles. Black bars indicate 1 cm.
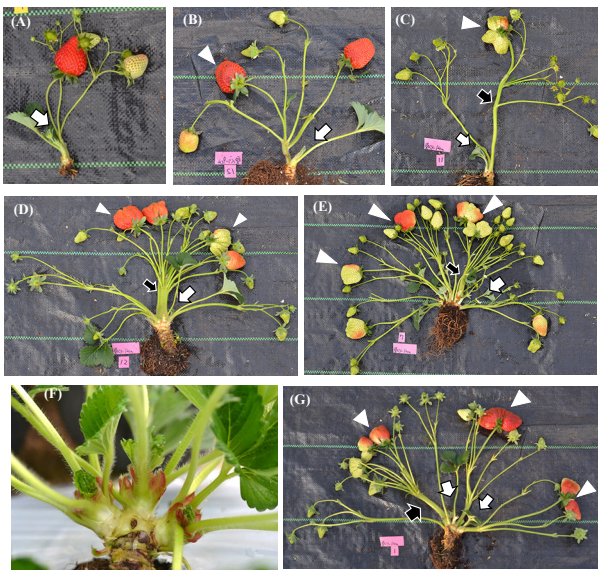
Fig. 2. Classification of fasciated inflorescences, based on levels from 0 to 4. (A) Level 0, Normal inflorescence; (B) Level 1, Slightly fasciated inflorescence with fasciated primary and/or other fruit (white arrowheads, see Fig. 1) with several secondary fruit and an oval-shaped cross-section of the main peduncle; (C) Level 2, Fasciated inflorescence with a fasciated peduncle (black arrows) and severely fasciated primary fruit; (D, E) Level 3, Severely fasciated inflorescence composed of a fused main peduncle or multiple clusters bearing a fasciated primary fruit for each (see Fig. 4); and (F, G) Level 4, Multiple apical shoots in a crown formed without sympodial branching (F and G are the same plant, see also Fig. S4). White arrows indicate lateral shoots.

Experiment 1. Fasciation development in 'Ai-Berry' and 'Nyoho'

Uniform plants of 'Ai-Berry' and 'Nyoho' grown in 12-cm pots were selected, and 60 of each cultivar were transplanted to 20 bowl planters as described before, and 2 g/plant of a 40-day-type slow-release fertilizer containing 13-9-11% of N-P_2O_5-K_2O (Eco long total 391; JCAM Agri Co., Ltd., Tokyo) was added on September 10, 2018. To observe the SAM, crowns of the six selected plants including the SAM were sampled by removing unfolded leaves and fixing them in acetic acid–alcohol solution (CH_3COOH:C_2H_5OH:H_2O = 5:45:50) on September 11. From September 15 to October 10, crown sampling of transplanted plants was similarly conducted to observe the differentiation and development of flower buds at 5-day intervals.

After October 18, before the fertilizer level became insufficient, 30% OAT A solution was supplied daily for the remaining 12 plants in preparation for flower bud, fruit, and inflorescence observation.

Experiment 2. Fasciation development in different-sized plants

On September 17, 2019, 18, 60, and 60 plants of 'Ai-Berry' grown in 12-, 10.5-, and 7.5-cm pots, respectively, and 60 plants of 'Sagahonoka' were planted on planters for flower bud, fruit, and inflorescence observation. For these plants, 7.5 g/plant of a 180-day-type slow-release fertilizer was added. From the day of transplanting, six crowns for 10.5- and 7.5-cm pot-grown 'Ai-Berry', and 10.5-cm pot-grown 'Sagahonoka' were fixed at 7-day intervals until November 5 as described previously. Flower, fruit, and inflorescence observations were conducted for the remaining 12–18 plants.

Experiment 3. Effects of artificial flower induction on flowering and fasciation development

More than 40 uniformly growing tray plants and 9-cm pot plants were selected for intermittent low-temperature storage (ILTS, 3 days of 15°C storage three times). From August 31 to September 15, 2019, ILTS was applied for 24 tray plants and 20 pot plants to induce early floral transition (Yoshida et al., 2012). Sixteen ILTS-treated and non-treated plants were planted on annual hills in an unheated plastic house on September 18. The effect of rapid floral transition induced by ILTS was examined by flower, fruit, and inflorescence observations, and compared with lagging transition under ambient conditions.

Experiment 4. Changes in TFL1 expression in vigorously growing 'Ai-Berry' plants

Expression of *TFL1*, the key floral repressor gene in seasonal flowering *F. × ananassa* (Mouhu et al., 2013; Nakano et al., 2015) was analyzed in the dissected meristem to demonstrate the implication of floral transition in the fasciation of meristematic tissues of 'Ai-Berry'. Around 40 of 9-cm pot-grown plants were continuously supplied with the same solution three times a week throughout the experiment. As biological replicates, five crown-tip samples were collected every 5 days from September 6, and trimmed to 3-mm-square of tissue. Total RNA was extracted by the hot borate method as described by Nakajima et al. (2014) based on the procedure of Wan and Wilkins (1994), treated with RNase-Free DNase (QIAGEN), and purified using a RNeasy Mini Kit (QIAGEN). Reverse transcription (RT) was performed using 300 ng of total RNA and PrimeScript RT Master Mix (Perfect Real Time) (TaKaRa Bio Inc., Kusatsu), according to the manufacturer's instructions. The cDNA of three of five biological replicates was used for quantitative real-time PCR (qPCR) with KOD SYBR qPCR Mix (Toyobo Co., Ltd., Osaka) in a LightCycler 96 System (Roche Diagnostics). The qPCR conditions were preheating at 98°C for 10 s, followed by 40 cycles of 60°C for 10 s and 68°C for 30 s. The relative expression was calculated using the thermal cycler real-time system software LightCycler 96. Primers used for analysis of *FaTFL1* (LC017718) and an actin gene (*FaACT*, LC017712) used as an internal control are listed in supplemental Table S1, as described by Nakano et al. (2015).

Results and Discussion

Experiment 1. Fasciation development in 'Ai-Berry' and 'Nyoho'

The incidence of inflorescence fasciation was much more common in 'Ai-Berry' compared to 'Nyoho' (Fig. 3). Less than 10% of 'Ai-Berry' plants developed normal inflorescence and well-shaped primary fruit, whereas only 10% of 'Nyoho' plants exhibited fasciated inflorescences (level 2), and 60% of the remaining plants developed fasciated primary fruit without inflorescence fasciation. The appearance of fasciation has been described in various plant organs as of different types including radial, linear, ring fasciation, or defasciation (Choob and Sinyushin, 2012). For 'Ai-Berry', linear fasciation was the most striking type directly affecting the shape of primary fruit and inflorescence architecture (Figs. 1 and 2). Darrow and Borthwick (1954) also reported similar symptoms in several US cultivars.

The vegetative SAM of 'Nyoho' successively differentiated leaf primordia (LP) following spiral phyllotaxis (Fig. 4A), and the floral initiation was inhibited by high temperature, long day-length, and continuous nutrient supply until mid-September (Yoshida and Nishimoto, 2020). Then, the floral transition of SAM was induced by the decrease in temperature and day length. SAM, in which LP differentiation was arrested, began to enlarge domically and developed into a primary IM (Fig. 4B) before initiating FM and developing into a flower (Chandler, 2012). This doming has long been recognized as the first morphological symptom of flower bud differentiation, or floral transition (Eguchi, 1932; Yoshida and Nishimoto, 2020). The domed IM was divided into two unequal-sized IMs by cleavage (Fig. 4C). 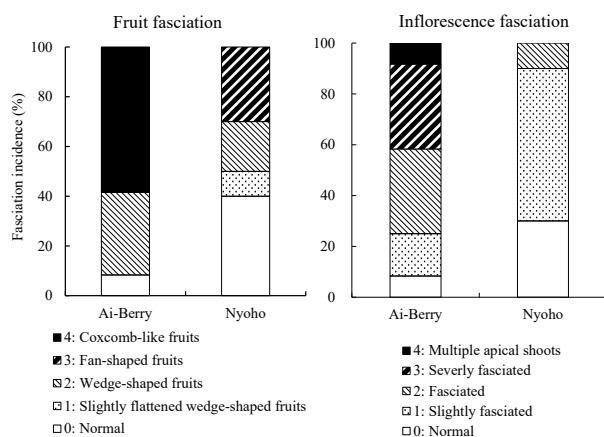The position of the smaller part appeared to follow the spiral phyllotaxis of leaves that had previously differentiated. The smaller part differentiated into a secondary IM and divided into three parts, which developed into an FM of the largest secondary flower (second flower of the inflorescence) and two tertiary IMs differentiated into the tertiary FM and quaternary IMs (Fig. 4F). Another larger part divided once (Fig. 4D), twice, or rarely three times (Fig. 4E), and the
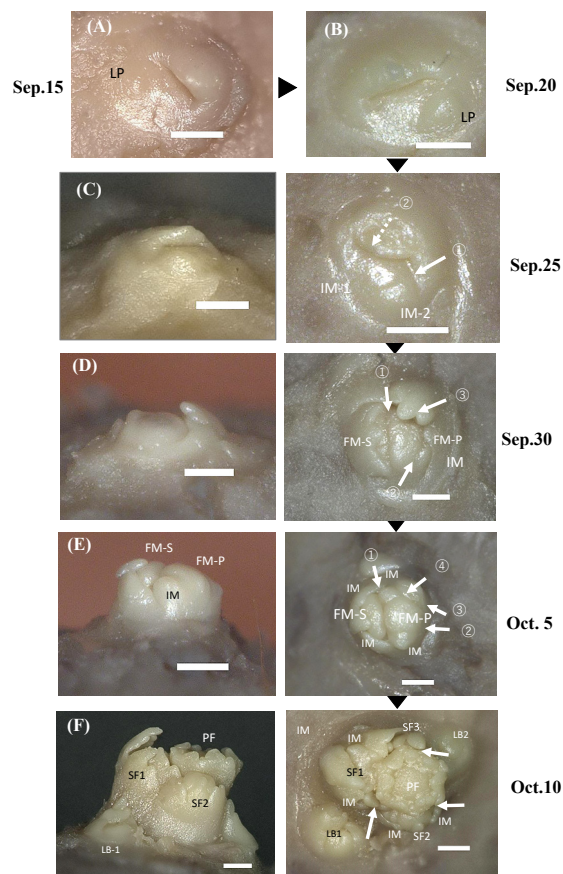

Fig. 4. Differentiation and development of non-fasciated normal flower buds in 'Nyoho'. Tissue samples were taken every 5 days from September 15 to October 10, 2018, after planting on 36-cm planters. (A) Vegetative SAM differentiating a new leaf primordium (LP); (B) SAM during floral transition just beginning to enlarge; (C) SAM divided into two inflorescence meristems (IMs) differentiating the largest secondary flower with inferior flowers (IM-2) and the primary flower with one or more secondary IMs (IM-1) following spiral phyllotaxis in a clockwise arrangement; dividing cleavages are indicated by white arrows with encircled numbers; (D) SAM divided into one lateral floral meristem (FM-S) differentiating into the largest secondary flower with two IMs differentiating inferior flowers and the primary FM (FM-P) with two IMs differentiating smaller secondary flowers following spiral phyllotaxis in a counter-clockwise arrangement; white arrows indicate separating cleavages; (E) SAM divided into one lateral FM with two IMs and a primary FM with three lateral IMs; and (F) SAM divided into three secondary FMs (SF1–SF3) with two IMs and a primary flower bud (PF) just before differentiating pistils on a receptacle, lateral buds developing on the uppermost axillar (LB1) and subsequent axillar (LB2) of a crown are visible. Scale bars indicate 200 μm.

Fig. 3. Fasciation in two strawberry cultivars grown in 12-cm pots, 2018 (n = 10–12). The susceptible cultivar 'Ai-berry' and less-susceptible cultivar 'Nyoho' were compared. The mean value of the crown diameter at transplanting was 1.37 and 1.22 cm in 'Ai-Berry' and 'Nyoho', respectively. See Figures 1 and 2 for classification of severity.

central part developed into an FM of the primary flower and the peripheral IMs developed into the subsequent secondary flowers (Fig. 4D–F). Each IM separated from the central FM was divided into three parts, including the FM of the secondary flower and two IMs developed into tertiary FMs on either side. The newly initiated IMs continued to initiate one FM and two IMs with an iterative pattern and developed into branches (Fig. 2A). The new IM initiation terminated in a specific order depending on the cultivar and/or environmental factors, thus determining the number of flowers in an inflorescence.

'Nyoho' SAM showed no symptoms of inflorescence fasciation but the primary flower was partly deformed (Fig. 3). On October 5, no deformation was observed in the FM of the primary flower except for one plant (Fig. S2A), but on October 10, most FMs began to differentiate pistil primordia, and oval-shaped primary flower buds were observed in two of six plants (Fig. S2B). The ratio of major and minor diameters of the two buds exceeded 1.2 (Figs. 3 and 4).

Before floral transition, SAMs of 'Ai-Berry' were larger than of 'Nyoho', but had similar plane and round shapes (Figs. 5A and 6). Then, SAMs became broad in the central zone in late September when floral transition was triggered by the decreased day length and temperature (Fig. 5B), and their appearance became substantially different from those of 'Nyoho' (Fig. 6). There were initial symptoms of linear fasciation and a marked increase was observed in the longitudinal diameter of SAMs after September 15. The enlarged 'Ai-Berry' meristems frequently had a widened central zone with a horizontal plane, and developed into oval-shaped or even linearly-elongated SAMs before bulging-out IMs. Such a SAM subsequently differentiated irregularly, with two or more LPs emerging simultaneously (Fig. 5C). The SAM symmetry following spiral phyllotaxis was also deformed. A similar deformation was reconfirmed in 2019 and delayed SAM deformation was also observed in the small 7.5-cm pot-grown plants (Fig. S5). The transforming process of flower bud differentiation from IMs to FMs was more complex and variable than the normal 'Nyoho' meristems. The linearly deformed meristem was often divided into several parts by various patterns, then several FMs of the primary flower were differentiated simultaneously, and each part developed into a separated and/or intensively fused cluster of a complex inflorescence (Figs. 2D–G and 5D, E).

The fasciation developmental model of strawberry meristem (Experiments 1 and 2)

An established developmental model of a linearly-fasciated SAM that differentiated four clusters in an inflorescence (Fig. 5E) is shown in Figure 7. During vegetative development, the SAM of strawberry has a highly organized stable structure. The central zone is
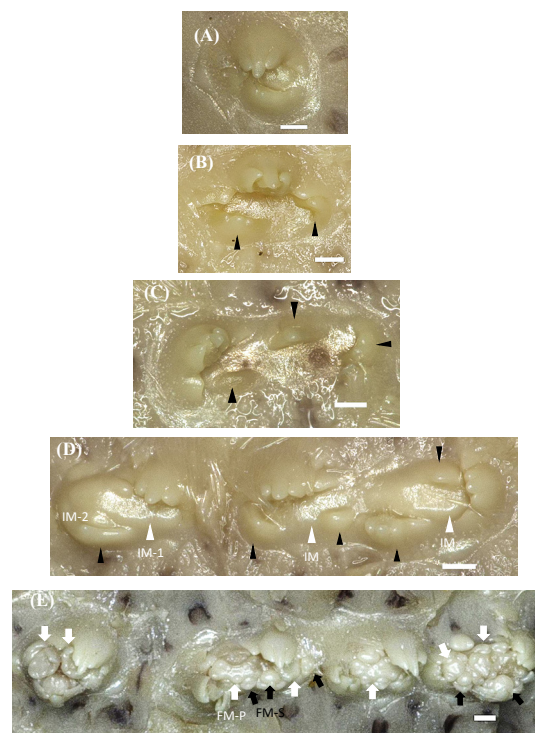

Fig. 5. Differentiation and development of flower buds in 'Ai-Berry'. Tissue samples were taken every 5 days from September 11 to October 10, 2018, just after planting on 36-cm planters. (A) Slightly flattened oval shape vegetative SAM initiating a new LP; (B) Flat and broad meristem during floral transition differentiating two leaf or bract primordia (black arrowheads, apical part apparently divided into three parts) almost simultaneously; (C) Flat and broad meristem during floral transition differentiating three leaf or bract primordia almost simultaneously; (D) Severely fasciated SAM during floral transition divided into three separated IMs and differentiating FMs developed into a fasciated broad primary flower (white arrowhead) in each cluster; and (E) Severely fasciated flower bud divided into four flower clusters differentiating one or more broad fasciated primary flowers forming sepals in a cluster (white arrows) and irregularly disposed a large number of lateral IMs developing secondary FM (black arrows). Abbreviations are the same as in Figure 4. Scale bars indicate 200 μm.
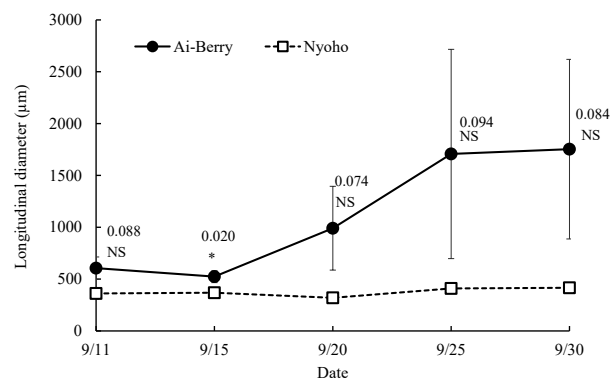

Fig. 6. Changes in the SAM longitudinal diameter in strawberry cultivars of 'Ai-Berry' (●) and 'Nyoho' (□). The longitudinal diameter of SAM including the youngest leaf primordia, 2018. Values indicate probability by *t*-test. Vertical bars indicate standard error (n = 6). Values were smaller than symbols in 'Nyoho'.
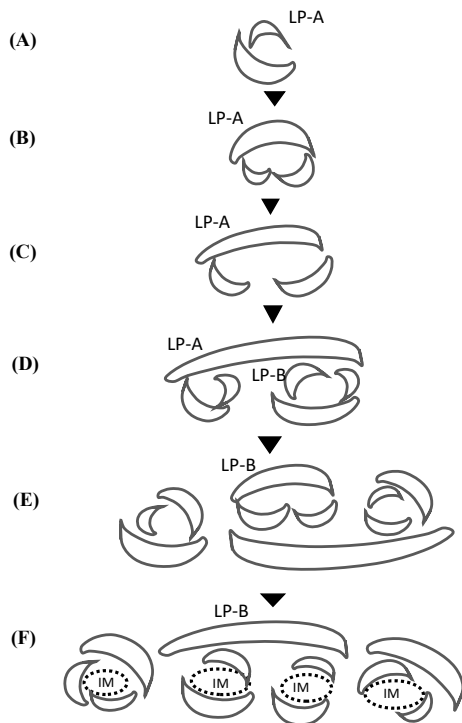
Fig. 7. Schemes illustrating the development of a severely fasciated SAM in 'Ai-Berry'. (A) Normal vegetative apical meristem; (B) expanding oval-shaped SAM initiating two LPs simultaneously; (C) continuously enlarging SAM where the angle and distance between LPs are changing; (D) SAM divided into two unequal parts; (E) separated smaller meristem differentiating into IM and a larger part dividing into two parts iteratively; (E) Separated smaller meristem differentiating into IM and a larger part dividing into two parts iteratively; and (F) IMs (dashed lines) differentiating FMs of the primary flower. LP-A and LP-B indicate the same LP, respectively.

small and flat, and maintained at a constant size despite the continuous differentiation of LP, similar to *Arabidopsis* and other species (Bernier, 1988). However, the vegetative SAM was larger in 'Ai-Berry' than in 'Nyoho' (Fig. 6). Such enlargement of the vegetative SAM in 'Ai-Berry' may cause multiple shoot formation before floral transition. Similar shoot branching was also observed in 2019 (Experiment 2, Fig. S4) in only two of the 18 12-cm pot-grown plants.

In late September, when most seasonal flowering strawberries initiate and consequently differentiate flower buds, the size and longitudinal diameter of SAM clearly increased. Two or more similar-sized LPs differentiated almost at the same time and consequently irregular phyllotaxis and linear fasciation developed (Figs. 5B, C and 7B, C). Although day-length was constantly decreasing, the environmental conditions were on the boundary between flower-inducing and -suppressing because the temperature was fluctuating under critical day-length, especially when accompanied by sufficient nutrient supply. In such conditions, floral transition, which is the change or shift in the developmental phase of SAM from vegetative to reproductive,

may have shifted slowly back and forth. A certain system that maintains the size of SAM-like *CLAVATA–WUSCHEL* feedback signaling in tomato (Somssich et al., 2016) may have lost stability, and triggered the irregular increase in SAM size. The simultaneous differentiation of multiple LPs may be indicated by the disruption of spatial (phyllotaxis) and/or temporal (plastochron) patterns in a well-organized SAM. Altered plastochron and phyllotaxis patterns were also reported in fasciated sunflower stems (Fambrini et al., 2006).

When an oval-shaped meristem directly differentiated an FM and IMs before multiple LP differentiation, slight fasciation symptoms appeared only in the primary flower and main peduncle. Otherwise, the oval-shaped meristems elongated further and could undergo irregular division patterns before initiating FMs and developing more severe fasciation symptoms. The flattened meristem, which differentiated two LPs at once (Figs. 5C and 7C), may have been unequally divided into two parts and continuously initiated new leaf or bract primordia (Fig. 7D). The smaller part sometimes directly initiated IM, while a similar iterative pattern occurred in the larger part, with it consequently dividing into four parts and differentiating into four IMs. The separated IMs were arranged linearly and initiated four FMs that developed into fasciated primary flowers of four clusters in the shoot apex of a crown (Fig. 5E).

Experiment 2. Fasciation development in different-sized plants

In 2019, the fasciation development in 'Ai-Berry' was confirmed by observing plants grown in 12-, 10.5-, and 7.5-cm pots compared with another non-susceptible cultivar 'Sagahonoka' grown in 10.5-cm pots; at transplanting, their crown diameters were 1.38, 1.25, 0.71, and 0.99 cm, respectively. The value for 12-cm pot-grown plants was similar to that in 2018 (Fig. 3), and that of 10.5-cm pot-grown 'Ai-Berry' was 25% larger than 'Sagahonoka'. The fasciation incidence was most common in 12-cm pot-grown 'Ai-Berry' in both inflorescences and primary fruit (Fig. 8). The severity in 12-cm pot plants was similar to that in 2018 and reduced with the decrease in pot size. The rate of plants developing normal primary fruit and inflorescences also increased with the decrease in pot size. Although more than 80% of 7.5-cm pot-grown plants developed normal inflorescences, severe fasciation symptoms still occurred in several plants, whereas 'Sagahonoka' developed normal inflorescences and slightly deformed receptacles were observed in less than 20% of primary flowers (Fig. 8). It is well known that root zone restriction often represses the vegetative growth of plants through water and nutrient stress (Peterson et al., 1991; Goto et al., 2001). The decrease in pot size may also have restricted water and nutrient availability (Massetani et al., 2014).

In 10.5-cm pot-grown 'Ai-Berry', the longitudinal

diameter of SAM was larger compared to those grown in 7.5-cm pots (Fig. S3). This increased slowly compared to that of 12-cm pot-grown plants in 2018 (Fig. 6) and the increase was much slower in 7.5-cm pots. The elongating deformation of SAM was first observed on September 24 in 10.5-cm pot plants when the mean of longitudinal diameter exceeded 500 μm. On October 1, severely fasciated SAMs were observed in around half of 10.5-cm pot plants. In 7.5-cm pot plants, clearly fasciated SAMs were first observed two weeks later only in a small proportion of the plants (Fig. S4). 'Sagahonoka' had a smaller SAM diameter (312 ± 31 μm) compared to 10.5-cm pot-grown 'Ai-Berry' (382 ± 14 μm) at transplanting and no deformed SAMs were observed, similar to 'Nyoho' in the previous year. These results may indicate that vigorously growing 'Ai-Berry' plants easily develop a thick crown and a large SAM compared to smaller plants or other non-susceptible cultivars. Such behavior of 'Ai-Berry' may relate to its high susceptibility to fasciation and vegetative SAM division resulting in multiple shoot formation without sympodial branching (Figs. 1E and S2).

Concerning the receptacle fasciation of individual fruit, severely fasciated 'Ai-Berry' fruit with a coxcomb- or fan-like shape may have developed from markedly flattened FMs differentiated in the linearly-fasciated SAM (Fig. 5). In 'Nyoho', we observed some oval-shaped FMs or flower buds of primary flowers and also some fasciated fruits with moderate symptoms. In 'Sagahonoka', no irregular-shaped FM or flower buds and a few slightly fasciated fruits were observed. These results may indicate that most of the irregular-shaped receptacles of fruits developed from irregular-shaped FMs. The fate of FM may have been decided at the
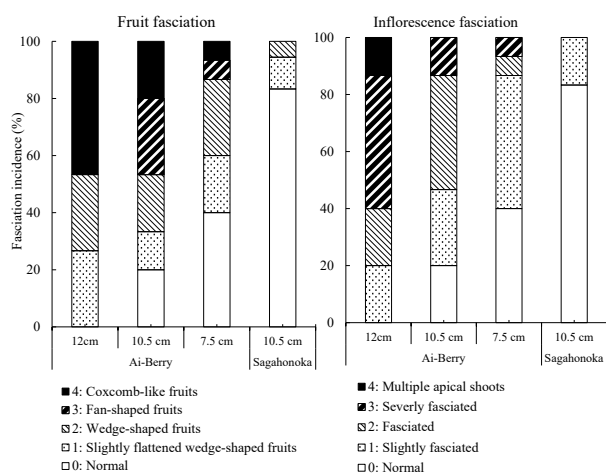
initiation, and then round and oval FMs developed into normal well-shaped and flat wedge-shaped fruits, respectively.

Experiment 3. Effects of artificial flower induction on flowering and fasciation development

The effect of ILTS, an artificial floral-inductive treatment established by Yoshida et al. (2012), on flowering is shown in Figure 9. We expected that the 9-cm pot plants and tray plants would respond differently to the treatment, because the pot plants which were grown with staggered arrangement before the treatment were more vigorous and old than the tray plants. However, there was no difference. Six ILTS-treated and one or zero non-treated plants in each plot of 9-cm pots and tray grown plants, around 40% and 3% of 32 plants in total, flowered before November 10, and the remaining plants flowered after December 8. The mean flowering date of ILTS-treated plants was 22 and 15 days earlier than for non-treated pot and tray plants, respectively. The number of non-fasciated plants was clearly greater in ILTS-treated than non-treated plants, but little difference was observed between pot and tray plants (Fig. S5). When the ILTS-treated plants were pooled and divided into two groups by flowering date, more than 80% of early flowering plants did not develop any fasciated primary fruit or inflorescences, whereas all of the late flowering plants developed fasciated primary fruit and/or inflorescences (Fig. 10).

Among the ILTS-treated plants, rapidly flower-induced plants developed well-shaped primary fruit and normal inflorescences. However, all remaining plants that did not respond to the treatment, and almost all non-treated plants, developed fasciated primary fruit and/or inflorescences. These results indicate that the rapid nutrient absorption of insufficiently flower-induced transplants caused vigorous vegetative growth and suppressed, delayed or half-finished floral transi-
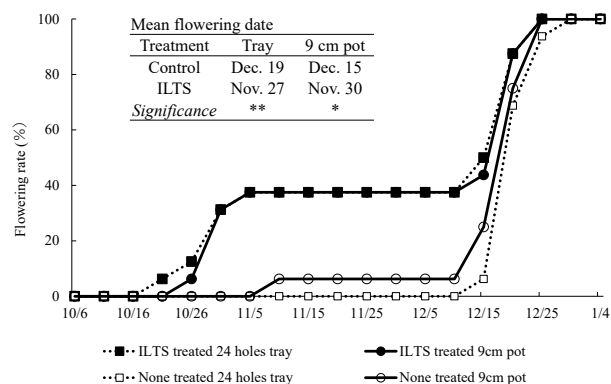

Fig. 8. Effect of propagating pot size on the incidence of fasciation in the primary fruit (left) and inflorescences (right) of 'Ai-Berry', 2019 (n = 15–18). A less-susceptible cultivar 'Sagahonoka' grown in 10.5-cm pots was also compared. The mean crown diameters were 1.38, 1.25, 0.71, and 0.99 cm, respectively (left to right). See Figures 1 and 2 for classification of severity.

Fig. 9. Effects of intermittent low-temperature treatment (ILTS) on flowering of 'Ai-Berry' transplants propagated in 24-hole trays (□, ■) and 9-cm pots (○, ●) (n = 16). Flowering of the primary flower was recorded from October 6, 2019, to January 4, 2020. *, **: Significant difference at $P < 0.05$ and $P < 0.01$ by t-test, respectively.
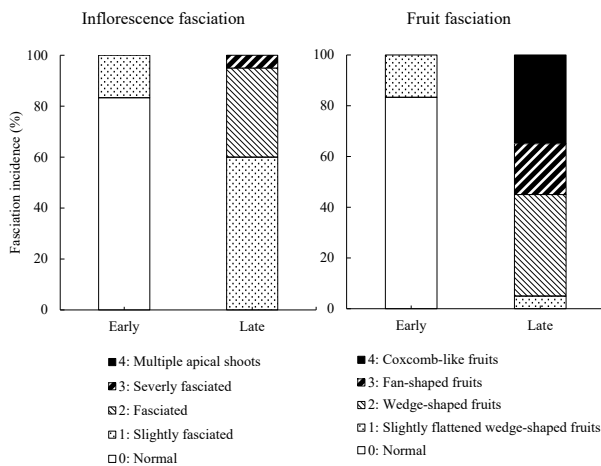
Fig. 10. Differences in fasciation occurrence between early- and late-flowering plants in ILTS-treated 'Ai-Berry' grown in 24-hole trays and 9-cm pots. ILTS-treated plants that flowered before November 10 and after December 10 are indicated as 'early' and 'late' flowering plants. Ten of twelve 'early' flowering plants did not develop any fasciated primary fruit or inflorescences, whereas all of the 'late' flowering plants developed fasciation in fruits and inflorescences. See Figures 1 and 2 for classification of severity.

Fig. 11. Changes in *FaTFL1* expression in the meristem of 'Ai-Berry' from September 6 to October 1, 2019. NS,*; non-significant and significant difference at $P < 0.05$ against September 6 by Dunnett's test; three biological and three analytical replications.

tion under natural conditions, i.e., the fluctuating temperature combined with the critical day-length in September may be the two major environmental factors affecting strawberry fasciation along with genetic susceptibility and vegetative growth vigor. Darrow and Borthwick (1954) also reported that the disorder was serious only in the Middle and South Atlantic States, where the temperature decrease in autumn is slower compared to northern or inland areas, and that fasciation became much more serious when unusually warm temperatures continued in the previous autumn. In the southern coastal area, floral transition in field-grown strawberries, especially in susceptible cultivars, may have progressed slowly or back and forth in autumn because of the warm climate.

Experiment 4. Changes in TFL1 expression in vigorously growing 'Ai-Berry' plants

Figure 11 shows changes in the expression of *FaTFL1* in the meristem of 'Ai-Berry'; *FaTFL1* is the key floral repressor gene in seasonal flowering *F. × ananassa* (Mouhu et al., 2013; Nakano et al., 2015). It was downregulated after September 16 and then fasciation symptoms were observed in the SAM (Fig. S5). This result supports the previously proposed hypothesis that fasciation of the strawberry inflorescences is induced during floral transition. 'Ai-Berry' appeared to be extremely susceptible to fasciation, and vigorously growing plants tended to develop a thick crown and large SAM. Large-sized plants, grown in 12- or 10.5-cm pots, sometimes developed several divided vegetative SAMs before transplanting and formed multiple shoots without sympodial branching (Figs. 2F and S3).
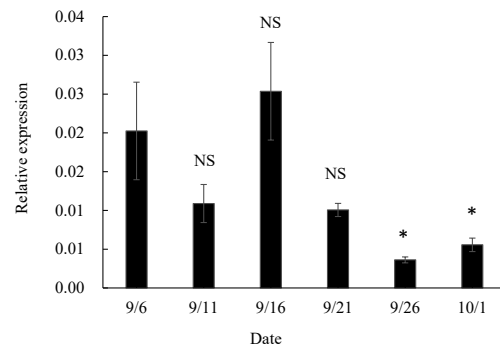
For such plants, we only observed severely fasciated inflorescences in one plant that rapidly developed multiple shoots (Fig. 2F, G). Under floral inhibitive conditions, a vegetative SAM that enlarged excessively in a vigorously growing plant, did not develop into a fasciated meristem but rather developed into divided multiple meristems as shown in Figure S4. Growth vigor of a crown may have been deconcentrated to multiple meristems. Then the size-maintaining system and stability of each SAM may have been resumed, and normal inflorescences consequently differentiated. However, when *FaTFL1* was downregulated slowly under incomplete floral-inductive conditions, the enlarged oval-shaped IM may have experienced irregular division patterns before differentiating FMs and developing fasciated inflorescences and/or primary flower(s) as shown in Figure 5C–E.

Conclusion

When the susceptible cultivar 'Ai-Berry' was propagated in large pots and grown with sufficient nutrient supply, the SAMs were clearly larger than for smaller plants or non-susceptible cultivars. Although division of vegetative SAMs resulting in non-sympodial branching was observed in some 'Ai-Berry' plants, enlarged oval-shaped SAMs were frequently observed after mid-September, when expression of *FaTFL1* just started to decrease. Such enlarged SAMs often showed disrupted spiral phyllotaxis as the initial symptom of inflorescence fasciation. When the nutrient supply was adequate, vigorously growing large plants clearly developed severely fasciated inflorescences compared to other non-susceptible cultivars or smaller plants. When early and rapid floral induction was achieved by ILTS, inflorescence or receptacle fasciation could be well controlled. Thus, the temperature fluctuating around boundary values between floral inhibition to induction may cause half-finished or slowly progressing floral transition in vigorously growing plants. Consequently, the development of deformed IMs and FMs in

SAMs may finally result in severe fasciation of the inflorescence and receptacle.

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