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主　論　文　の　要　旨

論文題目　Cultivated strawberry genes relating to transition between vegetative to reproductive phase
(栽培イチゴの栄養・生殖成長転換に関連する遺伝子群)

氏　　名　KHOEM Koembuoy

論　文　内　容　の　要　旨

Most cultivated strawberries in Japan are June bearing type of short-day and low temperature crops. In conventional cultivation, flower buds are differentiated in autumn, which is a short-day and low-temperature condition, and the fruit harvest period is from March to May of the following year after dormancy in winter. However, the demand for strawberries is high from late autumn to winter including Christmas time, so super forcing cultivation is being carried out to accelerate the fruit harvesting period. Specifically, in summer, controlling of short-day by covered with a dark curtain from 5 pm to 9 am and low temperature treatment by operated an air conditioner to keep the temperature at 15°C for about one month was carried out. Alternatively, intermittent refrigeration processing, such as every 3 days at 15°C for about 2 weeks is performed by moving out and putting in large refrigerators at outdoor environment. By these treatments, it is possible to artificially induce flower buds and adjust the fruit harvest period from late fall to winter, which is a high demand period. At this time, pot seedlings at the vegetative phase are grown under short-day and low temperature with a low nitrogen fertilizer in the summer to induce flowering. After the treatment, the plants need to be transferred in a greenhouse or a field under the long-day and high temperature with high nitrogen fertilizer. Thus, the confirmation of floral bud initiation condition at the end of the treatment is extremely important. That is, if transferring is performed before floral bud initiation, the initiation is suppressed and early fruit harvesting becomes impossible, and if seedlings continue to grow in pot seedlings after floral bud initiation, flower bud development is inhibited and fruit quality is reduced. Conventionally, floral bud initiation was confirmed based on a slight change in the shape of the shoot apical meristem (SAM) by a microscopic examination of the crown apical part, but this method is complicated and requires skill, and it also leads to loss of seedlings. Therefore,

development of a new method is needed. In this context, I will work to elucidate the floral bud initiation mechanism of cultivated strawberries and establish a super-forcing culture technology that uses a gene whose expression changes at floral bud initiation or at the switch from vegetative to reproductive phase as an indicator.

In *Arabidopsis thaliana* and rice, FLOWERING LOCUS T (FT) is a floral inducer, and the FT protein is transported from the leaves to the shoot apices, forming a complex with FLOWERING LOCUS D (FD) and activating the floral organ identity APETALA1 (AP1), which is known to cause floral differentiation.

In this study, I found that FaFT3 is a floral inducer of cultivated strawberries among the three FT genes present in the cultivated strawberry genome. FaFT3 in cultivated strawberry 'Tochiotome' was specifically up-regulated during floral bud initiation, and *Arabidopsis thaliana* overexpressing *FaFT3* gene exhibited early flowering phenotypes. In particular, RNA-seq analysis of shoot apical meristem using the laser microdissection (LM) method revealed that *FaFT3* is specifically expressed in a very limited area where floral buds are initiated in crown shoot apices during floral bud initiation. In addition, *FaFT3* was not expressed in the leaf tissue at the time of inducing flower bud differentiation, so it was considered that it is expressed and functions in the crown, which is a temperature-sensitive site, in cultivated strawberries. On the other hand, FaFT1 and FaFT2 are not involved in flower bud differentiation, and especially FaFT1 is highly expressed in the vegetative growth stage rather than in the reproductive growth stage, and *Arabidopsis thaliana* overexpressing *FaFT1* gene produced rosette-like leaves on main stems. Since it showed a unique leaf-forming phenotype, it was suggested to be involved in vegetative growth such as runner formation. This FaFT1 differs from FvFT1, which functioning as a floral inducer of wild strawberry, by only one amino acid, and it is considered that this one amino acid substitution is important for functional differentiation between FaFT1 and FvFT1. In addition, the structural features of them were clarified by protein modeling.

TERMINAL FLOWER 1 (TFL1), which belongs to the same PEBP family as FT, is a floral repressor that acts antagonistically with FT, and is expressed at the shoot apex and forms a complex with FD to suppress flower bud differentiation. In the cultivated strawberry 'Tochiotome', it was shown that decreased expression of *FaTFL2* was involved in floral bud initiation, but in this study, I found that the expression of *FaTFL1-1* was decreased in a limited area of shoot apex. In addition, since the *Arabidopsis thaliana tfl1* mutant introduced with 35S::*TFL1-1* restored the phenotype, it was confirmed that FaTFL1-1 is a major floral bud initiation repressor in cultivated strawberries.

Intermittent chilling treatment of cultivated strawberries 'Benihoppe' and 'Akihime' seedlings was performed to determine the shape of the shoot apical meristem (SAM) and examine the expression level of *FaFT3*, floral inducer, at the crown apex and *FaTFL1-1*, floral bud initiation repressor. The floral bud initiation state of each seedling was specified as an

indicator. Next, gene groups expressed in newly emerged leaves in each of the strains corresponding to before floral bud initiation or vegetative stage (stage 0), early floral bud initiation (stage A), and late floral bud initiation (stage B) were comprehensively analyzed by RNA-seq analysis. The 481 common genes which increased immediately after floral bud initiation occurs between 'Benihoppe' and 'Akihime' were obtained. Then the genes whose expression were down-regulated in stage B after floral bud initiation were excluded, and finally four genes with the highest expression fluctuations from stage 0 to stage A were used as candidate genes (biomarkers 1, 2, 3, 4). These candidate genes can be used to identify the time of floral bud initiation. From RNA-seq analysis, all were highly expressed in the stage after floral bud initiation as compared with before floral bud initiation, particularly, biomarker 2 was stably and highly expressed in all samples. In addition, when the expression analysis of biomarkers was performed on the cultivated strawberries that were subjected to the intermittent cold storage treatment in the next fiscal year, all of candidate genes (biomarker 1-4) were highly expressed specifically in the seedlings subjected to the intermittent cold storage treatment, indicating the usefulness of the biomarkers.

As described above, in this study, it was suggested that *FaFT3* is a common floral inducer gene in cultivated strawberries, and unlike model plants such as *Arabidopsis thaliana*, it is synthesized and functions not in leaves but in crown tissues. Furthermore, FaFT1, which was considered not to be involved in floral bud initiation and functioning in the vegetative growth stage, differed from the floral inducer gene *FvFT1* of wild strawberry by only one amino acid, and a difference in hydrogen bonding site was observed. In addition, it was confirmed that *FaTFL1-1* is a floral repressor gene. Using the expression level of these floral bud initiation-related genes at the crown site as an indicator, I found genes and biomarkers that are highly expressed in leaf tissues during floral bud initiation.