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論文題目	Study on identification of petaloid tepals in monocots （単子葉植物における花弁状花被形成機構に関する研究）
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## Background

Flowers, reproductive organs of plants, share principal points in common besides various shapes and colors. In higher eudicots, they consist of four types of organs, sepals, petals, stamens, and carpels. For explanation of floral organ identity, ABC model was proposed in two model plants, *Arabidopsis thaliana* and *Antirrhinum majus*. According to this model, sepal is identified by A function, petal is determined by A function and B function, stamen, by B and C function, and carpel, by C function (Fig. 1A).

However, morphology of plant flowers is full of diversity. Floral (and sometimes inflorescence that looks like a flower) diversity is found especially in petaloid organ. For example, non-grass monocot plants often have flower with petaloid organ instead of greenish sepal in whorl 1 and both of petaloid organs in whorls 1 and 2, called tepals. To explain the morphology of these flowers, modified ABC model was proposed with morphological analysis of wild type and mutant flowers in tulip. According to this model, class B genes are expressed in whorl 1 as well as whorls 2 and 3, therefore the organs of whorls 1 and 2 have the same petaloid structure (Fig. 1B).

I would like to study the relationship between the morphology of two whorls of petaloid perianth and expression patterns of MADS-box genes in monocots. Thus, I investigated *Alstroemeria ligtu* whose perianth consists of two types of petaloid tepals in chapter 1, and viridiflora tulip which have greenish tepals and expected as class B mutant in chapter 2.

## Chapter 1

*Alstroemeria* (Liliales) has two layers of petaloid tepals, in which the often spotted narrow inner tepals can be distinguished easily from the wider outer tepals (Fig.2). In order to explore this floral morphology in *Alstroemeria*, I investigated the tepal morphology and the expression patterns of three class B genes, whose homologs in eudicots have been shown previously to be involved in petal and stamen development.

Two *DEF*-like genes (*AlsDEFa* and *AlsDEFb*) and one *GLO*-like gene (*AlsGLO*) of *Alstroemeria ligtu* were isolated by rapid amplification of cDNA ends (RACE). Northern hybridization, reverse transcription-PCR (RT-PCR) and *in situ* hybridization analyses indicated that *AlsDEFb* and *AlsGLO* were expressed in whorls 1, 2 and 3 (outer tepals, inner tepals and stamens, respectively), whereas *AlsDEFa* expression was detected only in whorls 2 and 3 (Fig. 3).

These results suggest that in *A. ligtu*, *AlsDEFb* and *AlsGLO* would participate in determining the organ identity of two-layered petaloid tepals and stamens, which is in support of the modified ABC model.

Additionally, the distinctive expression patterns of *AlsDEFA* and *AlsDEFB* might be related to morphological differences between the two-layered tepals.

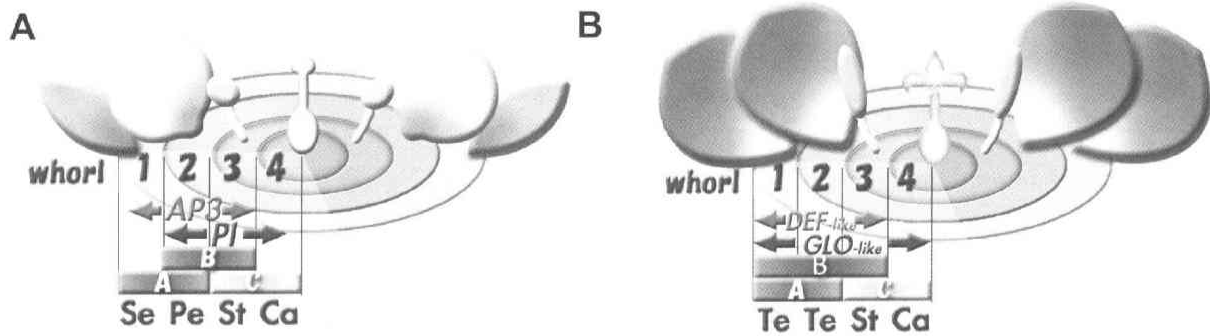
## Chapter 2

Viridiflora tulip, which is well-known cultivar group of tulips, has flower in which the tepals in whorls 1 and 2 has greenish stripe. This phenotype could be expected as class B mutant by the modified ABC model.

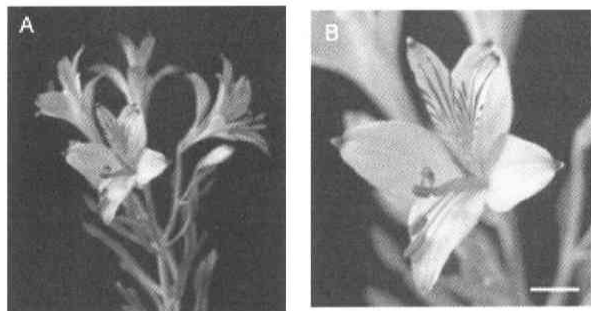
I observed the various strength phenotypes of viridiflora flower, and found out phenotype of tepals were varied each individual, and looked correlating with that of stamens (Fig. 4).

I isolated two class A genes (*TGSQA* and *TGSQB*) and one class C genes (*TGPLE*) from wild type tulip. Northern hybridization of class A, B and C genes showed that the expression of *TGDEFA* and *TGDEFB* in viridiflora tulip was weaker than that in wild type, whereas the expression pattern of *TGSQA*, *TGSQB*, *TGGLO* and *TGPLE* from wild type and viridiflora tulip were almost the same (Fig. 5). Comparison of amino acid sequences of class B genes from wild type ('White Dream') and viridiflora cultivars ('Spring Green') showed that two *DEF*-like genes, *TGDEFA* and *TGDEFB*, of 'Spring Green' had some amino acid differences; 119Ser->Asn in *TGDEFA*, 12Glu->Gly and 74Met->Ala in *TGDEFB*.

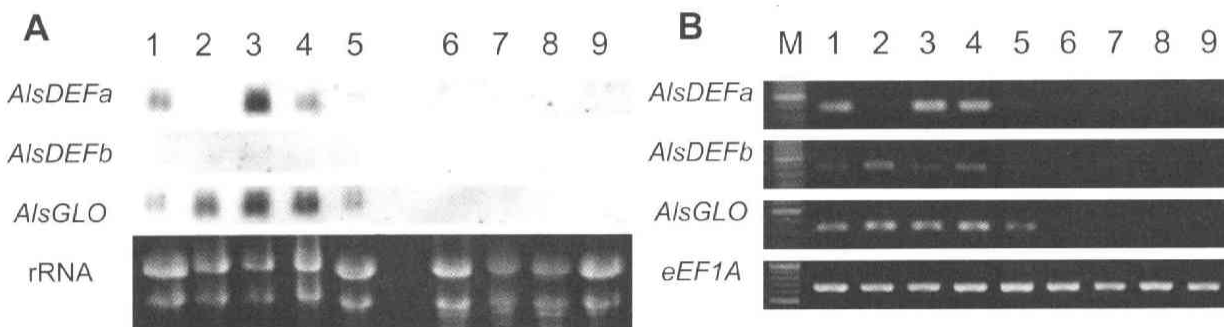
I found that the reduction of the expression level of two *DEF*-like genes is involved in the development of the viridiflora phenotype. This reduced expression of *DEF*-like genes might be caused by the amino acid difference at position 12 (Glu->Gly) found in *TGDEFB* gene.



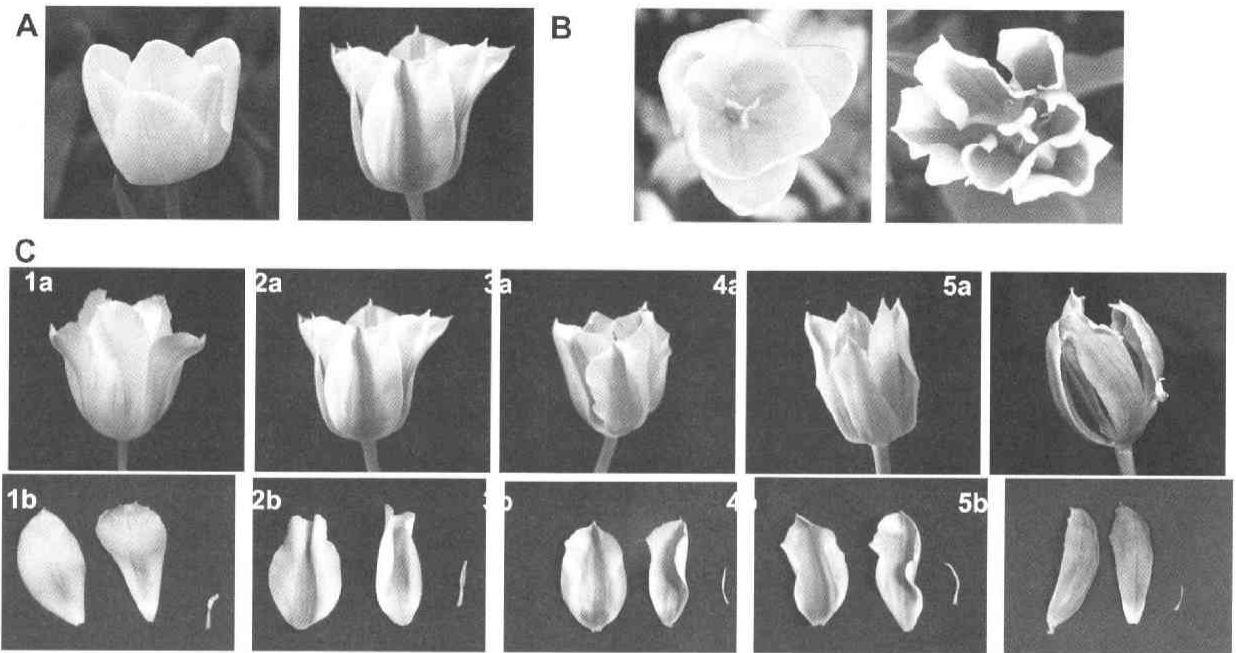
**Fig. 1 Models of floral organ identity**  
 A. The ABC model B. The modified ABC model  
 Se: sepal, Pe: petal, St: stamen, Ca: Carpel, Te: tepal



**Fig. 2 Morphology of *Alstroemeria ligtu***  
 A. Inflorescence of *A. ligtu* ssp. *ligtu*.  
 B. Mature flower of *A. ligtu*. Scale bar 1 cm.

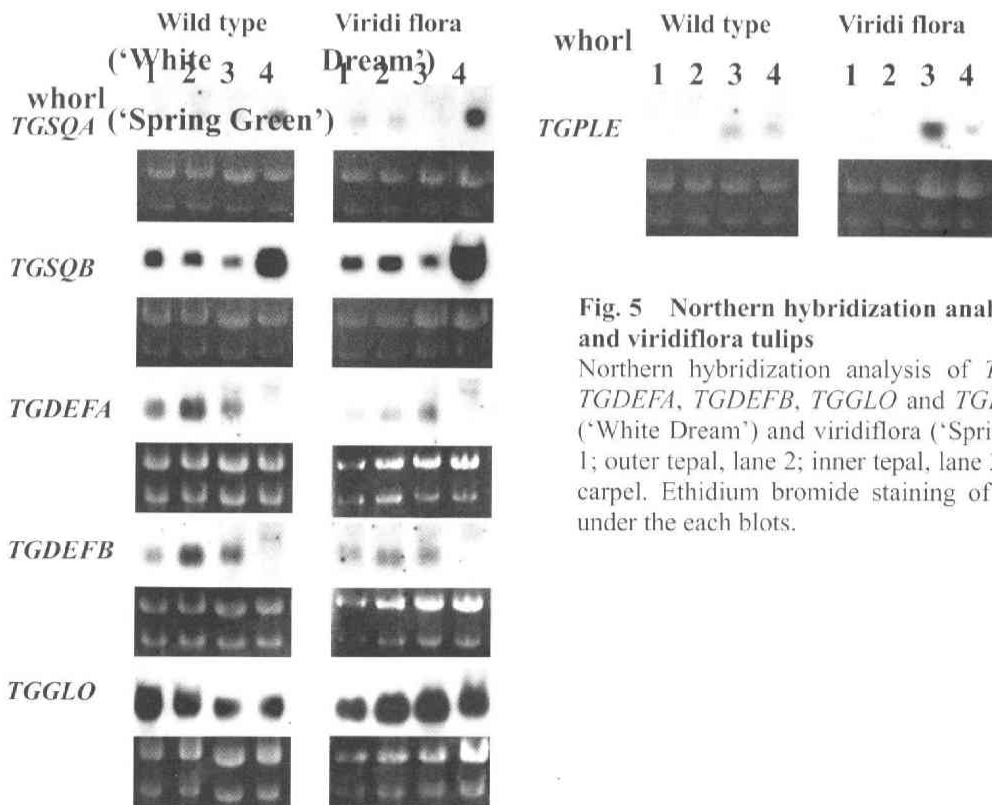


**Fig. 3 Expression analyses of class B genes of *A. ligtu***  
 Northern hybridization (A) and RT-PCR analyses (B) in each organ (1, young floral bud; 2, outer tepal; 3, inner tepal; 4, stamen; 5, carpel; 6, peduncle; 7, bract; 8, leaf; and 9, stem). Ethidium bromide staining of rRNA is shown under the blots (A). RT-PCR patterns are shown with 32 cycles. Amplification of the *eEF1A* fragment was used as positive control (B).



**Fig. 4** Flowers of wild type and viridiflora tulips

A. Side view of the wild type cultivar 'White Dream' (left) and the viridiflora cultivar 'Spring Green' (right). B. Upper view of 'White Dream' (left) and 'Spring Green' (right). The anthers of 'Spring Green' are smaller than those of the wild type. C. Variation in weak (1a, 1b), intermediate (2a to 4a, 2b to 4b) and strong (5a, 5b) phenotypes of the viridiflora cultivar 'Spring Green'. The weak phenotype flower has mostly white tepals and mature anthers (1a, 1b). The strong phenotype flower has sepaloid or leaf-like tepals, and degenerate stamens with filamentous anthers (5a, 5b). The viridiflora flowers have green and white tepals that appear to be a mosaic organ of tepal and sepal or leaf, (1a to 5a) and degenerate stamens with thin anthers (1b to 5b).



**Fig. 5** Northern hybridization analysis in wild type and viridiflora tulips

Northern hybridization analysis of *TGSQA*, *TGSQB*, *TGDEFA*, *TGDEFB*, *TGGLO* and *TGPLE* in wild type ('White Dream') and viridiflora ('Spring Green'). Lane 1; outer tepal, lane 2; inner tepal, lane 3; stamen, lane 4; carpel. Ethidium bromide staining of rRNA is shown under the each blots.

高等な双子葉植物の花器官形成ではモデル植物を用いた遺伝学的研究から ABC モデルが提唱されている。一方、単子葉植物にはユリのように花被が二層の花弁状器官から構成されている植物が多く存在している。本研究では、単子葉植物のアルストロメリアとチューリップを用い、ABC モデルに関わる遺伝子群が二層の花弁状花被形成にどのように働いているかを分子生物学的な手法を用いて明らかにした。

まず RACE 法によりアルストロメリアから 2 種類のクラス B 遺伝子である *GLO*-like (*AlsGLO*) と *DEF*-like (*AlsDEFA*, *AlsDEFb*) 遺伝子を単離した。次にノーザンハイブリダイゼーション法および RT-PCR 法による発現解析の結果、2 つの *DEF*-like 遺伝子は、外・内花被片および雄ずいで発現しており、*GLO*-like 遺伝子は外・内花被片、雄ずいおよび雌ずいで発現していた。さらに *in situ* ハイブリダイゼーション法を用いて、花器官原基におけるこれらの遺伝子の発現を解析した結果、原基においても同様の発現が観察された。このことから、アルストロメリアの二層の花被形成には 3 つの B クラス遺伝子 (*AlsGLO*, *AlsDEFA*, *AlsDEFb*) が関与しており、modified ABC モデルが適用されることが示唆された。

チューリップの *viridiflora* 系品種は花弁状花被片の中央に緑色の筋が入り、緑色が強ければ強いほど雄ずいが小さいことから、*viridiflora* の形質はクラス B 遺伝子の発現に変異があると考えられた。そこで、野生型および *viridiflora* 品種の花蕾から外・内花被片、雄ずい、雌ずいを分取し、各器官から全 RNA を抽出した。チューリップからすでに単離されている 3 つのクラス B 遺伝子 (*TGGLO*, *TGDEFA*, *TGDEFB*) をプローブとしてノーザンハイブリダイゼーションを行った結果、*viridiflora* 品種の外・内花被片、雄ずいにおいては 2 つの *DEF*-like 遺伝子の発現が抑制されていた。このことから *viridiflora* の形質は *DEF*-like 遺伝子の発現の抑制が直接的または間接的な原因であることが示唆された。

アルストロメリアおよびチューリップを用いた以上の結果より、これらの植物における花弁状花被の形成にはクラス B 遺伝子の発現が関与しており、modified ABC モデルに合致することが示唆された。本研究で得られた知見は高等植物における花の多様性の進化を分子生物学的に解明する上で重要であると考えられ、本研究を推進した平井雅代は自立して研究活動を行うに必要な高度の研究能力と学識を有し、平井の提出した論文は博士（生命科学）の博士論文として合格と認める。