Conversion of leaves into petals in *Arabidopsis*

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More than 200 years ago, Goethe proposed that each of the distinct flower organs represents a modified leaf [1]. Support for this hypothesis has come from genetic studies, which have identified genes required for flower organ identity. These genes have been incorporated into the widely accepted ABC model of flower organ identity, a model that appears generally applicable to distantly related eudicots as well as monocot plants. Strikingly, triple mutants lacking the ABC activities produce leaves in place of flower organs, and this finding demonstrates that these genes are required for floral organ identity [2]. However, the ABC genes are not sufficient for floral organ identity since ectopic expression of these genes failed to convert vegetative leaves into flower organs. This finding suggests that one or more additional factors are required [3, 4]. We have recently shown that *SEPALLATA* (*SEP*) represents a new class of floral organ identity genes since the loss of *SEP* activity results in all flower organs developing as sepals [5]. Here we show that the combined action of the *SEP* genes, together with the A and B genes, is sufficient to convert leaves into petals.

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Received: **8 December 2000**
Accepted: **18 December 2000**

Published: **6 February 2001**

Current Biology 2001, 11:182–184

0960-9822/01/$ – see front matter

Results and discussion

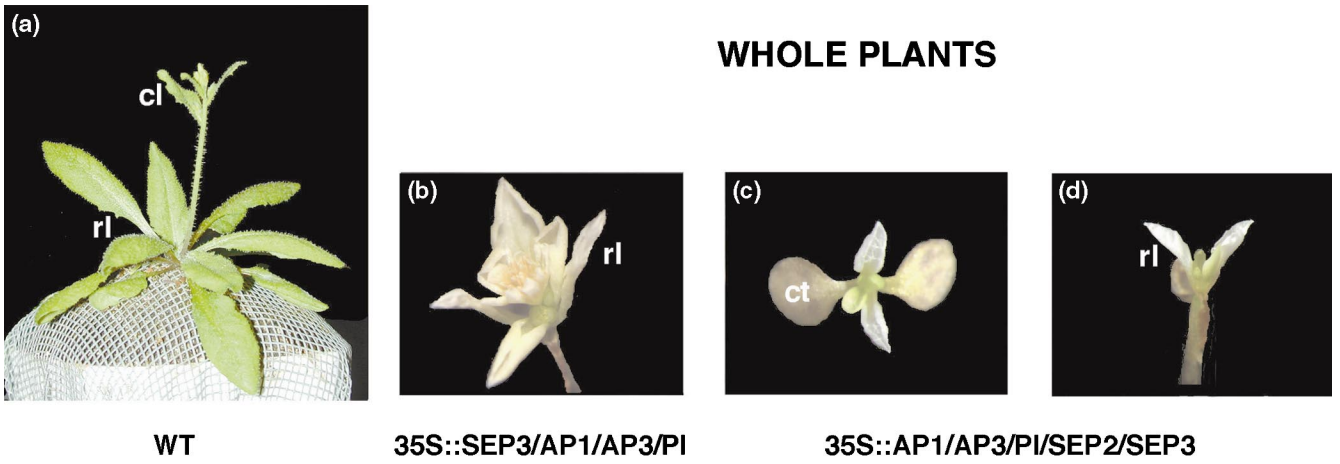
Arabidopsis plants produce a number of closely spaced rosette leaves during the vegetative phase. Upon the transition to flowering, the internode length increases, and this event causes a significant increase in the distance that separates the last few vegetative (cauline) leaves. The flowers are composed of four whorls of organs, with sepals in the first, or outermost, whorl, petals in the second whorl, stamens in the third whorl, and carpels occupying the fourth whorl, in the center. The simple and elegant ABC model of flower development was proposed to explain the activity of the floral organ identity genes [2, 6]. According to the model, the combined actions of three different functions, each one active in two adjacent whorls, is responsible for the development of the four types of organs of a typical eudicot flower. The model suggests that A alone specifies sepals, C alone specifies carpels, and the combined activities of AB and BC specify petals and stamens, respectively.

In *Arabidopsis*, *APETALA1* (*AP1*) and *APETALA2* (*AP2*) are the A function genes, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) are the B function genes, and *AGAMOUS* (*AG*) is the only C function gene. With the exception of *AP2*, all of these genes are members of the MADS-box family of transcription factors, whose expression patterns correspond to their domains of action (reviewed in [7, 8]). Triple mutants that carry mutations in the A, B, and C functions have a conversion of all floral organs into leaf-like organs, and this observation supports the idea that floral organs represent modified leaves [1] and that the ABC genes are necessary to convert leaves into flower organs [2]. However, ectopic expression studies indicate that the ABC genes are not sufficient to convert vegetative leaves into flower organs [3, 4].

A new class of floral organ identity MADS-box genes, *SEP1*, *SEP2*, and *SEP3*, was recently described [5, 9, 10]. This trio of largely redundant genes is required for the development of petals, stamens, and carpels since triple mutants lacking all three *SEP* activities produce flowers that consist only of sepals [5]. This phenotype is similar to that of mutants lacking both the B and C activities and suggests that the *SEP* gene products are required for B and C gene activity. To determine if the *SEP* genes are the missing factors required for the conversion of leaves into flower organs, we generated transgenic plants that ectopically express one or more of the *SEP* genes in combination with the A (35S::AP1) and B (35S::AP3, 35S::PI) organ identity genes. If the *SEP* genes represent the missing factors, then we would expect that the ectopic expression of the A and B genes, together with the *SEP* genes, will convert leaves into petals.

Previous studies in which both B genes were constitutively expressed showed a slight conversion of the cauline leaves toward petals, whereas the rosette leaves at the base of the plant remained largely unaffected [4]. Similarly, constitutive expression of the A function gene, 35S::AP1, alone did not alter the identity of leaves [11]. However, when the 35S::AP1 transgene, or any of the 35S::SEP

Figure 1



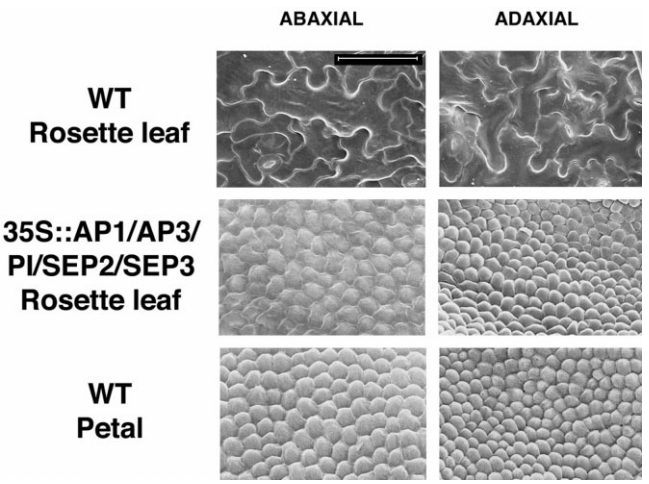
Conversion of leaves into petals. **(a)** Wild-type plant after the transition to reproductive development with numerous rosette leaves (rl) at its base and an extended inflorescence stem with cauline leaves (cl) and flowers arising at the apex. **(b)** Whole plant that has the 35S::SEP3/AP1/AP3/PI transgenes shows petaloid organs replacing all rosette leaves. **(c,d)** Whole plant with the 35S::SEP2/SEP3/AP1/AP3/PI transgenes shows petals in place of rosette leaves. "ct" indicates the cotyledon.

transgenes, is combined with the 35S::AP3 and 35S::PI transgenes, the conversion of cauline leaves toward petals is increased (data not shown). In fact, these cauline leaves are whiter and adopt a shape that is more similar to petals, although the cells of these cauline leaves retain many features common to leaves. In addition, the vegetative rosette leaves are only slightly affected; only a chalky color appears on the distal part of these curly leaves (not shown).

When the 35S::SEP2 and 35S::SEP3 transgenes (S. P., C. Gustafson-Brown, S. E. Kohalmi, W. L. Crosby, and M. F. Y., submitted) are combined with plants that constitutively express *AP1*, *AP3*, and *PI*, a complete conversion of rosette leaves into petals was observed, with the exception of three trichomes on the edges of these organs. Analyses of these bright-white rosette "petals" by scanning electron microscopy (SEM; [5]) revealed that cells on the abaxial (lower) surface of these converted organs closely resembled cells on the abaxial (outer) surface of normal petals (Figure 2). Similarly, cells on the adaxial (upper) surface of these converted organs closely resemble adaxial (inner) petal cells. Taken together, these results demonstrate that the combined action of the *SEP* genes, together with the A and B genes, is sufficient to convert vegetative rosette leaves into petals. We also found that the introduction of both 35S::SEP2 and 35S::SEP3 transgenes into plants that constitutively expressed the A and B transgenes produced a more complete transformation of rosette leaves into petals than did similar plants that contained only one of the two *SEP* transgenes (not shown). However, this likely reflects a difference in copy number and, hence, a difference in transgene expression levels since the genes appear to act in a largely redundant manner.

We have shown that the *SEP* genes, when combined with the A and B organ identity genes, are sufficient to convert vegetative rosette leaves into petals. Recent studies have indicated that ternary complexes of MADS-box proteins occur [12, 13], and this finding suggests that direct protein-protein interactions between the SEP proteins and the ABC organ identity gene products may determine organ

Figure 2



Scanning electron micrographs reveal the characteristic cell types on the abaxial and adaxial surfaces of wild-type (WT) rosette leaves and petals. Petal cells are very small and round, whereas leaf cells are comparatively large, irregular, and flat with interspersed stomata that are never present in petals. Cells on the abaxial and adaxial surfaces of the rosette leaves of 35S::SEP2/SEP3/AP1/AP3/PI transgenic plants closely resemble those on wild-type petals. The scale bar represents 50 μm.

fate. Because orthologs of the *SEP* genes have been described in eudicots [14–17], monocots [18], and gymnosperms [19], it seems likely that the interactions of the *SEP* genes with the ABC genes have been largely conserved in distantly related plant species.

Materials and methods

The 35S::SEP2 construct was generated by polymerase chain reaction from the *SEP2* cDNA by the use of primers with BamHI restriction sites. The primers used were the following: OEAB200, 5′-CCGGATCCATG GGAAGAGGAAGAGTAG-3′ and OEAB201, 5′-GGGGATCCTCAC AGCATCCAGCCAGG-3′. The PCR product was cloned into pGEM-T Easy (Promega) and then digested with BamHI for subsequent cloning into pBIN-JIT [20]. The constructs were confirmed by sequencing. The 35S::SEP2 construct was introduced into *Arabidopsis*, ecotype *Columbia*, by vacuum infiltration [21]. Transgenic plants were selected on kanamycin plates. Plants were grown under continuous light at 23°C–25°C.

Acknowledgements

We thank Pietro Ciceri and Lars Østergaard for comments on the manuscript; Beth Krizek for the 35S::AP3 and 35S::PI seeds; and Cheryl Wiley and Chika Kawashima for technical assistance. This work is supported by grants from the National Institutes of Health, the National Science Foundation, and the United States Department of Agriculture (to M. F. Y.) and by the Consejo Nacional de Ciencia y Tecnologia and the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (Universidad Nacional Autónoma de México) (to E. R. A.-B.). E. R.A.-B. was a PEW fellow, and R. T.-L. received Consejo Nacional de Ciencia y Tecnologia and Dirección General de Estudios de Posgrado (Universidad Nacional Autónoma de México) fellowships.

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