Plant Signaling & Behavior 4:9, 814-817; September 2009; © 2009 Landes Bioscience

# Floral organ size control

## Interplay between organ identity, developmental compartments and compensation mechanisms

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**Key words:** *Antirrhinum majus*, cell division, cell expansion, *COMPACTA ÄHNLICH*, compensation, floral size, *FORMOSA*, *NITIDA*, organ identity

Growth of lateral organs is a complex mechanism that starts with formation of lateral primordia. Basal developmental programs like polarity, organ identity and environmental cues influence the final organ size achieved via coordinated cell division and expansion. Recent evidence shows that the precise balance between these two processes, known as compensation mechanisms, seems to be influenced by the identity of the organ. Furthermore, studies of mutants affected in floral organ size suggest the existence of developmental compartments within different floral whorls that show distinct compensation behaviors.

### Global Aspect of Lateral Organ Growth

Formation of lateral organs starts with the recruitment of meristematic cells that acquire a primordial fate. Newly formed primordia start extending as a result of an organ polarity program.[1] A meristem signal is required to establish polarity within a developing organ.[2] How much an organ grows and what shape it achieves implicates two additional basal modulators, the organ identity imposed on the growing primordium and the level of interactions with the environment in terms of size and/or differentiation.

Flowers of angiosperms like *Arabidopsis thaliana*, *Antirrhinum majus* or Petunia sp. have in common the formation of concentric whorls of organs that include sepals, petals, stamens and carpels. Its precise arrangement is due to combinatorial genetic functions that give raise to the four identities found in the flower.[3-5] The formation of whorls requires the activation of the so-called organ identity genes that in Antirrhinum correspond mainly to *DEFICIENS, GLOBOSA* and *PLENA*.[6] Direct protein-protein interactions between these MADS-box genes give rise to heterodimers[7,8] and ternary complexes[9-11] that drive target gene expression required for floral morphogenesis. There is evidence that genes involved in polarity interact with early genes of SAM maintenance[1] and also with organ identity genes[12,13] affecting both organ growth and development.

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Submitted: 06/18/09; Accepted: 06/25/09
Previously published online:
http://www.landesbioscience.com/journals/psb/article/9394

Besides genetic developmental control, plants are able to change their morphogenesis adapting to environmental condition and modifying growth, flowering and sometimes survival.[14]

### Understanding Control of Floral Organ Size

Recent work has focused on understanding how floral size is controlled.[15] Two issues have been addressed; one is the existence of genes that control floral size in a specific way. Indeed, QTL affecting floral size have been found in Arabidopsis,[16] Petunia[17] and tomato.[18] The other issue is the genetic dissection of floral organ size control and its relation with floral organ identity genes.

Lateral organ growth can be divided in two phases: one, which includes cell mass increment coupled to cell proliferation and a second one, that takes place once cells exit the proliferative period and growth is mainly due to cell expansion.[19,20] Final organ cell number depends on the number of cells founding primordia, active dividing cells and cell proliferation rate and period.[19,21,22] Genetic analysis in Arabidopsis revealed several independent routes controlling plant organ size via changes in the cell division interval. For instance the auxin signaling through *ARGOS* and *AINTEGUMENTA* (*ANT*) extend the cell proliferation phase.[22,23] *KLUH*, which promotes growth through a non-cell autonomous signaling, seems to maintain cell division until primordia reach a determinate size.[24] *JAGGED* (*JAG*) and *NUBBIN* (*NUB*) act together to promote cell proliferation in marginal regions of lateral organs. Genes with opposite effects include *BIG BROTHER* (*BB*)[25] and *DA1*,[26] which restrict proliferation.

Cell expansion can induce organ size changes through various ways, including vacuolation, ploidy level, biosynthesis of cell wall components and cytoskeleton.[27,28] For instance the bHLH transcription factor *BIGPETALp* (*BPEp*), activated by floral identity genes, was shown to regulate petal size via cell expansion.[29]

### Organ Level Coordination between Cell Division and Expansion

In several higher organisms, normal organ size has been observed in mutants with aberrant or deficient cell division due to increased cell expansion.[30] This phenomenon is known as compensation mechanism and it adds another level of control on organ size by monitoring and coordinating cell division and expansion.[31]

The current hypothesis suggests that it could be a common procedure to control the size of determinate organs.[32] Recently published data suggest that reduction in cell expansion, by a decrease in endoreduplication, could also trigger compensation via increased cell proliferation to attain a normal leaf size.[33]

There are several studies in leaves showing reduction in cell number, which conduces to an increment in cell expansion. Mutation in *TANGLED* gene in corn shows aberrant cell division but no morphological defects,[34] mutations in *SHORT INTEGUMENT 2* gene also reduce cell number in integument but display almost normal morphology.[35] In Arabidopsis, null mutations in *ANGUSTIFOLIA3* restrict the proliferation period with a partial compensatory increment in cell expansion and narrower leaves.[36] Furthermore, several other mutations that induce compensation have been described as *struwelpeter*, *swellmap*, G-protein α-subunit1 and *deformed roots and leaves*.[37-40] A compensation mechanisms triggered by variations in cell division[41] has been identified in floral size mutants of Antirrhinum. The mutant *compacta ähnlich* (*coan*) shows a reduction in floral organ size in all floral whorls and this change is mainly due to a reduction in cell division (see below).[42] Although the mutants described in Arabidopsis and Antirrhinum show compensation mechanisms, lateral organs tend to be smaller than wild type.

The opposite compensation phenomenon has been documented in Arabidopsis integuments overexpressing *KNAT1*, with additional cell cycle rounds and reduced cell expansion, and also, in the mutant *swellmap*[38;43] and in the *Antirrhinum* mutant *formosa* (*fo*) with increased floral organ size due to higher cell number but a reduction in cell area.[41]

## Organ Identity Coming into Play

Several lines of evidence suggest that floral organ identity plays a key role as a regulator of organ size. Weak hypomorphic alleles of *DEFICIENS* (*DEF*)[44] in Antirrhinum, or single mutants of *PhDEF* and *PhGLO1* in petunia[45] where a full loss of organ identity is only achieved in the double mutant,[45] share a reduced petal growth as phenotype. These observations suggest that final organ size requires high expression of B function genes. Direct evidence has been obtained in temperature sensitive alleles of *DEF* that show decreased organ size under non-permissive temperatures.[46]

Several mutants affecting organ size do it in an organ-identity dependent fashion. Ectopic expression of *ANT* causes increased cell division in all floral whorls,[22] and also shows enhanced cell expansion in petals, stamens and pistils but not in sepals.[47] In tomato, the *OVATE* gene represses growth in fruits, but additional gene copy numbers expand the size of all lateral organs with a stronger influence in sepals and stamens than in petals and carpels.[48] Transgenic tobacco expressing dominant negative constructs of Cdc2 kinase display reduction in cell number in all lateral organs, that triggers compensation in leaves but not in flowers.[49] Mutations in the Antirrhinum *FO* gene increase cell division in the three inner floral whorls and displays compensation with a reduction in cell expansion in petals and pistils but not in stamens.[41] In contrast the mutant *coan* has reduced cell division in petals, stamens and pistils but compensation is triggered in petals via increased cell expansion. These differences in organ size control depending on identity is also supported by a double mutant between *fo*, affecting floral organ size, an a flower identity mutant, *plena* (*ple*), in a C function gene. The *ple fo* double mutant only shows size differences in petals of second whorl which have normal identity (**Fig. 1A and B**). Inner whorls with altered identity don't show any significant size variation between *ple* single mutant and *fo ple*. However *fo* effect over petal size in *ple* genetic background is stronger than in single mutant with an increment in dorsal petal expansion that doubles the one observed in single *fo* mutant. Cell compensation is not observed in *fo ple* petals anymore, showing an increment in conical cell expansion in contrast to the reduction observed in single *fo* mutant (**Fig. 1C and D**). This would suggest an implication of *PLE* in organ size control in a non-autonomous way. Several previous works in C function (*AGAMOUS*) mutation in Arabidopsis suggest its non-autonomous role in specifying the pattern of cell division in different layers of the second whorl,[50] intercellular communication between different whorls[50] and in petal development.[51] Moreover, the gene *BPEp* mainly expressed in petals, has been described to be regulated by identity genes including *AGAMOUS*.[29]

## Developmental Compartments Show Distinct Compensation Behaviors

Petals are highly specialized organs comprised of distinct regions like the tube or the petal blade. The latter has two cell types distinguished by shape and function since conical cells are involved in light scattering and scent production.[52,53] In *coan* cell behavior is complex as cell size is only affected in petal regions with epidermal conical cells while flat cells adjacent to the petal tube do not show this compensation process.[42] The opposite was observed in another floral size mutant of Antirrhinum, Nitida (*Ni*), where the reduction in floral size in petals also induces decreased expansion of flat cells while no variation was observed in conical petal cells.[42]

The evidences described confirm compensation could be triggered both in leaves and flowers but seems to depend on organ identity and even on different developmental compartments inside organs. This suggests the existence of a global control of organ size integrating cell division, expansion and development, but there is no mechanism clarifying communication between those programs yet. Hopefully forthcoming studies would reveal the intricate network controlling final organ size.

**Acknowledgements**

This work was supported by Biocarm (BANANASAI), Fundación Séneca de la Región de Murcia and MEC-AGL2007-61384. Grants from AECI to L.D.-B. Thanks to Izaskun Mallona for comments on the manuscript.

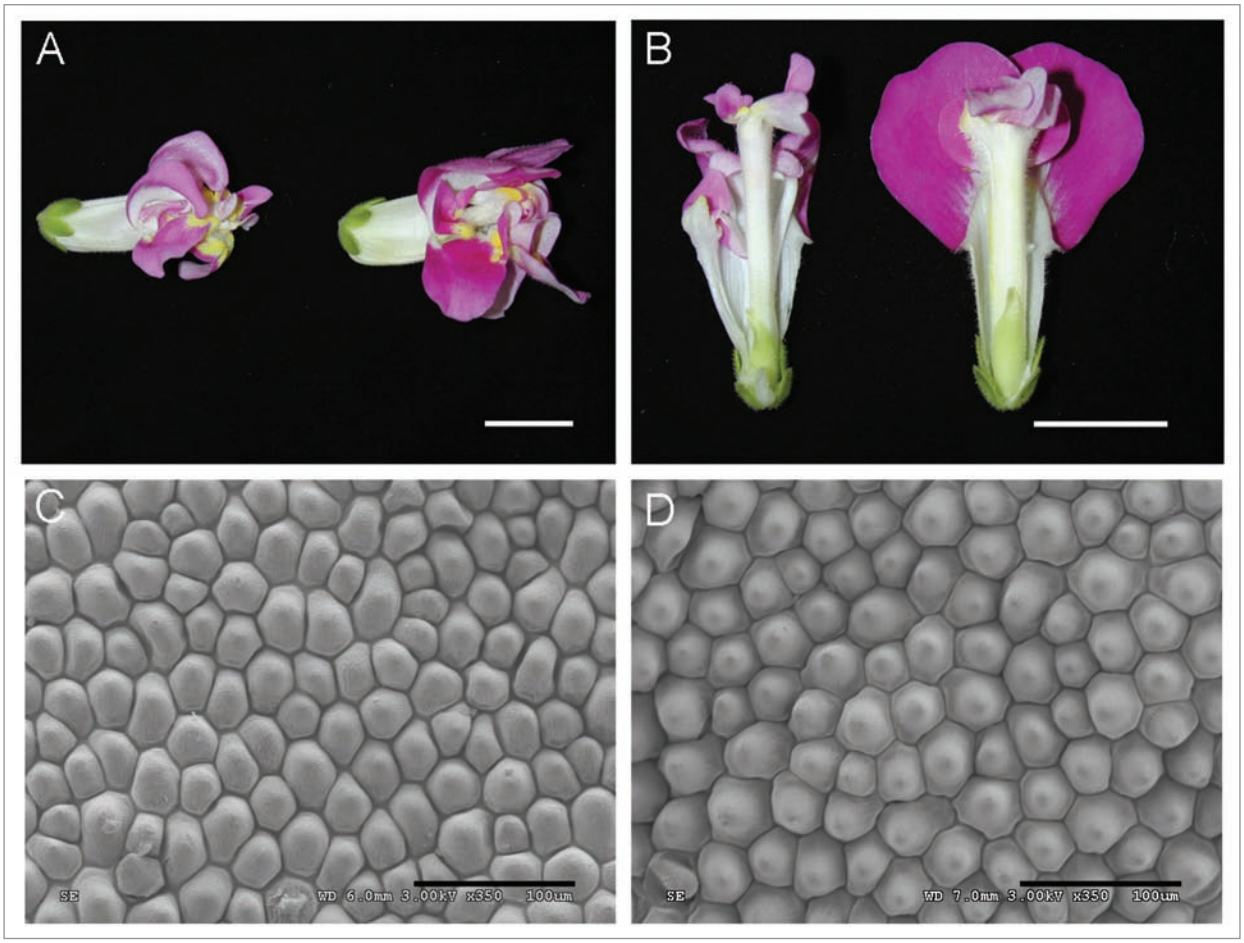


**Figure 1.** Pictures showing flowers of *ple* single mutant and *fo ple* double mutant (left and right respectively) (A) lateral view and (B) longitudinal section. White bars represent 1 cm. SEM images of petal conical cells (C) *ple* mutant and (D) *fo ple* double mutant. Black bars represent 100 μm.

## References


1. Kidner CA, Timmermans MCP. Mixing and matching pathways in leaf polarity. Curr Opin Plant Biol 2007; 10:13-20.
2. Reinhardt D, Frenz M, Mandel T, Kuhlemeier C. Microsurgical and laser ablation analysis of leaf positioning and dorsoventral patterning in tomato. Development 2005; 132:15-26.
3. Coen ES, Meyerowitz EM. The war of the whorls—genetic interactions controlling flower Development. Nature 1991; 353:31-7.
4. Egea Gutierrez-Cortines M, Davies B. Beyond the ABCs: ternary complex formation in the control of floral organ identity. Trends Plant Sci 2000; 5:473-8.
5. Rijpkema AS, Royaert S, Zethof J, van der Weerden G, Gerats T, Vandenbussche M. Analysis of the Petunia TM6 MADS box gene reveals functional divergence within the DEF/AP3 lineage. Plant Cell 2006; 18:1819.
6. Schwarz-Sommer Z, Huijser P, Nacken W, Saedler H, Sommer H. Genetic control of flower development by homeotic genes in *Antirrhinum majus*. Science 1990; 250:931-6.
7. Davies B, EgeaCortines M, Silva ED, Saedler H, Sommer H. Multiple interactions amongst floral homeotic MADS box proteins. EMBO J 1996; 15:4330-43.
8. Schwarz-Sommer Z, Hue I, Huijser P, Flor PJ, Hansen R, Tetens F, et al. Characterization of the Antirrhinum floral homeotic MADS-box gene deficiens: evidence for DNA binding and autoregulation of its persistent expression throughout flower development. EMBO J 1992; 11:251-63.
9. Causier B, Cook H, Davies B. An Antirrhinum ternary complex factor specifically interacts with C-function and SEPALLATA-like MADS-box factors. Plant Mol Biol 2003; 52:1051-62.
10. Egea-Cortines M, Saedler H, Sommer H. Ternary complex formation between the MADS-box proteins SQUAMOSA, DEFICIENS and GLOBOSA is involved in the control of floral architecture in *Antirrhinum majus*. EMBO J 1999; 18:5370-9.
11. Honma T, Goto K. Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature 2001; 409:525-9.
12. Franks RG, Wang CX, Levin JZ, Liu ZC. SEUSS, a member of a novel family of plant regulatory proteins, represses floral homeotic gene expression with LEUNIG. Development 2002; 129:253-63.
13. Meyerowitz EM. Genes and gene interactions that establish patterns in flower development. J Cell Biochem 1995; 329.
14. Hardtke CS, Torii KU. Plant growth and developmentùthe new wave. Curr Opin Plant Biol 2008; 11:1-3.
15. Weiss J, Delgado-Benarroch L, Egea-Cortines M. Genetic control of floral size and proportions. Int J Dev Biol 2005; 49:513-25.
16. Juenger T, Perez-Perez JM, Bernal S, Micol JL. Quantitative trait loci mapping of floral and leaf morphology traits in *Arabidopsis thaliana*: evidence for modular genetic architecture. Evol Dev 2005; 7:259-71.
17. Galliot C, Stuurman J, Kuhlemeier C. The genetic dissection of floral pollination syndromes. Curr Opin Plant Biol 2006; 9:78-82.
18. Frary A, Fritz LA, Tanksley SD. A comparative study of the genetic bases of natural variation in tomato leaf, sepal and petal morphology. Theor Appl Genet 2004; 109:523-33.
19. Anastasiou E, Lenhard M. Growing up to one's standard. Curr Opin Plant Biol 2007; 10:63-9.
20. Bogre L, Magyar Z, Lopez-Juez E. New clues to organ size control in plants. Genome Biol 2008; 9:226.
21. Mizukami Y. A matter of size: developmental control of organ size in plants. Curr Opin Plant Biol 2001; 4:533-9.
22. Mizukami Y, Fischer RL. Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. Proc Natl Acad Sci USA 2000; 97:942-7.
23. Hu Y, Xie Q, Chua NH. The Arabidopsis auxin-inducible gene ARGOS controls lateral organ size. Plant Cell 2003; 15:1951-61.
24. Anastasiou E, Kenz S, Gerstung M, MacLean D, Timmer J, Fleck C, et al. Control of plant organ size by KLUH/CYP78A5-dependent intercellular signaling. Dev Cell 2007; 13:843-56.
25. Disch S, Anastasiou E, Sharma VK, Laux T, Fletcher JC, Lenhard M. The E3 ubiquitin ligase BIG BROTHER controls Arabidopsis organ size in a dosage-dependent manner. Curr Biol 2006; 16:272-9.
26. Li Y, Zheng L, Corke F, Smith C, Bevan MW. Control of final seed and organ size by the DA1 gene family in *Arabidopsis thaliana*. Gene Dev 2008; 22:1331.
27. Joubes J, Chevalier C. Endoreduplication in higher plants. Plant Mol Biol 2000; 43:735-45.

28. Smith LG, Oppenheimer DG. Spatial control of cell expansion by the plant cytoskeleton. Annu Rev Cell Dev Biol 2005; 21:271-95.
29. Szecsi J, Joly C, Bordji K, Varaud E, Cock JM, Dumas C, et al. BIGPETALp, a bHLH transcription factor is involved in the control of Arabidopsis petal size. EMBO J 2006; 25:3912-20.
30. Horiguchi G, Ferjani A, Fujikura U, Tsukaya H. Coordination of cell proliferation and cell expansion in the control of leaf size in *Arabidopsis thaliana*. J Plant Res 2006; 119:37-42.
31. Tsukaya H. Interpretation of mutants in leaf morphology: Genetic evidence for a compensatory system in leaf morphogenesis that provides a new link between cell and organismal theories. Int Rev Cytol 2002; 217:1-39.
32. Tsukaya H. Controlling size in multicellular organs: focus on the leaf. PLoS Biol 2008; 6:174.
33. Larson-Rabin Z, Li Z, Masson PH, Day CD. FZR2/CCS52A1 Expression is a determinant of endoreduplication and cell expansion in Arabidopsis. Plant Physiol 2009; 149:874-84.
34. Smith LG, Hake S, Sylvester AW. The tangled-1 mutation alters cell division orientations throughout maize leaf development without altering leaf shape. Development 1996; 122:481-9.
35. Broadhvest J, Baker SC, Gasser CS. SHORT INTEGUMENTS 2 promotes growth during Arabidopsis reproductive development. Genetics 2000; 155:899-907.
36. Horiguchi G, Kim GT, Tsukaya H. The transcription factor AtGRF5 and the transcription coactivator AN3 regulate cell proliferation in leaf primordia of *Arabidopsis thaliana*. Plant J 2005; 43:68-78.
37. Autran D, Jonak C, Belcram K, Beemster GTS, Kronenberger J, Grandjean O, et al. Cell numbers and leaf development in Arabidopsis: a functional analysis of the STRUWWELPETER gene. EMBO J 2002; 21:6036-49.
38. Clay NK, Nelson T. The recessive epigenetic swellmap mutation affects the expression of two step II splicing factors required for the transcription of the cell proliferation gene STRUWWELPETER and for the timing of cell cycle arrest in the Arabidopsis leaf. Plant Cell 2005; 17:1994-2008.
39. Ullah H, Chen JG, Young JC, Im KH, Sussman MR, Jones AM. Modulation of cell proliferation by heterotrimeric G protein in Arabidopsis. Science 2001; 292:2066-9.
40. Nelissen H, Clarke JH, De Block M, De Block S, Vanderhaeghen R, Zielinski RE, et al. DRL1, a homolog of the yeast TOT4/KT112 protein, has a function in meristem activity and organ growth in plants. Plant Cell 2003; 15:639-54.
41. Delgado-Benarroch L, Causier B, Weiss J, Egea-Cortines M. *FORMOSA* controls cell division and expansion during floral development in *Antirrhinum majus*. Planta 2009; 229:1219-29.
42. Delgado-Benarroch L, Weiss J, Egea-Cortines M. The mutants *compacta ähnlich*, Nitida and Grandiflora define developmental compartments and a compensation mechanism in floral development in *Antirrhinum majus*. J Plant Res 2009; doi: 10.1007/s10265-009-0236-6.
43. Truernit E, Haseloff J. *Arabidopsis thaliana* outer ovule integument morphogenesis: Ectopic expression of KNAT 1 reveals a compensation mechanism. BMC Plant Biol 2008; 8:35.
44. Sommer H, Beltran JP, Huijser P, Pape H, Lonnig WE, Saedler H, et al. Deficiens, A homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*—the protein shows homology to transcription factors. EMBO J 1990; 9:605-13.
45. Vandenbussche M, Zethof J, Royaert S, Weterings K, Gerats T. The duplicated B-class heterodimer model: Whorl-specific effects and complex genetic interactions in *Petunia hybrida* flower development. Plant Cell 2004; 16:741-54.
46. Zachgo S, Silva E, Motte P, Trobner W, Saedler H, Schwarz-Sommer Z. Functional analysis of the Antirrhinum floral homeotic DEFICIENS gene in vivo and in vitro by using a temperature-sensitive mutant. Development 1995; 121:2861-75.
47. Krizek BA. Ectopic expression AINTEGUMENTA in Arabidopsis plants results in increased growth of floral organs. Dev Genet 1999; 25:224-36.
48. Liu JP, Van Eck J, Cong B, Tanksley SD. A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc Natl Acad Sci USA 2002; 99:13302-6.
49. Hemerly A, Engler JD, Bergounioux C, VanMontagu M, Engler G, Inze D, et al. Dominant-negative mutants of the Cdc2 kinase uncouple cell-dvision from iterative plant development. EMBO J 1995; 14:3925-36.
50. Jenik PD, Irish VF. Regulation of cell proliferation patterns by homeotic genes during Arabidopsis floral development. Development 2000; 127:1267-76.
51. Durfee T, Roe JL, Sessions RA, Inouye C, Serikawa K, Feldmann KA, et al. The F-box-containing protein UFO and AGAMOUS participate in antagonistic pathways governing early petal development in Arabidopsis. Proc Natl Acad Sci USA 2003; 100:8571-6.
52. Dudareva N, Murfitt LM, Mann CJ, Gorenstein N, Kolosova N, Kish CM, et al. Developmental regulation of methyl benzoate biosynthesis and emission in snapdragon flowers. Plant Cell 2000; 12:949-61.
53. Martin C, Bhatt K, Baumann K, Jin H, Zachgo S, Roberts K, et al. The mechanics of cell fate determination in petals. Philos Trans R Soc Lond B Biol Sci 2002; 357:809-13.