GENETIC CONTROL OF FLOWER MORPHOGENESIS IN PISUM SATIVUM (L.)

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Embryonary development in higher plants is characterized by the stablishment of meristems that give rise to an axis shoot/root after seed germination. These meristematic activities consist of cell divisions producing new meristematic undifferentiated cells and cells that differentiate to form the vegetative organs. Soon shoots form leaves and the body of the plant is constructed by repetition of units named phytomers. During the vegetative phase of growth all the plant cells are diploid and gametes are not produced (sporophyte generation). External factors such as temperature and photoperiod are necessary to modulate the expression of a set of genes that finally will lead to the production of reproductive organs and gametes characteristic of the gametophyte generation. To be able to understand and to control whether or not a plant will flower and even to decide which characters will display the floral organs is a common aim of developmental biologists and plant breeders. New strategies to understand floral morphogenesis based on the molecular genetic analysis of floral homeotic mutants have been developed[2]. Since then, many homeotic genes controlling processes such as meristem identity, floral organ identity and genes that regulate the spatial expression of these ones have been isolated from different plant systems. Most of them belong to the MADS-box gene family and code for transcription factors which show homology to others described in yeast and humans.The most complete analysis carried out deals with flower organogenesis in *A. thaliana* and has lead to the so called ABC model[3]. With a toolkit of A, B, and C loss-of- function mutations and B and C gain-of-function transgenic lines it is possible to decide the identity of the organ that will be form at any of the flower whorls. The question that remains open is whether or not this knowledge, obtained in the plant model systems, *A. thaliana* and *A. majus* is sufficient to explain flower development in other plants.The pea plant (*Pisum sativum* L.). is a classic experimental system for plant genetics, biochemistry and physiology. Many homeotic mutants are available. We have carried out extensive scanning electron microscopy studies of the ontogeny of the wildtype pea flower with the aim to select morphological markers that can be used to better characterise the alterations in flower morphogenesis in different homeotic mutants. This has allowed us to establish similarities and differences between the genetic control of flower morphogenesis in pea with respect to the model systems.

Plant material: We have used as wildtype cultivar Alaska n7 of pea. We selected four mutants: *frondosus* (*brac*)[4] ; n192[5]; *stamina pistilloida* (*stp*)[6] and *petalosus* (*pe*)[7] with phenotypes remembering the typical A, B, and C classes of mutations.

Scanning electron microscopy (SEM): Samples were fixed in ethanol: acetic acid: formaldehyde: water, 50:10:3,5:26,5 v/v/v/v, immediately after collection. They were vacuum infiltrated for 15 min, transferred to fresh fixing solution for 4h and stored in 70% ethanol at 4°C. Samples were maintained for 1h in absolute ethanol, mounted on stubs covered with activated charcoal and then were dried at critical point with CO_2 in an Autosamdri-84 Tousimis equipment. After that, the stubs and samples were covered with a shadow of gold-palladium particles (200 nm) in a Sputter Coater SC500 (Biorad). The micrographs were obtained with an Agfapan Apx25/120 (Agfa) film at a scanning speed of 200 s / image in an electron scanning microscope Hitachi S-2500 working at 15 KV and equipped with a Robinson retrodispersion detector.

Morphological description and ontogeny of the pea flower: The pea flower has five sepals, five petals, ten stamens and one carpel organised in four whorls (Fig. 1A, B, C). The sepals are fused at their bases forming a short tube. The corolla, also pentamerous, contains three types of petals, the standard or vexillum is the largest one and is situated at the adaxial position of the flower, two lateral petals, the alae or the wings, and two short petals, accreted, that form the keel at the abaxial position. Nine stamens are joined in a staminal tube around the carpel, and the tenth one at the adaxial position is free. The carpel is joined adaxially with one line of ovules on each side of the suture. The carpel grows out to a pod.

Extensive SEM observations allowed us to select morphological markers corresponding to eight different steps during the ontogeny of the pea flower. These are, 1. Differentiation of secondary inflorescent meristem, 2. Differentiation of floral meristem, 3. Abaxial sepal appears, 4. Common primordia to petals and stamens appear, 5. Differentiation of primordia of petals and stamens, 6. Sepals cover the floral bud, 7. Formation of locules in anthers and 8. Inflection of the carpel. These markers resulted to be very useful to characterise flower initiation in both wildtype and homeotic mutants. In the pea flower, after the initiation of the sepals, four common primordia are formed that delimit a central zone of the flower that differentiate the carpel primordium. Finally, after growing, the four common primordia will differentiate the petal and stamen primordia[8,9]. Fig.1D shows a diagram of the ontogeny of the pea flower. The ontogeny of the pea flower is drastically different from the one of *A. thaliana* and *A. majus* flowers. In these model systems the initiation of the primordia of the floral organs is a centripetal and sequential process. That is first the sepal primordia are formed, then they appear the primordia that will form petals and immediately the stamen primordia arise, Finally, the residual central part of the floral meristem forms the carpel primordium.

Ontogeny of pea floral homeotic mutants: The flowers of *frondosus* (Fig.1G), develop at their base a bract, an extra organ of foliar morphology. Sepals are also abnormal, appearing as a single organ with morphological similarities to the extra bract. The petals show alterations such as a reduction in number, fusions and green sectors. Often the flowers show intermediate characters between flower and inflorescence as it happens in the leafy mutant of *A. thaliana* that also forms bracts although *frondosus*flowers do not show carpelloidy. *Frondosus*, also shares phenotypic aspects with *apetala 1*, a class A mutation, therefore it could be implicated in both the suppression of bract formation, and the specification of floral meristems and A activity.

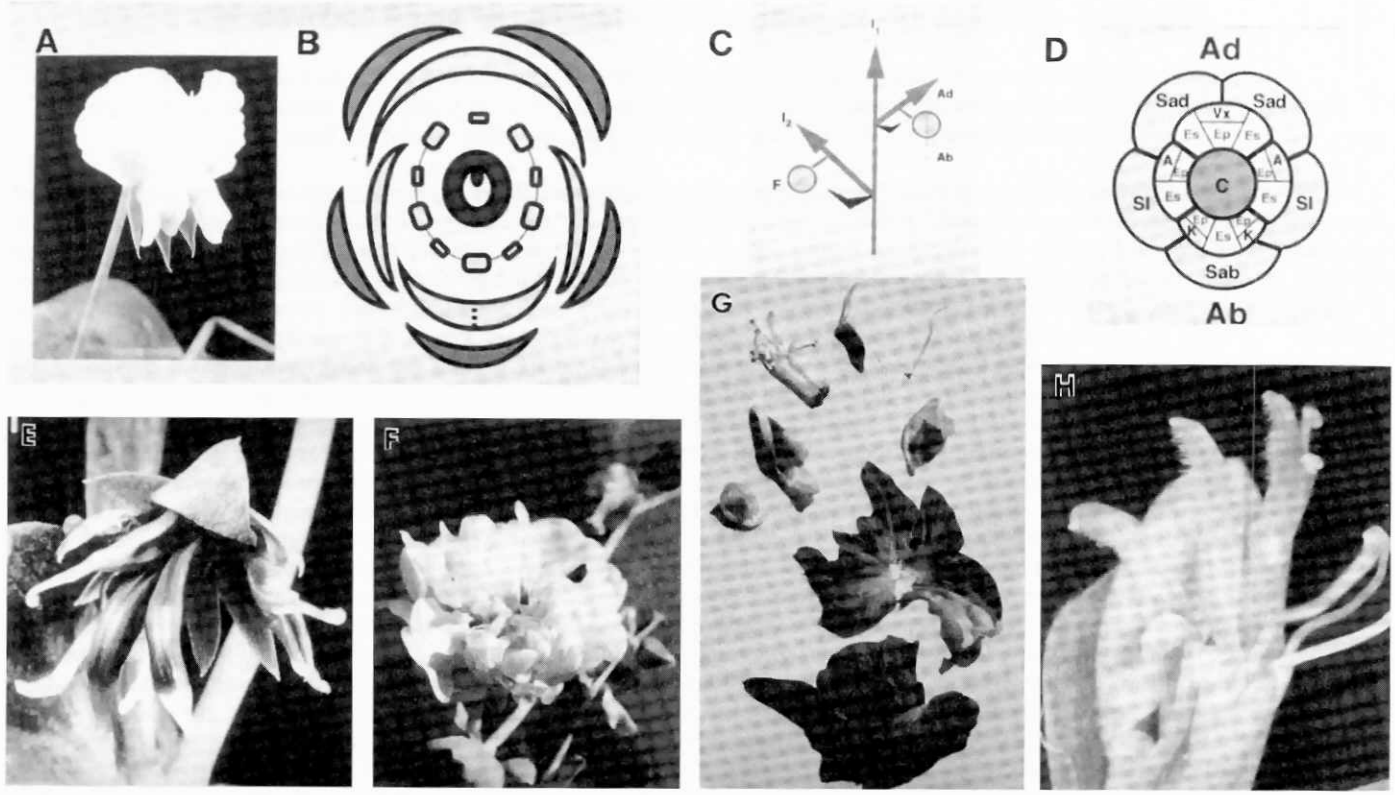

Figure 1. Ontogeny of the wildtype pea flower and phenotypes of pea homeotic mutants studied. **A**. wildtype flower.**B.** Diagram of a cross section. **C**. Orientation of flowers in the plant. **D.** Diagram showing the temporal formation of organ primordia as follows: Abaxial sepal (Sab); Lateral sepals (Sl); Adaxial sepals (Sad); Carpel (C); Abaxial common primordium; Lateral common primordia; Adaxial common primordium; Petals, K (keel), A (wing),and V_x (vexillum); Antesepal stamen primordia (Es); Antepetal stamen primordia (Ep); **E.** Phenotype of n192. **F.** Phenotype of *petalosus*. **G.** Phenotype of *frondosus* **H.** Phenotype of *stamina pistilloida* .

n192. The flowers of this mutant show the transformation of petals into sepals and stamens into carpels typical of the class B of mutations (Fig. 1E). However there are important differences. While in class B mutants of *A. thaliana* and *A. majus* the fourth whorl is suppressed in pea remains normal. Each stamen is transformed into a single carpel and still there is the wildtype carpel. In class B mutants of the model systems genes of class B might be implicated in maintaining the proliferating capability of the floral meristems so avoiding the termination of the meristem at the third whorl. According to the early appearance of the carpel primodium in pea this function could be unnecessary. Also, n192 flowers develop new flowers from the common primordia that remain undifferentiated longer than normal and have a delay in their subdivision to form petal and stamen primordia. This suggest that besides the typical B activities, n192 is implicated in controlling the organisation and determination of the common primordia.

Stp flowers display an interesting phenotype: the two stamen flanking the adaxial free one (antesepal stamen) are transformed into rudimentary carpels (Fig.1H).The petals forming the keel are not fused and show occasionally green sectors.The homeotic transformations of the third whorl in this mutant are positionally restricted to the adaxial antesepal stamen indicating that genes of class B in pea are spatially regulated and not only temporally regulated as other stamen differentiate before and after those ones.

Petalosus flowers show homeotic transformations affecting the 3th and 4th whorls. Most of the stamen present petaloid expansions or are converted into mosaic organs being petaloid at their base and staminoid at the apical position (Fig.1F). The carpels develops open and form different floral organs in the inside. These phenotypic alterations are characteristic of class C mutants. However in *petalosus* the indetermination of the central part of the floral meristem affects to the subdivision of the common primordia similarly to what happens in n192 mutant.This suggest that both genes could cooperate in the spatial organisation of the common primordia.

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