Understanding the function of *VEG1* in the identity of the I2 meristems

Marcos Serra Picó
Directors: Francisco Madueño Albi and Reyes Benlloch Ortiz
Programa de Doctorado en Biotecnología
* Instituto de Biología Molecular y Celular de Plantas (IBMCP), CSIC-UPV, Valencia (Spain) marserpi@etsii.upv.es

1. Introduction.

A striking characteristic of plants is the great variety of forms that can be found in nature. Plant architecture, an important agronomic trait that has a strong effect in yield, depends on the activity of the shoot apical meristem (SAM), which generates all the aerial parts of the plant. In the first developmental phases, the SAM is vegetative generating leaves and shoots. Later on, after the floral transition, the SAM becomes an inflorescence meristem and starts producing flowers.

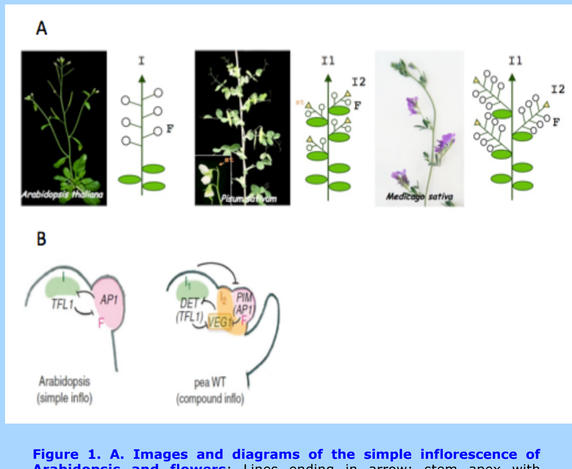

Figure 1. A. Images and diagrams of the simple inflorescence of Arabidopsis and flowers; Lines ending in arrow: stem apex with indeterminate growth; Orange triangles: "stubs" F: flower I: inflorescence. **B. Models of genetic networks of Arabidopsis (simple inflorescence) and pea inflorescence (compound inflorescence).** The expression domains of the regulatory genes and the interactions between them are indicated. The genetic model explains the phenotypes of mutant pea meristem identity. (Fig. Modified from Berbel et al., 2012. Nat Comms doi: 10.1038/ncomms1801)

The genetic network that controls the identity of the inflorescence meristems is best known in Arabidopsis, with a simple inflorescence. The formation of the inflorescence of Arabidopsis is mainly explained by the function of the genes *TERMINAL FLOWER 1* (*TFL1*), *APETALA 1* (*AP1*).

However, little is known about the genetic control of the development of the compound inflorescence of legumes, where the flowers are formed in secondary (I2) inflorescences. In legumes, especially in pea, several genes that control the identity of inflorescence meristems are known. Mutant alleles of these genes produce different architectures of the plant and inflorescence. Many of these architectures are the basis of varieties of great agricultural interest. The formation of secondary inflorescence meristem (I2) is crucial for the development of inflorescences of higher order and, therefore, for the formation of the compound inflorescence of legumes.

The formation of I2 depends on VEGETATIVE 1 (VEG1) which specifies the identity of the secondary inflorescence (I2) meristem, so that *veg1 (*mutant) plants are not able to form I2s, which are substituted by primary inflorescences (I1s). *VEG1* encodes a MADS transcription factor, without a functional equivalent in plants with simple inflorescence, such as Arabidopsis

2. Objectives.

Understand how VEG1 acts to specify the identity of the I2 meristems. The transcription factor VEG1, is central in the formation of the compound inflorescence of pea, the legume where the genetic network of the inflorescence is best known. VEG1 specifies the meristem identity of secondary inflorescence (I2) and is essential for the formation of compound inflorescences. Recent advances in the pea genome now allow us to address the study in detail of the genetic network regulated by VEG1.

Understand the function of AGL79, the VEG1 counterpart in Arabidopsis, where the development of the inflorescence and its gene regulatory network is different to that in legumes.

To achieve these objectives we will:

1) Identifying possible transcriptional targets of VEG1 we will perform transcriptome analysis of different pea mutants affected in the development of the inflorescence. We will use RNA-seq to compare the transcriptomes of inflorescence apices of wild-type plants with those of the veg1, pim and veg2-1 mutants.

2) For targets of VEG1, we will identify genes whose expression is responsive to the transient activation of the VEG1 transcription factor, using TARGET (Transient Assay Reporting Genome-wide Effects of Transcription factors) in pea protoplasts. The TARGET method has been recently developed and allows the identification of genes that respond to the activation of transcription factors.

3) Generate *agl79* mutants, using the CRISPr-Cas9 technology, and carry out a detailed functional charaterization of them.

3. Main stages of the current research

- Inflorescence genes with VEG1-dependent expression: We will select those genes that are expressed to a greater or lesser level in the inflorescences of the mutant *veg1* than in the wild and that show an opposite expression pattern in the inflorescences of pim.

- Identification of direct targets through TARGET method (genes that respond to VEG1-GR): We will use the TARGET system to analyze genes whose expression responds to the activation of VEG1-GR in pea protoplasts.

- We will compare the lists of possible targets obtained by the two previous activities, to develop a list of candidate genes of high confidence. A small number (2-4) of the target genes of greatest interest will be selected and characterization will begin. This will include expression analysis, and for the analysis of its possible function we will use VIGS in pea to silence its expression.

3.1 RNA-seq

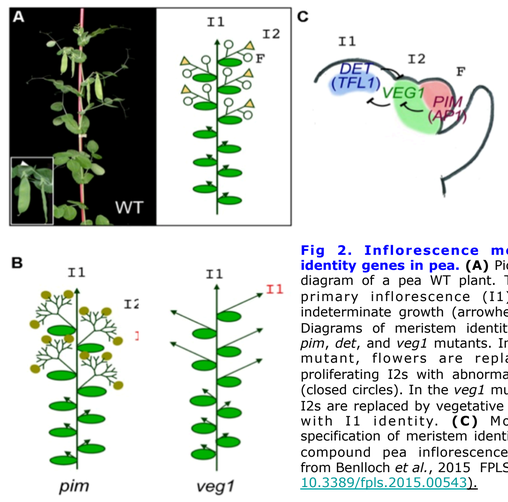

Fig 2. Inflorescence meristem identity genes in pea. (A) Picture and diagram of a pea WT plant. The main primary inflorescence (I1) shows indeterminate growth (arrowhead). **(B)** Diagrams of meristem identity of the *pim*, *det*, and *veg1* mutants. In the *pim* mutant, flowers are replaced by proliferating I2s with abnormal flowers (closed circles). In the *veg1* mutant, the I2s are replaced by vegetative branches with I1 identity. **(C)** Model for specification of meristem identity in the compound pea inflorescence (Figure from Benlloch *et al.*, 2015 FPLS doi: 10.3389/fpls.2015.00543).

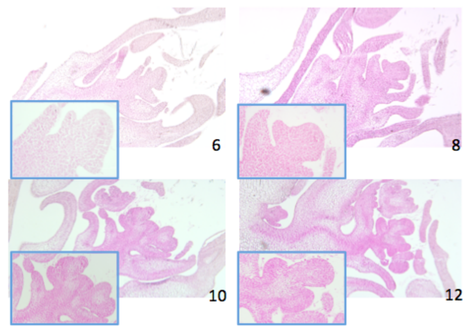

Figure 3. The formation of the I2 meristems occurs between nodes 8 and 10, probably at node 9 for the case of WT.

We will use RNA-seq to compare the transcriptomes of inflorescence apices of wild-type plants with those of the *veg1*, *pim* and *veg2-1* mutants.

-*veg*1 mutant: A transition occurs from the vegetative phase to the inflorescence but the secondary inflorescences I2 are replaced by stems of inflorescence I1.

-*pim* mutant: There are many I2 meristems since these proliferate, dividing repeatedly by the ectopic expression of *VEG1.*

-*Veg2* mutant: Has a similar phenotype that *veg1* but the molecular base is different, since it occurs as a consequence of the altered expression of several floral regulators.

3.2 TARGET system

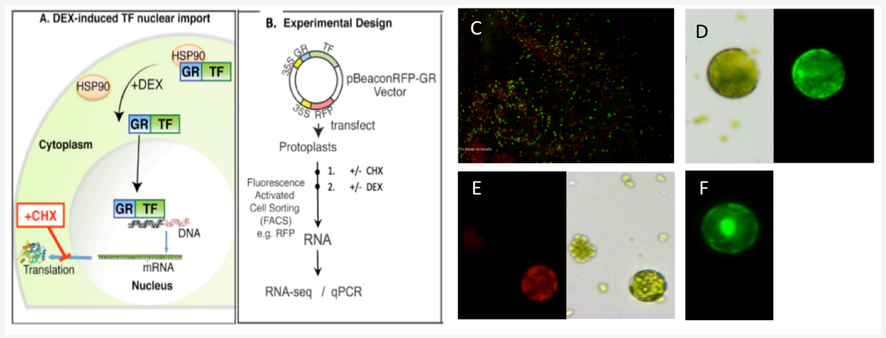

Figure 4. A, Basis of DEX activation of a transcription factor fused to GR. B, scheme of an experiment in the TARGET system. C. Protoplast transfection experiment with a vector containing the 35S: VEG1-GR construct and the caset with the GFP reporter gene (35S: GFP: Tnos). D. Staining with fluorescein diacetate to check the viability of transfected protoplasts. E. Staining with Propidium Iodide to check that the broken protoplasts were actually dead and were not transfected. F. Image of viable protoplast transfected.

TARGET system designed to analyze genes whose expression responds to the activation of VEG1-GR in pea protoplasts. We will use protoplasts, prepared from pea WT plants, which will be transfected with a construct into the pBeaconRFP-GR vector with a 35S::VEG1-GR cassette. We will compare protoplasts pretreated or not with CHX and later with DEX or mock. After purification of protoplasts by cell-sorting, we will analyze the gene expression by RNA-seq and qRT-PCR.

4. Expected results and possible profits

We expect to reach better knowledge of how the genetic network controls the development of the legume compound inflorescence. In particular, we expect to identify and understand the function of the main genes that are activated by VEG1 to direct the formation of the secondary inflorescences.

Legumes are the second most important family of crop species after grasses, and because of the high protein content of their seeds and low nitrogen fertilizer requirements are essential to develop a sustainable production of agricultural products of high nutritional value.

Our work should lead us to identify and understand the role of key genes regulating the development of the legume inflorescence. This should allow us to improve the number of flowers (fruits and seeds) and the position where they are formed by the plant. These characters are an essential determinant of productivity, and the knowledge and the biological material that will be generated in this project may be very useful for obtaining varieties of legumes with a higher and stable yield. For these reasons, our project is especially relevant in the context of improving and making more stable the yield of legume crops.

Summary

1.- Little is known about the genetic control of the development of the compound inflorescence of legumes. The formation of secondary inflorescence (I2) meristems is crucial for the development of inflorescences of high order and, therefore, for the formation of the legume compound inflorescence. The formation of the I2 depends on *VEGETATIVE 1* (*VEG1*), which specifies the identity of the I2 meristem.

2.- We aim to 1) understand how VEG1 acts to specify the identity of meristems I2 in the compound inflorescence of legumes and 2) understand the function of AGL79 , the *VEG1* counterpart in Arabidopsis, a specie with a simple inflorescence.

3.- To understand how VEG1 acts, we will study the trascriptome of different pea mutants affected in I2 development and will identify the genes whose transcription directly depends on VEG1, using the "TARGET" system in pea protoplasts. To undertand the function of AGL79 we will characterize agl79 mutants that we are developing through CRISPr.