How can we explain the phenotypic plasticity of adventitious root initiation?

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Introduction / Background

The root system of a plant is composed of the primary, lateral and adventitious roots. Lateral roots always develop from roots whereas adventitious roots (ARs) form from stem or leaf derived cells. Adventitious rooting is an essential step in artificial vegetative propagation of plants. In horticulture, agriculture and forestry, vegetative or clonal propagation is widely used to multiply elite genotypes obtained in breeding programs or selected from natural populations.

Data from a limited number of genetic studies suggest that competence to form ARs is controlled by many genes and as such is a complex genetic trait. In addition, for one given genotype, the adventitious root phenotype varies a lot (Figure 1B) most likely because very sensitive to any modification of internal regulatory factors such as hormones or environmental regulatory factors. This is why adventitious rooting is said to be a trait with "high phenotypic variability". Among the internal factors involved in the control of adventitious rooting auxin plays a central role. In this process the plant hormone auxin interacts either with other internal factors (mainly phytohormones) or environmental stimuli such as light.

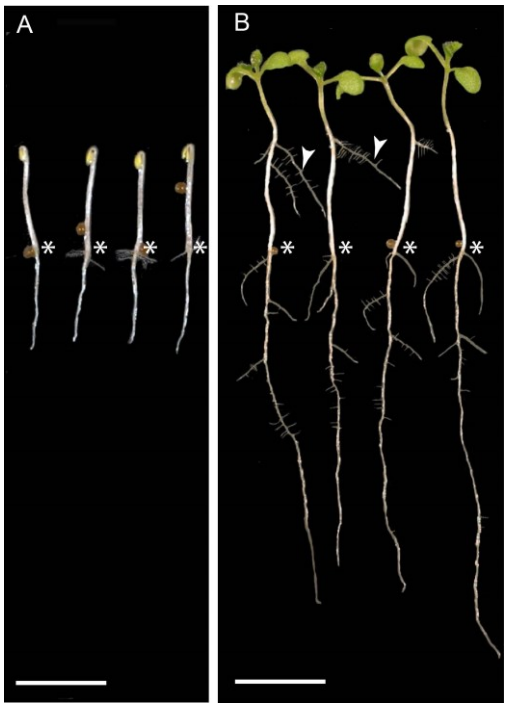


Figure 1: Adventitious rooting in Arabidopsis seedlings.
(A) Dark grown wild-type seedlings. Hypocotyls have reached 6 mm. (B) Wild-type seedlings 7 d after transfer to the light. Arrows indicate adventitious roots on the hypocotyls. Stars indicate hypocotyl-root junction. Bar = 5 mm.

We are interested in dissecting the genetic and molecular mechanisms that regulate the development of shoot-borne roots, also called adventitious roots, using the model plant Arabidopsis thaliana (Fig. 1). To induce adventitious roots, seedlings are grown in the dark until their hypocotyl has reached 6 mm length (Fig. 1A) and then transferred to the light for 7 days (Fig. 1B). Fig.1B illustrated the phenotypic plasticity. All seedlings are from the same genotype and were grown in the same conditions in vitro, but not all of them develop ARs.

By studying Arabidopsis mutants altered in adventitious root initiation (Sorin et al., 2005; 2006) we could identify transcription factors and their regulatory microRNA involved in the control of

adventitious rooting (Gutierrez et al., 2009) and could demonstrate that a subtle balance of activator and repressor *AUXIN RESPONSE FACTOR* (*ARF*) transcripts controls adventitious root initiation. Thus, *ARF17*, a target of miR160, is a negative regulator, and *ARF6* and *ARF8*, targets of miR167, are positive regulators of adventitious rooting (Fig. 2).

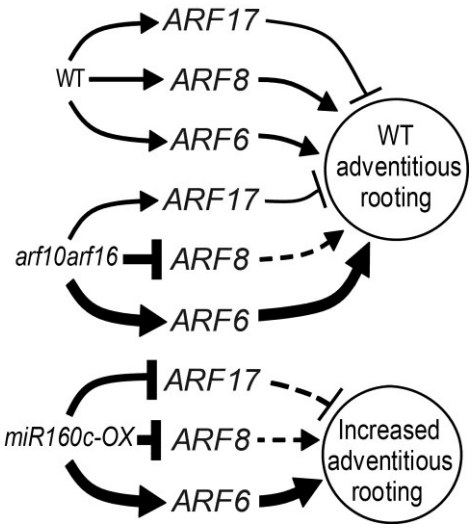


Figure 2: Comparison of *ARF6, ARF8*, and *ARF17* transcript levels found in the *arf10-3* mutant, *arf16-3* mutant, *arf10-3 arf16-3* double KO, and MIR160c-OX line showing the importance of the balance between these transcript levels for control of adventitious rooting (Gutierrez et al., 2009).

The three ARFs display overlapping expression domains, interact genetically, and regulate each other's expression at both transcriptional (Fig.3) and posttranscriptional levels by modulating miR160 and miR167 availability. This complex regulatory network includes an unexpected feedback regulation of microRNA homeostasis by direct and non-direct target transcription factors (Fig.4). These results allowed us to propose a model in which microRNA control of phenotypic variability by controlling the pool of transcription factor mRNAs (Fig. 5).

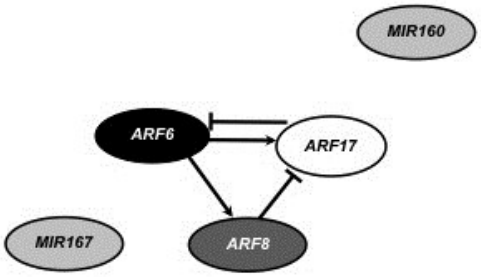


Figure 3: ARF6, 8 and 17 regulate each other expression at the transcriptional level.

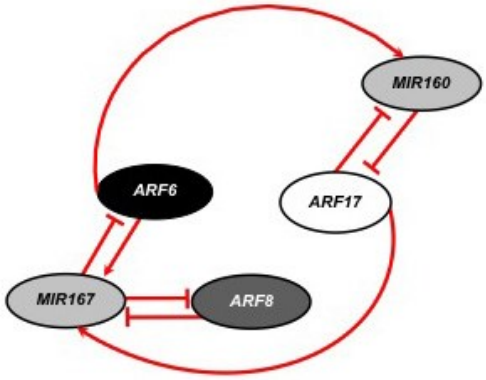
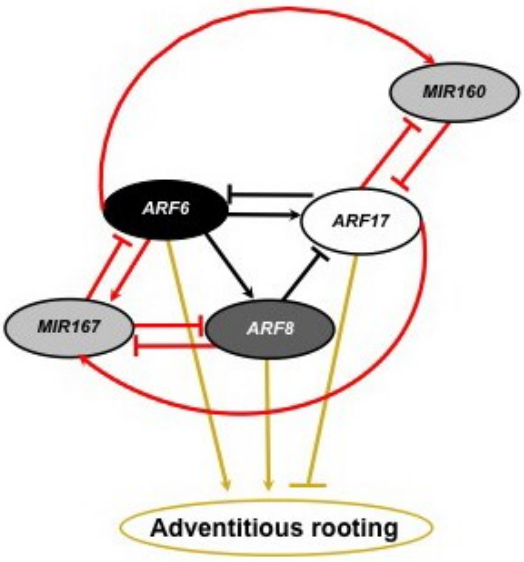


Figure 4: ARF6, 8 and 17 regulate each other expression at the post-transcriptional level by modulating, post-transcriptionally, the amount of their regulatory microRNA.



Black arrows indicate transcriptional regulation.
Red arrows indicate post-transcriptional regulations

Figure 5: A model integrating the regulatory loops between ARF and miRNA genes in the control of Adventitious Rooting based on results obtained in Gutierrez et al.(2009).

Adventitious root initiation is controlled by a subtle balance of activator and repressor ARF transcripts, which is maintained by a complex regulatory network. ARF6 has both a positive and a negative effect on ARF8 and ARF17 transcript levels. It regulates positively ARF8 and ARF17 total transcript levels, whereas it has a negative effect on their uncleaved transcript amount by modulating positively miR160 and miR167s abundance, which drives degradation of ARF17 and ARF8 transcripts, respectively. By regulating miR167s, it also regulates its own uncleaved transcript level. Moreover, ARF8 regulates negatively both ARF17 total transcript amount and miR167s abundance and by consequence ARF6 and its own uncleaved transcript level. In turn, ARF17 represses ARF6 total transcript abundance. In addition, ARF17 regulates positively the pool of miR167s and thereby has a negative effect on ARF6 and ARF8 uncleaved transcript abundance. ARF17 regulates its own uncleaved transcript abundance by feedback regulation of miR160 level.

The above models are based on quantitative data obtained from the characterization of the mutant phenotype (number of adventitious roots) and from the analysis, by quantitative real-time PCR, of the relative transcript amount of the different transcription factors and microRNAs, in the wild-type and mutant backgrounds. These quantitative data will be made available for the workshop.

Questions:

1 - Is the proposed model sufficient to explain the phenotypic variability observed in Arabidopsis hypocotyls?

2 – The ARFs cannot act directly on the microRNA. How can they control the pool of microRNA? Is the option of intermediate genes encoding mimicry microRAN targets a good (the best) option?