Targeting of Genes involved in Egg Cell Development via Artificial microRNAs in *Arabidopsis thaliana*

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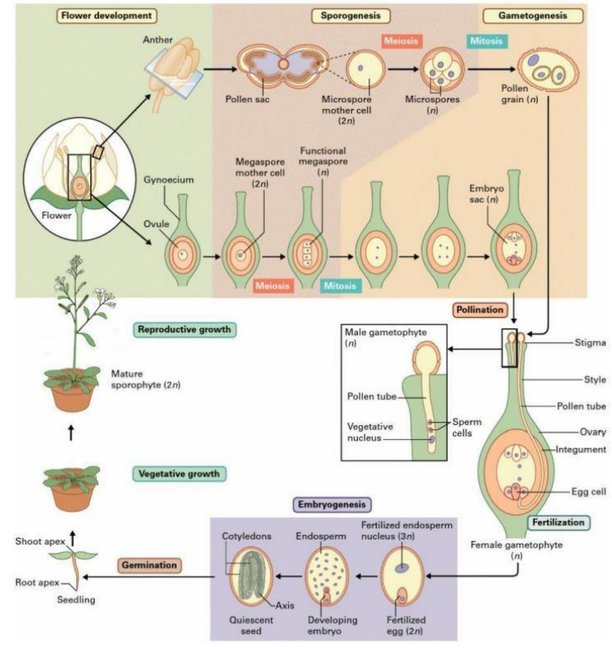
The formation of gametes is a crucial part of any sexually reproducing organism's life cycle. In plants, the gametes are formed in reproductive organs comprised of reprogrammed somatic cells. The female germ cells are generated in the female gametophyte, also called the embryo sac. Whilst the development of the female gametophyte has been morphologically characterized, the underlying molecular mechanisms are poorly understood. The RK domain (RKD) transcription factors have been associated with female gametophyte development in plants and are involved in egg cell formation, however their precise functions remain unknown. The hope is that understanding how these genes control egg cell development will enable engineered plant propagation through seeds without fertilisation (apomixis). To better understand the role of the RKD genes in female gametophyte development four different RKD transcription factors were knocked down in *A. thaliana* plants with the help of two genetic constructs. The downregulation of the RKD transcription factors is based on a knockdown strategy using artificial microRNAs, which are driven by a late-stage egg-cell specific promoter. The knockdown transformation lines showed no egg-cell phenotypes. A side-effect phenotype was observed in a single transformation line likely caused by T-DNA induced chromosomal translocation. This data provides novel insight into the regulatory control of female gametophyte development.

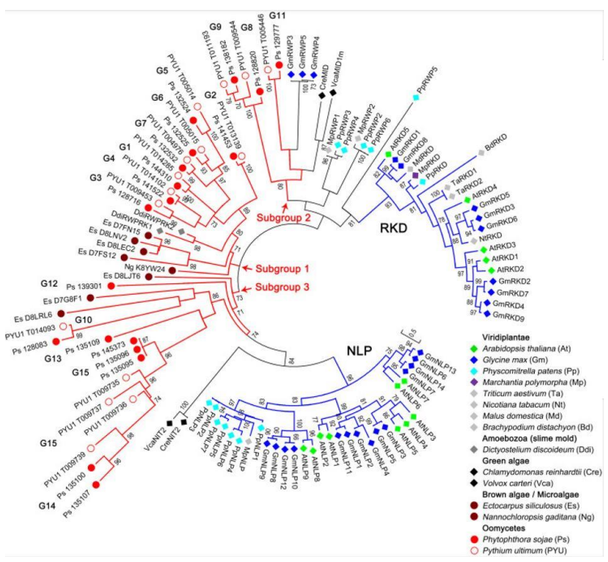
Arabidopsis thaliana

Arabidopsis thaliana is the most thoroughly studied plant species and has been established as the model organism of choice for research in plant biology. There are numerous reasons for this. It has a short life span of 6 weeks and produces a lot of off-spring. *A. thaliana* plants are small and can be easily cultivated in restricted space under various conditions. Furthermore, its entire genome has been sequenced and mapped. Arguably the most important characteristic is its simple, efficient transformation system utilising Agrobacterium tumefaciens, termed floral dip. Floral tissue can be dipped in solution containing sucrose and A. tumefaciens cells, holding a desired genetic construct. The floral tissue develops into seeds, of which a small percentage are transformants, which had a successful uptake of the construct.

Life cycle of *A. thaliana*

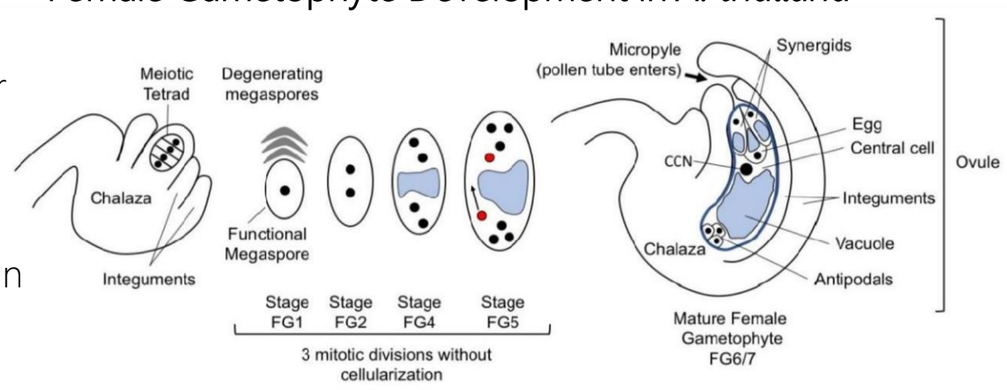


RKD in *A. thaliana*

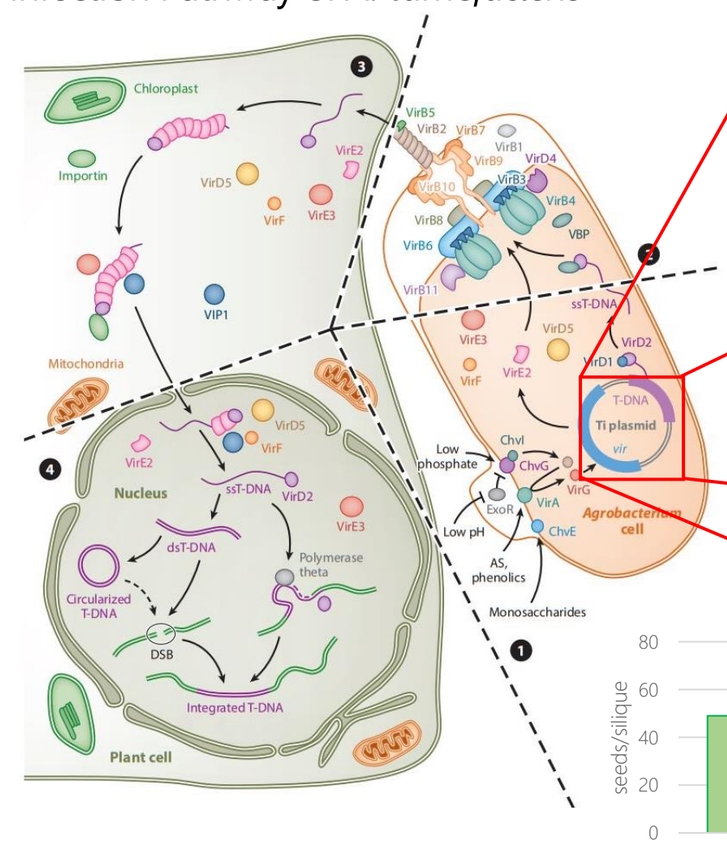


Five RKD genes have been identified in *A. thaliana* (see phylogeny tree on the left). Their exact functionality is still unknown and is of particular interest. Nevertheless the preferential time and location of their expression during the development of the female gametophyte (see on the right) have been identified. *AtRKD1* and *AtRKD2* mainly express in the egg cell during the later stages of female gametophyte development. *AtRKD3* and *AtRKD4* are present both in the early and later phases. *AtRKD5* has been found to be expressed in a more ubiquitous fashion as the transcript levels remain constant through ovule development.

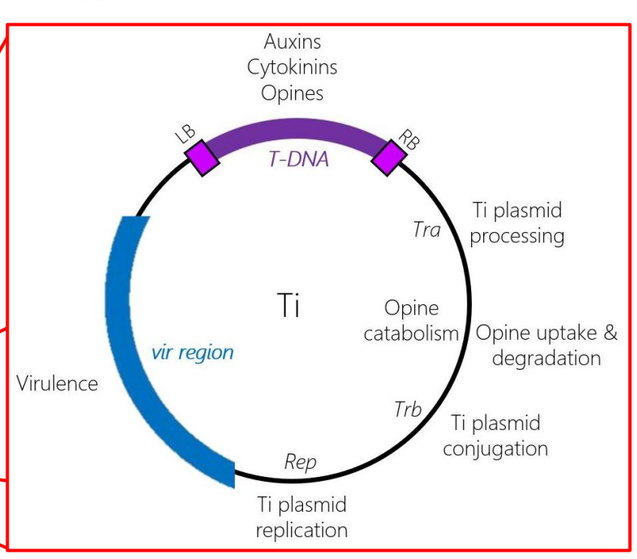
Female Gametophyte Development in *A. thaliana*



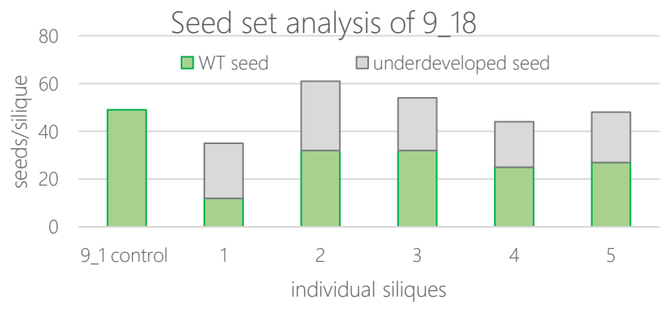
Infection Pathway of *A. tumefaciens*



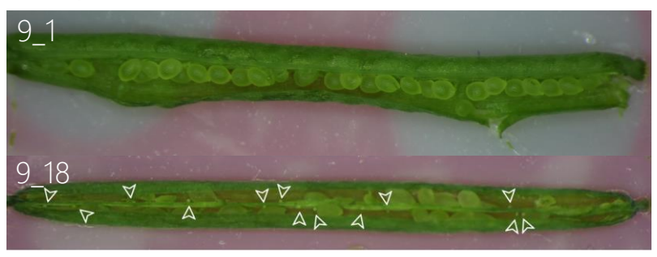
Ti Plasmid



The infection pathway of A. tumefaciens can be simplified into four steps. The Ti-plasmid contains two important regions the vir region, which initialises infection and the T-DNA, which is transferred into the host and contains the genes, which cause crown gall disease. These oncogenes however can be replaced with a desired gene of interest, which circumvents tumour growth in the plant. In the first step, the vir genes are activated when a low pH and acetosyringone are present. Virulence induction leads to the generation of the single-stranded T-DNA. Next, the ssT-DNA (single-stranded T-DNA) is exported out of the bacterium cell. It is then transported into the host cell and gets targeted to its nucleus. Once the T-DNA has arrived at the nucleus it is integrated into the host's genome.

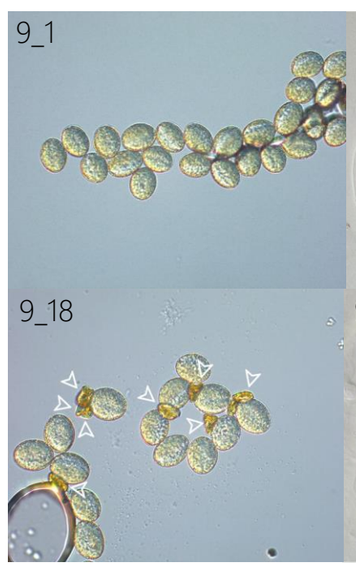


Siliques

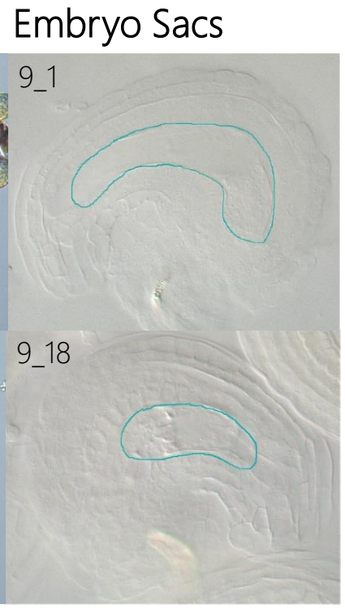


A singular pNSE9 plant (line 9_18) showed a phenotype with a high frequency of underdeveloped seeds. Line 9_1 acts as a control here. The remaining fourteen lines could not be differentiated from the wild-type phenotype. We looked at siliques of line 9_18 and counted the number of wild-type and arrested seeds. The abortion rate was roughly 50%. Half of the Pollen grains and female gametophytes were also arrested. This is a side-effect phenotype likely caused by T-DNA translation.

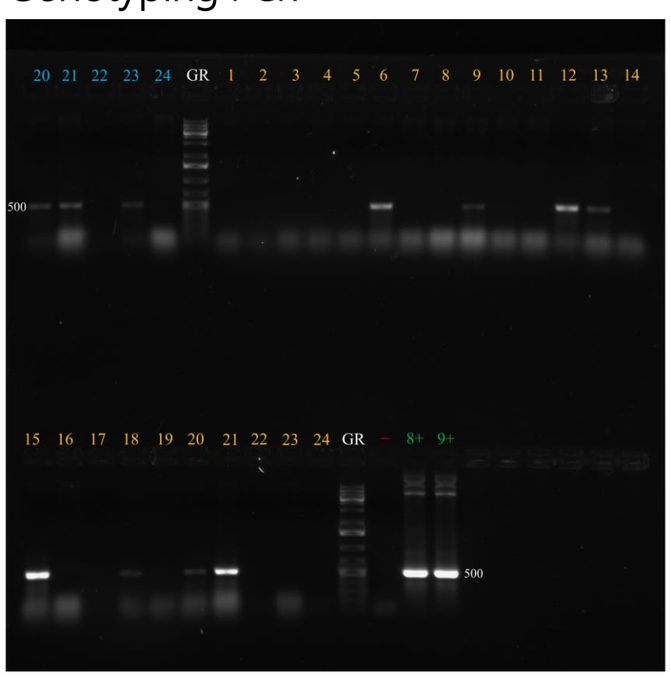
Pollen Grains



Embryo Sacs



Genotyping PCR



For each plasmid line 24 plants were selected, so in total we potted 48 plants onto soil. We conducted genotyping PCR on these plants to check for transformants. The PCR and electrophoresis of the DNA samples of the 48 *A. thaliana* plants showed that at least 15 plants have been successfully transformed, indicated by a band at around 500 bp The sequence between the primers utilised for PCR measured 512 bp.