Auxin production following fertilization drives early seed development in Arabidopsis

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Seed development in flowering plants is preceded by a double fertilization event, whereby one of the paternal sperm cells fertilizes the female egg cell, giving rise to the embryo, and a second sperm cell fertilizes the central cell, which originates the endosperm. The endosperm is a triploid tissue that supports embryo development and, in some species such as Arabidopsis, it is consumed as the embryo develops. Surrounding the two fertilization products is the seed coat, which derives from the ovule integuments and is of purely maternal origin. The successful establishment of a seed requires the fine tuning in the development of these three genetically-distinct structures. Seed development in most plant species is tightly coupled to fertilization, but this requirement can be bypassed in mutants for components of the FERTILIZATION INDEPENDENT SEED Polycomb Repressive Complex 2 (FIS-PRC2), which maintains gene repression by modifying chromatin structure. Lack of FIS-PRC2 function results in the formation of autonomous seeds that develop in the absence of fertilization. Our research shows that FIS-PRC2 represses the expression of auxin biosynthesis genes in the female gametophyte and we postulate that the paternal-specific expression of these genes drives endosperm development after fertilization. Furthermore, our most recent data indicates that auxin production is both necessary for the correct establishment of the seed and sufficient to initiate endosperm development in Arabidopsis.
Communicating within the seed: The endosperm-specific secreted peptide CERBERUS is necessary for embryonic cuticle integrity

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The production of a viable seed requires coordinated development of the embryo and the endosperm. Communication must occur via the embryonic epidermis, which also acts to surround the embryo with the cuticular material essential for seedling dessication resistance (Reviewed in 1). We aim to understand the process of embryonic cuticle formation, and embryo-endosperm crosstalk more generally, through the study of a signaling pathway involving both endosperm and embryo-specific components.

Embryonic cuticle integrity is controlled by heterodimers of the bHLH transcription factors ZOU & ICE1 located in the Embryo Surrounding Region (ESR) of the endosperm (2,3). These control the expression of ALE1, a secreted subtilisin-like serine protease, for which no substrate is known (4,5). Two redundantly acting LRR-RLKs: GSO1&GSO2, expressed in the embryo epidermis, are involved in the same genetic pathway (5,6). No ligands have been identified for these receptors. Based on our genetic analysis, and the identities and locations of the identified pathway components, we hypothesized that one or more apoplastically located peptides might act between ALE1 and GSO1&GSO2 in a signaling pathway bridging the interface between the endosperm and the embryo.

We identified CERBERUS (CRS), a unique cysteine-rich peptide, as a potential candidate for such a function. CRS is expressed specifically within the endosperm. Transient expression in tobacco supports an extracellular localization of CRS. Like ALE1, the expression of CRS is dependent upon the activity of the ZOU/ICE1 heterodimer. Phenotypic analysis of two Knock-Down insertion lines and of CRISPR-Cas9 generated Knock-Out lines reveals a role for CRS in both embryonic cuticle formation and more generally, in controlling the structure of the embryo-endosperm interface. Genetic and molecular interactions with the other components of the signaling pathway, and the effects of overexpression of CRS, are currently being investigated and will be discussed.

1 - Moussu et al, 2013, Plant Signalling and Behaviour
2 - Denay et al. 2014, Development,
3 - Yang et al. 2008, Development
4 - Tanaka et al. 2008, The Plant Journal
5 - Xing et al. 2013, Development
6 - Tsuwamoto et al. 2008, The Plant Journal
Genetic variation for triploid block in Arabidopsis thaliana


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Hybridization studies are important for understanding reproductive barriers to gene flow. They help to elucidate molecular mechanisms underpinning speciation and evolution of plants which could lead to biotechnological improvements in agriculture. Although polyploidy is a common phenomenon in flowering plants, interploidy crosses usually produce aborted or abnormal seeds. However, interploidy crosses using a range of A. thaliana ecotypes produce variable F1 lethality. A strong asymmetrical F1 lethality is seen in some crosses between diploid maternal A. thaliana ecotypes and certain tetraploid paternal ecotypes such as Col-0 which is not seen in reciprocal 4xX2x crosses. Certain ecotypes show a resilience to this asymmetrical F1 lethality such as C24 and Ler. This indicates that genetic variation in certain ecotypes and dosage-sensitive incompatibility play major roles in seed viability. Here, we report on the asymmetrical killer effect caused by a Col-0 tetraploid pollen parent using neotetraploid Col-0/Ler (recombinant inbred lines) RILs and the identification of three additive QTLs on chromosomes 1, 2 and 4 that affect post-zygotic F1 lethality. This information will facilitate the identification of paternal genes that cause F1 lethality and contribute to reproductive isolation.
The non-brittle rachis of domesticated barley independently evolved by loss-of-function mutations in \textit{Btr1} and \textit{Btr2}

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Grain crops were essential for humans to transition from hunter-gathering to agrarian societies. Barley (\textit{Hordeum vulgare} L.) was a founder crop in this process, and the most important step during its domestication was the occurrence and selection of plants that retained mature grains on their inflorescence and thus allowed effective harvesting. Classical genetic studies have established that the non-brittle rachis of domesticated barleys is associated with a genotype of either \textit{btr1Btr2} (\textit{btr1}-type) or \textit{Btr1btr2} (\textit{btr2}-type), suggesting that loss-of-function mutations in either of these two closely linked genomic loci were sufficient to prevent the formation of constriction grooves at the rachis nodes. These disarticulation zones themselves are a major prerequisite for the free dispersal of mature grains in barley's wild ancestor \textit{Hordeum vulgare} subsp. \textit{spontaneum}. In this study, the functional verification of candidate genes of the two \textit{btr} loci was achieved through transgenic complementation of \textit{btr1}-type barley using \textit{Btr1}-candidate ORF1 driven by its native promoter and, likewise, by complementation of a \textit{btr2}-type accession by \textit{Btr2}-candidate ORF3 driven by the maize \textit{Ubi1} promoter. Both approaches independently resulted in the brittle rachis character, which co-segregated with the respective transgene in progeny of the primary transgenic plants. The functionally confirmed \textit{Btr1} and \textit{Btr2} genes share no significant similarity with one another at either the nucleic acid or the amino acid sequence level, which supports the hypothesis that they act complementarily. We further conclude that the non-brittle rachis type independently evolved more than once during barley domestication.
Regulation of fruit growth: From small RNAs to Big Fruit

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One of the most remarkable features of angiosperm plants is the dramatic diversity of fruit size and shape. Whereas a wealth of information has been accumulated on fruit patterning, maturation, senescence and dehiscence, our understanding of the underlying mechanisms that control fruit growth and the genes involved remain a mystery. As a dynamic and multiscale process, growth requires a delicate balance between gene regulatory networks, signaling pathways and environmental inputs. We are employing integrative approaches combining forward and reverse genetics, genome-wide profiling methods, live imaging techniques together with new generation genome editing tools to identify and dissect the players orchestrating fruit growth in Arabidopsis thaliana. This strategy uncovered a number of miRNA-dependent regulatory modules playing key roles in controlling fruit growth. One such module includes the small RNA miR172. This riboregulator is crucial for promoting post-fertilization Arabidopsis fruit growth by integrating developmental and hormone signaling pathways. Recent studies identified that miR172 also influences fruit size in apple and tomato, which suggests that the miR172-dependent regulatory circuit is evolutionary conserved. We are currently investigating additional small-RNA-dependent regulatory modules and developing live imaging tools to monitor fruit cell size in a 4D scale.
Signaling and hormone-response pathways regulating peanut fruit development

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Peanut (Arachis hypogaea L.), characterized by geocarpic fruit development, forms flowers on monopodial inflorescences that emerge from leaf axils and produce 3-5 flowers each. Upon self-fertilization, the zygote initiates divisions until the embryo reaches 8-16 cells and enters a quiescent phase. A meristem at the base of the ovary becomes active, causing the gynophore to elongate and pushing the tip of the ovary underground, demonstrating both gravitropic and mechanical stimulus responses. Sensing its hypogeal position, the gynophore tip curves upward until approximately parallel with the soil surface and the embryo resumes growth. Genes underlying these responses were explored through gene expression profiling using RNA-seq data from reproductive stages of ‘Tifrunner’. Tifrunner is representative of tetraploid cultivated peanut, its two subgenomes derived from diploid A- and B-genome progenitors, A. duranensis and A. ipaensis, respectively. Of 827 transcripts that were specifically expressed during geocarpic development, 395 were B-genome derived and 432 were A-genome derived. Clustering of these genes using Self-Organizing Maps (SOM) revealed six co-regulated expression modules. Annotation of the geocarpic specific genes revealed 10 that were auxin-related, including a putative ortholog of the auxin biosynthetic enzyme YUCCA, an auxin transporter, auxin response factors, ARF6 and IAA9, and a putative ortholog of the auxin efflux regulator PINOID. Additionally, a putative ortholog of FERONIA, which has been proposed to regulate growth in response to mechanical stimuli is specifically expressed at pod swelling and during early pod development. Overall, these data represent a transcriptome map of the genetic regulation of geocarpic development in peanut.
Understanding the control of seed size and number in Brassicas

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Seed size and seed number are major yield components. We are investigating genes controlling these traits in Arabidopsis and Brassica to generate germplasm with increased seed yield. Investigations into the effect of parental gene dosage on seed development have revealed that MADS box transcription factors, particularly the AGAMOUS-like family play important roles in controlling endosperm proliferation and consequently, final seed size. *Brassica oleracea* displays strong parent-of-origin effects on seed development; triploid block due to lethal disruption of endosperm development was restricted to paternal excess. In addition, transcriptome analyses of Brassica homologs of Arabidopsis genes linked to parent-of-origin effects revealed conservation of some mechanisms controlling aspects endosperm behaviour in the two species. Data from Arabidopsis has shown that loss-of-function mutations in the transcription factor AUXIN-RESPONSE-FACTOR 2 (ARF2) result in extra cell divisions within the integument, in a larger seed cavity, and ultimately larger and heavier seeds. We have established that *Brassica rapa* possesses three ARF2 loci and we have obtained TILLING mutants in these lines for further analyses.

During early development of siliques, initiation of ovule primordia is a limiting factor determining the final seed number per silique. Genetic variation in the number of ovules per silique (and therefore seed number per silique) has been observed in Arabidopsis. We hypothesise that harnessing genetic variation in this trait is of key importance in optimising seed number per silique, and therefore seed yield.