



Advances in plant reproduction: from gametes to seeds

At a time of unprecedented human population growth, climate change, and losses in biodiversity, plant reproduction is a particularly strategic research topic. From the very moment that a sporophytic cell switches its developmental pathway to become the megasporocyte or microsporocyte until a seed is finally formed, an intricate network of tightly regulated signalling pathways is in action. In recent years our understanding of the plant reproductive system has evolved enormously, and at a great pace. This special issue includes a collection of reviews that present the current state of the art across several areas of research in plant reproduction.

Flowering plants (angiosperms) represent 90% of all living plant species (Paton *et al.*, 2008). Angiosperms are able to reproduce sexually or asexually, leading to the formation of seeds that enable them to survive to a new generation. Their mode of reproduction has allowed them to become the dominant species and biomass of most ecosystems.

Research into plant reproduction has never been so important for humankind. In a time of unprecedented population growth and losses in biodiversity it is crucial to rapidly and sustainably increase seed production for human and livestock consumption. Food security is a serious issue currently facing humankind. How to feed a growing population at a time of unpredictable climate change? Increasing crop productivity is imperative. There is an urgent and undeniable need to fully understand the fundamental molecular processes that finely regulate plant reproduction and lead to seed formation and development. This will enable increasing crop productivity and higher-quality seed production in a sustainable manner through crop breeding programs. This special issue focuses on diverse aspects of plant reproduction, including apomixis and sexual plant reproduction, germline identity, plant domestication, and fruit ripening. From the moment that a plant switches from vegetative to reproductive growth through to when a seed is finally formed, an intricate network of signalling and regulatory pathways are activated and finely controlled. In recent years remarkable progress has been made in the field of plant reproduction, mainly due to the use of the latest advanced technologies such as next-generation sequencing, high-throughput RNA sequencing (RNA-seq), cutting-edge genetics, live-cell imaging, new cellular isolation technologies, and molecular and quantitative approaches.

Is it all about sex or not?

The greatest evolutionary feature of angiosperms are flowers, which bear inside them the carpels and the stamens—the reproductive organs. This beautiful and highly advantageous structure has evolved a wide range of designs that allow different types of pollination to occur.

Whether in the female or male structures, germline formation depends on a single somatic cell that will shift its developmental program to initiate female or male gametogenesis. The whole process is well described for male gametogenesis but not so for the female gametogenesis, where the mechanisms are still poorly understood, mainly due to the position of the megaspore mother cell, which is deeply embedded in the ovular tissues. In this issue Lora *et al.* (2019) elegantly review this topic across several plant species and raise some interesting questions. Whilst in animals the male and female germlines are established very early in development, in plants this occurs in later stages of the life cycle. This implies that in plants, a pool of somatic cells must be ‘always ready’ to quickly switch from a sporophytic development to a gametophytic one. This whole process must be tightly regulated by gene repression and activation pathways. Lora *et al.* (2019) focus mainly on recent discoveries, for example describing how epigenetic pathways regulate germline development, and they conclude that AGO proteins and sRNAs have important roles in germline establishment. This is consistent with the need for a fast change of cell identity for germline establishment in plants, which may be supported by rapid changes in the histone code, which quickly silence and active the expression of specific genes.

Sexual plant reproduction starts with the landing of a pollen grain onto the surface of a receptive stigma. Here, a compatible pollen grain hydrates and germinates the pollen tube, which rapidly grows through the pistil tissues. Carrying two sperm cells, the pollen tube will grow along the style and the transmitting tract cells, following several cues that will guide it until it reaches an ovule. Near the funiculus, it will turn its direction of growth to the ovule entrance, reaching the filiform apparatus—a specialized structure formed by thickened cell-wall invaginations from the synergids. This is the entrance for the pollen tube into the embryo sac, which is deeply embedded in the ovular tissues. The pollen tube will be attracted to and enter the embryo sac by one of the synergids, which will degenerate afterwards. The two sperm cells released by the pollen tube when it bursts fuse with the egg cell and the central cell

(double fertilization), giving rise to the embryo and the triploid endosperm, respectively. At this point seed development finally starts. A seed may also be produced without meiosis and double fertilization, by asexual reproduction (apomixis), which generates a maternal clone (Hand and Koltunow, 2014). In this case, cells from the ovule adjacent to normal sexual cells may spontaneously assume the identity of germline cells, initiating a form of gametophyte development (Tucker *et al.*, 2012).

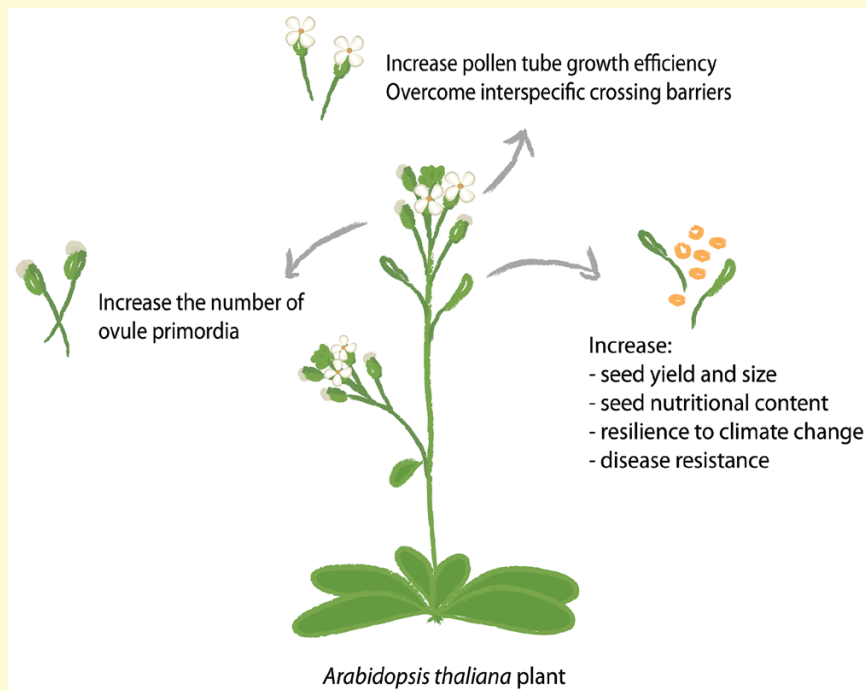
Both sexual reproduction and apomixis are advantageous for plant survival and have agricultural uses. Sexual reproduction allows plant to adapt to an ever-changing environment by generating genetic variability, offering also the possibility of breeding new varieties (Schmidt *et al.*, 2015). Apomixis is an important process for agriculture because it has great potential as a breeding technology, making it possible to generate clones of the mother plant, and thus fixing genotypes of interest in crop species (Hand and Koltunow, 2014). There is a very interesting debate about whether apomixis evolved from sex, or sex from apomixis. Albertini *et al.* (2019) consider this

topic in their review in this issue. They thoroughly review the different mechanisms of apomixis in plants, taking an evolutionary point of view. They suggest that neither sex evolved from apomixis nor apomixis from sex, but that apomixis and sexual plant reproduction are no more than polyphenisms of each other. Meaning that, faced with different environmental conditions, plants will switch on/off certain genes that affect the metabolic status of ovules, thus opting for an apomictic or sexual pathway for seed production. So, the same genome encodes both sex and apomixis. They further discuss how epigenetic mechanisms regulate these switches. However, it is not known which is the most primitive process: sex or apomixis. Apomixis has never been described in major seed crops, so the ability to manipulate the genome to turn on apomixis is of great importance in agriculture, and this can only be achieved by a deeper understanding of the epigenetic and genetic factors that control it.

All of these processes are possible only due to the plasticity of plant cells. Humans take advantage on this special trait by

Box 1. *Arabidopsis thaliana* as a model for crop improvement

A schematic representation of the model plant *Arabidopsis* is shown to illustrate several traits that can be improved for the production of better crops. During early flower development, when the ovule primordia are being developed, researchers can aim to regulate genetic pathways in order to increase the number of ovule primordia that are formed, in order to ultimately increase the number of seeds that will be produced. Later, when flowers are ready for pollination, researchers can manipulate pollen tube growth to increase its efficiency in targeting the ovules to fertilize them, and they can also use knowledge about the pollen tube's interactions with the female tissues to overcome interspecific crossing barriers, which is very useful for agriculture. For example, it allows genes of interest from wild species to be utilized for the benefit of some cultivated species. The ultimate aim is to use all the knowledge generated from plant reproduction research to produce better seeds and fruits, for example by increasing seed size and overall yield, by manipulating the seed nutritional content, and by increasing seed resistance to climate change and to pests. Most of the knowledge generated in studying *Arabidopsis* may easily be transferred into crop species.



using different parts of the plant to regenerate new organs and entire new plants. For example, in this issue [Testillano \(2019\)](#) thoroughly reviews the different factors that are essential for stress-induced microspore embryogenesis. Using *in vitro* embryogenesis we are able to regenerate an embryo and a whole new plant using any kind of somatic plant cell. *In vitro* microspore embryogenesis systems for plant production are advantageous in breeding and conservation programmes, even in recalcitrant crops, as some woody species such as Citrus ([Chiancone et al., 2015](#)). [Testillano \(2019\)](#) describes the advantages of this technique and how it may be improved by manipulating crucial steps of microspore embryogenesis.

Sometimes, it's all about sex

As described above, sexual plant reproduction involves the growth of the pollen tube along the pistil tissues to reach the embryo sac inside the ovule. In this issue, [Lopes et al. \(2019\)](#) review in detail each one of the steps of pollen tube growth and describe the major molecular players involved. The journey of the pollen tube may be divided in different phases: (1) pollen hydration and germination in the stigma; (2) growth along the stigmatic cells; (3) growth through the style and the transmitting tract; (4) growth along the funiculus; and finally (5) entrance into the embryo sac followed by bursting of the pollen tube and release of the sperm cells ([Dresselhaus and Franklin-Tong, 2013](#)). Afterwards, double fertilization takes place by the fusion of one sperm cell with the egg cell and fusion of the other with the central cell, generating the embryo and its nourishing tissue, the endosperm. Finally, seed development starts. During all these growth phases, a series of complex interactions occur between the pollen grain and pollen tube and the female sporophytic tissues and gametophyte. All these interactions are based on cell signalling mechanisms either for recognition, guidance, or attraction between the pollen and the female structures. Despite the enormous progress that has been made in recent years regarding the study of these important steps in plant reproduction, many questions remain unanswered. For example, it is known that *Arabidopsis* pollen tubes become more competent for growth and guidance after passing through a cut style ([Higashiyama et al., 1998](#)). Is it possible that other species possess a factor similar to AMOR, a sporophytic ovular arabinogalactan sugar chain that induces pollen tube competency to respond to female signals in *Torenia* ([Mizukami et al., 2016](#))? Another example that is still poorly understood is how the pollen tube abruptly turns its direction of growth from the transmitting tract into the funiculus. We know now that it requires the K⁺ transporters CHX21 and CHX23 ([Lu et al., 2011](#)), the mitogen-activated protein kinases MPK3 and MPK4 ([Guan et al., 2014](#)), and phytosulfokine (PSK) ([Ladwig et al., 2015](#)). Curiously, only PSK influences pollen tube guidance on both the male and the female side. K⁺ transporters and MPKs act only in the pollen tube. Clearly, there's still a lot to be discovered about this important stage of pollen tube growth.

In the end, it's all about seeds and fruits

Whether it's by sexual reproduction or apomixis, the final result leads to the development of a fruit that harbors the seed(s). The reproductive process goes beyond the double fertilization, after which big transformations take place. After fertilization all the flower structures will change: by a series of cell divisions and expansion the ovary will become the fruit, the ovules become the seeds, the integuments of the ovules will become the seed coat, and the sepals of the calyx and the petals of the corolla will fall. Just like seeds, fruits are an important source of food for humans, and they go through a ripening process. This process is essential for seed dispersal, but is not always favorable for human needs. Ripening will directly affect fruit quality and also its shelf life. In this issue, [Forlani et al. \(2019\)](#) make a critical analysis of this process and discuss the involvement of ABA and ethylene in relation to the interconnected biotic and abiotic phenomena that occur in ripening and senescence, as well as in relation to cell wall modifications. As the authors state, 'ripening, once started, is an irreversible process that can only be delayed'; through a better understanding how this biological process is regulated, we can aim to slow it down and to reduce its susceptibility to pathogen attack.

Most of the fundamental and applied research in plant reproduction has the ultimate goal of utilizing the knowledge obtained in crop plants, to bring about beneficial changes in terms of seed yield, nutrient contents, and adaptations to changing environments. Despite all the research and high-tech achievements that have resulted, many authorities in the world impose barriers to the production of new varieties that contain genetic modifications, or even to organisms that have only been subject to gene editing with no incorporation of foreign DNA, such as those obtained using the CRISPR/Cas9 technology. This is despite the reality that crops developed with gene-edited mutations are 'indistinguishable' from those produced by traditional breeding. In fact, the history of agriculture is full of examples where humans have actively selected for plant mutations, which may have occurred tens of thousands of years ago. The foundations of plant breeding are to take advantage of genetic modifications, regardless of whether they are caused by sunlight, X-rays, or Crispr. Every crop that we know today has already been radically modified from how it originally appeared in the wild. In this issue [Manrique et al. \(2019\)](#) describe the remarkable domestication processes that have led to alterations in the reproductive mechanisms of plants. Studies have revealed how domestication has affected many species at the genetic and molecular levels, demonstrating that humans have long been influencing the genetic modification of plants.

Conclusions

The different phases of plant reproduction where we can intervene to modify processes in order to produce improved crops are illustrated in [Box 1](#). The papers in this special issue not only describe the state of the art within the field, but also raise interesting new questions to be answered in the future. We cannot overstate the importance of research in plant reproduction in helping us to face all the challenges that the

coming years will bring to humankind. An interdisciplinary approach will surely help us to achieve better results and to rapidly transfer our knowledge into crop species in order to improve them. Fundamental and applied research must stand together as allies if our goals of sustainability are to be achieved.

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