Modelling anther opening and pollen release Zoe A Wilson

Introduction

Plant reproduction and the formation of viable gametes (reproductive cells) is critical to fertilisation and seed formation, and is thus essential to the production of most of the food that we eat. It plays a critical role in breeding systems and in the production of hybrid crops, which although difficult and costly to generate frequently out-yield inbred lines by 20-30%. The combination of population growth, climate change and the reduction of available agricultural land mean that it is essential that we develop sustainable, effective agricultural systems that give increased yield but are less damaging to the environment. Achieving these goals requires a deeper understanding of plant reproduction, particularly the formation and release of pollen, that will enable the development of strategies to aid hybrid development and thus food security.

The timing of pollen release is highly controlled to maximise the chances of fertilisation and is regulated partly by the developing pollen grains and partly by the developing maternal anther, which houses the pollen (Fig 1). Opening, or dehiscence, of the anther to release the pollen, involves a series of steps, involving differentiation of the cellular layers in the anther, the formation of secondary thickening of the plant cell wall within a specific cell layer in the anther (the endothecium), enzymatic digestion alongside differential expansion and dehydration. This causes weakening of specific cells combined with mechanical tension on the various tissues in the anther causing breakage and opening. Many mutants have been identified which fail to release their pollen (dehiscence mutants) due to defects in these various stages. Preliminary biological models have been developed to explain the processes and forces involved in anther opening, however no mathematical modelling of this process has been carried out and we currently do not know what are the critical aspects and physical features of this important developmental stage.

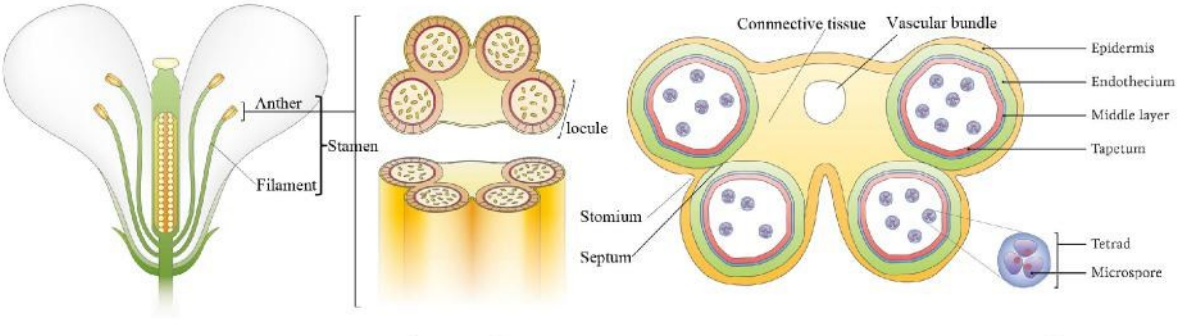

Figure 1. (A) Structure of the flower and anther. (B) Diagram of section through the anther showing the four cell layers of the anther, the outer epidermis, endothecium, middle cell layer, tapetum and the inner cells of the microspores (visible at this stage as tetrads), that will eventually form the mature pollen.

Anther formation

Pollen is formed within specialised organs, stamen, in the flower (Fig 1). These comprise of a wider upper region that forms the anther, containing the pollen, and a stalked region, the filament, containing the vascular bundles (transport system in higher plants), which extends to ensure that pollen is released away from the flower. The three cell layers of the floral meristem (L1, L2 and L3) initially undergo divisions to bring about the formation of the stamen primordium (organ or tissue in its earliest recognizable stage of development). Divisions in the L1 layer increase the surface area of the anther and form the epidermis, whilst L3 cells divide to form connective and vasculature tissues. Divisions in the L2 go to form the various anther cell types (Fig 2A). This results in a final characteristic anther structure comprising of four maternal cell layers; the outer epidermis, endothecium, middle cell layer, tapetum and the inner sporogenous cells which will form the pollen (Fig 2).

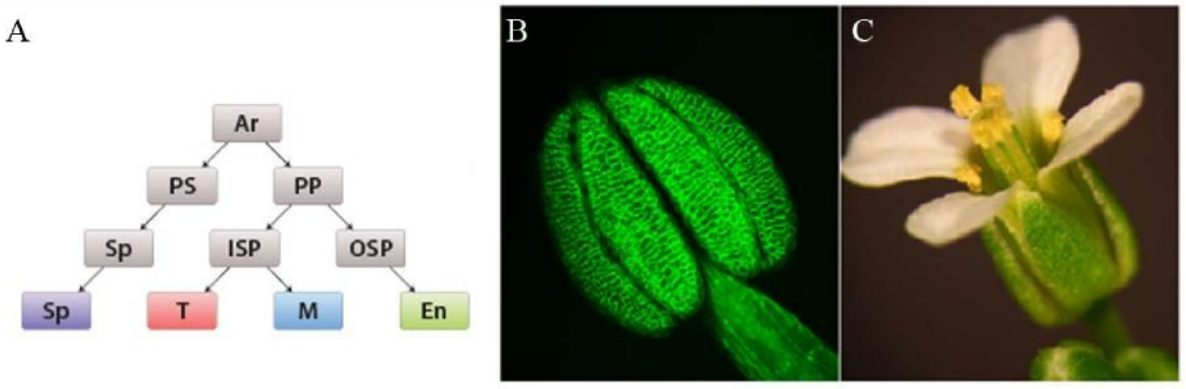

Figure 2. (A) Cell lineage model for the origin of the anther cell layers. Four clusters of archesporial cells (Ar) divide to form the primary parietal layer (PP) and the primary sporogenous layer (PS). The PP layer then goes through a further division to form two secondary parietal layers, the inner secondary parietal layers (ISP) and the outer secondary parietal layer (OSP). The outer (OSP) then divides again and differentiates to form the endothecium layer (En). Whilst the ISP divides and develops to form the tapetum (T) and middle cell layer (M).
(B) Mature anther showing network of secondary thickening in the endothecium. The stomium is beginning to split to reveal the mature pollen grains inside the locule.
(C). Fully dehisced anthers in an open *Arabidopsis* flower.

Process of anther dehiscence

Anther dehiscence (opening of the anther to allow pollen release) is a multi-stage process that involves localised cellular differentiation and degeneration, combined with changes to the structure and water status of the anther to facilitate complete anther opening and pollen release. This process involves the expansion and thickening of endothecial cells, the breakdown of specific cells in the anther wall, combined with anther dehydration and pollen swelling (Bonner and Dickinson, 1989; Keijzer, 1987; Scott *et al.*, 2004). Release of pollen occurs via the degeneration

and splitting of specific cells within the anther, the stomium and septum (Fig 1B, 2).

i) The stomium is formed by the differentiation of epidermal cells within the anther, early during anther development at the stage when the endothecium and connective walls are formed.

ii) After meiosis (cell divisions that result in the reduction (halving) of the chromosomal material to allow for sexual reproduction) and formation of the immature pollen the endothecium undergoes selective deposition of secondary thickening, whilst the stomium and septum region between the locules does not undergo thickening. This localised thickening is critical for subsequent anther opening. Endothecium development is coordinated with pollen maturation and the degeneration of the anther tapetum and middle layer.

iii) Initially degeneration of the septum occurs generating a bilocular anther, which is followed by stomium cell breakage and then retraction of the anther and pollen release. The importance of a functional stomium for dehiscence has been demonstrated by failure of tobacco dehiscence after specific cell ablation of the stomium. Prior to dehiscence the stomium undergoes cell death and splitting, this does not appear to require viable pollen to be present since splitting is still seen in male sterile lines without viable pollen. This breakage appears to be due to weakening of the septum and stomium due to enzymatic digestion, combined with expansion of the pollen and anther wall.

Degeneration of Cells in the Anther

i) Enzymatic breakdown of the septum

Anther dehiscence is thought to involve cell wall degrading enzymes which breakdown the pectin between cells. Several hydrolytic enzymes and proteins linked to cell wall loosening are thought to be involved, including polygalacturonases (PGs), -1,4-glucanases, and expansins. These enzymes are likely to be regulated by plant hormones (including Jasmonic Acid (JA), ethylene and abscisic acid (ABA)).

ii) Programmed Cell Death (PCD) of the septum and stomium

The anther septum and stomium breakdown is also thought to be via a PCD-related process. There have been a number of reports of dehiscence mutants resulting from changes to endothecium and stomium degeneration, which result in endothecium degeneration and indirectly cause failure of stomium region breakage, although the pollen appears normal.

Regulation of endothecium secondary thickening

The process of *Arabidopsis* pollen development has been separated into 15 stages based on anther development. In the *Arabidopsis* anther, the endothecium is first established during anther stage 5. It undergoes expansion during anther stage 6-10 and develops secondary cell wall

thickening during anther stage 11, at which point bar-like ligno-cellulose fibrous bands are deposited. Endothecium secondary thickening is essential for providing the mechanical force for anther dehiscence. We have demonstrated this experimentally with Arabidopsis *male sterile* mutants, for example *myb26* (Dawson et al., 1999) and the *NAC secondary wall thickening promoting factor1* (*nst1*)*nst2* double mutant (Mitsuda et al., 2007). In the *myb26* mutant, anther development appears normal up to anther stage 11, however during the later stages, the lingo-cellulosic wall thickenings seen in the wild type anther endothecium wall, do not form. Degradation of the septum and formation of stomium take place normally, however, the endothecial cells fail to expand, then collapse and the subsequent shrinkage of the anther walls doesn't occur, which results in failure of pollen release (Dawson et al., 1999).

Opening the anther

Observations of dehiscence in *Gasteria verrucosa* suggest that as pollen wall formation occurs, the epidermal and endothecium cells of the anther lose some of their starch, which may affect the osmotic potential within the cell, leading to water flow and changes in hydrostatic pressure. These cells then start tangential and radial expansion, which is followed by endothecial secondary thickening (Keijzer, 1987). However the stomium and septum region between the locules does not undergo secondary thickening. The septum undergoes enzymatic lysis reducing the adhesion between neighbouring cells, alongside the mechanical swelling of the bordering epidermal cells, facilitating stomium opening (Keijzer, 1987). Tangential swelling of the epidermis and endothecium increases the circumference of the locule wall, however because the enzymatic lysis reducing the adhesion between neighbouring cells, alongside the mechanical swelling of the bordering epidermal cells, facilitating stomium opening (Keijzer, 1987). Tangential swelling of the epidermis and endothecium increases the circumference of the locule wall, however because the endothecium walls have secondary thickening the inner locule wall dimensions are fixed. This outer enlargement combined with the inner fixed dimensions causes the locule wall to bend inwards causing disruptions to the stomium cells (Fig 3). The small epidermal cells facing the septum (stomium) are then mechanically broken by inward bending of the adjacent locule walls. The swelling of the pollen grains has also been proposed as a factor in generating the force required for final locule rupture in rice (Fig 3).

Dehydration of the anther wall

The final stages of anther dehiscence involve the dehydration of the endothecium and epidermal cells, which cause the locule to bend outwards (Fig 3). It has been suggested that this occurs, at least in part, as a consequence of evaporation but is more likely due to active removal of water from the anther. Observations of the water status of tomato anthers revealed differential regions of anther dehydration. Conversion of starch to sugar may serve to increase the osmotic potential of the anther tissues to

provide a mechanism for selective dehydration. This has been supported by data on localised sucrose transport around the anther tissues, which may serve to increase osmotic potential and induce selective dehydration within specific regions within the anther (Stadler et al., 1999).

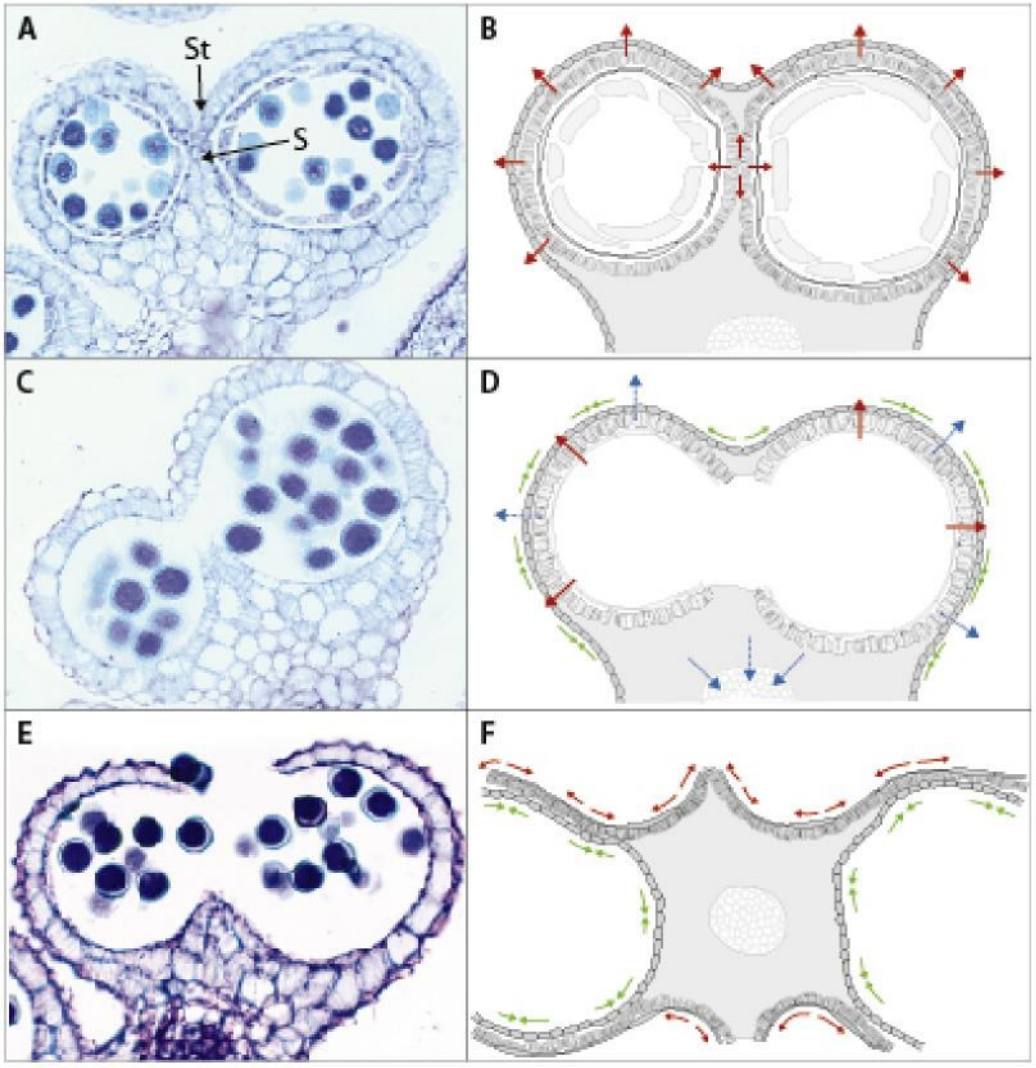

Figure 3. Anther dehiscence. (A,C,E): Light microscopy of the anther, (B,D,F): Diagram of the proposed forces on the anther during dehiscence.

(A) Microspore release, the tapetum starts to breakdown and the endothecium expands and secondary thickening is deposited. As the pollen expands there is an outward pressure (B, red arrows) exerted from the inside of the locule on the anther, which increases in size. However the bands of secondary thickening in the endothecium restrict expansion causing increased tension (D). (B) Enzymatic lysis of the stomium combined with the pressure from the expansion of the pollen causes the septum to break to form a single locule (C, D). At this point the anther walls begin to dehydrate due to evaporation and active water transport (D, blue arrows) causing shrinkage of the epidermal cells, resulting in an increased tension on the stomium

It has also been suggested that movement of potassium ions from the anther locule, prior to dehiscence, into the pollen grains may play a role in attracting water from the surrounding regions and causing the swelling of the endothecium and pollen prior to anther opening. This swelling of the pollen may be partly responsible for stomium rupture.

Water translocation frequently occurs via plasmodesmata connections between adjacent cells, however a large gene family encoding aquaporin proteins have been shown to mediate the passive movement of water between cells. Some aquaporins have been identified specifically within the anther and these are likely to influence cell wall permeability and water movement in the anther. It therefore seems likely that an active process of selected dehydration is occurring within the anther that acts to provide the final force for anther opening (Fig 3).

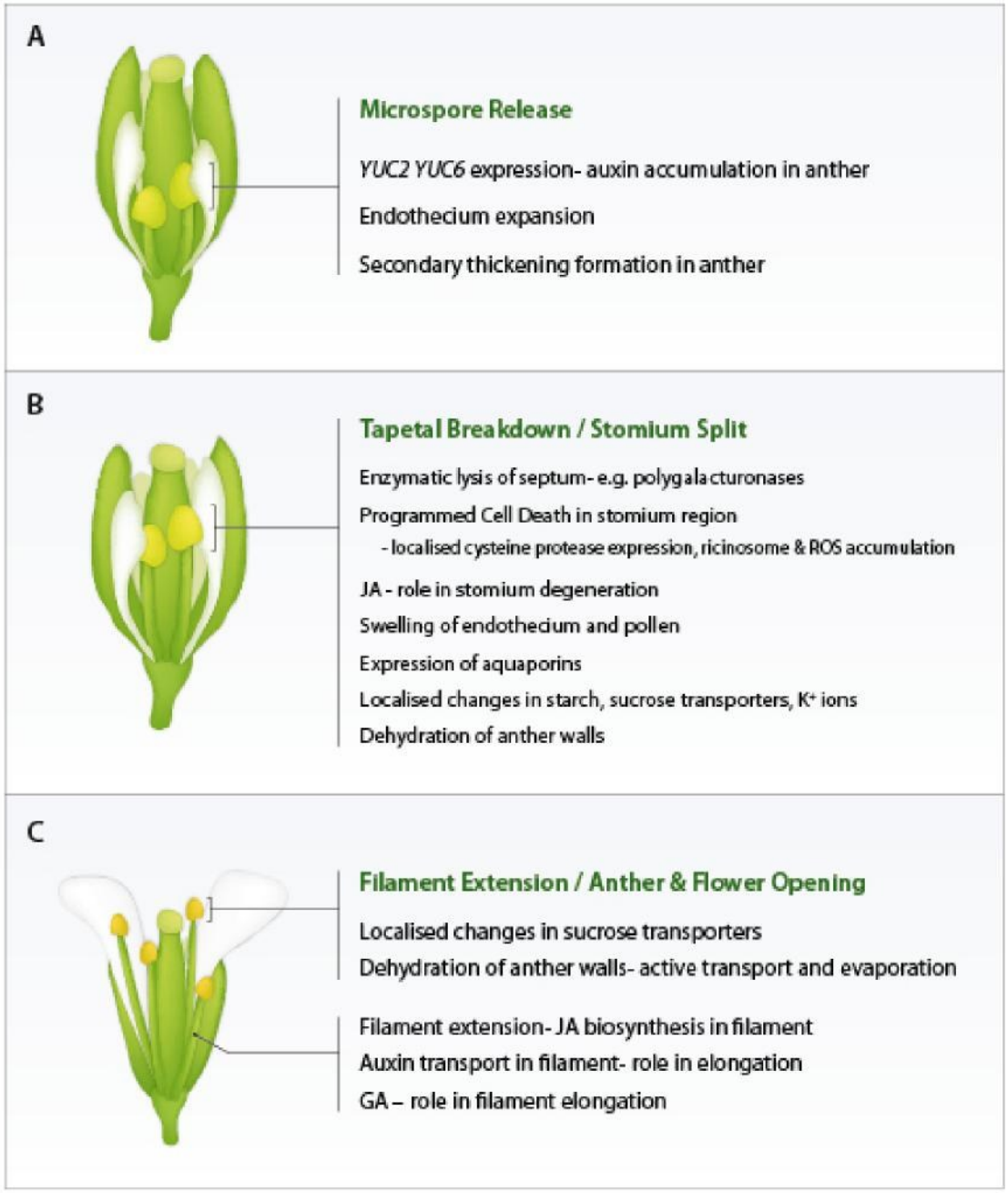

Figure 4. Key developmental events in anther dehiscence.

Summary

Clearly there are many processes involved in anther opening and pollen release, many of which are regulated by hormones. A summary of these stages and the key biological events at each stage is shown in Fig 4, however understanding of how these factors interact is currently very unclear. Understanding the mechanical aspects of anther opening would be extremely valuable, providing increased knowledge of pollen development and release, which has potential for the controlling the future regulation of fertility.

Key Questions

1. How does the regulation of osmotic potentials and water fluxes affect the tissue pressures?
2. Do these pressures lead to the tensions hypothesised in the literature?
3. What is the role of the cell wall secondary thickening in generating the tensions within the anther?
4. Is additional cell wall re-modification required to facilitate opening of the anther?

References

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