Int. J. Dev. Biol. 66: 349-358 (2022)
https://doi.org/10.1387/ijdb.220025mk

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Exploring the sister cells of embryo sac: developmental and functional attributes

INDERDEEP KAUR[1], MONIKA KOUL*[,2]

[1]*SGTB Khalsa College, University of Delhi, Delhi, India,*
[2]*Hansraj College DU and Fellow IoE, University of Delhi, Delhi, India*

ABSTRACT Synergids are metabolically dynamic cells of the egg apparatus and represent an important component of the female gametophyte. Besides directing the growth of the pollen tube towards the micropylar end of the embryo sac, these ephemeral structures make room for the pollen tube cytoplasm. Research carried out on model systems such as *Arabidopsis, Brassica, Capsella, Triticum* and *Torenia* has expanded our understanding of the molecular regulation of the pollen tube journey, its guidance and navigation in the pistil. Recently, the critical role of the central cell in fertilization and prevention of polytubey has also been thoroughly investigated. Sophisticated confocal microscopy, live cell imaging, and molecular tools have helped in furthering our knowledge of the functioning of synergids. Research using high throughput techniques has deciphered the role of various genes that regulate and govern the release of chemotropic substances, cell-to-cell interaction and synergid cell degeneration. Moreover, with the diversity displayed in form and function of organs in the angiosperms, and the switching of roles of the cells of egg apparatus, new insights have been provided into the involvement of synergids both pre- and post-fertilization. The present review provides a comprehensive account of synergids, their role in fertilization and the post fertilization events that have emerged using interdisciplinary approaches in recent years. We also discuss the variations observed in degeneration of synergids and the molecular mechanisms that govern the degeneration. Since environmental factors such as light and temperature have a significant impact on synergids and fertilization, it would be rewarding to study the role of chemo-attractants and other factors in elucidating the functional roles of synergids. Further studies into developing adequate protocols for manipulating synergid functions is certainly required. This research has enormous potential in the advancement of basic science and has potential applications in agriculture, horticulture, and bioprospecting.

KEYWORDS: degenerating synergid (DSY), filiform apparatus (FA), persistent synergid (PSY), programmed cell death (PCD)

Introduction

Synergids, important cells of the Female Gametophyte (FG), are the primary interface between male and female gametes (Sprunck, 2010). Earlier embryologists indicated that synergids have no role in fertilization but only provide guidance to the pollen tube (Maheshwari and Singh, 1967). Evidence regarding the role of synergids in fertilization was available only after exhaustive studies carried out on several taxa by Russell (1982), Kapil and Bhatnagar (1981) and Adhikari *et al.,* (2020). The advent of electron microscopy provided interesting insights into the cellular and subcellular organization of the synergids (Jensen,1965). These haploid cells arise as immediate sisters to the egg cell but exhibit reverse polarity vis-à-vis the egg cell (Sprunck and Groß-Hardt, 2011). Initial development of both the synergids follows the same pattern, but towards maturity, these show differences in structure and functions. One of the synergids (that degenerates first) (DSY) undergoes programmed cell death (PCD) as it prepares to receive the pollen tube. The trajectory of the DSY, also known as receptive synergid, has been monitored by scientists and is well-understood (Jensen *et al.,*.1977; Johri and Ambegaokar, 1984a). DSY attracts and guides pollen tube entry into the embryo sac/FG, besides facilitating pollen tube effusion. The other synergid, referred to as persistent synergid (PSY), has a short life span post fertilization, remains inflated and intact at the

Abbreviations used in this paper: DSY, degenerating synergid; ER, endoplasmic reticulum; FA, filiform apparatus; FG, female gametophyte; PCD, programmed cell death; PSY, persistent synergid; SC, synergid cell.

***Address correspondence to:** Monika Koul. Hansraj College DU and Fellow IoE, University of Delhi, Delhi, 110007, India. E-mail: drmkoul@gmail.com
https://orcid.org/0000-0002-6900-3344

Submitted: 25 March, 2022; Accepted: 31 July, 2022; Published online: 1 August, 2022.

ISSN: Online 1696-3547, Print 0214-6282

time of pollen tube entry (Coimbra and Salema, 1999). Completion of double fertilization triggers rapid elimination of the persistent synergid, an important event in checking polytubey. Interestingly, *Arabidopsis* can reverse polytubey block when the egg cell or central cell remain unfertilized, allowing the second pollen tube to recover from early fertilization failure (Beale *et al*., 2012; Kasahara *et al*., 2012; Maruyama *et al*., 2015). The synergids in apomictics and non-apomictics are also being examined to further elucidate their role in syngamy as well as double fertilization (Musiał and Kościńska-Pająk, 2013). Synergids show some anomalies and subcellular structures that are not characteristic of amphimictics. It is now well understood that the signaling molecules and specific phytohormones have a central role in striking a molecular dialogue between male and female gametophytes (Boisson-Dernier, 2011). Many ions such as calcium are known to mediate response (Higashiyama *et al*., 2003). More information has been added to the role of transcription factors, protein biosynthesis and metabolic pathways (Yue *et al*., 2014). The role of metabolome in governing the pollen tube pathway and delivery is equally important to understand the underlying mechanisms that govern pollen tube entry (Nägele *et al*., 2017).

The focus in recent years has been upon on the identification and elucidation of the role of various genes that govern fertilization via synergid mediated cascading events, making synergids an attractive system for genetic studies (Liu *et al*., 2016). Molecular tools have indicated a complex networking of genes between male and female gametophytes. The present review highlights recent insights at the cellular, ultrastructural, and molecular level into these important units of the female gametophyte. Here we provide a comprehensive account of processes operating at the molecular and transcriptome level. However, there are many aspects that require intricate experimentation. Despite the efforts made by many researchers, it remains unknown how synergids in angiosperm taxa, where genetic transformation/alteration is carried out, will respond to pollen stimuli and other phenoevents. Besides, many aspects of change in the cell fate vis-à-vis synergids in the female gametophyte need further investigation.

Synergids: structure, form, and functions

Synergids, generally two in number, are formed soon after megagametogenesis. The shape and size of synergids vary in different plants (Wilms, 1981). Dimorphic synergids are reported in *Allium tuberosum* (Deng *et al*., 2016) where the smaller one is the receptive or degenerative synergid and the larger one remains persistent. The number of synergids may be one, as in *Peperomia* type; or may be zero, as in *Plumbago* and *Plumbagella*. This variation is attributed to the early steps of megasporogenesis and divisions during megagametogenesis (Maheshwari and Negi, 1955). Synergids are characterized by the presence of wall ingrowths or the filiform apparatus (FA**),** which is a structurally and physiologically important part at the micropylar end. The site of attachment of the FA in synergids runs along the length of the common wall as seen in *Nicotiana* (Mogensen and Suthar, 1979), *Petunia* (Van Went, 1970), *Proboscidea* (Mogensen, 1978) and *Helianthus* (Newcomb, 1973; Yan *et al*.,1991). FA shows morphological diversity; in *Stipa*, the convolutions are spread out and the synergid cytoplasm is trapped between them; massive proliferation of wall material into the synergid has been observed in *Brassica* (Sumner and Van Caeseele, 1989), *Capsella* (Schulz and Jensen, 1968), Gossypium (Jensen, 1965), *Populus* (Russell *et al*., 1990) and *Spinacia* (Wilms, 1981). In *Jusione, Torenia* and *Saintpaulia*, filiform apparatus is exerted from the integuments and exposed directly to the interior of the ovary. The typical FA may be absent from the synergids of some members of the family Asteraceae, including *Calendula officinalis*, *Cichorium intybus, Crepis tectorum, Picris echioides* (Godineau, 1969) and *Crepis capillaris* (Kuroiwa, 1989). However, the thickenings of t he synergid wall in these genera may still carry out the functions of the FA. Histochemical localization has pointed to the presence of polysaccharides in *Capsella* (Schultz and Jensen, 1986) and hemicellulose in FA of *Paspalum* (Chao, 1971). Regarding the presence of FA in synergids of apomictic species, earlier it was hypothesized to be absent, but the work carried out by Płachno *et al.,* (2014) revealed that this was not true for Asteraceae.

Jensen *et al*., in their classical paper (1977), revealed that synergids have an uneven cell wall, which is thickest at the micropylar end, becoming discontinuous or almost absent at the chalazal end. This discontinuity makes it easier for the sperm nuclei to reach the female gametes (Willemse and van Went, 1984; Kasahara *et al.,* 2005). Russell (1993) and later Puwani and Drews (2008), while studying synergids, divided them into three subzones: the synergid hooks zone I; the neck-like zone II; and the head-like zone III. Zone I is the chalazal end of the synergid that is wrapped in central cell cytoplasmic protrusions, the synergid hooks or central cell apical pockets. Zone II, which is neck-like in shape, parallels the synergid hooks that are in the form of a complete ring around the two synergids. Zone III is the micropylar end and is most accessible to the advancing pollen tube, and the part that lies externally to the central cell pocket has a filiform apparatus.

Cytoplasmic organization

Synergids show reverse polarity with respect to egg cells in the same FG. The young synergid cell has numerous small vesicles or large vacuoles and dense cytoplasm (Folsom and Peterson, 1984). Gradually, the vesicles fuse to form a single large vacuole as in *Capsella* (Schulz and Jensen, 1968), several small ones as in *Zea* (Diboll and Larson, 1966), or one large and several small vacuoles spread uniformly, as seen in *Nicotiana*.

The vacuoles with high calcium content - up to 50% of their dry weight - provide chemical signals for pollen tube attraction and entry into the embryo sac (Jensen, 1965; Chaubal and Reger, 1992a, 1992b). Higashiyama (2002) observed that the mature synergid cytoplasm is densely occupied by endomembrane compartments (mitochondrion, ribosomes, dictyosomes, ER and plastids). This organization is reflective of a highly active secretion system generating messenger molecules towards the micropylar end, where chemotrophic attractants are synthesized. The organelles in mature synergids in some Poaceae members show a polarized distribution: plastids near the chalazal end and most of the mitochondria and dictyosomes at the micropylar pole, with ER and nucleus in close association and dispersed ribosomes (Jane, 1997). Vacuolation towards the chalazal pole of persistent synergid post pollination was also observed by Jane (1997). Ultrastructure of synergids in some apomictic species display changed orientation in microtubules (Greehaam and Chapman, 1990). Persistent synergids showed increase in number of mitochondria, plastids and ribosomes and facilitated nutrient transport in *Beta vulgaris* (Li, 2014). Plachno *et*

al., (2014) compared synergid morphology and ultrastructure of *Taraxacum tenuifolium* (normal amphimictic) with the apomictic tetraploid *Taraxacum brandenburgicum* and found that synergids in both species possessed a filiform apparatus. However, in *T. brandenburgicum*, both synergids were persistent even after the formation of embryo and endosperm, suggesting that some anomalies occur post-fertilization. Secretory structures in the vicinity of the filiform apparatus, including lipid bodies and starch grains, were observed in synergid cytoplasm of *S. rupestre* (Brzezicka and Kozieradzka, 2021)

Synergid haustoria

Development of haustoria and other haustorial structures arising as branches and buds from reproductive cells is a common feature in angiosperms. Synergid haustoria have been reported in Asteraceae members e.g., *Calendula, Cortedaria, Cotula*, *Mutsia,* and *Ursinia* (Davis 1962; Philipson, 1981). Presence of extensive synergid haustorial structures has been reported in Santalaceae e.g., *Quinchamalium*. In *Cortedaria,* the micropylar end encroaches into the nucellus and contains numerous transfer cell walls (Philipson, 1981). Crassulaceae members are also known to have elongated and extensive synergid haustoria. The nutritive role of persistent synergid haustorium with elaborate cell wall ingrowths after fertilization was observed by Huang and Russell (1992), 1994. In *Cotula australis*, synergid itself acquired haustoria-like structure, and the presence of finger- like projections in the haustoria, suggesting their role in transfer of nutrients (Johri and Ambegaokar, 1984b).

Functions

Synergids play a pivotal role in double fertilization, facilitating the fusion of one male gamete with the egg cell and the fusion of a second male gamete with the central cell (with either the polar nuclei or the fused product of polar nuclei, the secondary nucleus). The primary functions are attraction of the pollen tube towards the micropyle, its guidance to the female gametophyte, and intercellular communication during pollen tube reception (Higashiyama *et al*., 1998). The pollen tube grows along the placental surface, then towards the funicular surface, before it enters the micropyle and finally the female gametophyte. According to Punwani *et al.,* (2007), the female gametopyte guides the pollen tube at placental to funiculus and provides guidance from funiculus to the micropyle. According to Shimizu and Okada (2000), funicular guidance signals and micropylar guidance signals help the pollen tube to grow from the funiculus to the micropyle. Funicular guidance is controlled by both sporophytic and gametophytic tissues that operate through ovular signals (Dresselhaus and Franklin-Tong, 2013), and micropylar guidance is regulated by chemical signals that ensure a short-range pollen tube attraction. Higashiyama *et al.,* (2001) demonstrated that a single synergid cell was sufficient to generate attraction signal; however, the presence of two cells compounded the effect. T h e presence of active dictyosomes and their cisternae in synergids indicates their role in secretion. Several studies indicated FA as the site of pollen tube entry; however, Leshem *et al.,* (2013) have demonstrated that the pollen tube does not enter directly into the synergids through the filiform apparatus. It grows through cell wall invaginations beyond FA into a zone of SC (synergid cell) where the pollen discharge occurs.

Though the FG of sexually reproducing plants has been extensively investigated, the details of FG in plants with asexual seed formation have only just begun to emerge (van Baarlen *et al*., 2002). Synergid apogamy has been observed in a few taxa where specialized synergid cells give rise to embryos. In *Oryza sativa* (rice AP III), the role of synergids in embryo formation has been well explained (Mu *et al*., 2010). The nutritional role of synergids has also been studied, as these have active machinery for synthesis of a plethora of nutrients that support the growth of other cells of the FG as well (Płachno and Swiatek, 2012). Even in orchids where the endosperm is absent, the role of synergids in nutrition has been illustrated to be that of transfer cells (Alvarez and Sagawa, 1965).

Synergids: A fresh look and insights using molecular approaches

Recent molecular studies have increased understanding of the role of various genes responsible for morphogenesis, differentiation, functions, and degeneration of synergids. Many genes for secretion are expressed in synergids (Ohnishi *et al*., 2011). Research has revealed that cell-to-cell communication between the two synergids is extremely important for their proper functioning and subsequent fertilization. The studies are supported by the work on myb 98 mutants. In such mutants, one of the synergids acquires egg cell fate. It is the communication between two synergids that restricts only one synergid cell to becoming an egg cell; the other synergid continues to produce attractants for the pollen tube (Susaki *et al*., 2021). This cell-cell communication works fast and helps determine to which of the two synergids should acquire the egg cell fate. Genes coding extracellular signaling molecules expressed preferentially in the synergids is a characteristic of dicots (Jones-Rhoades *et al*., 2007). The sequence of fertilization involves the final entry of the pollen tube into the synergid, arrest of growth of pollen tube, mutual demise of pollen tube and receptive synergid, and finally, the delivery of male gametes. The precise crosstalk between male gametophyte and FG is a prodigious event involving several genes and pathways operating in a coordinated manner. The signaling system helps synergids and pollen tube to sense their mutual proximity, which is a fatal attraction leading to death of the two – pollen tube and the receptive synergid. The female gametophyte communicates with the incoming pollen tube via synergids, and this interaction regulates and slows down pollen tube growth, finally arresting the growth. The crosstalk between synergids (female gametophyte) and pollen tube (male gametophyte) culminates in the bursting of the pollen tube, which also witnesses a simultaneous degeneration of the receptive synergid. This act of a mutual demise is distinctive of angiosperms. Bursting of the PT (pollen tube) is a critical event in the sexual phase of the plant and must occur with great precision. The reproductive success depends on the integrity of pollen tube that is maintained through the style and its bursting at the right time and place upon its arrival in the receptive synergid. Bursting too soon, or failing to burst when it should, results in a reproductive failure. A few key female factors such as LURE peptides and FERONIA (receptor like kinase) and TFs controlling "pollen tube reception" that instruct the cessation and subsequent discharge of the penetrating pollen tube, leading to sperm release, have been identified (Johnson and Preuss, 2002; Kessler and Grossniklaus, 2011; Drews and Yadegari, 2002; Berger *et al*.,

2008). These factors, besides FERONIA (FER), probably the first factor to be involved in pollen tube reception (Escobar-Restrepo *et al*., 2007, Huck *et al*., 2003) are NORTIA (NTA) (Kessler *et al*., 2010), LORELEI (LRE) (Tsukamoto *et al.,* 2010), and early nodulin-like proteins (ENODLs) (Hou *et al*., 2016). Factors like HERCULES RECEPTOR KINASE1 (HERK1) and ANJEA (ANJ) are strongly localized at the filiform apparatus of the synergid cells and mediate pollen tube reception (Lopes *et al*., 2019). TURAN and EVAN are also synergid expressed genes required for pollen tube reception (Lindner *et al*., 2012). Though the role of MYB98 in FA formation is well documented, it is now known to affect morphology and cellular dynamics of the synergid cells (Kasahara *et al*., 2005). MYB98, which is seen in the synergid cell nuclei, is known to bind to specific sequence of the DNA. It acts as a transcriptional regulator with 16 downstream genes, of which at least one DD11 is reported to be a target of MyB98 (Punwani *et al*., 2007). DD11 can bind to MYB98 and thus activate the expression of synergid-gene regulatory network. This activation of a network of genes is responsible for guiding the pollen tube and for formation of the filiform apparatus. MYB98 is also required to produce chemoattractants for pollen tube (Kasahara *et al*., 2005). LRE and FER interact to receive pollen tube in the female gametophyte, FER encodes receptor like kinase LRE (Lorelei) and NTA (Nortia). LRE interacts with FER in the lumen of the endoplasmic reticulum, acting as a chaperone, and brings FER to filiform apparatus (Li *et al*., 2015a). In FA, LRE acts as a compressor with FER and perceives signals given by pollen tube. The changes in the calcium profile are then triggered, besides production of ROS (Ngo *et al*., 2014). One of the pollen tubes enters synergids, and LRE inhibits further growth of multiple pollen tubes through a signal cascade. The LRE participates in the pollen tube reception by both initiating and reducing its growth after it interacts with the synergids. According to Rotman *et al.,* (2008), the pause in pollen tube growth may then activate additional signaling between pollen tube and synergids, which then completes pollen tube reception. The LRE has two functions to play: chaperoning FER in the ER en route to FA; and acting as a co-receptor with FER in FA (Li *et al*., 2015a).

The pollination stimulus also brings about a ROS (reactive oxygen species) spike inside the female gametophyte (Martin *et al*., 2013). These reactive species from the female gametophyte bring about pollen tube rupture and are generated from NADPH oxidases (NOXs) in the female gametophyte (Duan *et al*., 2014). The interactions between RAC/ROPs (RHO-type GTPases; Ras homologous proteins) and FER (FER-ROPGEF-RAC/ROP complex) and participation of LRE mediate the activation of NADPH oxidase for ROS generation (Duan *et al*., 2014). A signaling pathway comprising FER-RAC/ROP-NADPH oxidase-ROS between the pollen tube and female gametophyte is required, and LRE is also a part of the signaling pathway, making it too intricate (Li, *et al*., 2015; Nissen *et al*., 2016). This interaction brings about formation of GTP from GDP. The RALF34 in the inner integument binds to the BUPS/ANX receptor complex in the pollen tube. RALF34-BUPS/ANX receptor complex ruptures pollen tube in the synergid cells. Some male transcription factors involved in pollen tube reception are MYB97, MYB101 and MYB120. Kasahara *et al*., (2005) and Marton *et al.,* (2005) identified genes MYB98 and ZmEA1 respectively in *Arabidopsis* and Maize. In *Arabidopsis*, MYB98 gene is expressed predominantly in the synergids and is known to encode R2R3-MYB transcription factor. Model systems such as *Nicotiana* and *Arabidopsis* are being studied to decode other attributes responsible for targeted entrance of pollen tube into the micropyle by plant scientists. A network of several molecules has been found to play an essential role in the pollen tube journey which includes pollen tube guidance and reception. The receptor molecules such as Buddha Paper Seal1and2 (BUPS1/2), FERONIA homologue ANXUR1 and ANXUR 2 (ANX1/2) guide the entry of the pollen tube and bring about its rupture, facilitating sperm delivery in the female gametophyte. Several small peptides known as Rapid Alkalinization Factors (RALF) 4, 19 and 34 as their ligand-molecules modulate the receptors' functions (Ge *et al*., 2019). While the receptors and RALF4 and 19 are required to maintain pollen tube integrity during the growth process, RALF34, expressed in the female, facilitates the bursting process (Somoza *et al*., 2021). However, the role of multitasking FERONIA appears to be a central part of the entire series of events (Liu *et al*., 2016). ROP/RAC activates tip growth by acting upstream of Ca^{2+} and may regulate the tip-localized influx of Ca^{2+} and the formation of the Ca^{2+} gradient. Localized activation of RHO GTPases of plants (ROPs) and downstream activation of Ca^{2+} signals have been reported by Malho and Trewavas (1996). In *Arabidopsis*, Takeuchi and Higashiyama (2012) reported specific receptor-like kinase 6, PRK6 in the pollen tube tip. PRK6 interacts with pollen expressed ROPGEFs (RHO of plant guanine nucleotide exchange factors), facilitating pollen tube growth by activating the RHOGTPase ROP1. Thus, PRK6 is the main receptor in the pollen tube which senses AtLURE1 and activates ROP signaling. Wang *et al.,* (2016) identified MDIS1-MIK (MALE DISCOVERER 1-MDIS1 and MDIS1- INTERACTING RLK 1 - MIK1), a cell surface receptor heteromer present on plasma membrane of the pollen tube. These are kinase containing extracellular leucine-rich repeats and an intracellular kinase domain which perceive/sense the AtLURE 1 attractant. AtLURE1 binds to the extracellular domains of MDIS 1- MIK. Two novel members *TURAN* (*TUN*) and EVAN (EVN) are also identified in the pollen tube reception pathway. These encode a uridine diphosphate (UDP)- glycosyltransferase superfamily protein and a dolichol kinase respectively, both required for N- glycosylation in ER present in the pollen tube (Lindner *et al*., 2012). Glycosylphosphatidylinositol-anchored protein LORELEI (LRE), LLG1 and early nodulin-like protein functions (ENODLs) seem to be the co-receptors for FER signaling at the entrance of female gametophyte. These regulate the activity of RBOHs and ROS generation in synergid. FERONIA interacts with ROP-guanine nucleotide exchange factors (RopGEFs, where Rop is Rho-like GTPases from plants) and brings about formation of GTP from GDP. This activates RAC/ROPs that direct the pollen tube growth. Accumulation of NTA in the Golgi apparatus is seen during synergid differentiation and in FA during pollen tube reception (Jones *et al*., 2017). It controls the synergid activity in response to extracellular ROS present in the micropylar end.

The role of the central cell in guiding the pollen tube functioning has also been better understood. A protein, central cell guidance (CCG) present in the central cell and not reported from t h e synergid or egg cell is involved in regulating the pollen tube guidance mechanism. It co-regulates CRPs through a set of other interacting genes, namely CCG BINDING PROTEIN1 (CBG1), mediator complex (MED), and central cell-specific AGAMOUS-transcription factors including LUREs (Li, *et al*., 2015b). According to Chen *et al.,* (2007), this protein alone is sufficient to provide pollen tube guidance, indicating the critical role of central cell in pollen tube

guidance. Recently, several members of the *Cr*RLK1L family have been identified as receptors for RALF peptides. While FER for RALF1 and RALF23; ANX1/2 and BUPS1/2 have been related to RALF19 activity, THE1 (THESEUS1) is a pH-dependent receptor for the peptide rapid alkalinization factor RALF 34 (Gonneau *et al*., 2018). This signaling module has a role in the fine-tuning pollen tube bursting, as THE1 also binds to ANX1/2 and BUPS1/2 receptor kinases, which form a complex in the pollen tube. RALF34 is expressed in the ovule and not in the pollen tube but competes with pollen-tube-specific RALF4 and RALF19 for binding to ANX1/2 and BUPS1/2 to regulate pollen tube growth and sperm cell release. RALF34, therefore, may be considered a spatial paracrine signal given from the female gametophyte. It interferes with the autocrine cell wall integrity maintenance system, triggering pollen tube rupture and release of sperm cells (Ge *et al*., 2017). The final step involves a signal from endosperm to synergid nucleus when the identity of the synergid cell completely disappears due to the nuclear disorganization during endosperm proliferation. This step is regulated by FIS-PRC2 (fertilization-independent seed-polycomb repressive complex 2), an endosperm-specific polycomb gene silencing complex specific to the central cell and the endosperm (Köhler *et al*., 2012). This implies that polytubey block is activated by central cell fertilization through the FIS-PRC2 pathway.

How and why of DSY demise

Synergids or the siren cells attract the pollen tubes once they have completed their journey in the style. This interaction leads to degeneration of the receptive synergid by programmed cell death, a key step during pollen tube reception (Russell, 1993; Higashiyama, 2002). The degenerated state sustains itself until cessation of pollen tube growth and the release of the pollen tube contents (van Went and Willemse, 1984). This has an evolutionary implication in terms of increasing control of the sporophyte over the gametophyte (Lora *et al*., 2016). In evolutionary-derived angiosperms, cues for pollen tube guidance toward the FG are provided by the outer integument (Herrero, 2000, 2003). Degeneration of synergids can also occur pre-pollination due to some ontogenetic changes (Li *et al*., 2009). Synergid degeneration in *Arabidopsis* (Leyden *et al*., 2015), barley (*Hordeum vulgare*) and pearl millet (*Pennisetum glaucum*) occurs in the absence of pollination (Engell, 1988; Chaubal and Reger, 1992a). However, in most plants, the programmed cell death (PCD) of the PT and one synergid occurs simultaneously, suggesting that synergid degeneration is influenced by the pollen tube signal (Russell, 1992; Drews and Yadegari, 2002). In a few plants however, synergid degeneration is triggered after coming in direct contact with the pollen tube (Russell 1992, Sandaklie-Nikolova *et al*., 2007). Therefore, it is still not completely confirmed whether or not the pollen tube discharge is an absolute requirement for receptive synergid degeneration. According to Russell (1992) and Higashiyama *et al.,* (2000), pollen tube discharge is mechanical and may occur due to massive increase in volume and pressure, which results in a bursting of the synergid membrane as seen in *T. fournieri* (Higashiyama *et al*., 2001). The synergid demise invariably involves a dramatic decrease in cell volume, collapse of the vacuoles, and complete disintegration of the plasma membrane and most cell organelles (Huang *et al*., 1993). However, several lines of evidence suggest that synergid degeneration does not result from mechanical breakdown of PT in *Arabidopsis*. Amien *et al.,* (2010) reported the presence of a synergid-expressed defensin-like (DEFL) protein, ZmES4, which interacts with the KZM1 (potassium channel) present in pollen tube in maize. Subsequent interaction between ZmES4 and KZM1 results in channel opening and K+ influx, which leads to water uptake and culminates in osmotic pollen tube burst. As ZmES4 is involved in pollen tube bursting, it is degraded soon after fertilization. According to Kessler and Grossniklaus (2011), the pollen tube and synergid coordinate their mutual demise, and in the process, male gametes are delivered for double fertilization. An elaborate machinery of the male gametophyte residing near the pollen tube tip is activated and establishes communication with the cells of FG, leading to its burst. In *Arabidopsis,* parallel to the FERONIA signaling pathway, molecular events involving AGPs play an active role in death of the receptive synergid. The AGPs are arabinogalactan proteins consisting of a large family of hydroxyproline-rich proteins, anchored to the plasma membrane and are extremely rich in sugars. The expression of AP1G, the γ-subunit of the tetrameric Adaptor Protein1 (Adaptor protein complexes are key regulators of cargo sorting into vesicles) is involved in acidification of the vacuole, an important mechanism in synergid degeneration (Wang *et al*., 2017*)*. AP1G is crucial for synergid-controlled pollen tube reception and mediates synergid degeneration through V-ATPases, the enzymes that mediate vacuolar acidification. According to Schumacher and Krebs (2010), the acid content in vacuoles initiates proton homeostasis, which further affects endomembrane trafficking through ROS and Ca^{2+} spiking. Direct evidence regarding the role of ROS in synergid cell degeneration is still to maintain high ROS at the micropylar end in the ovule. It has been seen that ROS accumulation occurs around the filiform apparatus, which also has FERONIA (Duan *et al*., 2014). FERONIA is known to induce RBOHD- dependent ROS production via the GEFs (Guanine nucleotide exchange factor) and ROP. Respiratory burst oxidase homologues H and J (RBOHH) and (RBOHJ) are regulated by receptor-like kinases (RLKs) such as ANXUR1 and ANXUR2, colocalized in the same plasma membrane domain at the pollen tube tip. They act downstream of ANXURs to control ROS production during PT growth (Boisson-Dernier *et al*., 2013). By regulating RBOHH and RBOHJ, pollen tube integrity is maintained. The synergid cell death module also requires a heterodimer VAL-VDD (VALKYRIE- VERDANDI). VDD and VAL are transcription factors of the family REM (Reproductive Meristem) as reported by Mantegazza *et al.,* (2014). These are direct targets of ovule identity complex - STK-SEP3 (SEEDSTICK-SEPALLATA3). VAL-VDD heterodimer is involved in both PCD of receptive synergid and pollen tube death by bursting (Mendes *et al*., 2016). This controls downstream expression of mitochondrial chaperon - GFA2 (gametophytic factor 2) which is responsible for the mitochondrial protein folding as pointed by Christensen, 2002). Thus, multiple factors are operating in the demise of DSY, each following its own pathway with downstream cascading events.

Persistent Synergid (PSY) - the journey forward

A single pollen tube delivers two sperms for double fertilization, resulting in embryo and endosperm formation. Once this is achieved, polytubey (the phenomenon of entry of multiple pollen tubes into the FG) is checked through polytubey block (Beale *et al*., 2012; Beale and Johnson, 2013). Upon successful fertiliza-

tion, the persistent synergid cell (PSY) is destined to die. The DSY undergoes PCD when receiving pollen tube discharge, while the persistent synergid cell undergoes nuclear degeneration within a few hours of successful double fertilization (Beale *et al*., 2012; Völz *et al.*, 2013). Chromosomal condensation and the loss of nuclear envelope integrity have been observed after the SE (synergid-endosperm) fusion by Maruyama *et al.,* (2015). Several researchers - Schulz and Jensen (1968), Beale *et al.,* (2012), Völz *et al.* (2013) - have indicated nuclear disorganization as a feature of synergid inactivation. Even though the milieu in which persistent synergid nucleus and the endosperm nuclei lie is common, only the PSY nucleus is selectively eliminated during the SE fusion. In many cell types where the programmed cell death occurs, nucleases are thought to have a role in nuclear degeneration (Ito and Fukuda, 2002; Furuta *et al*., 2014). However, the mechanism involved in death of the persistent synergid appears to be different as the elimination in this case is selective. Alternatively, the selective nuclear elimination may be caused by premature chromosome condensation and disorganizes synergid nucleus. Studies with artificial fusion between two cells at different stages demonstrated that M phase of one cell induces premature chromosome condensation and disorganizes synergid nucleus (Rao and Johnson, 1972; Szabados and Dudits, 1980). However, this aspect of premature chromosome condensation, and how selective elimination of persistent synergid is made possible, needs to be investigated. The precise quantification of DNA content in synergid nucleus before and after fertilization might shed some light on the process. It is, however, certain that for degeneration of the persistent synergid, cell completion of double fertilization is a pre-requisite. In *Arabidopsis thaliana*, fusion of the persistent synergid with the endosperm leads to cytoplasmic dilution of presecreted pollen attractants. This leads to nuclear degeneration, followed by rapid inactivation of persistent synergid. The entry of multiple pollen tubes is therefore discouraged into the female gametophyte. Maruyama *et al.,* (2015) traced the sequences as: i. Pollen tube attraction is terminated by the inactivation of persistent synergid ii. Persistent synergid is fused with the fertilized central cell or endosperm, iii. The fertilized egg cell regulates synergid nucleus degeneration via ethylene signaling and iv. Polycomb proteins and an AGP are required for synergid nucleus degeneration.

Calcium the Key Player

The 'male germ unit' in flowering plants is organized between the two sperm cells and the vegetative nucleus, forming a functional association (Dumas *et al*., 1984). This assemblage favours the transportation of the male gametes within the tube and ascertains their simultaneous delivery female gametes. But this assembly (association) must be disturbed, and the two sperms must be dissociated to enable their union with the egg cell and the central cell. Because the assemblage is presumably maintained by cytoskeletal elements (Palevitz and Tiezzi, 1992), calcium in the synergid may be involved in the breakdown of the cytoskeletal elements and the preparation of the sperm cell surface for fusion. The egg cell during the process retains consistently low levels of calcium (Chaubal and Reger, 1992b; Tian and Russell, 1997; Yu *et al*., 1998; Tian *et al*., 2000). However, in *Plumbago zeylanica* where synergids are absent, the egg has high calcium levels at maturity (Tian *et al*., 2000). This observation further elucidates the role of calcium in pollen tube attraction to the ovule and its entry into the FG *in vivo* (Cass and Karas, 1974; Russell, 1982). ACA9 is another Ca^{2+} transporter present in the pollen tube that presumably interacts with ZmES4, evidence of which is however, pending (Staiger *et al*., 2010). Live cell imaging studies by Ngo *et al.,* (2014) on *Arabidopsis* provide a conceptual framework for the molecular mechanism of the multistep programmed cell death. The live imaging studies have intricately revealed the role of Ca^{2+} pattern in three interacting cells – Pollen tube (PT), DSY and PSY during the phase of pollen tube discharge. These patterns have been traced to four stages of PT growth and sensing of the mutual proximity between PT and synergids. Phase I is slow PT growth, when the pollen tube grows slowly along micropylar region of the two synergids. This initiates calcium spike in both synergids but with different intensities. The pollen tube also shows local oscillations at the tip region. Phase II is marked by fast PT growth with elevation of Ca^{2+} at the tip. In PSY, oscillations continue, while in DSY, the cell is flooded with calcium. By the time the pollen tube reaches the chalazal pole of DSY, calcium spike is observed. At the chalazal pole of the degenerating synergid, the pollen tube stops growing for a while, but as it moves towards the micropylar pole of the DSY, pollen tube growth is fast. Here, pollen tube growth stops for a short while but is resumed as fast growth towards the micropylar pole of DSY. Phase III PCD is characterized by the rupture of the PT tip and the collapse of the DSY and marked increase in calcium in PT. The calcium level is higher in PSY than DSY, but soon subsides. Phase IV is marked by oscillation recovery in PSY. From here onwards, the calcium signatures in PSY (ready to degenerate) follow the same pattern as that of the DSY. Thus, calcium dynamics in the DSY in response to pollen tube growth distinguishes it from its genetically identical sister cell, the PSY.

Conclusions

The present review focuses upon the intricacies of the role of synergids in double fertilization. It highlights the role of genes and signaling cascades that lead to the PCD of degenerating synergid and elimination of the persistent synergid. The role of calcium signaling is exemplary, as calcium plays a key role in mitigating pollen tube growth and through the central cell, impacts the fertilization process. The interplay of small molecules and transcription factors also have an impact both pre- and post-fertilization. LRE, LLG1 and early nodulin-like protein functions (ENODLs) are the co-receptors for FER signaling at the entrance of female gametophyte. The responses triggered in PSY, the time lapse between the degeneration of the two synergids, and nuclear behaviour in PSY require further study. It is also clear that the cascading pathways and feedback loops involving many drivers lead to synergid demise that essentially ensure fertilization. Elimination of persistent synergid to circumvent polytubey is now seen as an important process that ensures proper embryo formation. Diversity in angiosperm taxa warrants extensive studies in pre- and post-fertilization behaviour of synergids.

Acknowledgments

Authors IK and MK thank Principals of their institutions for their continuous motivation. Both the authors thank the anonymous reviewers for improving the quality of the manuscript. MK also acknowledges Institute of Eminence, University of Delhi for the academic support.

References

ADHIKARI P. B., LIU X., WU X., ZHU S., KASAHARA R. D. (2020). Fertilization in flowering plants: an odyssey of sperm cell delivery. *Plant Molecular Biology* 103: 9-32. https://doi.org/10.1007/s11103-020-00987-z

ALVAREZ M. R., SAGAWA Y. (1965). A Histochemical Study of Embryo Sac Development in Vanda (Orchidaceae) . *Caryologia* 18: 241-249. https://doi.org/10.1080/00087114.1965.10796169

AMIEN S., KLIWER I., MÁRTON M. L., DEBENER T., GEIGER D., BECKER D., DRESSELHAUS T. (2010). Defensin-Like ZmES4 Mediates Pollen Tube Burst in Maize via Opening of the Potassium Channel KZM1. *PLoS Biology* 8: e1000388. https://doi.org/10.1371/journal.pbio.1000388

BEALE K. M., JOHNSON M. A. (2013). Speed dating, rejection, and finding the perfect mate: advice from flowering plants. *Current Opinion in Plant Biology* 16: 590-597. https://doi.org/10.1016/j.pbi.2013.08.005

BEALE K. M., LEYDON A. R., JOHNSON M. A. (2012). Gamete Fusion Is Required to Block Multiple Pollen Tubes from Entering an Arabidopsis Ovule. *Current Biology* 22: 1090-1094. https://doi.org/10.1016/j.cub.2012.04.041

BERGER F., HAMAMURA Y., INGOUFF M., HIGASHIYAMA T. (2008). Double fertilization – caught in the act. *Trends in Plant Science* 13: 437-443. https://doi.org/10.1016/j.tplants.2008.05.011

BOISSON-DERNIER A., FRIETSCH S., KIM T.H., DIZON M. B., SCHROEDER J. I. (2008). The Peroxin Loss-of-Function Mutation abstinence by mutual consent Disrupts Male-Female Gametophyte Recognition. *Current Biology* 18: 63-68. https://doi.org/10.1016/j.cub.2007.11.067

BOISSON-DERNIER A., LITUIEV D. S., NESTOROVA A., FRANCK C. M., THIRUGNANARAJAH S.GROSSNIKLAUS U. (2013). ANXUR Receptor-Like Kinases Coordinate Cell Wall Integrity with Growth at the Pollen Tube Tip Via NADPH Oxidases. PLoS Biology 11: e1001719. https://doi.org/10.1371/journal.pbio.1001719

BRZEZICKA E., KOZIERADZKA-KISZKURNO M. (2021). Developmental, ultrastructural and cytochemical investigations of the female gametophyte in Sedum rupestre L. (Crassulaceae). *Protoplasma* 258: 529-546. https://doi.org/10.1007/s00709-020-01584-z

CASS D. D., KARAS I. (1974). Ultrastractural organization of the egg ofPlumbago zeylanica. *Protoplasma* 81: 49-62. https://doi.org/10.1007/BF02055773

CHAO C. (1971). A periodic acid-schiff's substance related to the directional growth of pollen tube into embryo sac in paspalum ovules. *American Journal of Botany* 58: 649-654. https://doi.org/10.1002/j.1537-2197.1971.tb10015.x

CHAUBAL R., REGER B. J. (1992a). Calcium in the synergid cells and other regions of pearl millet ovaries. *Sexual Plant Reproduction* 5: 34-46. https://doi.org/10.1007/BF00714556

CHAUBAL R., REGER B.J. (1992b). The dynamics of calcium distribution in the synergid cells of wheat after pollination. *Sexual Plant Reproduction* 5: 206-213. https://doi.org/10.1007/BF00189813

CHEN Y.H., LI H.J., SHI D.Q., YUAN L., LIU J., SREENIVASAN R., BASKAR R., GROSSNIKLAUS U., YANG W.C. (2007). The Central Cell Plays a Critical Role in Pollen Tube Guidance in Arabidopsis . *The Plant Cell* 19: 3563-3577. https://doi.org/10.1105/tpc.107.053967

CHRISTENSEN C. A., GORSICH S. W., BROWN R. H., JONES L. G., BROWN J., SHAW J. M., DREWS G. N. (2002). Mitochondrial GFA2 Is Required for Synergid Cell Death in Arabidopsis. *The Plant Cell* 14: 2215-2232. https://doi.org/10.1105/tpc.002170

COIMBRA S. (1999). Ultrastructure of the Developing and Fertilized Embryo Sac of Amaranthus hypochondriacus L.. *Annals of Botany* 84: 781-789. https://doi.org/10.1006/anbo.1999.0978

DAVIS G.L. (1962). Embryological studies in the compositae, I. Sporogenesis, Gametogenesis, and Embryogeny in Cotula australis (Less.) Hook. F. *Australian Journal of Botany* 10: 1. https://doi.org/10.1071/BT9620001

DENG W., LI Q.G., TIAN H.Q. (2016). Dimorphism of Synergids in Allium tuberosum (Amaryllidaceae) . *Annales Botanici Fennici* 53: 267-275. https://doi.org/10.5735/085.053.0415

DIBOLL A. G., LARSON D. A. (1966). An electron microscopic study of the mature megagametophyte in Zea mays. *American Journal of Botany* 53: 391-402. https://doi.org/10.1002/j.1537-2197.1966.tb07351.x

DRESSELHAUS T., FRANKLIN-TONG N. (2013). Male–Female Crosstalk during Pollen Germination, Tube Growth and Guidance, and Double Fertilization. *Molecular Plant* 6: 1018-1036. https://doi.org/10.1093/mp/sst061

DREWS G. N., YADEGARI R. (2002). Development and Function of the Angiosperm Female Gametophyte. *Annual Review of Genetics* 36: 99-124. https://doi.org/10.1146/annurev.genet.36.040102.131941

DUAN Q., KITA D., JOHNSON E. A., AGGARWAL M., GATES L., WU H.M., CHEUNG A. Y. (2014). Reactive oxygen species mediate pollen tube rupture to release sperm for fertilization in Arabidopsis. *Nature Communications* 5: 3129. https://doi.org/10.1038/ncomms4129

DUMAS C., KNOX R. B., MCCONCHIE C. A., RUSSELL S. D. (1984). Emerging physiological concepts in fertilization. What's new in plant.. *Physiology* 15: 17-20.

ENGELL K. (1988). Embryology of Barley III: Synergids and Pollen Tube Before and After Fertilization. In *Sexual Reproduction in Higher Plants*, Springer, pp. 471-471. https://doi.org/10.1007/978-3-642-73271-3_83

ESCOBAR-RESTREPO J.M., HUCK N., KESSLER S., GAGLIARDINI V., GHEYSELINCK J., YANG W.C., GROSSNIKLAUS U. (2007). The FERONIA Receptor-like Kinase Mediates Male-Female Interactions During Pollen Tube Reception. *Science* 317: 656-660. https://doi.org/10.1126/science.1143562

FOLSOM M. W., PETERSON C. M. (1984). Ultrastructural Aspects of the Mature Embryo Sac of Soybean, Glycine max (L.) Merr.. *Botanical Gazette* 145: 1-10. https://doi.org/10.1086/337418

FURUTA K. M., YADAV S. R., LEHESRANTA S., BELEVICH I., MIYASHIMA S., HEO J., VATÉN A., LINDGREN O., DE RYBEL B., VAN ISTERDAEL G., SOMERVUO P., LICHTENBERGER R., ROCHA R., THITAMADEE S., TÄHTIHARJU S., AUVINEN P., BEECKMAN T., JOKITALO E., HELARIUTTA Y. (2014). Arabidopsis NAC45/86 direct sieve element morphogenesis culminating in enucleation . *Science* 345: 933-937. https://doi.org/10.1126/science.1253736

GE Z., CHEUNG A. Y., QU L.J. (2019). Pollen tube integrity regulation in flowering plants: insights from molecular assemblies on the pollen tube surface. *New Phytologist* 222: 687-693. https://doi.org/10.1111/nph.15645

GODINEAU C. (1969). Ultrastructure des synergids chez quelques Composees. *Revue de cytologie et de cytophysiologie vegetales* 32: 209-226.

GONNEAU M., DESPREZ T., MARTIN M., DOBLAS V. G., BACETE L., MIART F., SORMANI R., HÉMATY K., RENOU J., LANDREIN B., MURPHY E., VAN DE COTTE B., VERNHETTES S., DE SMET I., HÖFTE H. (2018). Receptor Kinase THESEUS1 Is a Rapid Alkalinization Factor 34 Receptor in Arabidopsis. *Current Biology* 28: 2452-2458.e4. https://doi.org/10.1016/j.cub.2018.05.075

GREENHAM J., CHAPMAN G. P. (1990). Ovule structure and diversity. In *Reproductive versatility in the grasses*. Cambridge University Press, Cambridge, pp. 52-75.

HERRERO M. (2003). Male and female synchrony and the regulation of mating in flowering plants. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 358: 1019-1024. https://doi.org/10.1098/rstb.2003.1285

HERRERO M. (2000). Changes in the Ovary Related to Pollen Tube Guidance. *Annals of Botany* 85: 79-85. https://doi.org/10.1006/anbo.1999.1014

HIGASHIYAMA T. (2002). The synergid cell: attractor and acceptor of the pollen tube for double fertilization. *Journal of Plant Research* 115: 149-160. https://doi.org/10.1007/s102650200020

HIGASHIYAMA T., KUROIWA H., KUROIWA T. (2003). Pollen-tube guidance: beacons from the female gametophyte. *Current Opinion in Plant Biology* 6: 36-41. https://doi.org/10.1016/S1369-5266(02)00010-9

HIGASHIYAMA T., KUROIWA H., KAWANO S., KUROIWA T. (1998). Guidance in Vitro of the Pollen Tube to the Naked Embryo Sac of Torenia fournieri . *The Plant Cell* 10: 2019-2031. https://doi.org/10.1105/tpc.10.12.2019

HIGASHIYAMA T., YABE S., SASAKI N., NISHIMURA Y., MIYAGISHIMA S., KUROIWA H., KUROIWA T. (2001). Pollen Tube Attraction by the Synergid Cell. *Science* 293: 1480-1483. https://doi.org/10.1126/science.1062429

HOU Y., GUO X., CYPRYS P., ZHANG Y., BLECKMANN A., CAI L., HUANG Q., LUO Y., GU H., DRESSELHAUS T., DONG J., QU L.J. (2016). Maternal ENODLs Are Required for Pollen Tube Reception in Arabidopsis. *Current Biology* 26: 2343-2350. https://doi.org/10.1016/j.cub.2016.06.053

HUANG B.Q., STROUT G.W., RUSSELL S.D. (1993). Fertilization in Nicotiana tabacum: ultrastructural organization of propane-jet-frozen embryo sacs in vivo. *Planta* 191: 256264. https://doi.org/10.1007/BF00199758

HUANG B.Q., RUSSELL S. D. (1992). Female Germ Unit: Organization, Isolation, and Function. In *International Review of Cytology*, Vol. 140. Elsevier, pp. 233-293. https://doi.org/10.1016/S0074-7696(08)61099-2

HUANG B. Q., RUSSELL S. D. (1994). Fertilization inNicotiana tabacum: Cytoskeletal modifications in the embryo sac during synergid degeneration. *Planta* 194: 200-214. https://doi.org/10.1007/BF01101679

HUCK N., MOORE J. M., FEDERER M., GROSSNIKLAUS U. (2003). The Arabidopsis mutant feronia disrupts the female gametophytic control of pollen tube reception . *Development* 130: 2149-2159. https://doi.org/10.1242/dev.00458

ITO J., FUKUDA H. (2002). ZEN1 Is a Key Enzyme in the Degradation of Nuclear DNA during Programmed Cell Death of Tracheary Elements. *The Plant Cell* 14: 3201-3211. https://doi.org/10.1105/tpc.006411

JANE W. (1997). The ultrastructure of megagametophyte before fertilization in Arundo formosana Hack. *Taiwania* 37: 85-103.

JENSEN W. A. (1965). The ultrastructure and histochemistry of the synergids of cotton. *American Journal of Botany* 52: 238-256. https://doi.org/10.1002/j.1537-2197.1965.tb06781.x

JENSEN W. A., SCHULZ P., ASHTON M. E. (1977). An ultrastructural study of early endosperm development and synergid changes in unfertilized cotton ovules. *Planta* 133: 179-189. https://doi.org/10.1007/BF00391917

JOHNSON M. A., PREUSS D. (2002). Plotting a Course. *Developmental Cell* 2: 273-281. https://doi.org/10.1016/S1534-5807(02)00130-2

JOHRI B. M., AMBEGAOKAR K. B. (1984a). Embryology: Then and Now. In *Embryology of angiosperms*. Springer, pp. 1-52. https://doi.org/10.1007/978-3-642-69302-1_1

JOHRI B. M., AMBEGAOKAR K. B. (1984b). Some unusual features in the embryology of Angiosperms. *Proceedings / Indian Academy of Sciences* 93: 413-427. https://doi.org/10.1007/BF03053092

JONES D. S., YUAN J., SMITH B. E., WILLOUGHBY A. C., KUMIMOTO E. L., KESSLER S. A. (2017). MILDEW RESISTANCE LOCUS O Function in Pollen Tube Reception Is Linked to Its Oligomerization and Subcellular Distribution. *Plant Physiology* 175: 172-185. https://doi.org/10.1104/pp.17.00523

JONES-RHOADES M. W., BOREVITZ J. O., PREUSS D. (2007). Genome-Wide Expression Profiling of the Arabidopsis Female Gametophyte Identifies Families of Small, Secreted Proteins. *PLoS Genetics* 3: e171. https://doi.org/10.1371/journal.pgen.0030171

KAPIL R. N., BHATNAGAR A. K. (1981). Ultrastructure and Biology of Female Gametophyte in Flowering Plants. In *International Review of Cytology*, Vol. 70. Elsevier, pp. 291-341. https://doi.org/10.1016/S0074-7696(08)61134-1

KASAHARA R. D., MARUYAMA D., HAMAMURA Y., SAKAKIBARA T., TWELL D., HIGASHIYAMA T. (2012). Fertilization Recovery after Defective Sperm Cell Release in Arabidopsis. *Current Biology* 22: 1084-1089. https://doi.org/10.1016/j.cub.2012.03.069

KASAHARA R. D., PORTEREIKO M. F., SANDAKLIE-NIKOLOVA L., RABIGER D. S., DREWS G. N. (2005). MYB98 Is Required for Pollen Tube Guidance and Synergid Cell Differentiation in Arabidopsis . *The Plant Cell* 17: 2981-2992. https://doi.org/10.1105/tpc.105.034603

KESSLER S. A., SHIMOSATO-ASANO H., KEINATH N. F., WUEST S. E., INGRAM G., PANSTRUGA R., GROSSNIKLAUS U. (2010). Conserved Molecular Components for Pollen Tube Reception and Fungal Invasion. *Science* 330: 968-971. https://doi.org/10.1126/science.1195211

KESSLER S. A., GROSSNIKLAUS U. (2011). She's the boss: signaling in pollen tube reception. *Current Opinion in Plant Biology* 14: 622-627. https://doi.org/10.1016/j.pbi.2011.07.012

KÖHLER C., WOLFF P., SPILLANE C. (2012). Epigenetic Mechanisms Underlying Genomic Imprinting in Plants. *Annual Review of Plant Biology* 63: 331-352. https://doi.org/10.1146/annurev-arplant-042811-105514

KUROIWA H. (1989). Ultrastructural examination of embryogenesis in Crepis capillaris (L.) Wallr.: 1. The synergid before and after pollination. *The Botanical Magazine Tokyo* 102: 9-24. https://doi.org/10.1007/BF02488109

LESHEM Y., JOHNSON C., SUNDARESAN V. (2013). Pollen tube entry into the synergid cell of Arabidopsis is observed at a site distinct from the filiform apparatus. *Plant Reproduction* 26: 93-99. https://doi.org/10.1007/s00497-013-0211-1

LEYDON A. R., TSUKAMOTO T., DUNATUNGA D., QIN Y., JOHNSON M. A., PALANIVELU R. (2015). Pollen Tube Discharge Completes the Process of Synergid Degeneration That Is Initiated by Pollen Tube-Synergid Interaction in Arabidopsis. *Plant Physiology* 169: 485-496. https://doi.org/10.1104/pp.15.00528

LI C., YEH F.L., CHEUNG A. Y., DUAN Q., KITA D., LIU M.C., MAMAN J., LUU E. J., WU B. W., GATES L., JALAL M., KWONG A., CARPENTER H., WU H.M. (2015a). Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in Arabidopsis. *eLife* 4: 06587. https://doi.org/10.7554/eLife.06587

LI D. X., LIN M. Z., WANG Y. Y., TIAN H. Q. (2009). Synergid: a key link in fertilization of angiosperms. *Biologia plantarum* 53: 401-407. https://doi.org/10.1007/s10535-009-0078-z

LI H.J., ZHU S.S., ZHANG M.X., WANG T., LIANG L., XUE Y., SHI D.Q., LIU J., YANG W.C. (2015b). Arabidopsis CBP1 Is a Novel Regulator of Transcription Initiation in Central Cell-Mediated Pollen Tube Guidance. *The Plant Cell* 27: 2880-2893. https://doi.org/10.1105/tpc.15.00370

LI W. (2014). Ultrastructure of Synergids in Sugar Beet. *Advanced Materials Research* 926-930: 1040-1044. https://doi.org/10.4028/www.scientific.net/AMR.926-930.1040

LIU X., CASTRO C., WANG Y., NOBLE J., PONVERT N., BUNDY M., HOEL C., SHPAK E., PALANIVELU R. (2016). The Role of LORELEI in Pollen Tube Reception at the Interface of the Synergid Cell and Pollen Tube Requires the Modified Eight-Cysteine Motif and the Receptor-Like Kinase FERONIA. *The Plant Cell* 28: 1035-1052. https://doi.org/10.1105/tpc.15.00703

LINDNER H., MÜLLER L. M., BOISSON-DERNIER A., GROSSNIKLAUS U. (2012). CrRLK1L receptor-like kinases: not just another brick in the wall. *Current Opinion in Plant Biology* 15: 659-669. https://doi.org/10.1016/j.pbi.2012.07.003

LOPES A. L., MOREIRA D., FERREIRA M. J., PEREIRA A. M., COIMBRA S. (2019). Insights into secrets along the pollen tube pathway in need to be discovered. *Journal of Experimental Botany* 70: 2979-2992. https://doi.org/10.1093/jxb/erz087

LORA J., HORMAZA J. I., HERRERO M. (2016). The Diversity of the Pollen Tube Pathway in Plants: Toward an Increasing Control by the Sporophyte. *Frontiers in Plant Science* 7: 107. https://doi.org/10.3389/fpls.2016.00107

MAHESHWARI P., NEGI V. (1955). The embryology of Dipteracanthus patulus (Jacq.) Nees. *Phytomorphology* 5: 465-472.

MAHESHWARI P., SINGH H. (1967). The female gametophyte of gymnosperms. *Biological Reviews* 42: 88-129. https://doi.org/10.1111/j.1469-185X.1967.tb01341.x

MALHO R., TREWAVAS A. J. (1996). Localized Apical Increases of Cytosolic Free Calcium Control Pollen Tube Orientation. *The Plant Cell* 8: 1935. https://doi.org/10.2307/3870403

MANTEGAZZA O., GREGIS V., CHIARA M., SELVA C., LEO G., HORNER D. S., KATER M. M. (2014). Gene coexpression patterns during early development of the native Arabidopsis reproductive meristem: novel candidate developmental regulators and patterns of functional redundancy. *The Plant Journal* 79: 861-877. https://doi.org/10.1111/tpj.12585

MARTIN M. V., DISTÉFANO A. M., BELLIDO A., CÓRDOBA J. P., SOTO D., PAGNUSSAT G. C., ZABALETA E. (2014). Role of mitochondria during female gametophyte development and fertilization in A. thaliana. *Mitochondrion* 19: 350-356. https://doi.org/10.1016/j.mito.2014.01.005

MARTIN M. V., FIOL D. F., SUNDARESAN V., ZABALETA E. J., PAGNUSSAT G. C. (2013). oiwa , a Female Gametophytic Mutant Impaired in a Mitochondrial Manganese-Superoxide Dismutase, Reveals Crucial Roles for Reactive Oxygen Species during Embryo Sac Development and Fertilization in Arabidopsis . *The Plant Cell* 25: 1573-1591. https://doi.org/10.1105/tpc.113.109306

MÁRTON M. L., CORDTS S., BROADHVEST J., DRESSELHAUS T. (2005). Micropylar Pollen Tube Guidance by Egg Apparatus 1 of Maize. *Science* 307: 573-576. https://doi.org/10.1126/science.1104954

MARUYAMA D., VÖLZ R., TAKEUCHI H., MORI T., IGAWA T., KURIHARA D., KAWASHIMA T., UEDA M., ITO M., UMEDA M., NISHIKAWA S., GROSS-HARDT R., HIGASHIYAMA T. (2015). Rapid Elimination of the Persistent Synergid through a Cell Fusion Mechanism. *Cell* 161: 907-918. https://doi.org/10.1016/j.cell.2015.03.018

MARUYAMA D., HAMAMURA Y., TAKEUCHI H., SUSAKI D., NISHIMAKI M., KURIHARA D., KASAHARA R. D., HIGASHIYAMA T. (2013). Independent Control by Each Female Gamete Prevents the Attraction of Multiple Pollen Tubes. *Developmental Cell* 25: 317-323. https://doi.org/10.1016/j.devcel.2013.03.013

MENDES M. A., GUERRA R. F., CASTELNOVO B., VELAZQUEZ Y. S., MORANDINI P., MANRIQUE S., BAUMANN N., GROSS-HARDT R., DICKINSON H., COLOMBO L. (2016). Live and let die: a REM complex promotes fertilization through synergid cell death in Arabidopsis . *Development* 143: 2780-2790. https://doi.org/10.1242/dev.134916

MOGENSEN H. L. (1978). Pollen tube-synergid interactions in Proboscidea louisianica (Martineaceae). *American Journal of Botany* 65: 953-964. https://doi.org/10.1002/j.1537-2197.1978.tb06160.x

MOGENSEN H. L., SUTHAR H. K. (1979). Ultrastructure of the Egg Apparatus of Nicotiana tabacum (Solanaceae) Before and After Fertilization. *Botanical Gazette* 140: 168-179. https://doi.org/10.1086/337073

MU X., JIN B., TENG N. (2010). Studies on the early development of zygotic and synergid embryo and endosperm in polyembryonic rice ApIII. *Flora - Morphology, Distribution, Functional Ecology of Plants* 205: 404-410. https://doi.org/10.1016/j.flora.2009.12.023

MUSIAŁ K., KOŚCIŃSKA-PAJĄK M. (2013). Egg Apparatus in Sexual and Apomictic Species of Taraxacum: Structural and Immunocytochemical Aspects of Synergid Cells. *Acta Biologica Cracoviensia Series Botanica* 55: 107-113. https://doi.org/10.2478/abcsb-2013-00011

NÄGELE T., FRAGNER L., CHATURVEDI P., GHATAK A., WECKWERTH W. (2017). Pollen Metabolome Dynamics: Biochemistry, Regulation and Analysis. In *Pollen Tip Growth*. Springer, pp. 319-336. https://doi.org/10.1007/978-3-319-56645-0_12

NEWCOMB W. (1973). The development of the embryo sac of sunflower Helianthus annuus after fertilization . *Canadian Journal of Botany* 51: 879-890. https://doi.org/10.1139/b73-110

NGO Q. A., VOGLER H., LITUIEV D. S., NESTOROVA A., GROSSNIKLAUS U. (2014). A Calcium Dialog Mediated by the FERONIA Signal Transduction Pathway Controls Plant Sperm Delivery. *Developmental Cell* 29: 491-500. https://doi.org/10.1016/j.devcel.2014.04.008

NISSEN K. S., WILLATS W. G.T., MALINOVSKY F. G. (2016). Understanding CrRLK1L Function: Cell Walls and Growth Control. *Trends in Plant Science* 21: 516-527. https://doi.org/10.1016/j.tplants.2015.12.004

OHNISHI T., TAKANASHI H., MOGI M., TAKAHASHI H., KIKUCHI S., YANO K., OKAMOTO T., FUJITA M., KURATA N., TSUTSUMI N. (2011). Distinct Gene Expression Profiles in Egg and Synergid Cells of Rice as Revealed by Cell Type-Specific Microarrays . *Plant Physiology* 155: 881-891. https://doi.org/10.1104/pp.110.167502

PAGNUSSAT G. C., YU H.J., SUNDARESAN V. (2007). Cell-Fate Switch of Synergid to Egg Cell in Arabidopsis eostre Mutant Embryo Sacs Arises from Misexpression of the BEL1-Like Homeodomain Gene BLH1 . *The Plant Cell* 19: 3578-3592. https://doi.org/10.1105/tpc.107.054890

PALEVITZ B. A., TIEZZI A. (1992). Organization, Composition, and Function of the Generative Cell and Sperm Cytoskeleton. In *International Review of Cytology*. Vol. 140. Elsevier, pp. 149-185. https://doi.org/10.1016/S0074-7696(08)61096-7

PHILIPSON M. N. (1981). The haustorial synergids of Cortaderia (Gramineae) at maturity. *Acta Societatis Botanicorum Poloniae* 50: 151-160. https://doi.org/10.5586/asbp.1981.022

PHILIPSON M. N. (1977). Haustorial synergids in Cortaderia (Gramineae) . *New Zealand Journal of Botany* 15: 777-778. https://doi.org/10.1080/0028825X.1977.10429647

PŁACHNO B. J., ŚWIĄTEK P. (2012). Actin cytoskeleton in the extra-ovular embryo sac of Utricularia nelumbifolia (Lentibulariaceae). *Protoplasma* 249: 663-670. https://doi.org/10.1007/s00709-011-0306-1

PŁACHNO B. J., MUSIAŁ K., ŚWIĄTEK P., TULEJA M., MARCINIUK J., GRABOWSKA-JOACHIMIAK A. (2014). Synergids and filiform apparatus in the sexual and apomictic dandelions from section Palustria (Taraxacum, Asteraceae). *Protoplasma* 251: 211-217. https://doi.org/10.1007/s00709-013-0539-2

PUNWANI J. A., DREWS G. N. (2008). Development and function of the synergid cell. *Sexual Plant Reproduction* 21: 7-15. https://doi.org/10.1007/s00497-007-0059-3

PUNWANI J. A., RABIGER D. S., DREWS G. N. (2007). MYB98 Positively Regulates a Battery of Synergid-Expressed Genes Encoding Filiform Apparatus–Localized Proteins. *The Plant Cell* 19: 2557-2568. https://doi.org/10.1105/tpc.107.052076

RAO P. N., JOHNSON R. T. (1972). Premature Chromosome Condensation: a Mechanism for the Elimination of Chromosomes in Virus-Fused Cells. *Journal of Cell Science* 10: 495-513. https://doi.org/10.1242/jcs.10.2.495

RUSSELL S. D. (1982). Fertilization in Plumbago zeylanica : entry and discharge of the pollen tube in the embryo sac . *Canadian Journal of Botany* 60: 2219-2230. https://doi.org/10.1139/b82-272

RUSSELL S. D. (1992). Double Fertilization. In *International Review of Cytology*. Vol. 140. Elsevier, pp. 357-388. https://doi.org/10.1016/S0074-7696(08)61102-X

RUSSELL S. D. (1993). The Egg Cell: Development and Role in Fertilization and Early Embryogenesis. *The Plant Cell* 5: 1349. https://doi.org/10.2307/3869787

RUSSELL S. D., ROUGIER M., DUMAS C. (1990). Organization of the early post-fertilization megagametophyte ofPopulus deltoides. *Protoplasma* 155: 153-165. https://doi.org/10.1007/BF01322625

SANDAKLIE-NIKOLOVA L., PALANIVELU R., KING E. J., COPENHAVER G. P., DREWS G. N. (2007). Synergid Cell Death in Arabidopsis Is Triggered following Direct Interaction with the Pollen Tube. *Plant Physiology* 144: 1753-1762. https://doi.org/10.1104/pp.107.098236

SANKARANARAYANAN S., JU Y., KESSLER S. A. (2020). Reactive Oxygen Species as Mediators of Gametophyte Development and Double Fertilization in Flowering Plants. *Frontiers in Plant Science* 11: 1199. https://doi.org/10.3389/fpls.2020.01199

SCHULZ R., JENSEN W. A. (1968). Capsella embryogenesis: the synergids before and after fertilization. *American Journal of Botany* 55: 541-552. https://doi.org/10.1002/j.1537-2197.1968.tb07410.x

SCHUMACHER K., KREBS M. (2010). The V-ATPase: small cargo, large effects. *Current Opinion in Plant Biology* 13: 724-730. https://doi.org/10.1016/j.pbi.2010.07.003

SHIMIZU K.K., OKADA K. (2000). Attractive and repulsive interactions between female and male gametophytes in Arabidopsis pollen tube guidance. *Development* 127: 4511-4518. https://doi.org/10.1242/dev.127.20.4511

SOMOZA S. C., SEDE A. R., BOCCARDO N. A., MUSCHIETTI J. P. (2021). Keeping up with the RALFs: how these small peptides control pollen–pistil interactions in Arabidopsis. *New Phytologist* 229: 14-18. https://doi.org/10.1111/nph.16817

SPRUNCK S. (2010). Let's get physical: gamete interaction in flowering plants. *Biochemical Society Transactions* 38: 635-640. https://doi.org/10.1042/BST0380635

SPRUNCK S., GROSS-HARDT R. (2011). Nuclear behavior, cell polarity, and cell specification in the female gametophyte. *Sexual Plant Reproduction* 24: 123-136. https://doi.org/10.1007/s00497-011-0161-4

STAIGER C. J., POULTER N. S., HENTY J. L., FRANKLIN-TONG V. E., BLANCHOIN L. (2010). Regulation of actin dynamics by actin-binding proteins in pollen. *Journal of Experimental Botany* 61: 1969-1986. https://doi.org/10.1093/jxb/erq012

SUSAKI D., SUZUKI T., MARUYAMA D., UEDA M., HIGASHIYAMA T., KURIHARA D. (2021). Dynamics of the cell fate specifications during female gametophyte development in Arabidopsis. *PLOS Biology* 19: e3001123. https://doi.org/10.1371/journal.pbio.3001123

SUMNER M. J., CAESEELE L. V. (1989). The ultrastructure and cytochemistry of the egg apparatus of Brassica campestris . *Canadian Journal of Botany* 67: 177-190. https://doi.org/10.1139/b89-026

SZABADOS L., DUDITS D. (1980). Fusion between interphase and mitotic plant protoplasts. *Experimental Cell Research* 127: 442-446. https://doi.org/10.1016/0014-4827(80)90450-4

TAKEUCHI H., HIGASHIYAMA T. (2012). A Species-Specific Cluster of Defensin-Like Genes Encodes Diffusible Pollen Tube Attractants in Arabidopsis. *PLoS Biology* 10: e1001449. https://doi.org/10.1371/journal.pbio.1001449

TIAN H. Q., ZHU H., RUSSELL S. D. (2000). Calcium changes in ovules and embryo sacs of Plumbago zeylanica L.. *Sexual Plant Reproduction* 13: 11-20. https://doi.org/10.1007/PL00009837

TIAN H.Q., RUSSELL S. D. (1997). Calcium distribution in fertilized and unfertilized ovules and embryo sacs of Nicotiana tabacum L.. *Planta* 202: 93-105. https://doi.org/10.1007/s004250050107

TSUKAMOTO T., QIN Y., HUANG Y., DUNATUNGA D., PALANIVELU R. (2010). A role for LORELEI, a putative glycosylphosphatidylinositol-anchored protein, in Arabidopsis thaliana double fertilization and early seed development. *The Plant Journal* 62: 571-588. https://doi.org/10.1111/j.1365-313X.2010.04177.x

VAN BAARLEN P., DE JONG H., VAN DIJK P. (2002). Comparative cyto-embryological investigations of sexual and apomictic dandelions (Taraxacum) and their apomictic hybrids. *Sexual Plant Reproduction* 15: 31-38. https://doi.org/10.1007/s00497-002-0132-x

VAN WENT J. L. (1970). The ultrastructure of the fertilized embryo sac of petunia. *Acta Botanica Neerlandica* 19: 468-480. https://doi.org/10.1111/j.1438-8677.1970.tb00672.x

VAN WENT J. L., WILLEMSE M. T. M. (1984). Fertilization. In *Embryology of angiosperms* (Ed. Johri B.). Springer, pp. 273-317. https://doi.org/10.1007/978-3-642-69302-1_6

VIJAYARAGHAVAN M. R., MISRA G., SAXENA P. (1988). Nutrient transfer during embryonic development of angiosperm plants. *Science Progress* 72: 467-480.

VÖLZ R., HEYDLAUFF J., RIPPER D., VON LYNCKER L., GROSS-HARDT R. (2013). Ethylene Signaling Is Required for Synergid Degeneration and the Establishment of a Pollen Tube Block. *Developmental Cell* 25: 310-316. https://doi.org/10.1016/j.devcel.2013.04.001

WANG J.G., FENG C., LIU H.H., FENG Q.N., LI S., ZHANG Y. (2017). AP1G mediates vacuolar acidification during synergid-controlled pollen tube reception. *Proceedings of the National Academy of Sciences* 114: E4877-E4883. https://doi.org/10.1073/pnas.1617967114

WANG T., LIANG L., XUE Y., JIA P.F., CHEN W., ZHANG M.X., WANG Y.C., LI H.J., YANG W.C. (2016). A receptor heteromer mediates the male perception of female attractants in plants. *Nature* 531: 241-244. https://doi.org/10.1038/nature16975

WILLEMSE M. T. M., VAN WENT J. L. (1984). The Female Gametophyte. In *Embryology of Angiosperms* (Ed. Johri B. M.). Springer, pp. 159-196. https://doi.org/10.1007/978-3-642-69302-1_4

WILMS H. J. (1981). Pollen tube penetration and fertilization in spinach. *Acta Botanica Neerlandica* 30: 101-122. https://doi.org/10.1111/j.1438-8677.1981.tb00390.x

YAN H., YANG H.Y., JENSEN W. A. (1991). Ultrastructure of the developing embryo sac of sunflower (Helianthus annuus) before and after fertilization . *Canadian Journal of Botany* 69: 191-202. https://doi.org/10.1139/b91-027

YU F.L., LIANG S.P., YANG H.Y., WANG Y. (1998). Ultra cytochemical localization of calcium in micropyle and embryo sac of Brassica napus before and after pollination.. *Acta Botanica Sinica* 40: 591-597.

YUE X., GAO X.Q., WANG F., DONG Y.X., LI X.G., ZHANG X. S. (2014). Transcriptional Evidence for Inferred Pattern of Pollen Tube-Stigma Metabolic Coupling during Pollination. *PLoS ONE* 9: e107046. https://doi.org/10.1371/journal.pone.0107046