



Pollen viability, longevity, and function in angiosperms: key drivers and prospects for improvement

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Abstract

Pollen grains are central to sexual plant reproduction and their viability and longevity/storage are critical for plant physiology, ecology, plant breeding, and many plant product industries. Our goal is to present progress in assessing pollen viability/longevity along with recent advances in our understanding of the intrinsic and environmental factors that determine pollen performance: the capacity of the pollen grain to be stored, germinate, produce a pollen tube, and fertilize the ovule. We review current methods to measure pollen viability, with an eye toward advancing basic research and biotechnological applications. Importantly, we review recent advances in our understanding of how basic aspects of pollen/stigma development, pollen molecular composition, and intra- and intercellular signaling systems interact with the environment to determine pollen performance. Our goal is to point to key questions for future research, especially given that climate change will directly impact pollen viability/longevity. We find that the viability and longevity of pollen are highly sensitive to environmental conditions that affect complex interactions between maternal and paternal tissues and internal pollen physiological events. As pollen viability and longevity are critical factors for food security and adaptation to climate change, we highlight the need to develop further basic research for better understanding the complex molecular mechanisms that modulate pollen viability and applied research on developing new methods to maintain or improve pollen viability and longevity.

Keywords Pollen · Abiotic stresses · Viability · Longevity · Storage · Chemical content · Stigma · Signaling · ROS · Calcium

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Introduction

The male gametophyte, pollen, has evolved to carry sperm cells to the female gametophyte for fertilization, which results in fruits and seeds, the basis of the human diet. To carry genetic information between individuals, pollen needs to remain viable in the environment for a period of time that varies greatly across species from short (minutes to days), intermediate (1 to 3 months), or long (over 6 months), defined in (Pacini and Dolferus 2019). Pollen viability is the capacity of the pollen grain to persist in the environment while maintaining its ability to germinate and to produce a tip-growing pollen tube on a receptive stigma/pistil (Edlund et al. 2004). The pollen tube carries its cargo of sperm cells to the ovule where fertilization initiates seed and fruit development. Pollen viability, longevity, and pollen tube performance are crucial factors for propagation of plant species in nature; for breeders who need to collect, store, and transport pollen across the globe to develop new

hybrids; and for farmers who depend on pollen performance to secure high yields of seed and fruit crops.

The Intergovernmental Panel on Climate Change (IPCC) predicts that most agricultural regions will experience more extreme environmental fluctuations, including severe variations of temperature, rain, wind speed, and direction in the coming decades (IPCC 2014). These rapid changes in the environment will impact pollen viability, longevity, performance, and transport between individuals for both wind- and animal-pollinated species. It is therefore important to understand the impact of climate change on the timing and quantity of pollen production and on pollen viability, longevity, and performance (Mercuri et al. 2016). More than twenty years ago, the last comprehensive review of pollen viability assessed methods to determine viability and discussed the intrinsic and extrinsic factors that determine this critical parameter for reproductive success (Dafni and Firmage 2000).

Here, we compare and contrast new and classical methods used to assess pollen viability and then analyze the data that have been gathered on the factors that determine pollen viability and performance (Fig. 1). We are particularly

interested in pointing to areas for future research into how molecular composition, signaling within and between cells, and interactions with the environment affect pollen function. We address fluxes in macromolecules and ions that determine pollen performance and how they are affected by cell–cell interactions within the floral reproductive tract. We also review data gathered on the impact of temperature, drought, mineral availability, and light stress on pollen viability and longevity (Fig. 1). Finally, several examples of methods are described for short- to long-term successful storage of pollen grains of different species that are important for academic researchers and companies.

There is no universal method to assess pollen viability

The ability to assess pollen viability is important for fruit, seed, and grain crop production; it is also critical for the plant conservation and breeding industries. Despite the obvious need to be able to rapidly determine whether collected pollen will be useful for multiple economically important applications, there is no universal viability/performance test

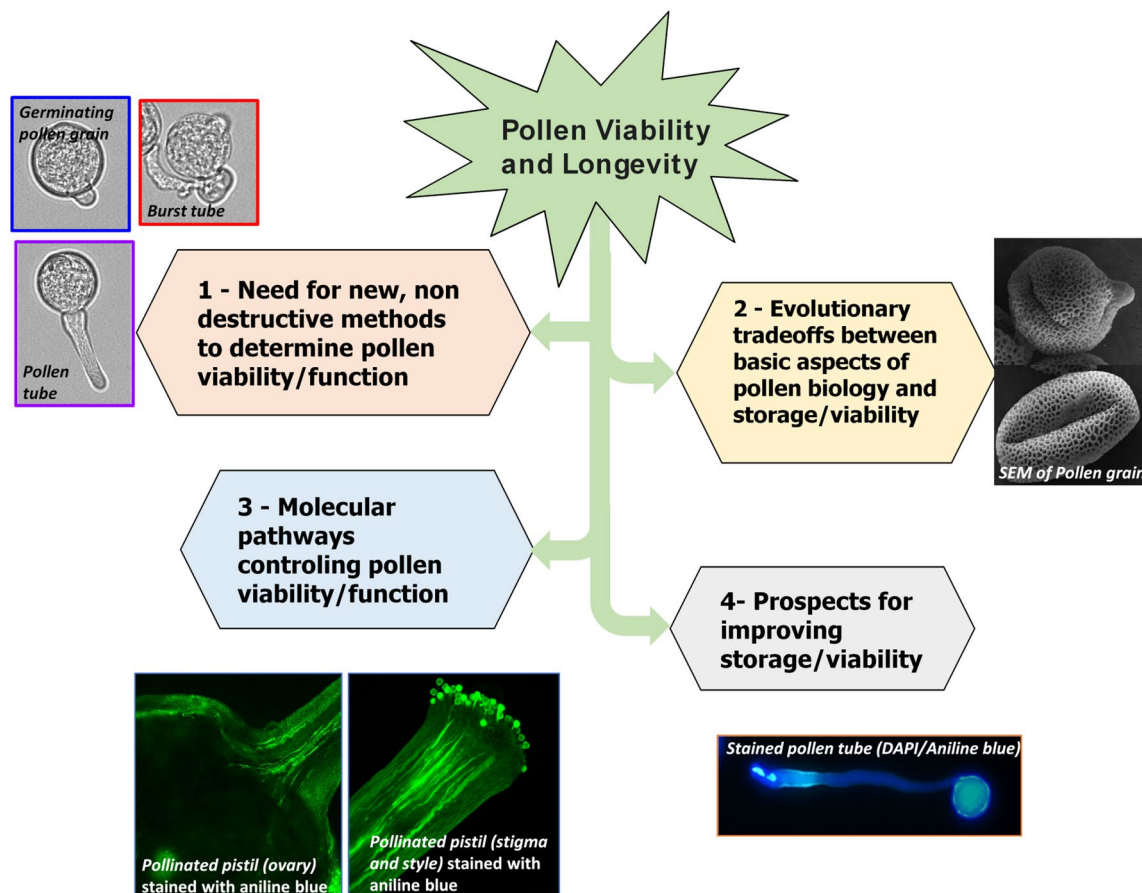


Fig. 1 Key challenges for understanding and optimizing pollen viability and longevity

(Dafni and Firmage 2000). Many methods have been developed (Table 1) that can be broken into two broad categories: 1) analysis of the pollen grain and 2) analysis of germination/tube extension. Ideally, the method would accurately predict the ability of pollen to fertilize ovules following pollination and would be nondestructive so that pollen deemed viable could be used directly for pollinations. In vitro pollen germination is the best option because it is relatively accessible/inexpensive and provides a direct measure of pollen performance. However, there is a lot of room for improvement and development of new methods that can be readily applied across species without the need for expensive instrumentation and could have an important impact.

Current methods for analyzing pollen grains are based on monitoring enzyme activity such as cytoplasmic esterases with fluorescein diacetate (FDA, Fig. 2a), ROS (Reactive Oxygen Species) production, or assessing cell integrity and quality using dyes such as neutral red, or by impedance flow cytometry (Table 1). Of these methods, only impedance flow cytometry is nondestructive; producing evaluated pollen that can be used for pollination. Methods for evaluating germination/tube extension include analysis of germination and pollen tube growth in vitro (Fig. 2b, c), in vivo (using aniline blue staining, Fig. 2d–f), in a *semi-in vivo* system in which pollen germinate on and grow through the stigma/style before analysis on defined media (*e.g.*, Palanivelu and Preuss

Table 1 List of approaches used to assess pollen viability

Type	Methods	References
Staining for enzyme activity or ROS production	–TCC (2,3,5-triphenyl tetrazolium chloride): dehydrogenase	Lakon (1949)
	–B5MTT or MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide: NAD(P)H-dependent oxidoreductase	Norton (1966); Khatun and Flowers (1995)
	–NBT (nitro blue tetrazolium, Tetrazolium, (3,3'-dimethoxy-4,4'-biphenylene)bis[2,(4-nitrophenyl)-5-phenyl-2H]-, chloride): ROS production	Hauser and Morrison (1964)
	–X-Gal: beta-galactosidase	Singh et al. (1985); Trognitz (1991)
	–Baker's reagent: alcohol dehydrogenase	Dafni (1992)
	–Benzidine test: catalase	King (1960)
	–Sigma peroxidase indicator	Rodriguez-Riano and Dafni (2000)
	–FDA (Fluorescein diacetate): esterase	Heslop-Harrison and Heslop-Harrison (1970)
	–Flow cytometry: H2-DCFDA (2',7'-dichlorodihydrofluorescein diacetate): esterase and ROS production	Luria et al. (2019); Impe et al. (2019); Langedijk et al. (2023)
	–Miscellaneous 'vital stains': methylene blue, neutral red, propionic carmine	Firmage and Dafni (2001)
Pollen integrity and quality	–Isatin test: proline content	Firmage and Dafni (2001)
	–Soluble sugar content: glucose, fructose, sucrose, raffinose, etc., by mid-infrared attenuated total reflectance (MIR-ATR) or ion-exchange chromatography coupled to pulsed amperometric detection	Jiang et al. (2015); Impe et al. (2020)
	–I2-KI staining method (Lugol): starch	Melloni et al. (2013)
	–Alexander's stain: pollen protoplasm and cellulose contained in the cell wall	Alexander (1969)
	–Microfluidic chips and impedance flow cytometry: cell size, cell number, membrane capacitance, cytoplasmic conductivity (Ampha Z32 by Amphasis, Switzerland)	Heidmann et al. (2016)
	–Pollen counter using Alexander's stain	Tello et al. (2018)
Automated image analysis	–Pollen profiler using a combination of FDA and propidium iodide	Ascari et al. (2020)
Germinability	–In vitro pollen germination and pollen tube length	Brewbaker and Kwak (1963); Boavida and McCormick (2007); Leroux et al. (2015); Tushabe and Rosbakh (2021)
	–In vivo pollen germination (aniline blue: callose, Fig. 2), calcofluor white: beta-glucan; auramine: cutin; periodic acid-Schiff reagent: insoluble carbohydrate; benzidine test: catalase, acetocarmine: nucleus)	Sanzol et al. (2003); Leroux et al. (2015); He et al. (2017); Impe et al. (2019)
Fertilization ability	–Fruit and seed/caryopsis set	Dafni and Firmage (2000)

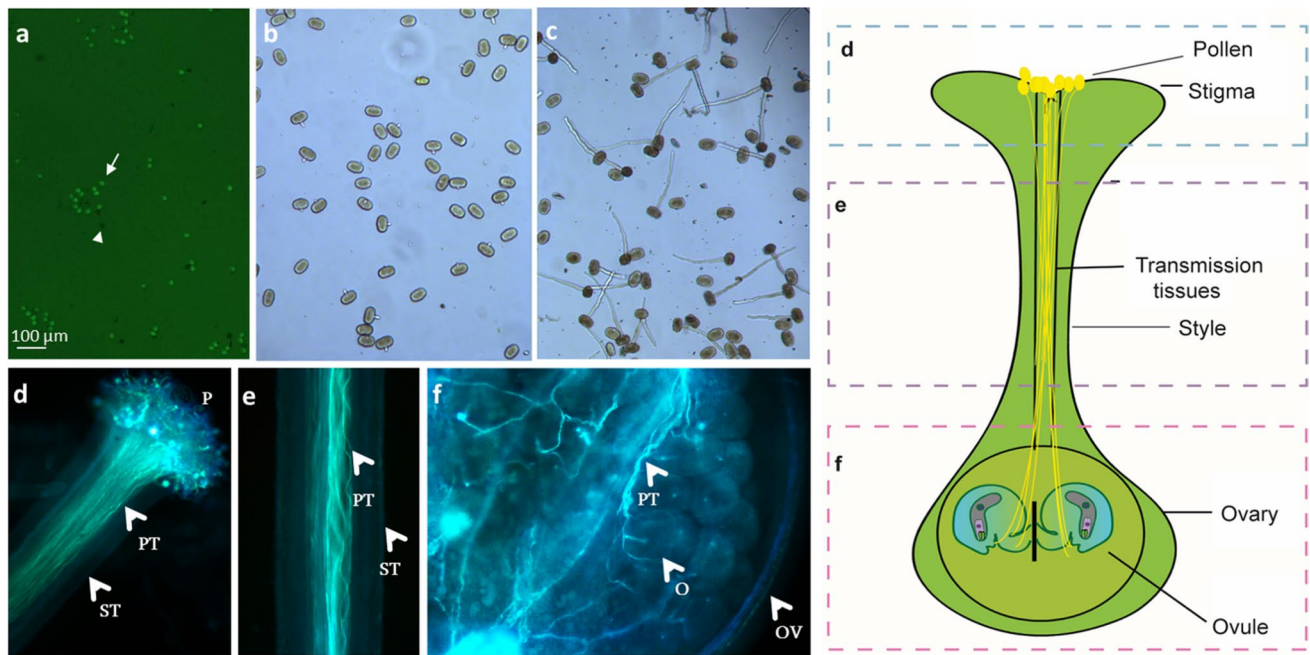


Fig. 2 Methods to assess pollen viability. **a** *Arabidopsis thaliana* pollen grains stained with FDA showing viable (green, arrows) and inviable (dark, arrowheads) pollen grains. **b–c** In vitro pollen germination of *Pisum sativum* after 1 h and 4 h of culture, respectively. **d–f** In vivo analysis of pollen germinability stained with aniline blue on

24 h pollinated tomato pistil. **d** Pollen grains on the stigma and **e** pollen tube elongation within the transmitting tract of the style. **f** Pollen tubes within the ovary and fertilization of ovules. P. Pollen grain, PT. Pollen tube, ST. Style, O. Ovule, OV. Ovary

2006), or estimating the fertilization ability by counting fruit/seed/grain set (Table 1). These methods provide direct measures of pollen performance but are all destructive, time-consuming, and dependent on optimization of culture conditions for each species. Pollen–pistil interactions and signaling are crucial for pollen germination (Johnson et al. 2019) and pistil factors may be required to overcome the failure of in vitro germination of pollen in recalcitrant species. Thus, in vivo methods may be preferred, however, the time from hand-pollination to seed/fruit maturity can vary from weeks (e.g., *Arabidopsis*) to months (e.g., tomato), to years (e.g., conifers, Breygina et al. 2021), and may not be a good measure of the quality of a pollen population in species with one or few ovules/flower where success is determined by a single pollen tube. Moreover, self-incompatibility mechanisms, stigma receptivity, and ovule maturity must be carefully considered (Gao et al. 2010).

A field test for pollen viability was conducted by (Dafni and Firmage 2000) on 17 species using four stain-based methods (X-Gal, MTT, Baker's solution and isatin, defined in Table 1) that were compared to in vitro pollen germination. The data showed that not all tests were suitable for all the species but MTT-based viability staining was a reasonable first choice as it best matched pollen viability in 10 out of the 17 tested species and was as good as the other tests in three additional species. Similarly, (Impe et al. 2019)

investigated several methods to evaluate pollen viability in wheat (*Triticum aestivum*). Among the tested methods (FDA, acetocarmine, Alexander's staining, impedance flow cytometry, in vitro germinability, semi-in vivo stigmatic germination, soluble sugar analysis), in vitro pollen germination was shown to be the best method and is generally the most widely used in laboratories. However, this method is only useful after pollen germination media have been optimized for each species to be evaluated—a significant investment (see Supplementary information Table S1 for a comprehensive listing of methods). Indeed, media optimized for wheat failed to support germination of pollen from closely related Poales: rye (*Secale cereale*), barley (*Hordeum vulgare*), and maize (*Zea mays*) (Impe et al. 2019).

Opportunities for new approaches to determining pollen viability

A universal method for analysis of pollen viability/performance is still a major challenge. Impedance flow cytometry is appealing because it is nondestructive and has shown to be predictive of pollen performance in the species that have been tested (Heidmann et al. 2016; Heidmann and Di Berardino 2017). The chief drawback is that the instrumentation is specialized, expensive, and needs to be calibrated for each species. In general, flow cytometry based methods for determining

pollen grain viability (Table 1) are attractive because they can be automated for high throughput analysis of pollen quality and can be adapted for screening to identify bioactive compounds that promote pollen viability and longevity either in the field or during storage (Luria et al. 2019; Rutley and Miller 2020).

Approaches that utilize artificial intelligence/machine learning to predict pollen viability/performance from images of recently collected pollen populations should be explored. These methods are being used to classify pollen (Viertel and König 2022; Olsson et al. 2021; Kubera et al. 2021), but have not yet been used to predict pollen functions. Training sets could be built by imaging pollen populations and then carefully analyzing pollen performance using the methods described here.

Germination of pollen and analysis of tube growth in vitro provide the opportunity to directly analyze several aspects of pollen performance (germination rate, tube growth rate, pollen tube integrity). However, optimization of media and development of automated analysis of live-imaging data need to be further developed (Ponvert et al. 2019; Palanivelu and Preuss 2006) and more broadly implemented.

Finally, integration of metabolic staining methods with pollen performance attributes is an exciting area of future research. For example, it was recently shown that *Arabidopsis* and tomato anthers produce populations of pollen with distinct levels of ROS that predict pollen performance (Luria et al. 2019). Further development of these techniques could lead to methods that allow one to sort for pollen that are primed to perform well under a particular set of conditions in the field.

Are there clear relationships between basic aspects of pollen biology and viability, longevity, and pollen performance?

The basic aspects of pollen biology include the number of apertures on the pollen grain (Fig. 3a–b), whether the mature pollen grain is bicellular or tricellular (Fig. 3c–d), whether the pollen is dispersed by wind or animal, and whether the pollen grain germinates on a wet (Fig. 3e–g) or dry stigma (Fig. 3f, h) (Pacini and Dolphus 2016; Tushabe and Rosbakh 2021). There is tremendous diversity in pollen morphology and floral structure across angiosperms (Fig. 4). In this section, we review the relationships that have been established between these fundamental aspects of pollen biology and viability and longevity (Dafni and Firmage 2000).

Number of apertures—a complex trade-off between pollen viability and pollen tube performance

Apertures are depressions or gaps in the pollen exine (the rigid external wall of the pollen grain) that are thought to

have two main functions: 1) facilitate pollen tube germination and 2) allow for changes in contraction and swelling of the pollen grain as it dehydrates during maturation and then rehydrates on the stigma (Katifori et al. 2010; Matamoro-Vidal et al. 2016; Wang and Dobritsa 2018; Halbritter et al. 2018; paldat.org). Angiosperms have evolved a wide range of aperture patterns (Fig. 4) and aperture number varies from 0 to 100. In contrast, pollen grains with a single aperture predominate in gymnosperms (conifers and Ginkgoales), basal angiosperms (Amborella, Magnoliales), and monocots (Fig. 4 panels 1–3) (Luo et al. 2015; Lu et al. 2015), suggesting that angiosperms have been subjected to selective pressures that have driven expansion in aperture number. Most eudicots have pollen grains with three furrow-like longitudinal apertures (Fig. 4 panels 4–8, 10–11, and 14) (Furness and Rudall 2004; Wang and Dobritsa 2018) like in tomato (*Solanum lycopersicum*) (Fig. 3a) and *Arabidopsis thaliana* (Fig. 3b). Other species have six or more apertures like *Hibiscus*, *Phlox*, *Plantago*, or *Borago* (Fig. 4 panels 9, 13, 16, 17, and 19).

Two approaches have been taken to determine the adaptive significance/function of apertures: analysis of mutants that alter aperture number and/or morphology and comparative approaches across species or within a species with individuals that produce pollen with variable aperture number (heteromorphic pollen) (Albert et al. 2022).

A comparison of six species with varying numbers of apertures and pollen wall thicknesses suggested that at least one aperture and a thicker pollen wall enabled pollen to withstand the stress of rapid hydration, which was associated with plasma membrane and cell wall rupture (Matamoro-Vidal et al. 2016). On the other hand, a study of *Arabidopsis* mutants with 0, 4, or 4 to 8 apertures (there are 3 in wild-type, Fig. 3b) found that pollen grains with the lowest aperture numbers (0 and 3) were able to better survive rehydration by minimizing plasma membrane rupture (Prieu et al. 2016). Together, these studies suggest that a low aperture number has been optimized to promote pollen grain wall integrity and longevity. Interestingly, using similar genetic approaches, it was also shown that the aperture number could also affect pollen germination and pollen tube performance (Albert et al. 2018). In a mixed pollination assay, Albert and colleagues found that triaperturate pollen grains produced pollen tubes that could outcompete those of inaperturate pollen; presumably because pollen germination and tube extension were facilitated by the presence of multiple apertures. These studies suggest a trade-off between high aperture numbers that promote pollen performance and low, but nonzero aperture numbers that accommodate rapid structural changes that accompany dehydration and rehydration.

Interestingly, in the highly heteromorphic pollen of the *Melanium* species within the genus *Viola* (pansies that producing pollen grains with 3–6 apertures), it was observed

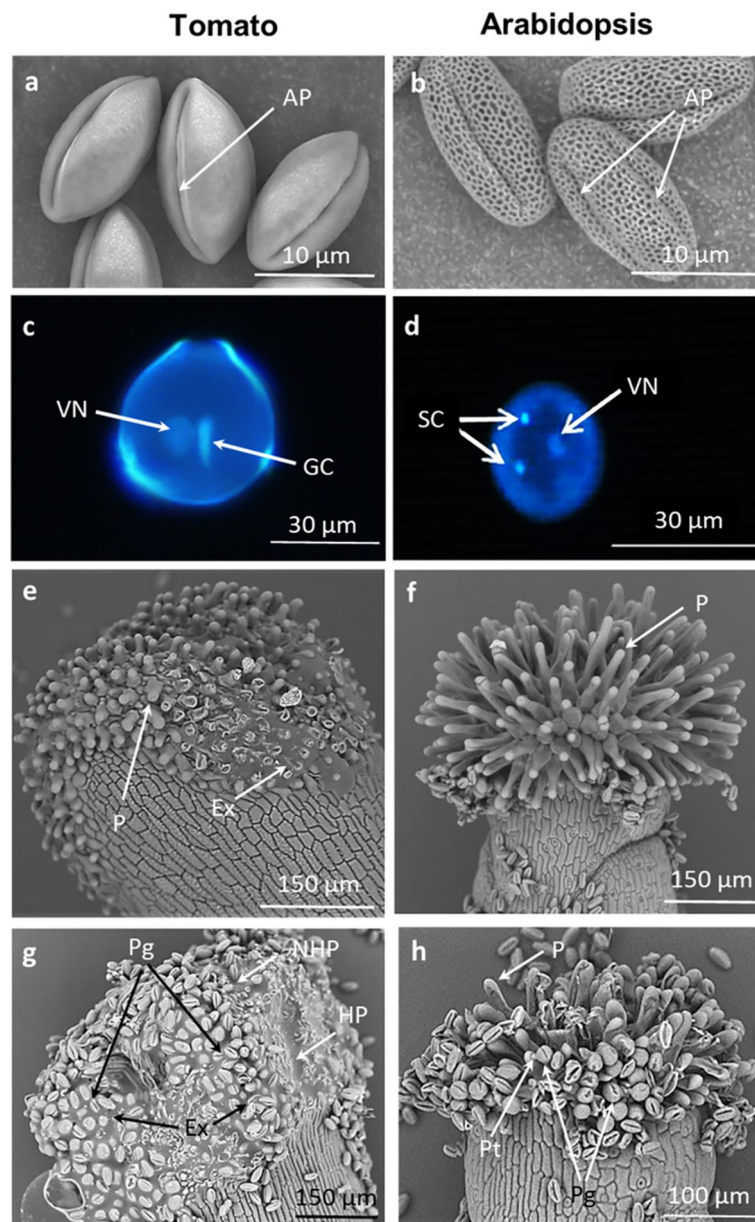


Fig. 3 *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*) represent species with contrasting aspects of pollen biology that affect pollen longevity and performance. **a, c, e, g** Tomato represents species with bicellular pollen and wet stigma; while **b, d, f, h** *Arabidopsis* represents species with tricellular pollen and a dry stigma. **a–b** SEM of pollen grains showing exine ornamentation and apertures. **c–d** DAPI staining showing the vegetative nucleus and the generative cell nucleus (tomato), or vegetative nucleus and two sperm cell nuclei (*Arabidopsis*). **e–f** SEM of unpollinated wet, exudate-covered tomato stigma with short papillae and the dry *Arabidopsis* stigma with long papillae. **g–h** SEM of pollinated tomato stigma showing pollen grains engulfed in exudate and *Arabidopsis* pistil showing pollen grains

adhering to papillae and germinating pollen tubes. Pollen grain and pistil images were acquired with a Hitachi TM3000 tabletop SEM at 5 kV in analytical and charge-up reduction modes and compositional imaging set up. Pistils were observed with an operating Peltier cooling stage (Deben) set up at -30°C . Pollen grains stained with DAPI were observed under UV illumination (absorption, 358; emission, 461 wavelength) with an inverted microscope (Leica DMI 6000B) equipped with the DFC450C camera. AP. Aperture; Ex. exudate; GC. generative cell nucleus; HP. hydrated pollen; NHP. non-hydrated pollen; P. papillae; Pg. pollen grain; Pt. pollen tube; SC. sperm cell nucleus; VN. vegetative cell nucleus

that environmental conditions that affect pollen viability (e.g., altitude) were not correlated with aperture number (Słomka et al. 2018). Further, in *V. diversifolia*, pollen grains with three apertures can be compared with those with four

or five. Triaperturate pollen has greater longevity and faster rates of pollen tube elongation in vitro, but pollen grains with four or five apertures germinate more rapidly (Dajoz et al. 1993; Till-Bottraud et al. 1999).

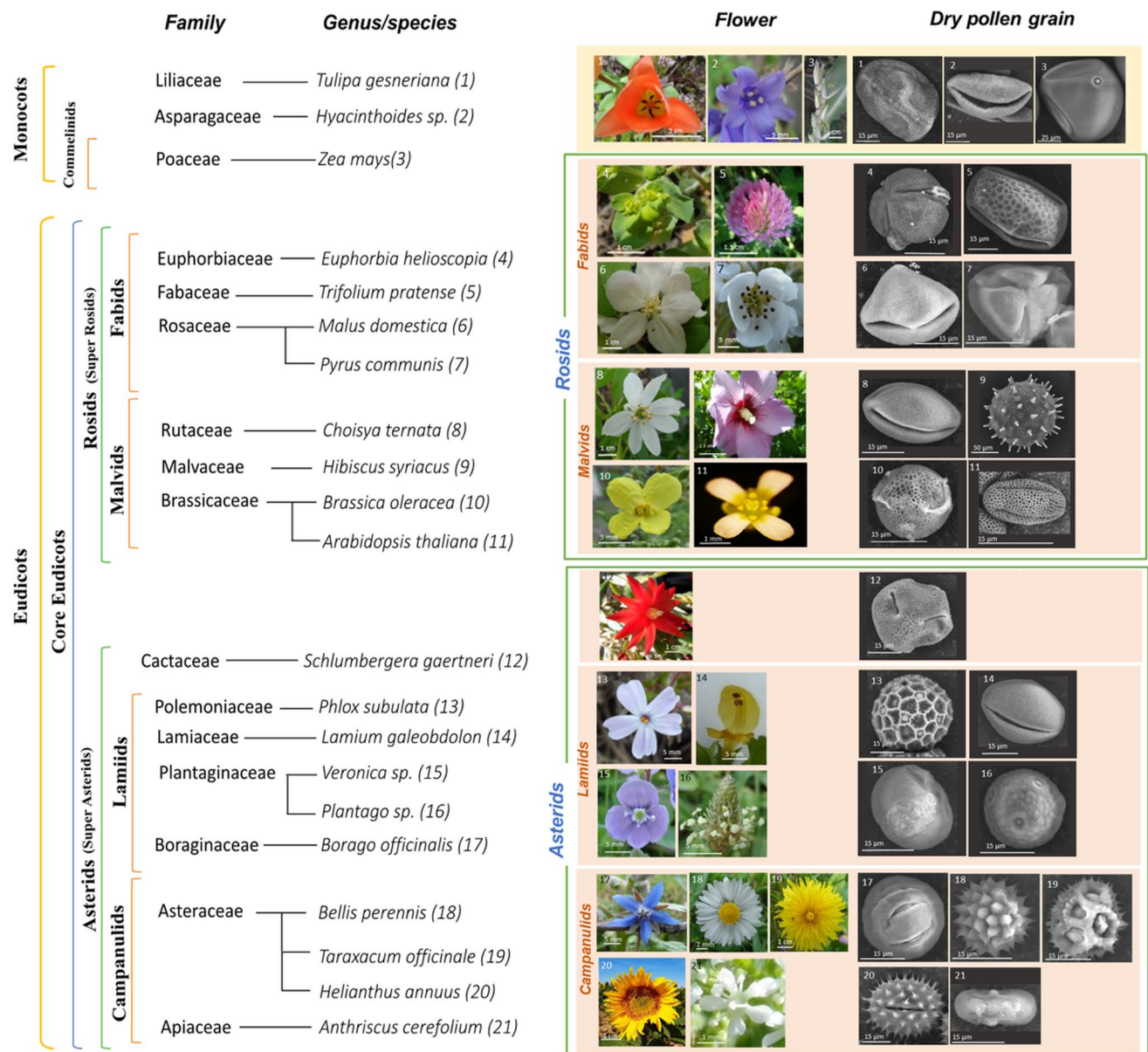


Fig. 4 Flower and pollen grain diversities across the angiosperm phylum. **a** Classification is according to the Angiosperm Phylogeny Group (2016). **b** Flower diversity. For *Zea mays*, only the male flower is shown. Flower pictures were obtained in a garden in Rouen with a WB150F Samsung digital camera in the macro mode. **c** Scanning

electron microscope (SEM) of dry pollen. Pollen grain images were acquired with a Hitachi TM3000 tabletop SEM at room temperature at 5 kV in analytical and charge-up reduction modes and compositional imaging set up

These studies all point to trade-offs between the number of apertures and pollen viability, longevity, and performance. The high frequency of triaperturate species may suggest optimization for rapid germination and pollen tube growth, while maintaining pollen grain integrity in the environment.

The extent of pollen dehydration and entry into developmental arrest varies across species and is significantly impacted by drought

Species that undergo extensive dehydration during

maturation in the anther (water content < 30% at dispersal) have been called ‘orthodox’ or partially dehydrated. On the other hand, species with pollen that remains partially hydrated have been called ‘recalcitrant’ and have water content > 30% at dispersal (Pacini and Dolferus 2019). Orthodox species tend to be heterogamous (anthers and pistils in separate flowers) and wind/animal pollinated—thus high levels of desiccation are thought to be adaptive because pollen must persist in the environment until it is deposited on a stigma and rehydrates (Franchi et al. 2011). Some orthodox species produce pollen that undergo developmental arrest—a period of metabolic quiescence that accompanies dehydration and is thought to extend viability in harsh environmental conditions until pollen is received by a receptive stigma (Pacini and Dolferus 2019; Footitt and Cohn 2001). Recalcitrant pollen has reduced longevity, is sensitive to desiccation that accompanies environmental exposure, and is common in homogamous species (anthers and pistils in the same flower) in which pollen can be rapidly transferred to the stigma without leaving the flower—pollen grains from these species have been found to germinate rapidly (Franchi et al. 2011).

Pollen rehydration initiates when pollen lands on the stigma, which can either be wet or dry. (Pacini and Franchi 2020). In wet stigmas (e.g., tomato, Fig. 3e, 3g), exudates are composed of a wide range of compounds including water, sugars, polysaccharides, lipids, proteins (including hydrolytic enzymes, defense and signaling proteins, and heat stress proteins), phenolics, amino acids, Ca^{2+} ions, and others (Swanson et al. 2004; Mollet et al. 2007; Rejón et al. 2013, 2014). Dry stigmas such as those of *A. thaliana* (Fig. 3f, 3h) typically have papillae covered by a primary cell wall, a waxy cuticle, and a proteinaceous pellicle (Swanson et al. 2004; Mollet et al. 2007). This characteristic is important for pollen adhesion, acceptance, and rehydration (Zinkl et al. 1999). Wet stigmas may capture and hydrate pollen grains non-specifically, whereas dry stigmas are considered more specific and may determine which pollen grains will be hydrated (Zinkl et al. 1999; Swanson et al. 2004; Doucet et al. 2016), thus influencing viability. Export of Ca^{2+} from papilla to germinating pollen via a calmodulin-activated calcium pump (ACA13, Autoinhibited Ca^{2+} -ATPase13, Iwano et al. 2014), exocyst-mediated polarized secretion (Safavian et al. 2015), actin focalization in stigmatic cells at the contact site with the pollen grain (Rozier et al. 2020) are thought to be required for pollen rehydration and germination.

In flowers with dry stigmas, water fluxes to the pollen grain are regulated spatially and temporally (Maurel et al. 2015; Di Giorgio et al. 2016b). Aquaporins facilitate passive water transport, including specific aquaporins found in sperm cells and others localized in tonoplast and endoplasmic reticulum membranes (Maurel et al. 2015). Four pollen-specific aquaporins have been identified in *A. thaliana*:

NIP4;1, NIP4;2 (Nodulin 26-like Intrinsic Proteins), TIP1;3, and TIP5;1 (Tonoplast Intrinsic Proteins) (Di Giorgio et al. 2016a). Expression peaks of NIP4;1 and NIP4;2 occur at different times, while TIP1;3 is expressed in the vegetative cell and TIP5;1 in the sperm cell within the pollen grain. The absence of both TIPs results in defects in pollen tube growth, resulting in an increase in unfertilized ovules and reductions in silique size and seed number, adversely affecting fitness (Di Giorgio et al. 2016a). Malfunctioning water fluxes thus have deleterious effects on pollen viability/function.

Water stress, including conditions of humidity and flooding, can have varying effects on pollen viability. The impact of flooding on pollen viability is not extensively studied, but researchers simulated flooding on bush lily (*Clivia miniata*), a xerophytic plant, by submerging pots in a water reservoir prior to anthesis (Yamburov et al. 2014). This study revealed that flooding primarily resulted in a reduction in the number of pollen grains per anther, followed by decreased pollen germination, and reduced pollen fertility, with minimal impact on pollen size. In addition, high humidity can rehydrate desiccated pollen, but it can also decrease pollen longevity (Pacini and Dolferus 2019). This effect has been observed in plants such as strawberry (*Fragaria × ananassa*) (Leech et al. 2002), *Cistus incanus*, and *Myrtus communis* (Aronne 1999).

Drought or water deficiency during the meiotic and mitotic stages causes disoriented nature and structure of the anthers (Yu et al. 2019a), leading to male sterility. Recent studies have pointed out the importance of the female counterpart. In wheat, high temperature and total water withdrawal for 5 d at gametogenesis altered the phenology of the plants (faster flowering and shortened microgametogenesis), a reduction of the relative water content, pollen viability and modifications of the morphology, and anatomy of the pistils (Fábián et al. 2019). This was associated with an enhanced generation of ROS and RNS (Reactive Nitrogen Species), decrease of NO, intensified lipid peroxidation in stigmatic papillae cells, and reduced fertility in sensitive genotypes (Fábián et al. 2019). The fertility loss was attributed for the reduced functionality of the male part at 66% and 34% of the female one (Fábián et al. 2019). In rice (*Oryza sativa*), drought stress was shown to dramatically affect male fertility. Altered starch distribution in the anthers was observed with an accumulation in connective cells and endothecium rather than in pollen, leading to a higher ratio of starchless pollen in the anther locule (Jin et al. 2013). This was accompanied by changes in carbohydrate metabolism and enzyme activities such as a reduction of acid invertase and soluble starch synthase leading to an inhibition of starch accumulation in pollen grains (Sheoran and Saini 1996).

Pollen development and regulated dehydration in the anther require accumulation of specific protective proteins and osmolytes and these processes are governed by

interactions between finely tuned hormone signal transduction pathways (increases in ABA and JA; along with decreases in GA and Auxin) (Parish and Li 2010; Parish et al. 2012). Drought and consequent water loss in the anther alters these pathways causing incorrect timing of tapetal programmed cell death (PCD) and significant increases in pollen abortion (Parish and Li 2010; Parish et al. 2012). Accumulation of protective proteins (*e.g.*, Late Embryogenesis Abundant, LEA) and osmolytes (amino acids, sugars, soluble pectin, polyols) is tightly regulated to accompany pollen dehydration and starts after the disappearance of the large central vacuole after the first pollen mitosis (Franchi et al. 2011; Pacini and Dolferus 2019; Pacini and Franchi 2020); these processes are also altered by drought. Finally, ROS levels are tightly regulated during normal pollen dehydration and development and are also altered by drought stress. ROS levels during pollen development and dehydration are increased during drought (Yu et al. 2019a). In rice, *Defective Tapetum Cell Death 1 (DTC1)* modulates ROS homeostasis and controls the degeneration of tapetal cells, ultimately affecting microspore and pollen development (Yi et al. 2016). DTC1 interacts with ROS scavengers suggesting a key regulator for tapetum PCD by inhibiting ROS-scavenging activity (Yi et al. 2016). This suggests that ROS homeostasis and the ability to inactivate excessive ROS also play an important role in male sterility during the reproductive stages. Using drought-tolerant and sensitive wheat cultivars, (Dong et al. 2017) showed that the tolerant lines displayed lower accumulation of ABA, higher superoxide dismutase (SOD), and peroxidase activities, leading to a higher caryopsis number compared to the sensitive cultivars (Dong et al. 2017).

Bicellular versus tricellular pollen grains

Efforts to understand the patterns of evolution of bicellular and tricellular pollen have prompted extensive analysis of this fundamental pollen characteristic across thousands of angiosperm species (Brewbaker 1967; Williams et al. 2014). Tricellular pollen is sexually mature and contains a pair of sperm cells within the pollen cell cytoplasm (Fig. 3 d, Arabidopsis). On the other hand, bicellular pollen contains a generative cell, a germline cell that must divide to produce a pair of sperm cells (Fig. 3 c, tomato). ~70% of angiosperm species are bicellular and this was thought to be the ancestral state (Brewbaker 1967), but recent work including many additional species and enabled by modern phylogenetic analysis tools calls this basic tenet into question and provides evidence that tricellular lineages produce bicellular species (and vice versa; Williams et al. 2014). Work in this area suggests that a trade-off between pollen longevity in the environment *versus* the ability to germinate rapidly on a receptive stigma may drive evolution of

this trait. Brewbaker noted that bicellular pollen was more viable *in vitro* and had enhanced longevity relative to tricellular and proposed that the mitotic division of the generative cell necessary to produce a pair of sperm cells depletes tricellular species of reserves that promote pollen longevity (Brewbaker 1967). Another proposal is that bicellular pollen undergoes greater levels of dehydration (see discussion above) and is more likely to undergo developmental arrest, while tricellular pollen tends to remain partially hydrated and shows more rapid germination on the stigma (Williams and Brown 2018). Williams and Brown suggested that tricellular pollen may be favored when viability during pollen transport is less critical than the ability to germinate rapidly and out compete other pollen on a receptive stigma. Interestingly, analysis of the basal angiosperm, *Annona cherimola*, shows that this species hedges this trade-off by tuning the timing of the division of the generative cell to environmental conditions (Lora et al. 2009). This species produces a mixture of bi- and tricellular pollen and it was found that the balance shifts in favor of tricellular pollen when development occurs at high temperature (Lora et al. 2009) or under desiccation (Lora et al. 2012). Moreover, tricellular pollen grains were found to germinate more rapidly *in vitro* (Lora et al. 2009, 2012). The balance of these studies suggests that trinucleate pollen grains have shorter longevity and are less able to persist in the environment, but they have an advantage in pollen competition owing to advanced maturity and better pollen performance in the pistil (see also Mulcahy and Mulcahy 1988).

The composition of the pollen coat

Pollen coat is a sticky mixture of substances found on the surface of the exine wall mostly composed of sporopollenin (Mackenzie et al. 2015). It mainly derives from the secretion and degeneration of the tapetum (Pacini 1997). During the rehydration process at the stigma surface, the pollen coat is essential for successful interactions between the stigma and pollen grains, leading to the activation of the basal compatible pollen response pathway (Doucet et al. 2016).

The amount of pollen coat found on the pollen grain depends on the species and is also related to the type of pollination (Teppner 2009). It is composed of many compounds, consisting mainly of hydrophobic substances such as lipid droplets with fatty acids, sterol esters, and triacylglycerols, with monogalactosyldiacylglycerol as the major polar lipid. It also includes proteins (*e.g.*, tapetal oleosins, caleosins, expansins, profilins, arabinogalactan proteins, Pollen Coat Proteins A-class and B-class (PCP-A and PCP-B), enzymes such as proteases, lipases, polygalacturonases, pectate lyases, xylanases, glucanases etc.), sugars, alkanes, alcohols, and other compounds (volatile organic compounds, triterpenoids, sterols, phenol amides, and flavonols) (Dobson

and Bergstrom 2000; Murphy 2006; Gong et al. 2015; Ischebeck 2016; Pacini and Dolferus 2016; Chichiricò et al. 2019; Xue et al. 2023).

In their review, Pacini and Hesse (2005) listed 20 functions for the pollen coat (pollenkitt) during pollen development and pollination. These functions encompass holding pollen within the anther until dispersal, protecting against water loss and UV radiation through flavonols in the pollen coat (which shield against UV-B radiation and safeguard the protoplasm from ROS damage), as well as facilitating adhesion and hydration on the stigma (Murphy 2006; Lin et al. 2013). For instance, *Arabidopsis* pollen coat components include the oleosin-domain protein GRP17 (glycine-rich protein 17) (Mayfield and Preuss 2000) and the lipase EXL4 (extracellular lipase 4), which aid in pollen hydration on the stigma (Updegraff et al. 2009).

The pollen coat has also been proposed to house sporophytic proteins responsible for pollen–stigma recognition, and to protect pollen from fungi and bacteria. Studies on *Campanula americana* and *Pisum sativum* have shown that pollen color, intine thickness, and pollen coat composition change in response to heat stress, suggesting that the pollen coat could be implicated in thermotolerance (Jiang et al. 2015; Koski and Galloway 2018). These observations all suggest that pollen coat is critical for pollen viability. A study by Chichiricò et al. (2019) on *Crocus vernus* and *Narcissus poeticus*, two species with dry stigmas, as in *A. thaliana* (Fig. 3 f, h), showed that removal of the pollen coat (via carbon disulfide treatment) did not affect in vivo or in vitro pollen germination, but reduced adherence to the stigmatic papillae (Chichiricò et al. 2019). Similarly, in rice, pollen coat mutants displayed a defective adhesion on the stigma, leading to a failure to rehydrate, thus affecting pollen function in vivo even though pollen grains were able to germinate in vitro (Yu et al. 2019b). This defect in adhesion and hydration could be rescued under high humidity conditions (Yu et al. 2019b), revealing that the pollen coat plays an important role in ensuring pollen–stigma interactions across a wide range of potential environmental conditions.

Future directions for research into the relationship between pollen type and pollen viability

The relationship between the pollen coat and the number/ placement of pollen apertures is a topic of research and no definitive relationships have been proposed. Some researchers propose a potential connection, suggesting that the pollen coat's composition and characteristics might influence aperture patterns. Wang and Dobritsa (2018) have suggested a correlation between the positions of apertures and the sites where callose forms during meiotic cytokinesis or where additional callose deposits occur post-cytokinesis.

Correlations have been established between the basic aspects of pollen biology (exine/aperture structure, extent of dehydration, timing of sperm maturation, and how these processes respond to environmental stress), pollen longevity, and pollen performance. An important goal for future research will be to continue to identify the genes and pathways that drive these critical pollen characteristics and to develop a mechanistic understanding of how each characteristic affects the ability of pollen to persist in the environment, germinate, and extend a pollen tube in the pistil. Identification of mutations that change these characteristics in a diversity of species as well as continued study of natural intra- and inter-specific variations, coupled with metabolomic studies will be important routes for further advances.

The molecular content of pollen grain is critical for viability and performance

The viability and longevity of pollen grains are determined by complex and interconnected metabolic processes that are regulated during pollen development and pollen tube growth and are strongly impacted by the environmental conditions occurring during flower development. Metabolism of macromolecules such as carbohydrates and lipids as well as ROS and ions regulate pollen hydration, germination, tube elongation, and pollen–stigma interactions.

Carbohydrates—fuel for development and growth and critical for osmotic control

Sucrose is transported to the anther from photosynthetic sporophytic sources where it is used to synthesize complex carbohydrates that serve as energy stores (*e.g.*, starch) and critical structural components of the pollen and pollen tube cell wall (*e.g.*, pectin). Hexokinase is the first committed step in building complex carbohydrates and OsHKK5 (HEXOKINASE5), which is pollen-specific and expressed late in pollen development was recently shown to be critical for pollen maturation, germination, and tube growth in rice (Lee et al. 2020).

Sugar uptake in pollen occurs in two stages: early during pollen development and late during anther development. In the early stage, a cytosolic invertase and the glucose exporter AtSWEET8 release monosaccharides from tapetum cells, which are actively taken up by transport proteins. In the late stage, AtSWEET13 and AtSWEET14 unload sucrose into the apoplast, with AtSUC1 facilitating its uptake into the pollen grain. Loss of AtSUC1 leads to sucrose deficiency and pollen germination defects. Additionally, vacuolar invertase AtVI2 plays a role in sucrose hydrolysis within pollen. AtVI2 cleaves vacuole-stored sucrose into monosaccharides that are subsequently transported to the cytosol. These monosaccharides play a crucial role in promoting

pollen germination and pollen tube growth. In *Atvi2* mutants, successful pollen germination is prevented (Seitz et al. 2023). The authors also mention the importance of various transporters in sugar metabolism and highlight that metabolic reprogramming can occur in the absence of apoplasmic sucrose, allowing for pollen germination but with altered energy source utilization (Seitz et al. 2023).

Carbohydrates are also critical for pollen water relations that affect viability and performance. Pollen grains have been divided into two groups depending on whether the mature pollen grain contains a starch reserve (in the form of amyloplasts), or whether accumulated starch has already been converted to soluble forms (Hoekstra et al. 1989; Pacini and Dolferus 2019). Starchless pollen grains have partially or totally hydrolyzed starch accumulated during development, resulting in high concentrations of sucrose and other low molecular weight carbohydrates along with high molecular weight polymers that play important structural roles for the pollen cell wall (Pacini et al. 2006). A comparison of the response to dehydration in corn and *Pennisetum* showed that high concentrations of sucrose was associated with protection of membranes during dehydration and it was proposed that differential sucrose concentrations could explain differential sensitivity to desiccation between these species (Hoekstra et al. 1989), see also (Pacini and Dolferus 2019).

Temperature stress affects carbohydrate metabolism with severe consequences for pollen development in the anther (Porch and Jahn 2001; Pressman et al. 2002; Frank et al. 2009; Santiago and Sharkey 2019). Importantly, thermotolerant genotypes have been shown to better maintain starch and sugar levels (Rieu et al. 2017). Interestingly, it was shown in wheat that elevated temperature during pollen development decreased pollen viability due to a decrease in photosynthetic capacity and a consequent reduction in starch synthesis and mobilization to the developing pollen (Dwivedi et al. 2017). Several studies have shown that tapetal cell invertase (hydrolysis of sucrose to produce glucose and fructose) activity decreases under heat stress, leading to reduced pollen viability (Zanor et al. 2009; Wang and Ruan 2016). In rice, heat-tolerant varieties displayed higher expression of cell wall acid invertase compared to the sensitive ones (Li et al. 2015). These data indicate that carbohydrate depletion can affect pollen viability but data are missing to clarify if reduced sugar levels at elevated temperature have a direct effect on pollen abortion and/or whether they reduce pollen function.

ROS: from cellular damage to vital signaling in plant reproduction and stress responses

ROS (e.g., $O_2^{\bullet-}$, H_2O_2 , and $OH^{\bullet}$) have traditionally drawn attention because of their deleterious effects on macromolecular structure and cellular viability, but it is now

appreciated that they also play key roles as signaling molecules (Huang et al. 2019). ROS control many physiological important events linked to plant stress responses and sexual plant reproduction including pollen development, pollen germination, pollen–stigma interactions, pollen tube growth, and fertilization (Fig. 5) (McInnis et al. 2006; Zhang et al. 2020). Accumulation of ROS was observed after pollen rehydration and just before pollen tube emergence (McInnis et al. 2006; Speranza and Scoccianti 2012; Gao et al. 2016). Generators of ROS, particularly NADPH oxidases (Respiratory Burst Oxidase Homologs, RBOH) that produce $O_2^{\bullet-}$, which is rapidly converted to H_2O_2 by SOD, were highlighted as a key element in pollen germination and pollen tube growth (Fig. 5). The use of diphenylene iodonium (DPI), a NADPH oxidase inhibitor, or ROS scavengers had a strong negative impact on pollen germination in angiosperms such as kiwi (*Actinidia deliciosa*) or olive (*Olea europaea*) (Speranza and Scoccianti 2012; Jimenez-Quesada et al. 2019) and in gymnosperms (Maksimov et al. 2018). Eighty percent of *rbohH rbohJ* double-mutant pollen ruptured immediately upon germination in vitro (Boisson-Dernier et al. 2013) and have reduced fertility due to precocious pollen tube growth arrest in the pistil (Kaya et al. 2014). Similarly, loss of function of the tapetum-expressed *RBOHE* reduced ROS production in tapetal cells, resulted in a delay in tapetal PCD, promoted pollen abortion, and significantly reduced pollen germination (Xie et al. 2014). Moreover, it has been suggested that ROS regulate pollen rehydration. In the *Arabidopsis* mutant *kinβγ* (*kinaseβγ*), the ROS levels in pollen grains were reduced, and pollen adhesion and hydration on the stigma surface were impaired, while they were able to germinate normally in vitro (Gao et al. 2016). The mutant also displayed reduced numbers of mitochondria and peroxisomes, suggesting that KINβγ may be involved in their biogenesis. Excessive exogenous application of H_2O_2 or chemically generated OH almost completely abolished tobacco pollen germination without affecting pollen viability (Smirnova et al. 2014). The data suggested that $OH^{\bullet}$ triggered local loosening of the intine wall at the germination aperture, whereas H_2O_2 was implicated in the cell wall stiffening in the rest of the wall through oxidative coupling of feruloyl-polysaccharides. UV light, particularly UV-B radiation, is a factor that regulates ROS accumulation. In maize, UV radiation reduces pollen viability and tube growth, leading to increased production of ROS ($O_2^{\bullet-}$ and H_2O_2), lipid peroxidation, and decreased activities of protective enzymes such as SOD and catalase (Wang et al. 2010). At the stigma, H_2O_2 accumulation was found in many species (Fig. 5) (McInnis et al. 2006; Hiscock et al. 2007; Kaya et al. 2014; Zafra et al. 2016; Lan et al. 2017). Interestingly, when pollen grains were in contact with the stigma, ROS levels decreased at the stigma interface suggesting a possible signaling cross talk between NO produced

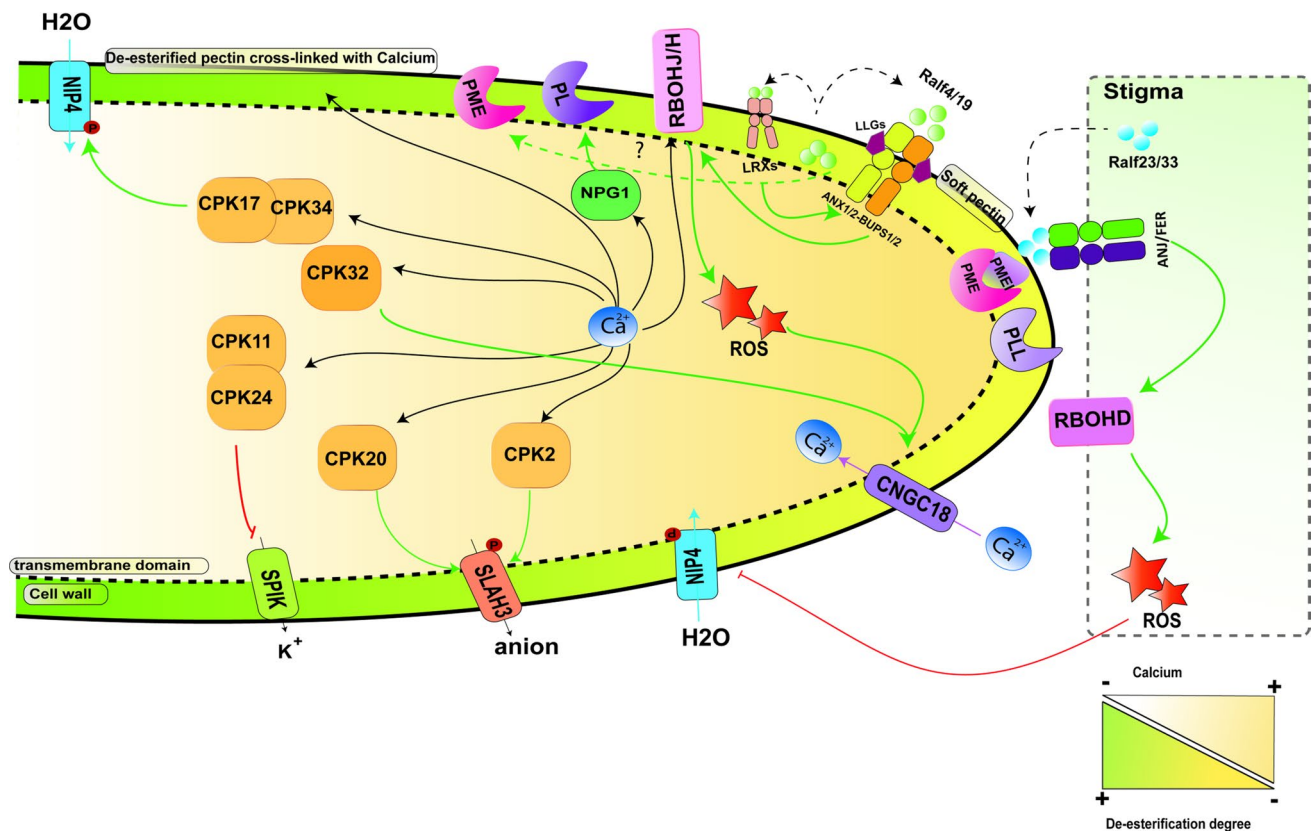


Fig. 5 Key molecular signals in the pollen tube, several CPKs (Ca^{2+} -Dependent Protein Kinases) regulate the pollen tube. Seven *A. thaliana* CDPKs were shown to regulate pollen tube growth, some being positive and other negative regulators. CPK17 and CPK34 phosphorylate the aquaporin NIP4 (Nodulin 26-like Intrinsic Protein 4), CPK20 and CPK2 activate SLAH3 (SLow Anion cHannel-associated3), and CPK32 phosphorylates the Ca^{2+} channel CNGC18 (Cyclic Nucleotide-Gated Channel 18), while CPK11 and CPK24 inhibit SPIK (Shaker Pollen Inward K^{+} channel) (Yip Delormel and Boudsocq 2019b). The NPG1 (No Pollen Germination1), a pollen-specific calmodulin-binding protein, interacts with PL (pectate lyase) to modify cell walls and promote PT growth (Shin et al. 2015). Stigmatic RALF23/33 (Rapid Alkalization Factor 23/33) peptides interact with ANJ-FER (ANJEA-FERONIA) receptors and induce ROS (Reactive Oxygen Species) via RBOHD (Respiratory Burst Oxidase Homologue D) to prevent pollen hydration (Lan et al.

2017). This mechanism can be unlocked by PCP-B (Pollen Coat Protein B)-class peptides, which compete with RALF23/33 for binding to the ANJ-FER complex, leading to a decline of stigmatic ROS, facilitating pollen hydration (Liu et al. 2021). On the other hand, pollen RALF4/19 peptides interact with LRX 8/9/10/11 (Leucine-Rich Extensin 8/9/10/11) monitoring cell wall changes (Mecchia et al. 2017), which are communicated to the pollen tube via the ANX1/2-BUPS1/2-LLG2/3 (Anxur1/2-Buddha's Paper Seal 1/2-LORELEI-Like-Glycosylphosphatidylinositol (GPI)-anchored proteins 2/3) (Feng et al. 2019; Ge et al. 2019) coreceptor complex to maintain pollen tube integrity and growth by activating ROS via RBOHJ/H, which leads to calcium channel activation (Somoza et al. 2021). The red line shows the inhibition process, and the green line shows the activation process. PME: Pectin MethylEsterase, PMEI Pectin MethylEsterase Inhibitor, PLL: pectate lyase-like

by the pollen and H_2O_2 generated in the stigma (McInnis et al. 2006). All the data reveal that ROS control the fine-tuning of pollen viability and manipulating this equilibrium could improve pollen viability.

A recent study in *A. thaliana* (Liu et al. 2021) highlighted this intricate cross talk between the stigma and the pollen allowing pollen hydration (Liu et al. 2021). RALF23/33 (RAPID ALKALINIZATION FACTOR23/33) peptides from the stigma trigger ROS production via RBOHD, preventing hydration of incompatible pollen (Fig. 5). In contrast, when compatible pollen landed on the stigma, Pollen Coat Protein B-class peptides compete with RALFs for the

binding site of the ANJEA-FERONIA complex, leading to a reduction of ROS production, thus promoting pollen hydration. However, this may not be a general pattern and changes in ROS levels may have different effects in other species. Indeed, in ornamental kale (*Brassica oleracea*), a decrease of ROS in the stigma reduced the adhesion and germination of compatible pollen (Lan et al. 2017). Nonetheless, these findings reveal that the stigma can precisely control pollen acceptance, hydration, pollen germination, and pollen tube growth by modulation of ROS levels.

Polyamines (PAs) (spermidine, putrescine, spermine etc.) are key components during pollen development. A reduction

of endogenous free PAs or lowering the activity of their biosynthetic enzymes affected pollen viability upon rehydration (Falasca et al. 2010) and strongly impaired pollen germination (Antognoni and Bagni 2008). In addition, moderate exogenous application of PAs was able to restore the germination rate of long-term stored tomato pollen grains (Song and Tachibana 2007). As such, spermidine (0.1 mM) was introduced in an *Arabidopsis* in vitro culture medium to promote pollen germination despite the fact that it slightly reduced pollen tube length after 5 h (Rodríguez-Enriquez et al. 2013). In contrast, high concentrations of natural PAs led to a strong reduction of pollen tube length and cell survivals (from 100 to 20% and 0% with 100 and 500 μ M spermine after 60 min, respectively), which was correlated with a decrease of ROS in germinating pollen and a strong increase of SOD and catalase activities (Aloisi et al. 2015). Similarly, a recent report has shown that the reduction of *Paeonia lactiflora* pollen viability after cryopreservation and long-term storage was correlated to PCD and ROS accumulation (Ren et al. 2020), presumably occurring during the freezing or thawing steps. Exogenous application of antioxidants such as ascorbic acid or glutathione could minimize the loss of pollen viability (Ren et al. 2020).

ROS levels also respond to heat stress and are likely critical for maintaining pollen function. (Frank et al. 2009) linked tomato microspore thermotolerance to higher basal expression of heat shock proteins and ROS scavengers. Several studies have shown that heat stress can also affect the female reproductive organ by modifying the turgor of papilla cells, inducing ROS production and membrane lipid peroxidation and a reduction of ROS-scavenging enzymes, soluble carbohydrate content and NO production, thus altering the pollen–pistil interactions, rehydration, and fertility (Snider and Oosterhuis 2011; Djanaguiraman et al. 2018; Fábian et al. 2019; Lohani et al. 2020).

Calcium: modulating pollen rehydration and germination

An increase of Ca^{2+} was observed at the germination site of the pollen tube and in pollinated papilla cells, three spikes were observed at three important pollen–stigma interaction stages: at the adhesion site of the pollen grain, during rehydration, and when the pollen tube penetrated the cell wall of the papilla (Iwano et al. 2004). This fine-tuning of water and Ca^{2+} exchanges between the stigma and the pollen grain can modulate pollen rehydration and germination.

As Ca^{2+} is a major ion governing pollen tube growth, research has focused on signaling molecules that sense and respond to fluctuations in Ca^{2+} concentration (Yip Delormel and Boudsocq 2019). At least seven *A. thaliana* CDPKs (Calcium-Dependent Protein Kinases) were shown to either positively or negatively regulate pollen tube

growth: CDPK2, CDPK11, CDPK17, CDPK20, CDPK24, CDPK32, and CDPK34 (Fig. 5). Another protein important for Ca^{2+} signaling, calmodulin, was shown to be essential for pollen germination (Golovkin and Reddy 2003). These authors identified a pollen-specific calmodulin-binding protein, called NPG1 (No Pollen Germination1) that is a regulator of pollen germination. Interestingly, the same group showed that NPG1 was regulating pollen germination through its interaction with pectate lyases, which modify pollen cell walls and regulate pollen tube emergence and growth (Fig. 5) (Shin et al. 2015). Moreover, a recent study in *Arabidopsis* showed that pectate lyases-like were required for intine loosening during early events of pollen germination and were secreted in the liquid culture medium during semi-in vivo pollen tube growth, suggesting that *in planta* they may be released from the pollen tube tip to soften the cell wall and/or middle lamellae of the transmitting tract (Chebli and Geitmann 2023). Leroux et al. (2015) found a link between Ca^{2+} signaling and pollen hydration when they showed that a *PME48* (*Pectin MethylEsterase48*) loss-of-function mutant caused a decrease of PME activity with higher levels of highly methylesterified pectins and possibly lower levels of Ca^{2+} in the intine. It strongly affected pollen rehydration and induced a strong delay in in vitro pollen germination especially on solid medium. Interestingly, the phenotype could be restored when additional Ca^{2+} was added to the germination medium, suggesting that in wild-type plants, Ca^{2+} in the intine wall may be a source for the intracellular Ca^{2+} signaling during pollen grain germination (Leroux et al. 2015).

Future directions: main molecular composition and pathways affecting pollen viability

Several critical pollen tube signaling pathways are not yet fully understood. For example, the downstream events of the RALF signaling pathway are still unclear. How does RALF signaling maintain pollen tube integrity? What are the connections between RALF signaling, calcium signaling, and the enzymes like PMEs that directly modify the cell wall? PME function during pollen tube extension has been described, but there is much to learn like 1) whether individual PMEs have specific substrates, 2) how the two major groups of PMEs function together (type 1: PME with a PME Inhibitor pro-region, which is supposed to be removed in the Golgi by subtilisin-like proteases allowing the secretion of PMEs to the cell wall and type 2: PME without pro-region (Röckel et al. 2008; Wolf et al. 2009), and 3) what are the regulatory mechanisms of PME–PMEI interaction during pollen germination and at the pollen tube tip, which control the fine-tuning of the degree of methyl esterification of HG (HomoGalacturonan).

Finally, most of the signaling pathways are identified in model plants such as *Arabidopsis*, while fewer are reported in others, which limits our understanding of responses and pathways specific to each plant species. More studies should be carried out on other species, both domesticated and wild, to determine which of the major signaling systems are universal and which are unique to a given species.

Short- to long-term storage of pollen grains

The ability to store, transport, and use pollen is critical for growers, breeders, conservationists, and academic researchers. Pollen viability and longevity are highly species-dependent and are affected during storage. Orthodox or PD pollen is generally more suitable for long-term storage as they can better sustain dehydration (Franchi et al. 2011; Pacini and Dolferus 2019). Storage at low temperatures (−20, −30, −60, −76, or −196 °C) with or without desiccant and under controlled conditions can extend pollen viability: for up to 1 year as shown with date palm (*Phoenix dactylifera*) (Mesnoui et al. 2018), tomato (Song and Tachibana 2007), and others (Supplementary information Table S2); to up to 6 years with some orchids (Marks et al. 2014). In other species, pollen viability can remain reasonably high for several weeks/months like in agave (*Agave tequilana*) (Díaz and Garay 2008) and others (Supplementary information Table S2). In other cases, pollen viability dropped significantly with storage: cannabis (*Cannabis sativa*) pollen cannot be stored for 4 months; amaryllis (*Hippeastrum sp.*) pollen germination decreased from ~50–70% at storage day 0 to ~0 to 40% at storage day 125 (de Almeida et al. 2019). Jasmine pollen stored at −60 °C decreased from ~90% to ~40% viability after 48 weeks (Perveen and Sarwar 2011). Several wheat cultivars illustrate the challenge with germination rates that approach 0 after only 7 h of storage at either 5 or −20 °C (Baninasab et al. 2017) (Supplementary information Table S2). For economically important species like these, there will be great interest in development of new storage approaches. For example, moderate exogenous application of polyamines (PAs) or antioxidants such as ascorbic acid, SOD, or malate dehydrogenase could improve pollen germination of long-term stored pollen (Song and Tachibana 2007; Ren et al. 2020). The rehydration step of pollen grains is also critical as it can cause plasma membrane damage if water uptake is too rapid. Thus, a slow rehydration of pollen grains in a humid chamber can be beneficial in maintaining viability (Volk 2011). Knowing the physiological state at the time of pollen harvest and the need for standardized handling methods after collection will definitely affect the success for long-term storage under optimized conditions.

Research directions to improve pollen storage

In the case of crop species, like wheat, improving pollen storage would have great economic impact. Application of antioxidants is a promising approach, but further analysis of the molecular pathways that govern pollen dormancy and rehydration could also bring genetic approaches that will improve pollen storage. In addition, artificial pollination is increasingly important for crop production, especially when populations of pollinators are threatened by climate change. Emerging technologies such as drone- or robotic-pollination devices may help to address this challenge. However, implementation of methods could influence pollen viability due to factors such as physical stress, temperature variations during transport, storage conditions, drying processes, transfer mechanisms, and compatibility issues with target plant species. To maintain pollen viability in artificial pollination, it is crucial to manage these factors meticulously and leverage technological advancements and research to mitigate any adverse effects.

Conclusions and perspectives

Pollen viability and function are determined by a complex interplay between the particular pollen biology of a species and the environment. Understanding the molecular mechanisms responsible for pollen biology discussed here will be critical for efforts to understand how climate change is affecting the distribution of plant species and to develop resilient crops. Selection has resulted in a tremendous diversity of pollen across species that uniquely balances the trade-offs between maintenance of structural integrity in the environment and the ability to rapidly germinate a pollen tube on a receptive stigma.

The molecular composition of pollen grains holds paramount importance in determining their viability and overall performance. The viability and longevity of pollen grains are governed by intricate metabolic processes that involve the regulation of carbohydrate metabolism, ROS, and calcium signaling. Carbohydrates play a crucial role as both a source of energy for growth and development and as regulators of osmotic balance. ROS, once seen as strictly detrimental, are now appreciated as essential signaling molecules guiding multiple stages of pollen development, germination, and interactions with the stigma. Calcium signaling is intricately linked with pollen rehydration and tube growth, mediated by Ca^{2+} -Dependent Protein Kinases and other Ca^{2+} -binding proteins.

The interaction between these factors provides a multifaceted perspective on pollen viability and function and many challenges for future research. Temperature stress, a significant environmental factor, impacts carbohydrate metabolism

Table 2 Effects of temperature stress on pollen viability of several species

Species / Common name	Temperature range tested	Effects	References
25 herbaceous species	0–40 °C	Large spectrum of plants for which the adaptation of pollen germination and tube growth to a broad range of temperatures determined their geographic distributions	Rosbakh and Poschlod (2016)
<i>Triticum aestivum</i> : wheat	25–35 °C	Study over three seasons. Reduced pollen viability at high temperature due to late sowing,	Dwivedi et al. (2017)
<i>Oryza sativa</i> : rice	30–40/24 °C	Salicylic acid reversed pollen abortion caused by heat stress	Feng et al. (2018)
<i>Phaseolus vulgaris</i> : common bean	32/27 °C	The heat-sensitive genotypes displayed reduced pollen viability	Porch and Jahn (2001)
<i>Pisum sativum</i> : pea	35/18 °C	Heat stress reduced pollen viability	Jiang et al. (2019)
<i>Cicer arietinum</i> : chickpea	3, 7, 12, 15, or 25 °C	Low temperature (less than 15 °C) affected both the development and function of reproductive structures in the chickpea flower	Clarke and Siddique (2004)
<i>Glycine max</i> : soybean	15/10 °C	Low temperature caused abnormal pollen grain formation	Ohnishi et al. (2010)
<i>Arachis hypogaea</i> : groundnut	10–47.5 °C, at 2.5 °C intervals	Optimal temperatures for pollen viability and pollen tube growth, in 21 groundnut genotypes, were observed around 30 and 34 °C, respectively. Some genotypes showed shifts of optimal temperatures by 10 °C	Kakani et al. (2002)
	32/22 °C, 36/26 °C, 40/30 °C & 44/34 °C	Pollen germination decreased from 82% (36/26 °C) to 14% (44/34 °C) when exposed to high temperatures	Prasad et al. (2011)
<i>Sorghum bicolor</i> : sorghum	32/22 °C, 36/26 °C, 40/30 °C & 44/34 °C	Pollen longevity in sorghum was shorter (4 h) at 36/26 °C than at 32/22 °C (6 h)	Prasad et al. (2011)
<i>Citrus</i> sp.: mandarin, lemon, pomelo, clementine	10, 15, 20, 25 & 30 °C	Optimal temperature for pollen germination was around 25 °C, pollen tube growth relied on particular male–female interaction and ranged between 15 and 25 °C. Temperature has a role on self-incompatibility	Distefano et al., (2012)
<i>Solanum lycopersicum</i> : tomato	32/26 °C	Reduced number of pollen grains per flower and decreased viability	Pressman et al. (2002)
	43–45 °C for 2 h	Microspore thermotolerance was associated to heat shock proteins, among other factors, analyzed by microarray	Frank et al. (2009)
	38 °C for 1 h	Transcriptomics and proteomics revealed large sets of genes and proteins linked to tomato microsphere thermotolerance	Keller et al. (2018)
<i>Medicago sativa</i> : alfalfa	25–37 °C	Slightly reduced pollen viability at high temperature. The most important factor was the time after removal from the anthers	Brunet et al. (2019)
<i>Chenopodium quinoa</i> : quinoa	40/24 °C	Heat stress affected pollen viability: effects were more dramatic in the heat-sensitive genotypes	Hinojosa et al. (2019)

and ROS levels, highlighting the vulnerability of pollen to changing climatic conditions (Table 2). Intraspecific variations in pollen viability highlight the importance of genetic diversity and the potential to breed more resilient plant varieties. A study compared two pea (*Pisum sativum*) cultivars, ‘CDC Golden’ and ‘CDC Sage,’ under heat stress conditions. After exposure to 35 °C for 4 or 7 days, ‘CDC Golden’ saw a 50% decline in pollen viability at both flower bud and anthesis stages, while ‘CDC Sage’ only experienced reduced viability at anthesis. This indicates ‘CDC Sage’ is more heat tolerant (Jiang et al. 2019). Similarly, heat stress affected 14 rice cultivars from temperate and tropical regions, leading to reduced spikelet fertility due to decreased pollen production, viability, and reception on the stigma (Prasad et al. 2006).

Climate change will alter the geographic distribution of plants and pollinators. Selection of new plant varieties, able to produce pollen that tolerates abiotic stresses, will minimize crop losses. Indeed, it has been shown that plants that are resistant/tolerant to abiotic stresses or to reduced resources produce pollen that outperforms pollen from sensitive plants (Delph et al. 1997). Research on biostimulants alleviating the deleterious effects of environmental stresses on pollen viability and longevity or improving pollen storage could be an alternative. For example, Pohl et al. (2019) showed that temperate zone eggplants treated with Göemar BM-86, an *Ascophyllum nodosum* extract, had improved pollination efficiency: higher numbers of pollen tubes in the style and more fertilized ovules. Similarly, a recent study has shown that addition of carbohydrate-based extracts to tomato pollen could promote in vitro pollen germination and pollen tube growth under cold temperatures (8 and 13 °C) and NADPH oxidase (*RBOH*) gene expression was correlated with a higher number of viable pollen tubes in biostimulated pollen tubes compared to the control (Laggoun et al. 2021).

Pollen develops in the anther and functions in the pistil. To improve pollen storage, viability, and function in a dynamic climate, we must understand the complex pathways that govern the onset of dehydration and dormancy and how these processes are reversed. Continued progress in this area will provide key insights into plant adaptation as well as leading to new approaches to improve agricultural productivity.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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