Pollen–stigma interactions in Brassicaceae
Bosch, Maurice; Wang, Ludi

Published in:
Journal of Experimental Botany
DOI:
10.1093/jxb/eraa117
Publication date:
2020

Citation for published version (APA):
Bosch, M., & Wang, L. (2020). Pollen–stigma interactions in Brassicaceae: Complex communication events regulating pollen hydration. Journal of Experimental Botany, 71(9), 2465–2468. https://doi.org/10.1093/jxb/eraa117

**Document License**
CC BY

**General rights**
Copyright and moral rights for the publications made accessible in the Aberystwyth Research Portal (the Institutional Repository) are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Aberystwyth Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Aberystwyth Research Portal

**Take down policy**
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

tel: +44 1970 62 2400
e-mail: is@aber.ac.uk
Pollen–stigma interactions in Brassicaceae: complex communication events regulating pollen hydration

Maurice Bosch* and Ludi Wang

Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Plas Gogerddan, Aberystwyth, SY23 3EE, UK

*Correspondence: mub@aber.ac.uk

This article comments on:
Rozier F, Riglet L, Kodera C, Bayle V, Durand E, Schnabel J, Gaude T, Fobis-Loisy I. 2020. Live-cell imaging of early events following pollen perception in self-incompatible Arabidopsis thaliana. Journal of Experimental Botany 71, 2513–2526.

The process of plant reproduction is responsible for much of our food supply, with fertility and seed set critical for crop yield and thus food security. The first stage of male–female recognition in flowering plants takes place when pollen lands on the surface of the stigma. In Brassicaceae, compatible pollen will hydrate and germinate, leading to successful fertilization, while these processes are severely impaired in incompatible pollen, preventing fertilization. Rozier et al. (2020) have developed a semi-in vivo live-cell imaging system to investigate early pollen–stigma interactions in both compatible and incompatible pollinations in Arabidopsis thaliana.

Successful fertilization in flowering plants involves multiple communication events between the pollen and the pistil (Dresselhaus and Franklin-Tong, 2013). In Brassicaceae, this communication starts soon after pollen is captured on stigmatic papilla cells. A captured desiccated pollen grain has to hydrate first to become metabolically active before it can germinate and develop a pollen tube that penetrates the stigmatic cell wall and grows through the apoplastic space down to the ovary for fertilization (Kandasamy et al., 1994). The dry surface of stigmas in the Brassicaceae represents an important checkpoint for selective pollen hydration (Dickinson, 1995). A compatible pollination triggers cellular responses in the stigma to transfer water to the desiccated pollen while in the Brassicaceae species that exhibit the sporophytic self-incompatibility (SI) system, pollen hydration is significantly inhibited following an incompatible pollination (Dickinson, 1995). Even when incompatible pollen manages to by-pass the first checkpoint, for instance under conditions of high humidity, these tubes are arrested at the point of stigmatic cell wall penetration (Zuberi and Dickinson, 1985).

Although we have good knowledge of the SI-induced signalling events leading to self-pollen rejection in the Brassicaceae (briefly discussed later; see also Box 1), studies on the cellular and molecular responses of stigmatic papilla cells following both compatible and self-incompatible pollination are challenging, with many aspects remaining unclear.

Several studies have used imaging techniques to investigate changes in the stigmatic papilla cells during self- and cross-pollination (e.g. Dickinson, 1995; Iwano et al., 2007; Hiroi et al., 2013; Safavian and Goring, 2013). The new system described by Rozier et al. (2020), utilizing engineered Arabidopsis SI lines in combination with an elegant imaging set-up, allows live-cell imaging of morphological and physiological changes in Arabidopsis following compatible and self-incompatible pollinations.

What controls pollen recognition and hydration?

Following capture of pollen grains on the stigmatic surface, pollen coat material mainly composed of proteins and lipids forms a ‘foot’ that establishes continuity at the pollen–papilla cell interface (Kandasamy et al., 1994). Rozier et al. (2020) carried out semi-in vivo assays using live-cell imaging to record early pollination events. Using the ratio of the long and wide pollen axis (L/W) as a proxy for pollen hydration, they showed that compatible pollen need to pass below a threshold value of L/W=1.4 before they start germinating. As shown before in Brassica (Dickinson, 1995; Hiroi et al., 2013), the hydration of compatible pollen starts within a few minutes after pollen deposition. The kinetics of compatible pollen hydration turned out to be biphasic—a rapid hydration phase during the first 10 min is followed by a phase where there is little further hydration and pollen germination occurs (Rozier et al., 2020). Most incompatible pollen showed little sign of hydration (Rozier et al., 2020). As suggested by Dickinson (1995), this indicates that the SI response operates within the first few minutes after deposition of incompatible pollen and that one of the main consequences is blocking of pollen hydration.
Secreted cysteine-rich proteins (CRPs) found in the pollen coat have been shown to be involved in cellular recognition/communication during the earliest stages of pollen–stigma interaction (reviewed by Marshall et al., 2011). The S-locus cysteine-rich/S-locus protein 11 (SCR/SP11) is a pollen coat-derived CRP involved in controlling recognition of incompatible pollen. SCR/SP11 acts as the ligand in Brassicaceae SI, binding to the allelic S-locus receptor kinase (SRK) in the stigmatic papillae to activate a signalling cascade that inhibits hydration and germination of incompatible pollen (Schopfer et al., 1999; Takayama et al., 2000). Another class of CRPs, called PCP-Bs (pollen coat protein B class), have been identified as key
regulators of compatible pollen hydration (Wang et al., 2017). Knockouts of PCP-B-encoding genes exhibited severely impaired pollen hydration. To obtain a better understanding of pollen–stigma communication that establishes compatibility, it will be important to identify the stigma receptor of the PCP-B proteins. Such a ‘compatible’ receptor–ligand module may be responsible for triggering a signalling cascade in the papilla cells that leads to polarized secretion at the pollen contact site.

One of the early cellular regulators of pollen hydration and germination is vesicle trafficking in the papilla cells (Box 1; reviewed in Goring, 2017). Studies in Brassica oleracea showed vesicle-like structures in the papilla cell wall following compatible pollination (Dickinson, 1995; Elleman and Dickinson, 1996). More recent studies illustrated that multivesicular bodies (MVBs) in papillae fuse to the plasma membrane beneath the contact point with compatible pollen (Safavian and Goring, 2013). In response to incompatible pollen, MVBs as well as autophagic bodies were found in the vacuole, which may indicate disruption of vesicle trafficking and secretory activity (Safavian and Goring, 2013; Indriolo et al., 2014).

Exocyst-mediated secretory vesicle trafficking in stigmatic papilla cells was shown to be important for compatible pollen hydration (Samuel et al., 2009; Safavian et al., 2015). EXO70A1, a subunit of the exocyst complex acting as a tethering mediator of polarized secretions, has been shown to be essential for the acceptance of compatible pollen and to be ubiquitinated by the ARM repeat-containing protein ARC1 E3 ligase in the SI annotation (Samuel et al., 2012, 2014; Box 1). Based on L/W ratios reported by Rozier et al. (2020), the pollen volume between 2 min and 10 min after compatible pollen deposition increases with ~1500 μm³ (equivalent to a 44% increase). This further illustrates the extent of vesicle trafficking and targeted secretion required at the papilla–pollen contact site to accommodate the rapid pollen hydration process in Brassicaceae.

A function of actin focalization in hydration and germination of compatible pollen?

Remodelling of the actin cytoskeleton, leading to actin focalization at the contact site with compatible pollen, appears to be a hallmark feature in compatible pollen–stigma interactions in Arabidopsis and Brassica (Iwano et al., 2007; Rosier et al., 2020). Rosier et al. (2020) hypothesize that actin remodelling in stigmatic papillae is triggered by the mechanical pressure produced by the pollen tube at the site of cell wall penetration. Indeed, interaction with microbes and application of physical stimuli have both been shown to cause aggregation of actin microfilaments beneath the contact point in leaf epidermal cells or cotyledons (Jayaraman et al., 2014). How local mechanical stimulation is translated to localized actin remodelling remains to be determined. As an alternative to the mechanical pressure hypothesis, the observed actin focalization may be a result of a signalling cascade initiated by a compatible pollen–stigma recognition event. Calcium plays an important role in controlling actin dynamics (Hepler, 2016) and may, perhaps by altering the properties of specific actin-binding proteins (ABPs), be involved in actin remodelling leading to focalization. Compatible pollinations induce increases in papilla cell Ca²⁺, just below the contact site, with the highest increase observed when the pollen tube penetrates the papilla cell wall (Iwano et al., 2004). Interestingly, a subsequent study showed that compatible pollination triggers the export of Ca²⁺ from papilla cells to germinating pollen, a process promoting successful fertilization and mediated by ACA13, a calmodulin-activated calcium pump, accumulating at the plasma membrane near the pollen attachment site (Iwano et al., 2014).

The function of actin focalization remains to be determined. The fact that the actin cytoskeleton is important for the delivery of secretory vesicles and knowledge that there is polarized delivery of secretory vesicles in the papilla cells to the pollen contact site to promote localized exocytosis in the papilla cells as well as autophagic bodies were found in the vacuole, which may indicate disruption of vesicle trafficking and secretory activity (Safavian and Goring, 2013; Indriolo et al., 2014).

Penetrating the cell wall barrier

Early electron microscopy studies have shown that, prior to pollen tube penetration, a compatible pollen–stigma interaction triggers an expansion of the outer papilla cell wall layer at the site of interaction (Dickinson, 1995). Following penetration of the papilla cell cuticle and cell wall, the pollen tube grows toward the base of this cell through the cell wall matrix along the surface of the plasma membrane. Papilla cell wall expansion and/or loosening, pollen tube penetration, and subsequent growth through the cell wall matrix clearly require major enzymatic modifications of the papilla cell wall. Yet, besides a functional role in pollen tube penetration implicated for pollen polygalacturonases and cutinases (Hiscock et al., 2002), there are surprisingly few data on cell wall-related enzymes involved in these early pollen–stigma interaction steps. As previously mentioned, a compatible interaction triggers polarized exocytosis in the stigmatic papillae. It will be important to identify the content of these secretory vesicles that are delivered to the pollen–stigma interaction site as the presence of molecules involved in pollen hydration and penetration. In agreement with earlier studies (e.g. Zuberi and Dickinson, 1985), Rozier et al. (2020) showed that even when an incompatible pollen manages to germinate and grow a tube, for instance under high humidity conditions, these invariably fail to penetrate the papilla cell wall. This suggests that stigma-derived enzymes required for pollen tube penetration have not been delivered to the stigmatic surface, implicating the existence
of an additional checkpoint that prevents the penetration of germinated incompatible pollen when the earlier hydration checkpoint has been breached.

Two interacting signalling pathways have been suggested to operate in the stigma of Brassicaceae that exhibit SI: the ‘basal compatible pollen response pathway’ regulating the acceptance of compatible pollen, and the extensively studied self-incompatible pollen pathway leading to self-pollen rejection (Doucet et al., 2016; Box 1). Future work, utilizing proteomic and transcriptome profiling approaches, will undoubtedly reveal further components playing a part in the early pollen–stigma recognition events involving these two pathways. The availability of Arabidopsis plants harbouring the Brassica SI system, along with other genetic tools that can be combined with live-cell imaging approaches utilizing the set-up described by Rozier et al. (2020), provides exciting opportunities to further dissect the molecular and physiological mechanisms involved in the early pollen–stigma communication events.

Acknowledgements

L.W is supported by a grant from the Biotechnology and Biological Sciences Research Council (grant no. BB/P005489/1) to M.B.

Keywords: Actin cytoskeleton, Brassicaceae, cellular communication, hydration, pollen recognition, pollen–stigma interaction, self-incompatibility

References

Dickinson H. 1995. Dry stigmas, water and self-incompatibility in Brassica. Sexual Plant Reproduction 8, 1–10.

Doucet J, Lee HK, Goring DR. 2016. Pollen acceptance or rejection: a tale of two pathways. Trends in Plant Science 21, 1058–1067.

Dresselhaus T, Franklin-Tong N. 2013. Male-female crosstalk during pollen germination, tube growth and guidance, and double fertilization. Molecular Plant 6, 1018–1036.

Elleman CJ, Dickinson HG. 1996. Identification of pollen components regulating pollination-specific responses in the stigmatic papillae of Brassica oleracea. New Phytologist 133, 197–205.

Goring DR. 2017. Exocyst, exosomes, and autophagy in the regulation of Brassicaceae pollen-stigma interactions. Journal of Experimental Botany 69, 69–78.

Hepler PK. 2016. The cytoskeleton and its regulation by calcium and protons. Plant Physiological 170, 3–22.

Hiroi K, Sone M, Sakazono S, Osaka M, Masuko-Suzuki H, Matsuda T, Suzuki G, Suwabe K, Watanabe M. 2013. Time-lapse imaging of self- and cross-pollinations in Brassica rapa. Annals of Botany 112, 115–122.

Hiscock SJ, Bown D, Gurr SJ, Dickinson HG. 2002. Serine esterases are required for pollen tube penetration of the stigma in Brassica. Sexual Plant Reproduction 15, 65–74.

Indriolo E, Safavian D, Goring DR. 2014. The ARC1 E3 ligase promotes two different self-pollen avoidance traits in Arabidopsis. The Plant Cell 26, 1525–1543.

Indriolo E, Tharmapalan P, Wright SI, Goring DR. 2012. The ARC1 E3 ligase gene is frequently deleted in self-compatible Brassicaceae species and has a conserved role in Arabidopsis lyrata self-pollen rejection. The Plant Cell 24, 4607–4620.

Iwano M, Shiba H, Matoba K, et al. 2007. Actin dynamics in papilla cells of Brassica rapa during self- and cross-pollination. Plant Physiology 144, 72–81.

Iwano M, Shiba H, Miwa T, Che FS, Takayama S, Nagai T, Miyawaki A, Isogai A. 2004. Ca2+ dynamics in a pollen grain and papilla cell during pollination of Arabidopsis. Plant Physiology 136, 3562–3571.

Iwano M, Igarashi M, Tarutani Y, et al. 2014. A pollen coat-inducible autoinhibited Ca2+-ATPase expressed in stigmatic papilla cells is required for compatible pollination in the Brassicaceae. The Plant Cell 26, 636–649.

Jayaraman D, Gilroy S, Ané JM. 2014. Staying in touch: mechanical signals in plant-microbe interactions. Current Opinion in Plant Biology 20, 104–109.

Kandasamy MK, Nasrallah JB, Nasrallah ME. 1994. Pollen–pistil interactions and developmental regulation of pollen tube growth in Arabidopsis. Development 120, 3405–3418.

Marshall E, Costa LM, Gutierrez-Marcos J. 2011. Cysteine-rich peptides (CRPs) mediate diverse aspects of cell-cell communication in plant reproduction and development. Journal of Experimental Botany 62, 1677–1686.

Rozier F, Riglet L, Kodera C, Bayle V, Durand E, Schnabel J, Gaude T, Fobis-Loisy I. 2020. Live-cell imaging of early events following pollen perception in self-incompatible Arabidopsis thaliana. Journal of Experimental Botany 71, 2519–2526.

Safavian D, Goring DR. 2013. Secretory activity is rapidly induced in stigmatic papillae by compatible pollen, but inhibited for self-incompatible pollen in the Brassicaceae. PLoS One 8, e84286.

Safavian D, Zayed Y, Indriolo E, Chapman L, Ahmed A, Goring DR. 2015. RNA silencing of exocyst genes in the stigma impairs the acceptance of compatible pollen in Arabidopsis. Plant Physiology 169, 2526–2538.

Samuel MA, Chong YT, Haasen KE, Aldea-Brydges MG, Stone SL, Goring DR. 2009. Cellular pathways regulating responses to compatible and self-incompatible pollen in Brassica and Arabidopsis stigmas intersect at Exo70A1, a putative component of the exocyst complex. The Plant Cell 21, 2655–2671.

Schopfer CR, Nasrallah ME, Nasrallah JB. 1999. The male determinant of self-incompatibility in Brassica. Science 286, 1697–1700.

Takayama S, Shiba H, Iwano M, Shimosato H, Che FS, Kai N, Watanabe M, Suzuki G, Hinata K, Isogai A. 2003. The pollen determinant of self-incompatibility in Brassica campestris. Proceedings of the National Academy of Sciences, USA 97, 1920–1925.

Wang L, Clarke LA, Eason RJ, Parker CC, Qi B, Scott RJ, Doughty J. 2017. PGP-B class pollen coat proteins are key regulators of the hydration checkpoint in Arabidopsis thaliana pollen-stigma interactions. New Phytologist 213, 764–777.

Zuberi MI, Dickinson HG. 1985. Pollen-stigma interaction in Brassica. Ill. Hydration of the pollen grains. Journal of Cell Science 76, 321–336.