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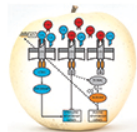
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SHORT COMMUNICATION



KATANIN and cortical microtubule organization have a pivotal role in early pollen tube guidance

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ABSTRACT

Following pollen deposition on the receptive surface of the stigma, pollen germinates a tube that carries male gametes toward the ovule where fertilization occurs. As soon as it emerges from the pollen grain, the pollen tube has to be properly guided through the pistil tissues so as to reach the ovule and ensure double fertilization. Chemical attractants, nutrients as well as receptor kinase-dependent signaling pathways have been implicated in this guidance. Recently, we showed in *Arabidopsis* that the microtubule severing enzyme KATANIN, by acting both on cortical microtubule (CMT) dynamics and cellulose microfibril (CMF) deposition, conferred particular mechanical properties to the papilla cell wall that act as active guidance factors. Here we confirm the importance of KATANIN and CMT orientation in pollen tube directionality by examining another *katanin* mutant.

ARTICLE HISTORY

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Pollen tube guidance; stigma papilla; plant reproduction; katanin; microtubule; arabidopsis

Sexual reproduction in flowering plants ends up with the discharge of male gametes into the embryo sac of the ovule, where the double fertilization takes place. Male and female gametophytes are physically distant. Whereas ovules are embedded in the ovary, male gametes contained in the pollen grain are conveyed to the ovule by a tube, which emerges following hydration and germination of the pollen on the receptive surface of the stigma. The use of *in vitro* pollen germination as well as semi-*in vivo* fertilization assays, together with the analysis of mutants defective in male or female functions, have given a comprehensive image of the mechanisms implicated in pollen tube growth, its guidance through the pistil tissues and its entrance into the ovule to deliver the male gametes^{1–3}. Many molecules have been identified as playing a role in pollen tube guidance and growth, including lipids, sugars, arabinogalactan proteins, secreted peptides, receptor-like kinase proteins, calcium ions, and reactive oxygen species (see reviews^{4,5}). More recently, but independently of any interactions with the female tissues, mechanical constraints imposed to pollen tubes growing *in vitro* were also reported to directly act on pollen tube directionality^{1,6}. In line with these latter examples, we recently showed that KATANIN (AT1G80350), a microtubule severing enzyme, confers particular mechanical properties to the stigma papilla cells and contributes to the orientation of pollen tube growth in *Arabidopsis*⁷. Indeed, in the absence of KATANIN in the stigma of the *ktn1-5* mutant, wild-type pollen tubes grow by making turns around the papilla cells and exhibit signs of misguidance, sometimes even growing in the opposite direction to the ovules. Thus, KATANIN, by acting on papilla cell wall mechanics, appears as a stigmatic factor mediating proper orientation of the germinating pollen tube as early as it emerges

from the pollen grain. Whether chemical cues might also participate in early orientation of pollen tubes in the stigma remains largely unknown. In *Lilium*, chemocyanin, a plantacyanin-like protein, was described as a stigma chemotropic factor for pollen tube guidance⁸. Interestingly, overexpression of the plantacyanin gene in *Arabidopsis* stigmas⁹ was shown to lead to aberrant pollen tube growth that resembles that of the coiling phenotype we observed in the *ktn1-5* mutant. Thus, we wondered whether plantacyanin might be up-regulated in *ktn1-5*. By quantitative RT-PCR, we found no up-regulation of plantacyanin in the mutant, which even exhibited a weaker expression level than wild-type stigmas (Figure 1). This result, together with the fact that no defect in pollen tube growth directionality was detected in a knock-down plantacyanin mutant⁹, question the actual role of this protein as a chemoattractant in *Arabidopsis*.

To further ascertain the function of KATANIN in pollen tube growth, we examined the behavior of pollen tubes on stigmas from plants of a different genetic background. Our former analysis was carried out on the *ktn1-5* mutant in Col-0 and we asked whether a knock-out mutant for the KATANIN gene in the Wassilewskija (WS) ecotype *Botero1-7* (*bot1-7*)¹² would display a similar aberrant growth of wild-type pollen tubes. To this end, we pollinated stigma papillae of *bot1-7* with WS pollen grains and imaged the pollen tube path one hour after pollination using scanning electron microscopy. Whereas wild-type WS pollen tubes grew mainly straight to the base of the WS papilla cells, they made a striking high number of turns on *bot1-7* stigma papillae with, in several cases, tubes growing up to the top of papillae (Figure 2a, b). The reason why the coiled pollen tube phenotype was stronger in *bot1-7* stigmas than in *ktn1-5* stigmas remains obscure. We then confirmed,

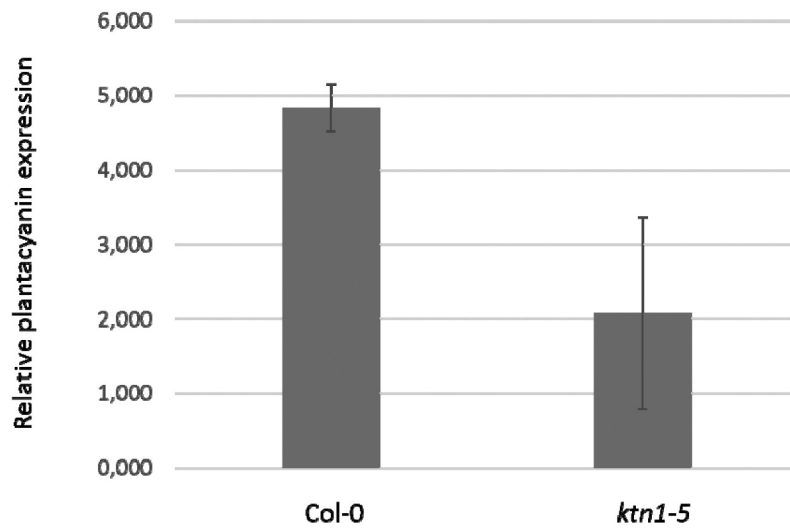


Figure 1. Analysis of plantacyanin gene expression. Plantacyanin expression in Col-0 and *ktn1-5* stigmas at stage 13-early 14¹⁰ is determined by quantitative real-time RT-PCR as described¹¹. Transcript level is normalized by the geometric mean of the two housekeeping genes used as endogenous references, Actin8 and Ubiquitin conjugate enzyme. Standard deviation is based on two replicates for Col-0 and four replicates for *ktn1-5*.

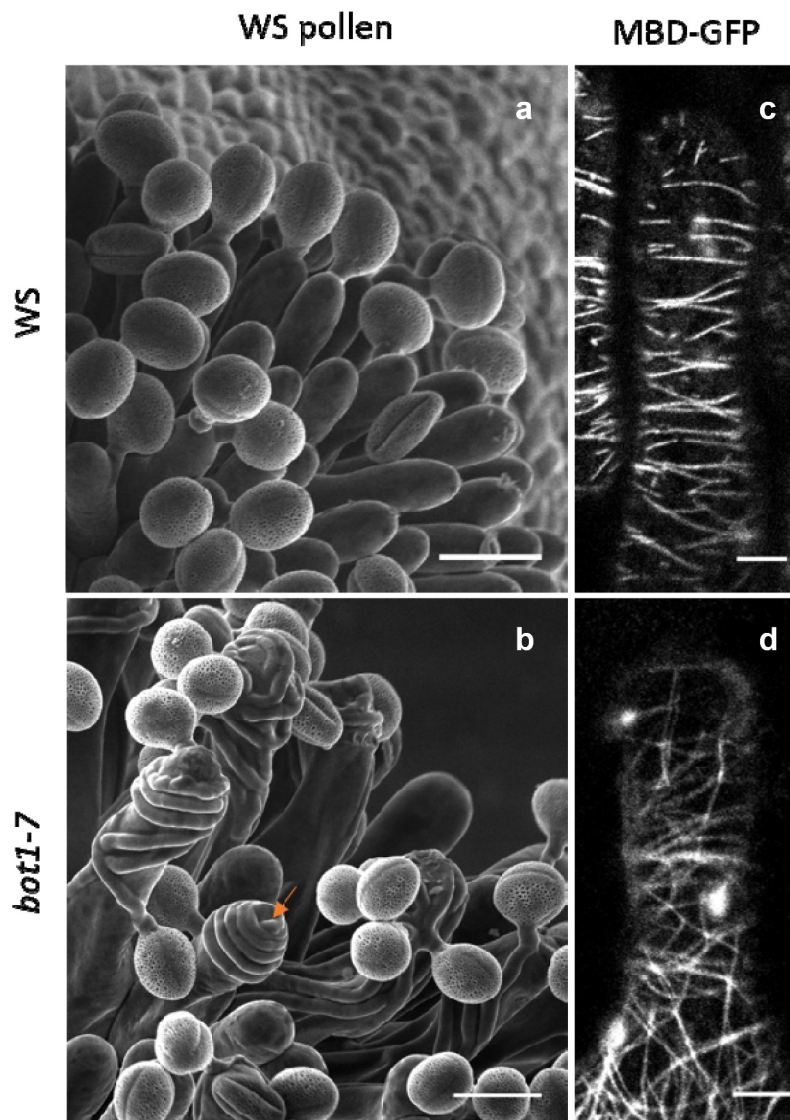


Figure 2. Effect of CMT organization on *botero1-7* pollen tube path. (a) WS and (b) *bot1-7* papillae pollinated with WS pollen, one hour after pollination. Arrow indicates a pollen tube growing upward and arrested at the top of the papilla. Scale bar, 20 μ m. Confocal images of WS (c) and *bot1-7* (d) papilla cells expressing MBD-GFP. Scale bar, 5 μ m.

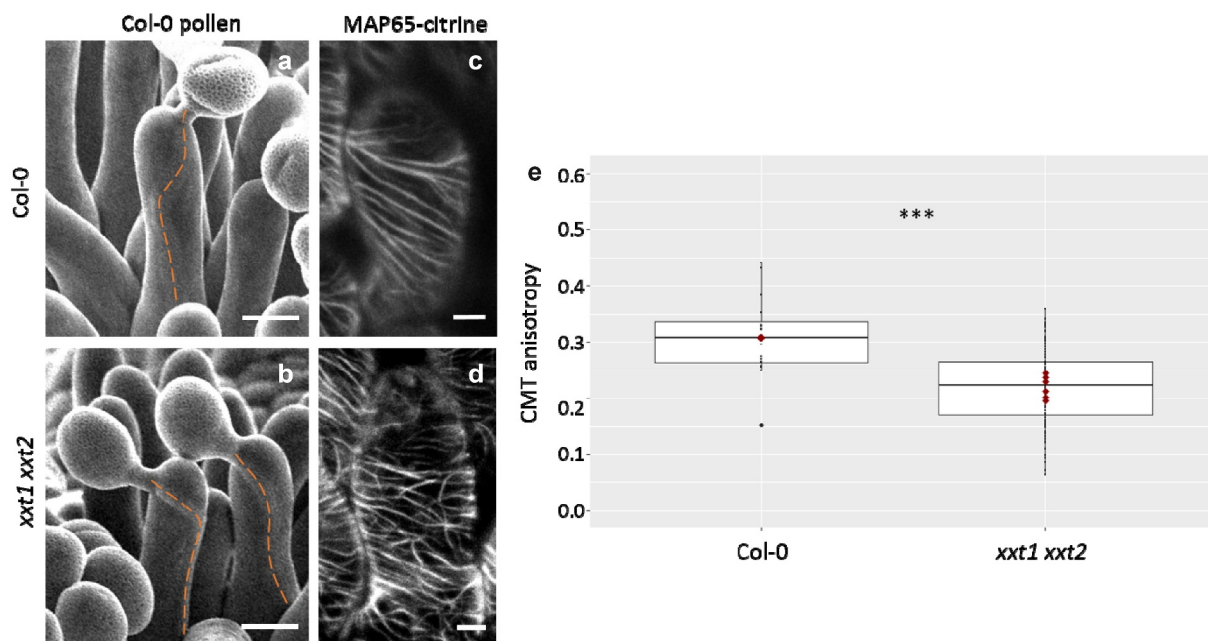


Figure 3. Effect of CMT organization on pollen tube path. (a) Col-0 and (b) *xxt1 xxt2* (in Col-0 background) papillae pollinated with Col-0 pollen. Pollen tube trajectory is underlined by a dashed line. Scale bar, 10 μ m. Confocal images of WT Col-0 (c) and *xxt1 xxt2* (d) papilla cells expressing MAP65-1-citrine. Scale bar, 5 μ m. (e) CMT anisotropy of Col-0 and *xxt1 xxt2* papilla cells. N(Col-0) = 1 stigma, n(Col-0) = 16 papillae, N(*xxt1 xxt2*) = 7 stigmas, n(*xxt1 xxt2*) = 188 papillae. Statistical differences were calculated using a Shapiro-Wilk test to evaluate the normality and then a T-test. *** $p < 0.01$.

using *bot1-7* expressing a GFP-tagged microtubule binding domain (Map4 Binding Domain (MBD)-GFP)¹³, that like in *ktn1-5*, the cortical microtubule (CMT) network was severely affected in *bot1-7* papillae, which exhibited isotropic reorientation of microtubules (Figure 2c, d).

We previously showed that stigmas of the *xxt1 xxt2* double mutant, impaired in xylosyltransferase activity and hemicellulose biosynthesis, allowed normal growth and guidance of wild-type pollen tubes (⁷ and Figure 3a, b). As we found that the coiled pollen tube phenotype was correlated with isotropic reorientation of CMTs in katanin (*ktn1-5* and *bot1-7*) mutants⁷, we looked at the organization of CMTs in *xxt1 xxt2* papillae. We suspected that CMT network would be different from *katanin* mutants. To this end, we crossed the *xxt1 xxt2* mutant with the CMT marker line MAP65-1-citrine and quantified the CMT anisotropy using the ImageJ plugin FibrilTool¹⁴. Although different from wild-type stigmas, the CMTs in *xxt1 xxt2* were not as isotropic as those described in *katanin* mutants (compare Figure 2c, d with Figure 3c–e, and ⁷). These data underscore the particular role of KATANIN in organizing the CMT network of papilla cells and in mediating early pollen tube guidance on the stigma surface.

Our recent work⁷ revealed that KATANIN mediates a specific organization of CMTs and cellulose microfibrils associated with particular mechanical properties of the papilla cell wall. More generally, it is striking that, whatever the position of the flower and hence the pistil on the stem, the pollen tube grows to the base of the papilla in the direction of the ovules. Our results suggest that the mechanical constraints imposed by the KATANIN activity force the emerging pollen tubes to take the right direction, preventing them to get lost on top of the papillae. Hence, KATANIN has likely played an essential role in the

success of flowering plants on earth by promoting pollen tube progression and hence fertilization. Further studies are required to better understand how KATANIN finely mediates the papilla cell wall mechanics while the pollen tube is growing. Similarly, how the pollen tube senses the resistance forces of the papilla wall and reacts accordingly to progress along the papilla remains a fascinating question to unravel.

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