Two aquaporins, SIP1;1 and PIP1;2, mediate water transport for pollen hydration in the Arabidopsis pistil

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Abstract Pollination is the crucial initial step that brings together the male and female gametophytes, and occurs at the surface of the stigmatic papilla cell in Arabidopsis thaliana. After pollen recognition, pollen hydration is initiated as a second critical step to activate desiccated mature pollen grains for germination, and thus water transport from pistil to pollen is essential for this process. In this study, we report a novel aquaporin-mediated water transport process in the papilla cell as a control mechanism for pollen hydration. Coupled with a time-series imaging analysis of pollination and a reverse genetic analysis using T-DNA insertion Arabidopsis mutants, we found that two aquaporins, the ER-bound SIP1;1 and the plasma membrane-bound PIP1;2, are key players in water transport from papilla cell to pollen during pollination. In wild type plant, hydration speed reached its maximal value within 5 min after pollination, remained high until 10–15 min. In contrast, sip1;1 and pip1;2 mutants showed no rapid increase of hydration speed, but instead a moderate increase during ∼25 min after pollination. Pollen of sip1;1 and pip1;2 mutants had normal viability without any functional defects for pollination, indicating that decelerated pollen hydration is due to a functional defect on the female side in sip1;1 and pip1;2 mutants. In addition, sip1;1 pip1;2 double knockout mutant showed a similar impairment of pollen hydration to individual single mutants, suggesting that their coordinated regulation is critical for proper water transport, in terms of speed and amount, in the pistil to accomplish successful pollen hydration.

Key words: Arabidopsis thaliana, live imaging, pollination, time-lapse, water channel.

Introduction

In angiosperms, sexual reproduction is a complex series of events that are regulated by multiple physiological processes for successful seed production. Pollination is an important initial step for sexual plant reproduction to bring together the male and female gametophytes, and is initiated after a mature pollen grain released from the anther lands on the stigma. Subsequently, pollen grains obtain water and other resources from the stigma for germination, and elongate pollen tubes towards the ovule by guidance signals (Adhikari et al. 2020; Cheung et al. 1995; Franklin-Tong 1999; Lord 2000; Shimizu and Okada 2000; Takeuchi and Higashiyama 2012). Two sperm cells in the pollen tube finally fertilise an egg cell and central cell in the ovule, termed double fertilisation (Dickinson and Elleman 1994).

Brassicaceae species have the dry type of stigma (Heslop-Harrison and Shivanna 1977), and pollination events occur on the stigmatic surface cell, the papilla cell, which is a large single cell that has two layers of cell wall, namely the cuticle and the pectocellulosic layer (Elleman et al. 1988, 1992). When pollen is captured and adheres to a papilla cell on the surface of the stigma, a strong interaction occurs between pollen and papilla cell in which proteins and lipids of the pollen coat and papilla surface form a structure called the foot (Elleman and Dickinson 1990; Gaude and Dumas 1984; Kandasamy et al. 1994; Zinkl et al. 1999). During foot formation, proteins and lipids of the pollen coat reorganise to form a capillary system (Elleman and Dickinson 1986; Murphy 2006), through which pollen receives the resources

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necessary for hydration, germination and tube growth from vacuoles in the stigma (Dickinson 1995; Zuberi and Dickinson 1985). The subsequent process of pollination is divided into four steps: pollen recognition, pollen hydration, pollen germination, and tube elongation; these events occur sequentially and are regulated by particular physiological mechanisms in the papilla cell, although most of their molecular mechanisms are still under investigation. As their pollen recognition system, many Brassicaceae species have sporophytic self-incompatibility (SI), a sophisticated system for pollen selectivity to prevent self-fertilisation, which is genetically controlled by a single highly polymorphic S-locus (Bateman 1995). S-locus protein 11/S-locus cysteine-rich protein and S-locus receptor kinase (SRK) in the S-locus are male and female S-determinant factors, respectively (Schoof et al. 1999; Stein et al. 1991; Suzuki et al. 1999; Takasaki et al. 2000; Takayama et al. 2000), and their physical interaction in an allele-specific manner initiates the subsequent incompatible reaction to reject self (genetically related) pollen (Takayama et al. 2001), through an incompletely understood downstream system. The SI response in Brassicaceae is manifested as the prevention of pollen hydration and the inhibition of pollen tube penetration (Dickinson 1995; Rozier et al. 2020; Zuberi and Dickinson 1985), and pollen hydration is thought to be regulated by a balanced combination of multiple components of hydration, dehyration, and rehydration, from the viewpoint of morphology and cytology (Hiroi et al. 2013). Thus, an effective water transport system in the papilla cell is critical for successful pollination, and vesicle trafficking via Exo70A1-mediated exocytosis (Samuel et al. 2009) and vacuolar dynamics in the papilla cell (Iwano et al. 2007) have been previously predicted to underlie pollen hydration.

Aquaporins are a family of small (24–30kDa) pore-forming integral membrane proteins that are categorised as major intrinsic proteins (MIPs), and they are required for water conductance and other physiological processes in bacteria, fungi, animals, and plants (Tyerman et al. 1999). Water is the major component of all organisms, and a variety of aquaporin isoforms have been identified as channel proteins for the regulation of water permeability, with different tissue specificity and subcellular localization in multicellular organisms (Heymann and Engel 1999). Some members of the family also facilitate transport of gas and small molecules, such as CO₂, glycerol, NH₃, NO, and urea, in addition to water, for developmental and physiological regulation (Gaspar et al. 2003; Hanba et al. 2004; Kruse et al. 2006; Loque et al. 2005; Maurel et al. 2002; Niemietz and Tyerman 2000; Uehlein et al. 2003). The plant aquaporins comprise four major groups: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), Nodulin 26-like intrinsic proteins (NIPs), and small basic intrinsic proteins (SIPs). In Arabidopsis thaliana, there are 35 aquaporin genes in the genome (Johanson et al. 2001), and the diversity of Arabidopsis aquaporins reflects their critical and specialised roles in water transport and physiological processes. These roles have been well addressed in the context of leaf and root water transport systems. For example, AtPIP1;2 contributes to hydrostatic, not osmotic, water transport in Arabidopsis leaf and root (Postaire et al. 2010), whereas AtPIP2;2 has a specialised role in osmotic, not hydrostatic, water uptake in Arabidopsis root (Javot et al. 2003). Examples in legumes include GmNOD26, belonging to the NIP family, and MtTIP1g, which are channel proteins in the membrane of legume–rhizobia symbiotic root nodules and mediate the exchange of metabolites between the host plant and the microsymbiont (Fortin et al. 1987; Gavrin et al. 2014; Sandal and Marcker 1988). These representatives illustrate the distinct and divergent roles of aquaporin isoforms in different tissues, and in physiological and developmental processes, as well as the importance of aquaporin channels in plants. Nevertheless, a comprehensive understanding of water transport and developmental processes is still lacking.

So far, two independent water transport systems of vesicle trafficking via exocytosis and vacuolar dynamics have yielded insights regarding water transport from pistil to pollen on pollination, as mentioned above. However, the overall molecular mechanisms underlying water transport in pollination remain inconclusive, although aquaporin-mediated water transport is a strong candidate for pollen hydration because of its specialised capability in water conductance. In this study, we report that the ER-bound SIP1;1 and the plasma membrane-bound Pip1;2 are essential for temporal and quantitative control of water transport from pistil to pollen in pollination, using a combination of time-series imaging of pollination and reverse genetic analysis of aquaporin using T-DNA insertion Arabidopsis mutants (sip1;1 and pip1;2). We elucidate morphological characteristics and molecular factors in pollination, in terms of water transport and secretion in the pistil.

Materials and methods

Plant materials, growth conditions, and genotyping

Seeds of Arabidopsis thaliana ecotype Columbia (Col-0) and T-DNA insertion mutants of aquaporin genes expressed in the papilla cell (Supplementary Table S1) were obtained from the Arabidopsis Biological Resource Center. All plants used in this study were grown in pots filled with vermiculite, in a growth chamber (BIOTRON LPH300, NK System, Osaka, Japan), at 22°C under a 16-h light/8-h dark photoperiod and general (50–60%) humidity conditions. One fully expanded leaf of a 6–8-week-old plant was used to
extract genomic DNA. To determine plant genotypes, SIP1;1 and PIP1;2 gene-specific primers and the left border of T-DNA primer were used in genomic PCR (Supplementary Table S2).

The sip1;1 pip1;2 double knockout mutant plants were developed by crossing between the sip1;1 and pip1;2 single mutant lines.

**RT-PCR analysis**
mRNAs were extracted from the stigma, at anthesis stage (flower development stage 14–15), using a FastTrack 2.0 mRNA Isolation Kit (Invitrogen, San Diego, CA, USA), and first-strand cDNAs were synthesised from 150 ng of mRNA using a High-Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific, Waltham, MA, USA). After the RT reaction, cDNAs were subjected to the following PCR with Ex Taq DNA polymerase (TaKaRa Bio, Shiga, Japan): 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min. A cytosolic cyclophilin, ROC1 (Beecikman et al. 2002), was used as a control. Primers used are listed in Supplementary Table S2.

**Observation of pollen viability**
Pollen grains from anthers of freshly opened flowers in each line were placed on a glass slide, and stained with Alexander staining solution (Alexander 1969), 1% (v/v) I2 in 3% (v/v) KI, or 0.05 mg/ml fluorescein diacetate (FDA). The stained pollen grains were observed using a stereoscopic microscope (Axio Imager A2, Carl Zeiss, Jena, Germany), and the percentage of viable pollen grains was calculated by counting the number of stained and non-stained pollen grains.

**Semi-in vivo pollen tube growth assay**
Cut styles at the anthesis stage were mounted on solid pollen germination medium and hand-pollinated with pollen grains from freshly opened flowers. After pollination for 8 h, pollen tubes growing through the cut style were observed using a stereoscopic microscope (Axio Imager A2) and measured using digital image processing software (AxioVision 4.8, Carl Zeiss).

**In vivo observation of pollen tube growth**
Flower buds at flower development stage 12 were emasculated and incubated until anthesis. Emasculated flowers then were pollinated with pollen, from freshly opened flowers, of the appropriate genotype in each experiment and incubated for 6 h. Pollinated pistils were then collected, fixed in a 9:1 mixture of ethanol and acetic acid, and stained with aniline blue solution (0.1% aniline blue in 0.1% K3PO4) for 6 h. The stained pistils were observed by UV fluorescence microscopy (Axio Imager A2), according to Tsuchimatsu et al. (2010) and Sudo et al. (2013).

**Time-course imaging of pollen hydration**
Flower buds at flower development stage 12 were emasculated and incubated until anthesis. Emasculated flowers were then put on 1% agar medium and pollinated with pLAT52::GFP pollen collected from freshly opened flowers. After pollination, time-course images were recorded at 2-h intervals using a UV fluorescence microscope (Axio Imager A2), for an overview of pollination behaviour in the wild type and mutant pistils.

**In vivo time-lapse imaging of pollen hydration**
A cuboid plastic case was mounted on a glass slide and held in place with double-sided tape. Excised pistils, at the anthesis stage, from emasculated flowers were attached to the upper side of the plastic case with double-sided tape, and a small piece of 1% solid agar was placed on one side of the plastic case to provide water to the pistil. After approximately 2 h, 10 to 30 wild-type pollen grains collected from freshly opened flowers were placed on the top side of the stigma, and time-lapse imaging of pollen behaviour during pollination was recorded for 60 min, with image capturing at one-minute intervals, using a stereoscopic microscope (SterEo Discovery V20, Carl Zeiss) and digital image processing software (AxioVision 4.8). Because the rate of water flow into pollen is accurately correlated with the change of pollen shape (Hiroi et al. 2013; Zuberi and Dickinson 1985), to assess pollen hydration the short axis (width) pollen grain diameter was measured at 5-min intervals with AxioVision software. Pollen hydration was expressed relative to the value at 0 min. In this study, we defined pollen whose width increased 1.2- and 1.4-fold after pollination as “partially hydrated” and “completely hydrated”, respectively, and measured the time required.

The speed of pollen hydration was calculated by the following formula,

\[
\text{Hydration speed (μm/min)} = \frac{(W_{t+5} - W_{t})}{5},
\]

where \(W\) is the pollen width at \(x\) and \(x+5\) min after pollination.

**Complementation of sip1;1 and pip1;2 mutants**
To generate the complementation construct, 3,765- and 3,199-bp genomic fragments of the SIP1;1 and PIP1;2 genes, respectively, containing the 5’ promoter region (1,504 bp in SIP1;1 and 1,511 bp in PIP1;2) and the coding region (2,261 bp in SIP1;1 and 1,688 bp in PIP1;2), were amplified by PCR, using genomic DNA of Col-0, with primers shown in Supplementary Table S2. After verifying their nucleotide sequence, the resulting SIP1;1- and PIP1;2-harbouring plasmids were introduced into Agrobacterium tumefaciens strain GV3101 and transformed into sip1;1 and pip1;2 mutant lines, respectively.

**Results**

**Confirmation of T-DNA insertion mutants of SIP1;1 and PIP1;2**
From the Arabidopsis papilla cell gene expression profiles (Matsuda et al. 2015; Osaka et al. 2013), we first selected 23 aquaporin genes which are expressed in the papilla cell, and then 14 genes for which T-DNA insertion mutants are available in the Arabidopsis Biological Resource Center. Among them, we here focused
two aquaporin genes, SIP1;1 and PIP1;2, which have higher gene expression in the papilla cell and obvious phenotypic disruptions in T-DNA insertion mutants, as candidates for controlling water transport from pistil to pollen upon pollination (Supplementary Table S1). The T-DNA insertion in sip1;1 (SALK_069427) and pip1;2 (SALK_019794) was confirmed by genomic PCR, with a combination of gene-specific and T-DNA-specific primers (Supplementary Table S2). Homozygotes for T-DNA insertion were obtained by self-pollination in each line and confirmed by genomic PCR (Figure 1A). The expression of SIP1;1 and PIP1;2 was assessed by reverse transcription (RT)-PCR of stigma cDNAs of wild type and mutant lines, at anthesis stage (flower development stage 14–15, Smyth et al. 1990), and we confirmed complete suppression of SIP1;1 and PIP1;2 in the sip1;1 and pip1;2 homozygous mutants, respectively, whereas both genes were expressed in wild type (Figure 1B). Thus, each homozygous mutant line is a null mutant for either sip1;1 or pip1;2.

**sip1;1 and pip1;2 mutants have defects in seed development**

In the sip1;1 and pip1;2 mutants, no obvious phenotypic disruptions were observed in vegetative organs or floral morphology, compared to wild type plants. However, the silique length and the number of seeds in each silique differed between wild type and mutant lines. Siliques were shorter in mutant plants (Figure 1C), and average numbers of seeds per silique were 35.0 in wild type but 23.5 in sip1;1 and 18.1 in pip1;2 (Figure 1D) (p<0.05).

Sato and Maeshima (2019) reported that the sip1;1 mutant has normal siliques with a total seed number similar to wild type; our and their findings are thus contradictory. This may be attributable to the different methods used to count seed number: per silique in this study and per plant in Sato and Maeshima (2019), and/or growth conditions. Our results suggest that suppression of SIP1;1 and PIP1;2 affects function in reproduction processes including pollination.

**sip1;1 and pip1;2 mutant pollens have normal viability and function in pollination**

Because shorter siliques with reduced seed number were observed in sip1;1 and pip1;2 mutants, pollen viability and growing ability of pollen tubes were evaluated. There were no obvious differences between wild type and mutant lines, in terms of morphology, number of pollen grains in the anther, or the degree of staining by Alexander, KI and FDA solutions (Figure 2A–D), and 98.5% and 93.7% of pollen grains in sip1;1 and pip1;2 mutants, respectively, had normal viability, equivalent to that in wild type plants (87.1%) (Figure 2E).

To observe pollen tube growth, self-pollinated pistils were fixed, stained with aniline blue solution, and observed by microscopy (Figure 3A). In the case of wild type, pollen tubes arrived at the basal part of the pistil at 12h after pollination, with an average length of 1,272.3 µm, indicating that most pollen tubes reach the ovules for fertilisation within 12h after pollination. In contrast, in sip1;1 and pip1;2 mutants, the tips of most pollen tubes were at the middle part of the style at 12h.
after pollination, with an average pollen tube length of 794.0 \(\mu m\) in sip1;1 and 820.3 \(\mu m\) in pip1;2. Thus, the pollen tube length in sip1;1 and pip1;2 was reduced to approximately 60% that of wild type. An equivalent reduction of pollen tube growth was observed when sip1;1 and pip1;2 mutant pistils were pollinated with wild type pollen (Supplementary Figure S1). To evaluate the growing ability of the pollen tube in sip1;1 and pip1;2 mutants, a semi-in vivo pollen tube growth assay was performed by in vitro crossing experiments between wild type and mutants (Figure 3B). When pollen grains of wild type and mutant lines were pollinated with wild type pistils, pollen tubes growing through the cut style at 8h after pollination averaged 952.2 \(\mu m\) in wild type, 860.3 \(\mu m\) in sip1;1, and 953.2 \(\mu m\) in pip1;2, and there was no significant difference in length between wild type and either mutant line. Hydration of pollen grains on in vitro pollen germination medium in sip1;1 and pip1;2 mutants was also equivalent morphologically to that in wild type plants (Figure 3C). These results indicate that pollen of sip1;1 and pip1;2 mutants has normal viability without any functional defects for pollination.

**sip1;1 and pip1;2 mutants show delayed pollen hydration and germination**

Because sip1;1 and pip1;2 mutants had shorter siliques with fewer seeds but their pollen had normal viability and growing ability of the pollen tube, an overview of pollination behaviour on the wild type and mutant pistils was acquired by time-course imaging with 2-h intervals, using pLAT52::GFP pollen (Col-0 background) (Figure 4, Table 1). Generally, GFP protein expressed under the LAT52 promoter localizes in the pollen cytosol and GFP fluorescence can be observed inside the pollen grain (Ottenschläger et al. 1999). During pollination, once pollen germination and tube elongation are initiated, GFP protein migrates towards the tip of the growing pollen tube, together with movement of the cytosol, whereas the GFP signal disappears in the remnant
pollen grain without cytosol on the stigma. In the time-course observation of pollination (Figure 4), GFP fluorescence in the pollen grain was visible initially and then disappeared at 2 h after pollination in wild type (as exemplified by the circled area). Meanwhile, in sip1;1 and pip1;2 mutants, GFP fluorescence persisted in some pollen grains during 24 h of observation (arrowhead), and delayed initiation of hydration was also observed in others (white arrows; the delayed initiation is indicated by yellow arrows). In wild type, most pollen grains (93.9%) completed successful hydration within 2 h after pollination and 57.1% quickly started germination, with the maximum value of 73.5% germination reached by 8 h after pollination. However, in each mutant, pollen hydration and germination were lower: 64.5–74.2% hydration and 25.8–58.1% germination in sip1;1, and 53.8–61.5% hydration and 11.5–42.3% germination in pip1;2. Hydration and germination patterns differed slightly between sip1;1 and pip1;2: maximal hydration was reached in pip1;2 within 4 h after pollination, whereas a moderate increase in hydration continued up to 8 h in sip1;1. Germination occurred at a low level throughout the observation period in both mutants, probably due to this retarded or incomplete hydration. These results suggest that a delay of pollen hydration and

Table 1. Percent pollen hydration and germination in wild type and mutant plants during pollination.

| After pollination (h) | Hydrated | Germinated | Hydrated | Germinated | Hydrated | Germinated |
|-----------------------|----------|------------|----------|------------|----------|------------|
|                       | WT       | sip1;1     | pip1;2   |
| 2                     | 93.9     | 64.5       | 53.8     |
| 4                     | 93.9     | 67.7       | 61.5     |
| 6                     | 93.9     | 67.7       | 61.5     |
| 8                     | 93.9     | 74.2       | 61.5     |
| 10                    | 93.9     | 74.2       | 61.5     |
| 12                    | 93.9     | 74.2       | 61.5     |
| 24                    | 93.9     | 74.2       | 61.5     |

Table 2. Reciprocal crossing between wild type and mutant lines.

| Female parent | Male parent | No. of seeds |
|---------------|-------------|--------------|
| WT            | WT          | 33.7±4.2     |
| WT            | sip1;1      | 35.8±5.3     |
| WT            | pip1;2      | 34.1±6.1     |
| WT            | sip1;1 pip1;2 | 32.1±5.0   |
| sip1;1        | WT          | 20.2±5.0*    |
| pip1;2        | WT          | 18.8±5.2*    |
| sip1;1 pip1;2 | WT          | 20.7±4.9*    |

*Indicates a significant difference between self-pollination of WT and each crossing (t-test, p<0.05).
germination is due to a functional defect on the female side in sip1;1 and pip1;2 mutants. This interpretation was supported by reciprocal crossing between wild type and mutant lines: when mutant pistils were pollinated with wild type pollen, the resulting seed number per silique was reduced while the normal seed number, as seen in self-pollinated wild type siliques, was observed in a cross between wild type pistil and mutant pollen (Table 2).

To evaluate pollination behaviour in more detail, time-lapse images of pollen behaviour during pollination were recorded, with images captured for 60 min at 1-min intervals, in wild type and mutant lines (Figure 5, Supplementary Movies S1–S3). In this in vivo pollination experiment, wild type pollen grains collected from freshly opened flowers were pollinated to wild type and mutant pistils at the anthesis stage, to focus on an effect of sip1;1/pip1;2 in the pistil. In both wild type and mutant pistils, initial pollen width was uniform, approximately 14 \( \mu \text{m} \), and we defined pollen whose width increased 1.2- and 1.4-fold after pollination as “partially hydrated” and “completely hydrated”, respectively. We observed that pollen hydration began soon after pollination and was complete within 20 min on wild type pistils, whereas mutant pistils showed a reduced rate of hydration and never completed during the 60-min observation period. Representative images were taken at 5-min intervals, from 0 to 60 min after pollination. Scale bars = 10 \( \mu \text{m} \).

When the change of pollen width during pollination was compared between wild type and mutant lines, it was calculated at as 0.35 \( \mu \text{m/min} \), at the maximum, on wild type pistils and less than 0.25 \( \mu \text{m/min} \) on both mutant pistils (Figure 7). Transition patterns were also apparently different between wild type and mutant pistils. In wild type, hydration speed reached its maximal value within 5 min after pollination, remained high until 10–15 min, and then rapidly declined. In the mutants, there was no rapid increase of hydration speed, but instead a moderate increase was observed during 25 min after pollination, followed by a gradual decrease. Taken together, these results indicate that suppression of SIP1;1 and PIP1;2 interferes with water supply from pistil to pollen, resulting in decelerated and...
Phenotypic disruption in sip1;1 and pip1;2 is recovered by gene complementation

To achieve complementation of sip1;1 and pip1;2, each single mutant line was transformed with the SIP1;1 promoter::SIP1;1 and PIP1;2 promoter::PIP1;2 construct, respectively. In each transformant, we confirmed the recovery of normal pollen hydration and pollen tube growth in pollination, and normal resultant seed number in siliques (Supplementary Figure S2, Supplementary Movies S5, S6). Thus, we conclude that disruption of either SIP1;1 or PIP1;2 caused a deceleration of the water supply to pollen on the pistil.

sip1;1 pip1;2 double knockout mutant shows a similar impairment of pollen hydration to sip1;1 and pip1;2 single mutants

SIP1;1 and PIP1;2 are aquaporin family proteins that regulate water transport in different parts of the cell, and their single mutants showed similar deceleration of water supply to pollen from the papilla cell during pollination. To assess a genetic effect and association of SIP1;1 and PIP1;2 in water transport in the papilla cell, we generated sip1;1 pip1;2 double knockout mutant plants by crossing between sip1;1 and pip1;2 single mutants. After confirmation of the complete knockout of both SIP1;1 and PIP1;2, phenotypic aspects including reduced seed number in the silique and decreased hydration speed were observed, and showed no greater severity in sip1;1 pip1;2 double knockout mutants than in the individual sip1;1 and pip1;2 single mutants (Figures 1–3, 5–7). This suggests that SIP1;1 and PIP1;2 act in the same water transport pathway, in the papilla cell, for pollen hydration.

Discussion

From the 1980s onwards, cytological analyses have revealed intracellular structures of the papilla cell and pollen tube dynamics on pollination (Dickinson 1995; Elleman and Dickinson 1986; Gaude and Dumas 1984; Hiroi et al. 2013; Zuberi and Dickinson 1985), but the underlying molecular mechanisms still remained to be elucidated. After pollen recognition, pollen hydration is the second critical step in the pollination process to activate desiccated mature pollen grains for germination and tube elongation, and it is clear that water transport from pistil to pollen is essential for this process, from morphological and cytological viewpoints (Rozier et al. 2020). While aquaporin family members such as NIP4;1, NIP4;2, TIP1;3, TIP5;1, and SIP2;1 have been reported to selectively control water uptake in pollen, and aquaporins are also predicted to regulate water transport and supply in the pistil (Pérez Di Giorgio et al. 2016; Sato and Maeshima 2019; Soto et al. 2008), the female control system that governs water transport and permeability in papillae, as a source for pollen hydration, has not yet been elucidated. In this study, we found a novel aquaporin-mediated water transport process in the papilla cell for pollen hydration, and that two membrane-localized aquaporins, SIP1;1 and PIP1;2, are essential for temporal and quantitative control of water transport from pistil to pollen in pollination.

Compared to wild type, the speed of hydration in both mutants was lower, less than 0.25 μm/min, with no rapid increase during pollination (Figure 7). This observation indicates that suppression of SIP1;1 and PIP1;2 results not in an inhibition of water supply but in impeded water transport from papilla to pollen. SIP1;1 and PIP1;2 are plant-specific aquaporins belonging to the MIP family (Boudichevskaia et al. 2015; Kumar et al. 2018; Ishikawa et al. 2005; Postaire et al. 2010). Plant aquaporins display a variety of isoforms that have functions in various tissues/organs, in terms of water transport (Kruse et al. 2006). PIP1;2 is one of the most abundantly expressed PIP1 isoforms in Arabidopsis and localizes to the plasma membrane (Santoni et al. 2003). It has a water channel.
whose activity contributes to hydrostatic water transport, and not to osmotic hydraulic conductivity (Postaire et al. 2010). SIP1;1 is also a water channel that promotes intracellular water transport in the rough ER, underlying the plasma membrane (Hanton et al. 2005; Ishikawa et al. 2005). These results suggest that water efflux from the papilla cell for pollen hydration is controlled as follows: when water is released from the rough ER by the SIP1;1 water channel, it exits the cell through the PIP1;2 water channel for transcellular water flow. In this mechanism, SIP1;1 regulates the amount of water leaving the rough ER and PIP1;2 controls the hydrostatic transport of water to the outer surface of the cell, as aquaporin channels in the water transport system in the papilla cell.

Exo70A1, a subunit of the exocyst complex that is involved in targeted vesicle trafficking to the plasma membrane for exocytosis, is reported to be involved in water transport from papilla to pollen in pollination, and Arabidopsis exo70A1 mutants exhibit reduced pollen hydration, with only 45% of pollen grains partially hydrated (Samuel et al. 2009). In this system, Golgi-derived stigmatic resources including water are transported to the plasma membrane for vesicle tethering and fusion by the exocyst complex. Multivesicular bodies, small vesicles surrounded by a membrane (also known as exosomes), are also observed at the papillary plasma membrane under the pollen attachment site (Safavian and Goring 2013). We thus propose that there are two pathways for water transport from pistil to pollen in pollination: the Golgi-derived polarized secretion system and the ER-derived hydrostatic water transport system. Since components of the exocyst reportedly associate with intracellular vesicles carrying aquaporins to fuse with the plasma membrane and thus to increase water permeability, and the rough ER serves to target proteins to their proper destinations, in concert with the Golgi apparatus (Barile et al. 2005; Levine 2004), it is of interest to determine whether the exocytosis system and the hydrostatic aquaporin system are integrated, or independent, as a sophisticated water transport system in papilla cells for pollen hydration in pollination.

In summary, we have identified two membrane-localized aquaporins, SIP1;1 and PIP1;2, as key regulators of transcellular water efflux from pistil to pollen during pollination. Their coordinated regulation is critical for proper water transport in the pistil, in terms of speed and amount, to accomplish successful pollen hydration. This is a component of a sequential and complex series of processes in plant reproduction, and plays a key role in mid-pollination for successful seed production.

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