Abscisic acid-mediated developmental flexibility of stigmatic papillae in response to ambient humidity in Arabidopsis thaliana

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Stigmatic papillae develop at the apex of the gynoecium and play an important role as a site of pollination. The papillae in Brassicaceae are of the dry and unicellular type, and more than 15,000 genes are expressed in the papillae; however, the molecular and physiological mechanisms of their development remain unknown. We found that the papillae in Arabidopsis thaliana change their length in response to altered ambient humidity: papillae of flowers incubated under high humidity elongated more than those under normal humidity conditions. Genetic analysis and transcriptome data suggest that an abscisic acid-mediated abiotic stress response mechanism regulates papilla length. Our data suggest a flexible regulation of papilla elongation at the post-anthesis stage, in response to abiotic stress, as an adaptation to environmental conditions.

Key words: abscisic acid, Arabidopsis thaliana, humidity, stigmatic papillae

INTRODUCTION

Reproduction is a central process for all organisms to pass on their genome to the next generation. Plants have evolved fertilization systems between male and female gametophytes (Suwabe et al., 2010). In angiosperms, the male gametophyte is produced as pollen, which develops in the anther of the stamen. Female gametophytes are generated within the gynoecium, which consists of a stigma, a style, an ovary within which ovules develop, and a gynophore that connects the gynoecium to the base of the flower (Smyth et al., 1990; Sessions and Zambryski, 1995; Roeder and Yanofsky, 2006). These tissues develop along the apical-basal and radial axes, and this patterning is mainly dependent on the polar transport of auxin, as shown in Arabidopsis thaliana (Larsson et al., 2013, 2014; Moubayidin and Østergaard, 2014).

In Brassicaceae plants, reproduction comprises a complex and orchestrated series of events, including capture and adhesion of pollen grains onto a surface of stigmatic papillae, foot formation to strengthen the pollen-stigma interaction, hydration and germination of pollen grains, pollen tube elongation into the stigma and style, pollen tube guidance towards the ovule, and fertilization of egg

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cells and central nuclei, termed double fertilization. The stigma in Brassicaceae is classified as dry type and unicellular, in terms of morphological and cytological properties (Heslop-Harrison and Shivanna, 1977), and in most diploid species such as *Brassica rapa* it plays a critical role in the selection of compatible or incompatible pollen to avoid self-pollination (Elleman and Dickinson, 1986; Dickinson, 1995; Watanabe et al., 2012), in addition to the sequence of pollination events described above.

In *A. thaliana*, a self-compatible species in Brassicaceae, stigmatic papillae arise from the apex of the gynoecium at floral stage 11 and elongate rapidly within a few days to accept pollen upon flower opening at stage 13 (Smyth et al., 1990). To understand the molecular mechanism of pollination in stigmatic papilla cells, transcriptome analyses have been performed using papillae from three different Brassicaceae species. A combination of laser microdissection and RNA-sequencing has revealed that 17,240, 19,260 and 21,026 genes are expressed in the three different Brassicaceae species. A combination of laser microdissection and RNA-sequencing has revealed that 17,240, 19,260 and 21,026 genes are expressed in the stigmatic papillae of *A. thaliana*, *A. halleri* and *B. rapa*, respectively (Osaka et al., 2013). Approximately 70% of the genes are commonly expressed in all three species. In *A. thaliana*, one-third of the genes expressed in papilla cells are papilla-specific. Another transcriptome analysis has shown that pre-pollination and pollinated papillae express 15,475 and 17,360 genes, respectively, among which 14,392 genes are commonly expressed (Matsuda et al., 2015). These comprehensive gene expression analyses have revealed the functional commonalities of Brassicaceae papillae. They form a foundation for exploring molecular systems in development and pollination, and they can increase our knowledge of the mechanisms of physiological, cellular, developmental and reproductive processes in papillae.

Despite their importance as the initial site of sexual reproduction in plants, the developmental and physiological mechanisms in papillae remain largely unknown. Because stigmatic papillae are exposed to changing external conditions after anthesis, they must quickly adjust to the surrounding environment to maintain their functional properties during reproduction. We found that papilla length changes under different humidity conditions at the post-anthesis stages, and our data suggest that the abscisic acid (ABA)-mediated abiotic stress response mechanism regulates papilla elongation.

**MATERIALS AND METHODS**

**Plant growth conditions** The Columbia (Col) ecotype of *A. thaliana* was used as the wild type. T-DNA insertion mutants were obtained via the SIGnAL website (http://signal.salk.edu/) and from the Arabidopsis Biological Resource Center (https://abrc.osu.edu/). Plants were grown on soil or agar medium under long-day conditions (16 h light and 8 h dark) with white light at 22 to 24 °C in BIOTRON LPH300 (NK system, Japan) or MLR-350 (SANYO, Japan) growth chambers.

**Measurement of stigmatic papilla length** For semi-*in vitro* measurement of papillae, either whole inflorescences, flowers at stage 12, or dissected gynoecia at the same stage were incubated on medium containing 1% sucrose [w/v] and 1% agar [w/v] in 6-cm-diameter Petri dishes with or without lids under long-day conditions (16 h light and 8 h dark) with white light at 22 to 24 °C. The approximate humidity in plates with lids was ~100%, while it was ~50–60% in plates without lids, as measured by a hygrometer (THI–HP, AS ONE, Japan) in an MLR-350 growth cabinet (SANYO, Japan). Papilla length was analysed when flowers reached stage 14 (flower incubation) or after 24 h (dissected gynoecia). Images of stigmatic papillae were captured using an S8AP0 stereomicroscope and an EC3 digital camera (Leica, Germany), and the length of papilla cells was measured using ImageJ software (http://imagej.nih.gov/ij/). For clarification of floral tissues, inflorescences were fixed in FAA (3.7% [v/v] formaldehyde, 5% [v/v] acetic acid, 25% [v/v] ethanol), hydrated through an ethanol series, and cleared in clearing solution (40 g chloral hydrate, 10 ml glycerol and 5 ml distilled water) overnight. Cleared stigmata were observed using a DM2500 optical microscope (Leica, Germany). For ABA treatment, stage 12 flowers were immersed in 5 μM ABA solution or water control for 22 h (Sharp et al., 2000; Sharp and LeNoble, 2002; LeNoble et al., 2004), and papillae were observed and analysed using a stereoscopic microscope (StereO Discovery.V20, Carl Zeiss, Germany) and digital image processing software (AxioVision 4.9.1, Carl Zeiss).

**Genotyping and quantitative RT-PCR** DNA was isolated as previously described (Edwards et al., 1991). Oligonucleotide primers used for genotyping the T-DNA insertion mutants are listed in Supplementary Table S2. For PCR amplification, Ex Taq (TaKaRa, Japan) and 2720 or 9700 thermal cyclers (Applied Biosystems, USA) were used. For CDNA synthesis for RT-PCR, RNA from three replicate samples, each of which contains two inflorescences of *A. thaliana*, was extracted by RNeasy Plant Mini Kit (QUIGEN, Germany), and reversed-transcribed by ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO, Japan). For quantitative RT-PCR, THUNDERBIRD SYBR qPCR Mix and the Thermal Cycler Dice Real Time System Lite was used (TaKaRa, Japan). Oligonucleotide primers used for qRT-PCR are listed in Supplementary Table S2.

**Processing of transcriptome data for the stigmatic papillae** To curate information regarding ABA and ethylene biosynthesis/response genes, the corresponding gene locus IDs were obtained from the *Arabidopsis*
information resource (TAIR: http://www.arabidopsis.org/), and their transcription profiles were sorted using RNA-sequencing data for the Arabidopsis papilla cell (Osaka et al., 2013; Matsuda et al., 2015).

**Observation of pollen and pollination status under different humidity conditions** For observations of pollen viability, pollen grains were placed on a glass slide, stained with Alexander solution (Alexander, 1969) or 1% (v/v) I$_2$ in 3% (v/v) KI (Hiroi et al., 2013), and observed using a stereoscopic microscope (SteREO Discovery.V20, Carl Zeiss). For observations of pollination, pollinated pistils were stained with aniline blue solution (100 mM K$_3$PO$_4$, 0.1% [w/v] aniline blue), and observed using a UV fluorescence microscope (SteREO Discovery.V20, Carl Zeiss). For evaluation of pollination and subsequent seed setting, the number of seeds per siliquae was counted. All samples were collected from Arabidopsis plants grown in a humidity-controlled incubator (BIOTRON LPH300, NK system).

**RESULTS**

**Stigmatic papillae show greater elongation under high humidity** While growing A. thaliana plants in different laboratories (i.e., different growth chambers), we observed that papilla length can change under different growth conditions (Fig. 1). Since this difference seemed to be caused by ambient humidity conditions, as suggested previously (Safavian et al., 2014), we measured papilla length from soil-grown plants that were incubated under 100% or 50% humidity in the a humidity-regulating growth chamber and found that papillae became longer under high humidity (Fig. 2A). To maintain consistent growth conditions for stigmatic papillae, we incubated inflorescences of A. thaliana (Col) on agar medium under high (~100%) or normal (~50–60%; see Materials and Methods) humidity conditions in the growth chamber. The length of developing papillae did not differ up to the anthesis stage (Fig. 3, A1–A8 and B1–B8) but increased more under high humidity after anthesis (Fig. 3, A9 and B9), indicating that papillae change their length after anthesis in response to elevated ambient humidity.

For subsequent semi-in vitro analysis, to quantify papilla length under different humidity conditions, flowers at stage 12 were incubated on agar medium with different humidity conditions as shown above, and papilla length was measured when the flowers had developed to around stage 14. We found that the papilla length was greater for flowers incubated under high humidity than for those incubated under normal humidity (Fig. 2B), which was consistent with the observations of soil-grown plants in growth chambers with different humidity conditions (Fig. 2A). Together, the data suggest that papillae elongate more under high humidity at the post-anthesis stage.

**Papilla response is gynoecium-autonomous** High humidity delays and inhibits anther dehiscence in Allium triquetrum (García et al., 2006); indeed, we found that anthers were cleaved under normal humidity, whereas they failed to dehisce under high humidity (Supplementary Fig. S1). This finding suggests that the difference...
in papilla length may be due to delayed pollination or delayed anther dehiscence. To evaluate this hypothesis, we removed floral organs, excluding the gynoecium, from stage 12 flower buds and incubated them on medium under high or normal humidity. Longer papillae were generated only under high humidity conditions after 24 h (Fig. 2C). We also examined the effect of pollination on papilla length by hand pollination onto papillae that were dissected from flowers, and found that their length was almost the same with or without pollination (Fig. 2D), while there were no significant differences in pollen viability between different humidity conditions (Supplementary Fig. S2).

Together, these data indicate that stigmatic papillae demonstrate greater elongation under high humidity and that the mechanism is gynoecium-autonomous.
Papilla development under normal and high humidity

To understand the molecular mechanism of papilla elongation, we examined the function of genes that are highly expressed in papilla cells according to transcriptome data (Osaka et al., 2013). First, we selected the 18 genes that were most highly expressed in the stigmatic papilla cells of *A. thaliana*, *A. halleri* and *B. rapa* (Table 1). Next, we chose the top 24 genes that were highly and preferentially expressed in papilla cells of *A. thaliana* (Table 2). To examine whether they are involved in papilla elongation, we analysed insertion mutants, in which T-DNA was inserted within or close to their coding sequences. T-DNA insertion mutants were available for 13 genes from the first gene list, three of which did not segregate into homozygous mutants (Table 1). From the second list, 12 T-DNA insertion mutants were available (Table 2).

We examined the length of papillae in these 22 T-DNA insertion mutants under high humidity conditions. Among them, we found that two lines, SALK_086251 and SALK_048417, developed significantly longer stigmatic papillae, and two others, SALK_056141 and SALK_116062, developed shorter stigmatic papillae than those in wild type plants (Fig. 4), suggesting that the corresponding genes are involved in papilla elongation. The difference of length was significant but not drastic, suggesting that these genes are not involved in papilla initiation but rather in controlling the later elongation process.

### Table 1. Top 18 genes that are highly expressed in stigmatic papillae of *A. thaliana*, *A. halleri* and *B. rapa*

| AGI code     | Category | ID       | Term                                      | Description (TAIR database)                                                                 | Insertion line |
|--------------|----------|----------|-------------------------------------------|-------------------------------------------------------------------------------------------|----------------|
| AT1G19910    | F        | GO:0016887 | ATPase activity                           | AtVHA-C2, vacuolar H⁺-pumping ATPs 16 kDa proteolipid (AVA-P2)                            | n.a.          |
| AT1G25330    | F        | GO:0003677 | DNA binding                               | CESTA, a positive regulator of brassinosteroid biosynthesis                              | SALK_082100   |
| AT1G53070    | F        | GO:0030246 | carbohydrate binding                      | Legume lectin family protein                                                              | n.a.          |
| AT1G53130    | P        | GO:0042742 | defence response to bacterium             | GRI, involved in the regulation of cell death induced by extracellular ROS                | SALK_121701   |
| AT1G54630    | F        | GO:0000036 | acyl carrier activity                     | ACP3, an acyl carrier protein expressed in leaves, roots and dry seeds                   | SALK_123023   |
| AT1G61560    | F        | GO:0005516 | calmodulin binding                        | AtML06, a homologue of the barley mildew resistance locus o                                | SALK_031197   |
| AT2G03440    | P        | GO:0009738 | abscisic acid mediated signalling pathway  | AtNRP1, induced by *Pseudomonas syringae* pv. tomato infection                           | SALK_056141C  |
| AT2G41800    | P        | GO:0008150 | biological_process                        | unknown                                                                                   | SALK_067403   |
| AT3G14060    | P        | GO:0008150 | biological_process                        | unknown                                                                                   | SALK_022855C  |
| AT4G22240    | P        | GO:0008150 | biological_process                        | FBN1B, plastid-lipid associated protein PAP                                               | n.a.          |
| AT5G14000    | C        | GO:0005575 | cellular_component                        | ANAC084, NAC domain containing protein 84                                                 | n.a.          |
| AT5G17920    | F        | GO:0003871 | 5-methyltetrahydropteroyl-triglutamate-homocysteine S-methyltransferase activity | AtCIMs, a cytosolic cobalamin-independent methionine synthase                             | SALK_048417   |
| AT5G22980    | P        | GO:0006508 | proteolysis                               | SCPL47, serine carboxypeptidase-like 47                                                    | SALK_073712   |
| AT5G24770    | F        | GO:0003993 | acid phosphatase activity                 | AtVSP2, with acid phosphatase activity dependent on the presence of divalent cations     | n.a.          |
| AT5G38320    | P        | GO:0008150 | biological_process                        | unknown                                                                                   | SALK_039762   |
| AT5G42020    | C        | GO:0005783 | endoplasmic reticulum                     | BIP2, luminal binding protein                                                            | SALK_073202   |
| AT5G53300    | C        | GO:0005575 | cellular_component                        | UBC10, a ubiquitin conjugating enzyme                                                     | SALK_033033   |
| AT5G60390    | F        | GO:0005516 | calmodulin binding                        | GTP binding elongation factor Tu family protein                                          | SALK_035228C  |

*C*, cellular component; *F*, molecular function; *P*, biological process.

*n.a.*, not available.

**Genes involved in regulating papilla length**

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in response to the environment.

The finding of T-DNA mutants with longer papillae suggested that At5g21020 and At5g17920 are involved in suppressing papilla elongation. At5g17920, down-regulated in the T-DNA mutants (Fig. 5), encodes the cytosolic cobalamin-independent methionine synthase ATCIMS/AtMetE. Proteomic analysis has shown that ATCIMS/AtMetE is down-regulated in 18-day-old plants that were treated with 150 mM NaCl for 48 h (Jiang et al., 2007). This suggests that papilla elongation is regulated by an abiotic stress response mechanism. Because At5g21020 was not down-regulated in the T-DNA insertion mutants (Fig. 5), its genetic effect is not clear.

On the other hand, the occurrence of T-DNA mutants with shorter papillae suggests that At2g03440 and At3g53040 positively regulate papilla elongation. Both genes were significantly down-regulated in the T-DNA mutants (Fig. 5). At2g03440 encodes the nodulin-related
protein AtNRP1. Overexpression of AtNRP1 results in reduced synthesis of ABA and lower resistance to heat stress in seedlings (Fu et al., 2010). This result suggests that papillae express factors involved in ABA synthesis, regulating the response to environmental stresses, such as drought, heat and salinity (Cutler et al., 2010; Golldack et al., 2014). SALK_116062, in which At3g53040 is disrupted, has been shown to have higher desiccation tolerance in seeds than wild type (Costa et al., 2015). At3g53040 encodes a putative late embryogenesis abundant (LEA) protein, a member of the ABA-responsive proteins. Together, these results suggest that factors involved in stress responses against the environment regulate a flexible change of papilla length in response to fluctuations in ambient humidity.

**ABA affects papilla length** The above data suggest that environmental stresses influence papilla length, and that the plant hormone ABA plays a key role in such developmental and environmental responses. To test this hypothesis, we examined the effects of ABA on papilla development. First, we applied ABA solution directly to flower buds of soil-grown plants, but they wilted the next day and we could not analyse the phenotype of papillae. Therefore, stage 12 flowers were immersed in 5 μM ABA solution and, after incubation for 22 h, the length of

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**Fig. 4.** Papilla length of mutants grown on medium under high humidity conditions. Stage 12 flowers were incubated on medium and papilla length was measured when they developed to around stage 14. Lower-case ‘s’ stands for SALK of T-DNA insertion lines. Values are means ± SDs. N = 35 (5 papillae from 7 flowers for each mutant line). *P < 0.05, **P < 0.01 (t-test).

**Fig. 5.** qRT-PCR of four candidate genes for papilla length regulation. Expression level was normalized by TUB4. *P < 0.05, **P < 0.01 (t-test).
## Table 3. Gene expression profiles in ABA synthesis, signalling and response pathways

| Category & Other | Gene name | AGI code | Relative expression value (RPKM)* |
|-----------------|-----------|----------|----------------------------------|
| **ABA synthesis** | ABA1 | AT5G67030 | 16.90 |
| | ABA4 | AT1G67080 | 13.41 |
| | NCED2 | AT4G18350 | 0.01 |
| | NCED3 | AT3G14440 | 0.00 |
| | NCED5 | AT1G30100 | 0.00 |
| | NCED6 | AT3G24220 | 0.02 |
| | NCED9 | AT1G78390 | 0.03 |
| | ABA2 | AT1G52340 | 0.02 |
| | AAO3 | AT2G27150 | 0.00 |
| **ABA signalling** | PYR1 | AT4G17870 | 140.89 |
| | PYL1 | AT5G46790 | 1.79 |
| | PYL2 | AT2G26040 | 0.04 |
| | PYL3 | AT1G73000 | 0.04 |
| | PYL4 | AT2G38310 | 5.87 |
| | PYL9 | AT1G01360 | 8.91 |
| | PYL12 | AT5G45870 | 0.03 |
| | RCAR1 | AT1G01360 | 8.91 |
| | PP2C-HA (HAB1) | AT1G72770 | 0.40 |
| | PP2CG1 | AT2G33700 | 1.60 |
| | PP2C5 | AT2G40180 | 0.01 |
| | PP2CA | AT3G11410 | 1.31 |
| | PP2C52 | AT4G03415 | 0.01 |
| | PP2C74 | AT5G36250 | 2.70 |
| | SnRK2.1 | AT5G08590 | 2.67 |
| | SnRK2.2 | AT3G50500 | 2.42 |
| | SnRK2.3 | AT5G66880 | 1.23 |
| | SnRK2.5 | AT5G63650 | 4.88 |
| | SnRK2.6 | AT4G33950 | 1.53 |
| **ABA response & other** | ABA3 | AT1G16540 | 0.73 |
| | ABAP1 | AT5G13060 | 0.07 |
| | ABAK | AT5G13630 | 3.41 |
| | ABD1 | AT4G38480 | 0.19 |
| | ABAF2 (AREB1) | AT1G45249 | 4.52 |
| | ABAF4 (AREB2) | AT3G19290 | 2.02 |
| | ABAH1 | AT2G13540 | 0.56 |
| | ABI1 | AT4G26080 | 1.58 |
| | ABI2 | AT5G50500 | 1.08 |
| | ABI3 | AT3G24650 | 0.02 |
| | ABI4 | AT2G40220 | 0.02 |
| | ABI5 | AT2G36270 | 0.26 |

### Pollen
- **pre-pollination**: Papilla
- **compatible pollinated**: Pollen compatible
- **incompatible pollinated**: Pollen incompatible

### References
- ABA1
- ABA4
- NCED2
- NCED3
- NCED5
- NCED6
- NCED9
- ABA2
- AAO3
- PYR1
- PYL1
- PYL2
- PYL3
- PYL4
- PYL9
- PYL12
- RCAR1
- PP2C-HA (HAB1)
- PP2CG1
- PP2C5
- PP2CA
- PP2C52
- PP2C74
- SnRK2.1
- SnRK2.2
- SnRK2.3
- SnRK2.5
- SnRK2.6
- ABA3
- ABAP1
- ABAK
- ABD1
- ABAF2 (AREB1)
- ABAF4 (AREB2)
- ABAH1
- ABI1
- ABI2
- ABI3
- ABI4
- ABI5
### Papilla development under normal and high humidity

| Gene | Accession | RPKM Normal | RPKM High |
|------|-----------|-------------|-----------|
| ABI8 | AT3G08550 | 1.60        | 1.42      |
| ABO1 | AT5G13680 | 0.25        | 0.40      |
| ABO3 | AT1G66600 | 0.00        | 0.03      |
| ABO4 | AT1G08260 | 0.13        | 0.21      |
| ABO5 | AT1G51965 | 0.06        | 0.04      |
| ABO6 | AT5G04895 | 0.05        | 0.05      |
| ABO8 | AT4G11690 | 0.45        | 0.19      |
| ABR  | AT3G02480 | 0.04        | 0.99      |
| ABR1 | AT5G64750 | 0.02        | 0.04      |
| AHG1 | AT5G51760 | 0.03        | 0.06      |
| AHG2 | AT1G55870 | 0.27        | 0.29      |
| AIB  | AT2G46510 | 0.43        | 0.85      |
| ABR1 | AT5G62300 | 0.16        | 0.08      |
| AHG11| AT2G44880 | 0.02        | 0.02      |
| AHG2 | AT1G55870 | 0.03        | 0.06      |
| AIB  | AT2G46510 | 0.43        | 0.85      |
| AIP1 | AT3G62300 | 0.16        | 0.08      |
| AIRP1| AT4G23450 | 0.05        | 0.06      |
| AIRP2| AT5G01520 | 0.16        | 0.04      |
| AIRP3| AT3G09770 | 2.02        | 2.32      |
| AIRP4| AT5G58787 | 1.34        | 0.77      |
| AIRP5| AT1G69850 | 0.03        | 0.03      |
| AKS1 | AT1G51140 | 0.86        | 0.71      |
| AKS2 | AT1G05805 | 3.06        | 1.07      |
| AKS3 | AT2G42280 | 0.11        | 0.02      |
| APX1 | AT1G07890 | 0.01        | 0.07      |
| APX2 | AT3G09640 | 0.01        | 0.02      |
| ARPK1| AT4G11890 | 0.02        | 0.02      |
| AREB3| AT3G06850 | 5.60        | 3.93      |
| ARP1 | AT3G54770 | 0.00        | 0.04      |
| ARS1 | AT3G02860 | 3.35        | 1.59      |
| CCS  | AT1G12520 | 1.74        | 0.91      |
| CSD3 | AT5G18100 | 11.10       | 7.32      |
| EIN2 | AT5G03280 | 0.66        | 0.36      |
| ERA1 | AT5G66470 | 0.33        | 0.10      |
| ERA2 | AT1G30960 | 0.42        | 0.28      |
| FTB  | AT5G40280 | 0.69        | 0.44      |
| KAT1 | AT5G46240 | 0.11        | 0.06      |
| actin1| AT2G37620 | 1.84        | 4.88      |
| actin2| AT3G18780 | 1.62        | 2.13      |
| actin3| AT3G53750 | 2.28        | 3.89      |
| actin4| AT5G59370 | 0.08        | 18.82     |
| actin7| AT5G09810 | 12.15       | 19.44     |
| actin8| AT1G49240 | 10.73       | 15.73     |
| actin9| AT2G42090 | 0.04        | 0.01      |
| actin11| AT3G12110 | 5.57        | 21.40     |
| actin12| AT3G46520 | 0.13        | 18.76     |

*Expression values are normalized by reads per kilobase of exon per million mapped reads (RPKM). Original expression data are available from Matsuda et al. (2015).
papillae was measured. The papillae of flowers treated with ABA showed greater elongation than those of water-treated flowers (Fig. 6), supporting our hypothesis that papilla elongation is regulated by an ABA-mediated response mechanism at the post-anthesis stages.

We then examined the expression of genes related to the ABA biosynthesis and signalling pathways using papilla transcriptome data (Osaka et al., 2013; Matsuda et al., 2015). We found that genes involved early in the pathway of ABA biosynthesis, i.e., *ABA1* and *ABA4*, were highly expressed, whereas later downstream genes in the pathway, including *NCED*, *ABA2*, *AAO3* and *ABA3*, were not (Table 3). The majority of genes in the ABA signalling pathway, including *PYR/PYL/RCAR*, *PP2Cs* and *SnRK2*, and ABA-responsive genes, such as *AKSs* and *AREBs*, were expressed in papillae. These expression profiles indicate that the primary biosynthesis pathway and signalling/response systems for ABA are active in papilla cells for a rapid response against abiotic stress. This result further supports the hypothesis that there is an ABA-mediated abiotic stress response mechanism for papilla elongation.

**Fig. 6.** ABA elongates stigmatic papillae. (A) Papillae treated with 5 μM ABA for 22 h. (B) Papillae treated with water for 22 h. Bars: 100 μm. (C) Papilla length after treatment with ABA or water. Values are means ± SDs. N = 20 (1 papilla from 20 flowers). ** P < 0.01 (t-test).

In guard cells, ABA produced in response to drought stress initiates a cascade, including calcium and protein...
phosphorylation, leading to water efflux that closes stomata (Bauer et al., 2013; Lim et al., 2015; Munemasa et al., 2015). This effect led us to speculate on the role of ABA in papilla elongation. In our ABA treatment experiment, flowers were immersed in ABA solution in water, which reflected a well-watered condition with ABA rather than a drought condition. The result therefore suggests that another system is acting in response to exogenous ABA. Under well-watered conditions, inhibition of ABA biosynthesis or ABA-deficient mutants suppresses shoot growth in Arabidopsis, tomato and maize, and these defects are overcome by exogenous ABA (Sharp et al., 2000; Sharp and LeNoble, 2002; LeNoble et al., 2004). ABA in well-watered conditions may repress the accumulation of ethylene, which represses shoot growth. Therefore, papilla elongation by ABA under high humidity may be mediated by the suppression of ethylene accumulation, which leads to cell growth promotion, although the effects of ABA and ethylene at the cellular level have not yet been demonstrated. One hypothesis is that the cell elongation of papillae is mediated by a change in microtubule orientation, as the appropriate amount of ethylene regulates microtubule orientation during the triple response in the Arabidopsis hypocotyl (Woeste and Kieber, 1998; Ma et al., 2016). However, most known factors involved in ethylene biosynthesis, signalling and response are not highly expressed in developing papillae (Supplementary Table S1), suggesting a role for an ethylene-independent response, or post-transcriptional ethylene regulation, in papillae elongation. This indicates that the ABA response differs among cell types and environments; in papillae, it acts as a positive regulator of elongation in response to high humidity.

Ambient humidity affects another aspect of pollination. EXOCYST SUBUNIT EXO70 FAMILY PROTEIN A1 (Exo70A1), interacting with the ARM repeat-containing 1 (ARC1) protein in B. napus, is involved in vesicle secretion at the plasma membrane at the site of pollen attachment, leading to pollen hydration (Safavian and Goring, 2013), and is required for compatible pollen acceptance in stigmatic papillae (Samuel et al., 2009). The exo70A1 mutation in A. thaliana inhibits pollen germination on papillae, and this defect is rescued by high humidity (Safavian et al., 2014). Therefore, ambient humidity affects at least two processes during plant reproduction: papilla elongation, which we discovered, and pollen hydration after the pollen lands on papillae.

Pollination and fertilization is a central process for plant reproduction. The sessile style of plant life has led to the evolution of a high ability of adaptation to the environment. Since high humidity suppresses anther dehiscence (Supplementary Fig S1), elongation of papillae under high humidity may increase the chance of pollination. Our finding represents an example of how plants adapt to an ever-changing environment.

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