RESEARCH PAPER

High temperature causes breakdown of S haplotype-dependent stigmatic self-incompatibility in self-incompatible Arabidopsis thaliana

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Abstract

Commercial seeds of Brassicaceae vegetable crops are mostly F1 hybrids, the production of which depends on self-incompatibility during pollination. Self-incompatibility is known to be weakened by exposure to elevated temperatures, which may compromise future breeding and seed production. In the Brassicaceae, self-incompatibility is controlled by two genes, SRK and SCR, which function as female and male determinants of recognition specificity, respectively. However, the molecular mechanisms underlying the breakdown of self-incompatibility under high temperature are poorly understood. In this study, we examined the self-incompatibility phenotypes of self-incompatible Arabidopsis thaliana SRK-SCR transformants under normal (23 °C) and elevated (29 °C) temperatures. Exposure to elevated temperature caused defects in the stigmatic, but not the pollen, self-incompatibility response. In addition, differences in the response to elevated temperature were observed among different S haplotypes. Subcellular localization revealed that high temperature disrupted the targeting of SRK to the plasma membrane. SRK localization in plants transformed with different S haplotypes corresponded to their self-incompatibility phenotypes, further indicating that defects in SRK localization were responsible for the breakdown in the self-incompatibility response at high temperature. Our results provide new insights into the causes of instability in self-incompatibility phenotypes.

Keywords: Brassicaceae, F1 hybrid, high temperature, protein transporting, receptor kinase, self-incompatibility.

Introduction

Plant growth and development are affected by variability in environmental conditions. Both higher average and peak temperatures caused by climate change have the potential to affect the performance of crop plants worldwide. The Brassicaceae family contains a number of agriculturally and horticulturally significant crops. Commercial cultivation of Brassicaceae vegetables generally employs F1 hybrids, which show superior crop performance relative to their parents (Lippman and Zamir, 2007; Fujimoto et al., 2018). F1 hybrid seeds of Brassica vegetables are produced by mutual crossing of two inbred self-incompatible parent lines, and also by crossing a cytoplasmic male-sterile line as a female parent with a male-fertile parent line. However, the self-incompatibility is known to be erratic, as external factors such as high temperature (Nasrallah and Wallance, 1968; Richards and Thurling, 1973; Visser, 1977; Hirosaki and Niikura, 2008) as well as internal factors can cause breakdown of the self-incompatibility response (Carter and McNeilly, 1975; Ockendon, 1978; Hirosaki and Niikura, 2008).
As a result, F1 hybrid seed populations obtained by using self-incompatibility often contain some self-fertilized and sibling-fertilized seeds, and this is an important issue for the production of F1 hybrid seed.

The Brassicaceae family has a sporophytic self-incompatibility mechanism that is governed by haplotypes of the S locus, which contains two tightly linked polymorphic genes: S-locus receptor kinase (SRK) and S-locus cysteine-rich protein/S-locus protein 11 (SCR /SP11, hereafter referred to as SCR). SRK encodes a plasma membrane-localized receptor kinase that is expressed in stigma papilla cells (Stein et al., 1991; Takasaki et al., 2000), and SCR encodes a pollen coat-localized peptide ligand for SRK (Schoffler et al., 1999; Takayama et al., 2000). SRK can only interact with SCR of the same S haplotype (Kachroo et al., 2001; Takayama et al., 2001). Allele-specific SRK–SCR interactions trigger the self-incompatibility response, causing inhibition of pollen germination and of pollen tube penetration into the stigma epidermal cell wall.

Several SRK+SCR gene pairs, including the Ss haplotype in Arabidopsis lyrata, which is a closely related self-incompatible species to A. thaliana, have been previously shown to confer an intense self-incompatibility phenotype in the A. thaliana C24 ecotype (Nasrallah et al., 2004; Boggs et al., 2009a, b). The AlSRK+AlSCR A. thaliana transformant provides a very useful model system for analysis of the self-incompatibility mechanism in the Brassicaceae, which is difficult to examine using Brassica crops. These transformants have provided new insights into a variety of molecular mechanisms, such as SRK–SCR interactions (Boggs et al., 2009a), SRK biogenesis (Yamamoto et al., 2014), and self-incompatibility signaling (Kitashiba et al., 2011; Yamamoto and Nasrallah, 2013; Ivano et al., 2015).

Breakdown of self-incompatibility in Brassicaceae as a result of high temperatures has been known for more than 50 years. Nasrallah and Wallace (1968) examined the effects of temperature on the degree of self-incompatibility by cultivating cabbage plants in growth chambers set at 15, 21, and 27 °C. They found that a high-temperature (27 °C) pre-treatment for 6 d before pollination caused an increase in the number of seeds after selfing, and that the differences of sensitivity of self-incompatibility to high temperature were likely to be determined by the genetic background of the plants. Brussels sprouts grown in glasshouses controlled at 23/14 °C and 26/14 °C (day/night, daylength 11 h) have been shown to produce more seeds than those grown at 17/14 °C and 20/14 °C (Visser, 1977). Both studies reported that plants showing a high degree of the self-incompatibility response were not affected by the high-temperature treatment (Nasrallah and Wallace, 1968; Visser, 1977). Richards and Thruling (1973) found that Brassica napus plants treated at 30 °C in a temperature-controlled growth cabinet showed lower self-incompatibility levels than those at 25 °C. However, since S genes had not been identified in the 1960s and 1970s, the roles of the SRK and SCR proteins in self-incompatibility instability under high temperature conditions and their molecular mechanisms remained unclear.

In this study, the molecular mechanisms behind the breakdown of self-incompatibility resulting from high-temperature treatment was investigated using self-incompatible A. thaliana plants transformed with SRK and SCR gene pairs derived from A. lyrata. Self-incompatible transgenic A. thaliana can be cultivated in growth chambers under strictly controlled temperatures, thus serving as a suitable model for the analysis of molecular mechanisms of high temperature effects. We examined the self-incompatibility of self-incompatible A. thaliana transformants under normal (23 °C) and elevated temperature (29 °C) by pollination assays and seed production, and monitored the subcellular localization of SRK proteins by biochemical analysis. We found that high temperature perturbed the stigmatic self-incompatibility response. Our results indicated that defects in plasma-membrane localization of SRK are responsible for the breakdown in self-incompatibility at high temperature.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana C24 plants (obtained from the Arabidopsis Biological Resource Center, https://abrc.osu.edu/) were used in this study. All plants were grown at a constant temperature of 23 °C under long-days (100 µmol photons m–2 s–1, 16/8 h light/dark). The relative humidity was not controlled. To avoid plant age-related effects on seed fertility and self-incompatibility phenotypes when counting seed numbers, at least eight siliques were collected between 1–3 weeks after flowering and seeds per silique were counted. For the high-temperature treatment, plants were grown at 23 °C until bolting and then transferred to growth chambers set at a constant temperature of 29 °C under long days as detailed above.

Plasmid construction and production of transgenic plants

AlSRKb-FLAG+AlSCRa A. thaliana transformants have been described previously by Yamamoto et al. (2014). AlSRKb+AlSCRa, AlSRKb+AlSCRb, and AlSRKb-FLAG+AlSCRb A. thaliana transformants were developed as follows. Arabidopsis lyrata plants carrying the Ss and Sb haplotypes were grown from seeds kindly provided by Professor Barbara Mable, University of Glasgow, UK and were identified by nucleotide sequence analysis of PCR products amplified with the 13F1 (tcgcaggttaacctgtctc) and SLGR (atgscataaagatctgcc) primers (Schierup et al., 2001) using a GenomeLab DTCS Quick Start Kit (SCIEX, Framingham, MA) and a Beckman Coulter CEQ2000XL DNA sequencer (SCIEX). A 360-bp fragment corresponding to the AtS1 (At3g12000) promoter, a stigma papilla cell-specific promoter (Yamamoto et al., 2014), was used to control expression of the AlSRKb and AlSCRa genes. The AtS1 promoter and a ~350-bp DNA fragment corresponding to the A. lyrata SRKb terminator were introduced into the EorRI and KpnI sites and the BankHI and Xhol sites of pCambia1300, respectively, and the resulting plasmid was designated as pMYC290. The AlSRKb, AlSRKb, and AlSRKb coding sequence fragments were amplified by PCR using the primers AlSRKb-F and AlSRKb-R for AlSRKb, primers AlSRKa-F and AlSRKa-R for AlSCRa, and primers AlSRKb-F and AlSRKb-R for AlSRKb (see Supplementary Table S1 at JXB online). The amplified AlSRKb coding sequence fragment was introduced into the KpnI site of pMYC290 by InFusion cloning (Takara Bio), followed by introduction of the AlSCRb genomic fragment containing ~580 bp and ~220 bp regions corresponding to the AlSCRb promoter and terminator, respectively, into the Xhol site. A 2680-bp AlSCRb genomic fragment and a 2952-bp AlSCRb genomic fragment, which contained 600 bp and 300 bp of the promoter and terminator, respectively, were amplified by PCR with the primers AlSCRa-F and AlSCRa-R for AlSCRa, and primers AlSCRa-39-F and AlSCRa-39-R for AlSCRa (Supplementary Table S1). The AlSRKb fragment was introduced into the KpnI site of pMYC290 by InFusion cloning (Takara Bio), followed by introduction of the AlSCRb fragment into the Xhol site. The AlSRKb and AlSCRb fragments were similarly introduced into the KpnI and PstI sites, respectively, of pMYC290 by InFusion cloning (Takara Bio). For construction of AlSRKb-FLAG+AlSCRb, the AtS1 promoter–Stud
site-3×FLAG-ALSRKb-terminator DNA cassette was produced by the recombinant PCR method (Pont-Kingdon, 1994). Genomic DNA prepared from the $\text{ALSRK}_b$-FLAG-ALSCR$_b$ A. thaliana transformant was used as the template, using the primers AtS1 StuI-F and AtS1 Stuf-R for the AtS1 promoter and the primers AlSRKb term StuI-F and AlSRKb term Stuf-R for the AlSRKb terminator (Supplementary Table S1) in the first-round PCR. Next, the AtS1 promoter-Stu site-3×FLAG-ALSRKb-terminator fragment was amplified using the first-round PCR products as templates with the AtS1 StuI-F and AlSRKb term Stuf-R primers (Supplementary Table S1). The resulting AtS1 promoter-Stu site-3×FLAG-ALSRKb-terminator fragment was introduced into the EcoRI site of pCambia1300, and the construct was designated as pMYC190. The AlSCR$_b$ promoter and AlSCR$_b$ terminator were amplified by PCR using the primers AlSCRb pro-F and AlSCRb pro-R and the primers AlSCRb term-F and AlSCRb term-R, respectively (Supplementary Table S1). The amplified fragments were introduced into the Sad and KpnI sites and the Psfl and HindIII sites of pMYC190, respectively, and the resulting construct was designated as pMYC192. The $\text{ALSRK}_b$ and $\text{ALSCR}_{19}$ genomic fragments were amplified by PCR using the primers $\text{ALSRK39}$-FLAG-F and $\text{ALSRK39}$-FLAG-R and the primers $\text{ALSCR39}$-F2 and $\text{ALSCR39}$-R2, respectively (Supplementary Table S1). The $\text{ALSCR}_{19}$ fragment was inserted into the KpnI and Sad sites of pMYC192, followed by introduction of the $\text{ALSRK}_b$ fragment into the Sgt site by InFusion cloning (Takara Bio). The resulting plasmids were used for transformation of A. thaliana plants by the floral dip method (Clough and Bent, 1998) using Agrobacterium tumefaciens strain GV3101.

Pollination assays
We carried out the pollination assays in the morning (usually between 2–3.5 h after the beginning of the light period). Emasculated flowers at flower stage 13 (Smyth et al., 1990), which is 1 d before anthesis in A. thaliana, were collected and placed on a 0.5% agar plate, and stigma was manually pollinated under a stereomicroscope with pollen grains from mature flowers. They were then left for 2 h at 23°C under continuous light (relative humidity was not controlled) prior to fixation in an ethanol:acetic acid (3:1) solution for 10 min at 55°C. The fixed stigmas were treated with 8 M NaOH for 15 min at room temperature, then washed with water, and stained with decolorized Aniline Blue. Stained stigmas were observed using fluorescence microscopy as previously described (Kho and Bear, 1968). Each pollination assay was performed with at least three stigmas, and was repeated on at least two different dates for each transformant line. Pollen tubes were counted, and self-incompatibility was classified according to the number of tubes per pollinated stigma as follows: incompatible, <10; partially incompatible, >10–29; compatible, >30. Images of pollinated stigmas were captured using a C-terminal FLAG-tagged AlSRKb and AlSCRb transgenic plants grown at 23°C were self-pollinated, and the pollinated pistils were incubated for 16 h at 29°C. No self-pollen germination or pollen tube growth was observed in $\text{ALSRK}_b$-FLAG-ALSCR$_b$ A. thaliana transplants that had not been exposed to elevated temperatures (Fig. 1A). Stigmas treated at 29°C for 6 h showed some self-pollen germination but no pollen tube growth. In contrast, treatment at 29°C for 16 h resulted in self-pollen germination and pollen tube penetration into the stigma tissues.

To investigate the effect of high temperature after pollination on self-incompatibility, stigmas of $\text{ALSRK}_b$-FLAG-ALSCR$_b$ A. thaliana transformants grown at 23°C were self-pollinated, and the pollinated pistils were incubated for 16 h at 29°C. Exposure to high temperature was not responsible for the lack of pollen germination since wild-type (WT) pollen grains germinated and pollen tubes elongated in the stigmas of WT plants (Fig. 1B). These experiments indicated that high-temperature treatment for 16 h before, but not after, pollination caused defects in self-incompatibility.

We also investigated whether high temperature affected the self-incompatibility response in the stigmas, the pollen, or both stigmas and pollen. When the stigmas of $\text{ALSRK}_b$-FLAG-ALSCR$_b$ A. thaliana transformants treated at 29°C were pollinated with pollen from untreated transformants, numerous pollen tubes were observed in the pistils (Fig. 1C), as was also observed with pollen from WT plants. By contrast, pollen from $\text{ALSRK}_b$-FLAG-ALSCR$_b$ A. thaliana transformants treated at 29°C did not germinate on the stigmas of transformants grown at 23°C. Germination and pollen tube growth of pollen from $\text{ALSRK}_b$-FLAG-ALSCR$_b$ A. thaliana transformants treated at 29°C were observed on untransformed WT stigmas, indicating that the high-temperature treatment did not disrupt the viability of AlSCRb-bearing pollen. These results indicated that the high-temperature treatment did not affect self-incompatibility in the pollen.

Protein electrophoresis
Ten flower buds at flower stage 13 were collected from each of the untreated plants (23°C) and plants exposed to 29°C for 16 h and homogenized in extraction buffer [100 mM Tris-HCl, pH 8.0, 0.2% (w/v) SDS, 5.7 mM β-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride]. The extracted proteins were precipitated using the trichloroacetic acid/acetone method and dissolved in SDS-PAGE sample buffer (Laemmli, 1970). Some of the protein samples were treated with endoglycosidase H (New England BioLabs) at 37°C for 1 h. The proteins were separated on a 7.5% SDS-PAGE gel and transferred to Immobilon-P membranes (Millipore) as previously described (Towbin et al., 1979). For detection, primary anti-FLAG antibody (Sigma-Aldrich) was used at 1:1000 dilution, and secondary HRP-labeled anti-mouse IgG (GE Healthcare) was used at 1:3000 dilution. The protein membranes were subsequently stained with Coomassie Brilliant Blue R250 and detected proteins were quantified using the ImageJ software (https://imagej.nih.gov/ij/).

Results and discussion
Breakdown of the self-incompatibility response in A. thaliana AlSRKb+AlSCRb stigmas upon exposure to elevated temperature
To examine the effect of high temperature on self-incompatibility in self-incompatible plants, A. thaliana C24 plants expressing a C-terminal FLAG-tagged AlSRKb and AlSCRb (hereafter $\text{ALSRK}_b$-FLAG-ALSCR$_b$), which had previously been found to exhibit an intense self-incompatibility response (Yamamoto et al., 2014), were subjected to high temperature (29°C) for 6 h or 16 h prior to pollination. Self-incompatibility was generally assessed following manual pollination of stage-13 flower buds (Smyth et al., 1990) as the number of pollen tubes per pollinated stigma. No pollen germination or pollen tube growth was observed in $\text{ALSRK}_b$-FLAG-ALSCR$_b$ A. thaliana transplants that had not been exposed to elevated temperatures (Fig. 1A). Stigmas treated at 29°C for 6 h showed some self-pollen germination but no pollen tube growth. In contrast, treatment at 29°C for 16 h resulted in self-pollen germination and pollen tube penetration into the stigma tissues.

To investigate the effect of high temperature after pollination on self-incompatibility, stigmas of $\text{ALSRK}_b$-FLAG-ALSCR$_b$ A. thaliana transformants grown at 23°C were self-pollinated, and the pollinated pistils were incubated for 16 h at 29°C. No self-pollen germination or elongation was observed on the stigmas (Fig. 1B). Exposure to high temperature was not responsible for the lack of pollen germination since wild-type (WT) pollen grains germinated and pollen tubes elongated in the stigmas of WT plants (Fig. 1B). These experiments indicated that high-temperature treatment for 16 h before, but not after, pollination caused defects in self-incompatibility.
Fig. 1. Self-incompatibility phenotypes of AlSRKa+AlSCRa Arabidopsis thaliana transformants at high temperature. Plants were grown at 23 °C. For the high-temperature treatment, plants were transferred to 29 °C for either 6 h or 16 h before being hand-pollinated. Schematic diagrams above the images indicate the procedures used for the pollination assays. Aniline Blue staining was used to monitor pollen germination and pollen tube growth. (A) Images of self-pollinated AlSRKb-FLAG+AlSCRb [AlSRKb-FLAG+AlSCRb] stigmas. Numerous self-pollen tubes were observed in AlSRKb-FLAG+AlSCRb stigmas treated at 29 °C for 16 h. (B) Pistils of wild-type (WT) and AlSRKb-FLAG+AlSCRb transformants grown at 23 °C were self-pollinated and incubated at 29 °C for 16 h. Self-pollen tubes were not observed in AlSRKb-FLAG+AlSCRb stigmas. (C) Upper images show stigmas of AlSRKb-FLAG+AlSCRb transformants treated at high temperature and pollinated with pollen from AlSRKb-FLAG+AlSCRb transformants (left) or WT plants (right) grown under normal conditions (i.e. 23 °C). Lower panels show the stigmas of AlSRKb-FLAG+AlSCRb transformants (left) and the WT (right) grown under normal conditions and pollinated with pollen from AlSRKb-FLAG+AlSCRb transformants treated at high temperature. Numerous pollen tubes elongated in the stigmas of AlSRKb-FLAG+AlSCRb transformants treated at 29 °C. By contrast, pollen from the AlSRKb-FLAG+AlSCRb transformants treated at 29 °C exhibited an intense incompatible response on the stigmas of AlSRKb-FLAG+AlSCRb transformants. Scale bars are 100 μm. (This figure is available in colour at JXB online.)

Table 1. Pollination phenotypes of transgenic Arabidopsis thaliana expressing A. lyrata S genes at 23 °C

| Transgenes | Total no. of transformants | No. of self-pollen tubes observed per stigma* |
|------------|---------------------------|--------------------------------------------|
|            |                           | <10 | 10–29 | >30 |
| AlSRKb+AlSCRb | 15                       | 9   | 0     | 6   |
| AlSRKb+AlSCRa | 10                       | 4   | 0     | 6   |
| AlSRKb+AlSCRb | 15                       | 14  | 0     | 1   |
| AlSRKb-FLAG+AlSCRb | 11                   | 6   | 0     | 5   |

* Number of pollen tubes observed in stigma 2 h after pollination with self-pollen: <10, incompatible; 10–29, partially incompatible; >30, compatible.
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significantly fewer (Fig. 2B). Although the AlSRKb+AlSCRb A. thaliana transformants exhibited self-incompatibility, the number of seeds per silique produced under normal conditions in AlSRKb+AlSCRb transformants #5 and #7 did not differ significantly from that of the WT plants, and AlSRKb+AlSCRb and AlSRK39+AlSCR39 transformants exhibited complete breakdown of the self-incompatibility response after exposure to elevated temperature prior to pollination, whereas transformants expressing AlSRK39+AlSCR39 retained the self-incompatibility response. Scale bar is 100 µm. (B) Box-plots of seed number per silique for plants grown at 23 °C or 29 °C. Data are 10 siliques for AlSRKb+AlSCRb transformant #1 grown at 23 °C, and from 8 siliques for the other transformants. Different letters indicate significant differences between means as determined using the Tukey–Kramer method (P<0.05). (This figure is available in colour at JXB online.)

Since high temperature affected the stigmatic self-incompatibility response in AlSRKb-FLAG+AlSCRb transformants (Fig. 1C), we examined the accumulation and subcellular levels of plasma membrane-localized AlSRKb but not AlSRK39 under high-temperature conditions.
localization of AlSRK proteins under normal (23 °C) and high-temperature (29 °C) conditions. Transformants were developed that expressed AlSCR39 under the control of the AlSCRb promoter and AlSRK39-FLAG (AlSRK39 tagged at the C-terminus with three tandem FLAG tags) under the control of the AtS1 promoter. Six of eleven AlSRK39-FLAG+AlSCR39 transformants inhibited self-pollen germination and pollen tube growth at 23 °C (Table 1 and Fig. 3A). Two independent AlSRK39-FLAG+AlSCR39 transformants showed an intense self-incompatibility response under high-temperature conditions. These results showed that the C-terminal FLAG tag did not affect AlSRK39 function.

Total proteins prepared from the buds of AlSRKb-FLAG+AlSCRb and AlSRK39-FLAG+AlSCR39 transformants grown at 23 °C and 29 °C were analysed by immunoblotting with anti-FLAG antibody. The accumulation of the AlSRKb-FLAG and AlSRK39-FLAG proteins was comparable in transgenic plants grown at 23 °C and at 29 °C (Fig. 3B), indicating that the elevated temperature did not affect their accumulation in the stigmas.

We then used endoglycosidase H (Endo H) treatment to examine the subcellular localization of AlSRKb-FLAG and AlSRK39-FLAG. Glycans in the endoplasmic reticulum (ER) are high in mannose and Endo H-sensitive, whereas those in the Golgi apparatus are further modified and become Endo H-resistant. Endo H treatment can thus be used to distinguish plasma membrane-targeted glycoproteins retained in the ER from those that entered the Golgi apparatus before transport to the cell surface, and it has previously been used to monitor the plasma membrane localization of AlSRKb (Yamamoto et al., 2014). Consistent with this previous study, 52±5.3% of AlSRKb-FLAG was Endo H-resistant in plants in the normal temperature treatment, as demonstrated by its slower migration relative to the Endo H-sensitive form during SDS-PAGE analysis (Fig. 3C). Most of the AlSRK39-FLAG (85±5.7%) occurred in an Endo H-resistant form. The proportion of Endo H-resistant AlSRKb-FLAG was substantially lower at 29 °C than at 23 °C (11±0.9% at high temperature versus 52±5.3% at normal temperature), indicating that the high temperature affected the transport of AlSRKb-FLAG to the plasma membrane. Conversely, a large proportion (75±1.1%) of AlSRK39-FLAG was Endo H-resistant at high temperature, suggesting that elevated temperature did not affect the transport of AlSRK39-FLAG to the plasma membrane, consistent with the results of the pollination assays.

Fig. 3. Determination of subcellular localization of AlSRKb and AlSRK39 in stigmas of Arabidopsis thaliana at high temperature. (A) Self-pollinated AlSRKb-FLAG+AlSCRb A. thaliana transformants at normal temperature (23 °C, left panel) or treated at 29 °C for 16 h before pollination (right panel). Plants expressing C-terminus-FLAG-fused-AlSRK39 inhibited pollen germination, indicating that the C-terminus FLAG fusion did not affect AlSRK39 function. Scale bar is 100 μm. (B) Total proteins from flower buds of wild-type (WT) plants, and transformants expressing AlSRKb-FLAG and AlSRK39-FLAG at 23 °C, and transformants treated at 29 °C for 16 h prior to bud collection were analysed by immunoblotting (IB) with anti-FLAG antibody (upper panel). The membrane used for immunoblot analysis was stained with Coomassie Brilliant Blue (CBB) as a loading control (lower panel). (C) Total flower-bud proteins were treated (+) with Endo H at 37 °C for 1 h; untreated proteins are indicated by ‘−’. The upper panel shows IB analysis with anti-FLAG antibody. Glycosylated (arrowhead) and deglycosylated (arrow) forms of AlSRKb-FLAG and AlSRK39-FLAG are indicated. The lower panel shows the membrane stained with CBB as a loading control. The graph shows the percentage of AlSRKb-FLAG and AlSRK39-FLAG in the Endo-H resistant form, which indicates that AlSRK proteins entered the Golgi apparatus (see text for details). Data are means (±SE) of three replicates; dots indicate the value of each replicate. Different letters indicate significant differences between means as determined using the Tukey–Kramer method (P<0.05). (This figure is available in colour at JXB online.)
Conclusions

High temperature affected the stigmatic but not the pollen self-incompatibility response in AISRKb-FLAG+AlSCRb A. thaliana transformants. The levels of plasma membrand-localized AISRKKb-FLAG were significantly lower at elevated temperature (29 °C) than at normal temperature (23 °C). Conversely, AISRKKb+AlSCRb transformants retained the self-incompatibility phenotype at high temperature and the plasma membrane localization of AISRKK39-FLAG was not affected. These results suggest that the breakdown in the self-incompatibility response induced by high temperature is caused by a defect in AISRK transport to the plasma membrane. Since proteins are transported to the plasma membrane after forming their tertiary structures in the ER, we speculate that AISRKK39 is able to form its tertiary structure more effectively than AISRKb, and that the efficiency of formation may reflect the differences observed in the high-temperature tolerance of the self-incompatibility function between S haplotypes. Identification of S haplotypes that produce stable SRK proteins may be useful for plant breeding in the coming decades, over which period temperatures are expected to increase as a consequence of climate change. Stable production of SRK proteins at elevated temperatures would facilitate the production of parental lines that exhibit consistent self-incompatibility phenotypes for F1 seed production in vegetable Brassicaceae.

Supplementary data

Supplementary data are available at JXB online.

Table S1. PCR primers used for plasmid construction.

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