

Supplemental Data. Indriolo et al. (2014). Plant Cell 10.1105/tpc.114.122879

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AlARC1  MVTE-AIFASLRRRSSPSLDAYLFTTVDLSDVPLLQTLASTSAELVSCFRGVRFSFQRRN 59
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BnARC1  SPSSLSDFFHDLKDYSTLLDVFLPVDSLCLNDDVREQVQLLHMQHYVD-----DNDLETL 174

AlARC1  RDRFYSFLDGFEEKGQIPNSEELRSFFVEKLEISDPKSCRDEIEFLLEEQIVNHDCLDLEPT 238
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BnARC1  RSVINGFIDITRYVMFLFKIEDG-----NEIKKQRKRLISEEIENTETTLPKDFICS 287

AlARC1  ISLSLMKDPVIVSTGQTYDRSSIVRWF-EEGHSTCPKTGQKLVDS SCIVANLALRNLITR 356
BnARC1  ISLNLMDPVIISTGQTYDRISIARWIHQEGRSTCPKTGQKLVDS-FVSNLALRHLITL 346

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BnARC1  TVTEIRLIVETGAIPYLRSLKSNVAQENAVASIFNLSIDEANRSLIVEEHDCLEPI 462

AlARC1  VSVLISGLTLEAQNAAATLYSLSTVHEYKRIANVDGCIKSLASISRNCKPRGKKDALN 535
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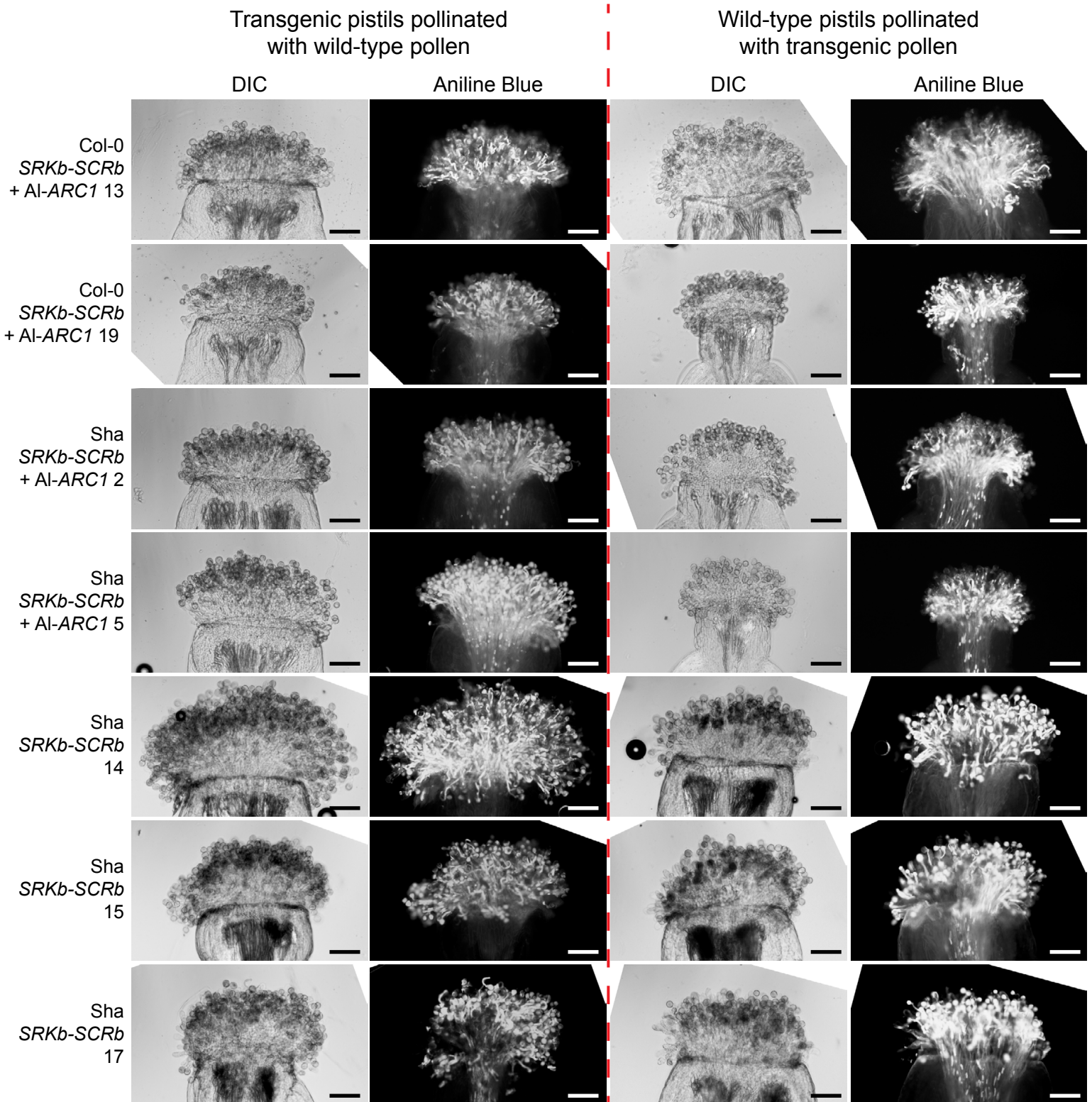
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AlARC1  SAVVAGLIELMRCGTPRGKENAVATLLHLCINGGTVVVEKVVVPALSDLTOKLLLTGTNR 655
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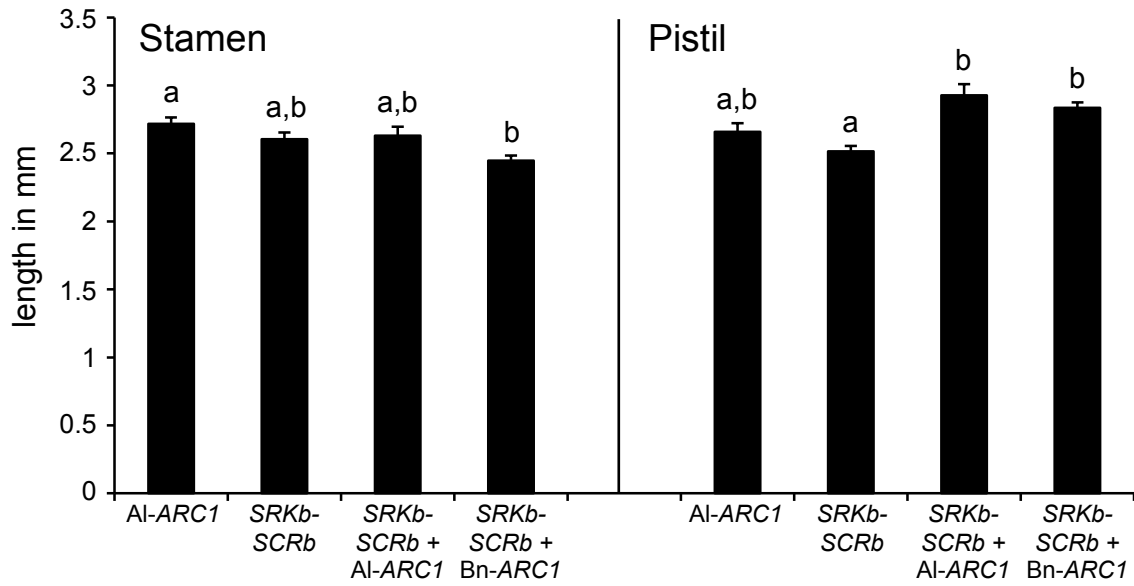
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BnARC1  AKRKAVS-LSKVCKGCQNTQR----- 661

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Supplemental Figure 1. Alignment of *A. lyrata* ARC1 and *B. napus* ARC1 amino acid sequences. The alignment was performed with Clustal W2 with a slow pairwise alignment. The protein weight matrix was PAM, the gap open was set to 10 and the gap extension to 0.1.



Supplemental Figure 2. Pollen grain attachment and pollen tube growth in reciprocal crosses between the transgenic *A. thaliana* Col-0 and Sha lines with wildtype Col-0 and Sha plants, respectively. Functional compatible pollen responses in the transgenic Col-0 and Sha lines were confirmed by reciprocal pollinations with wildtype plants. Following pollinations, pistils were stained with aniline blue to visualize the pollen tubes. All transgenic lines showed fully compatible responses with abundant pollen grain adhesion (DIC images) and pollen tube growth (aniline blue images) in these reciprocal crosses. Scale bars = 50 μ m.



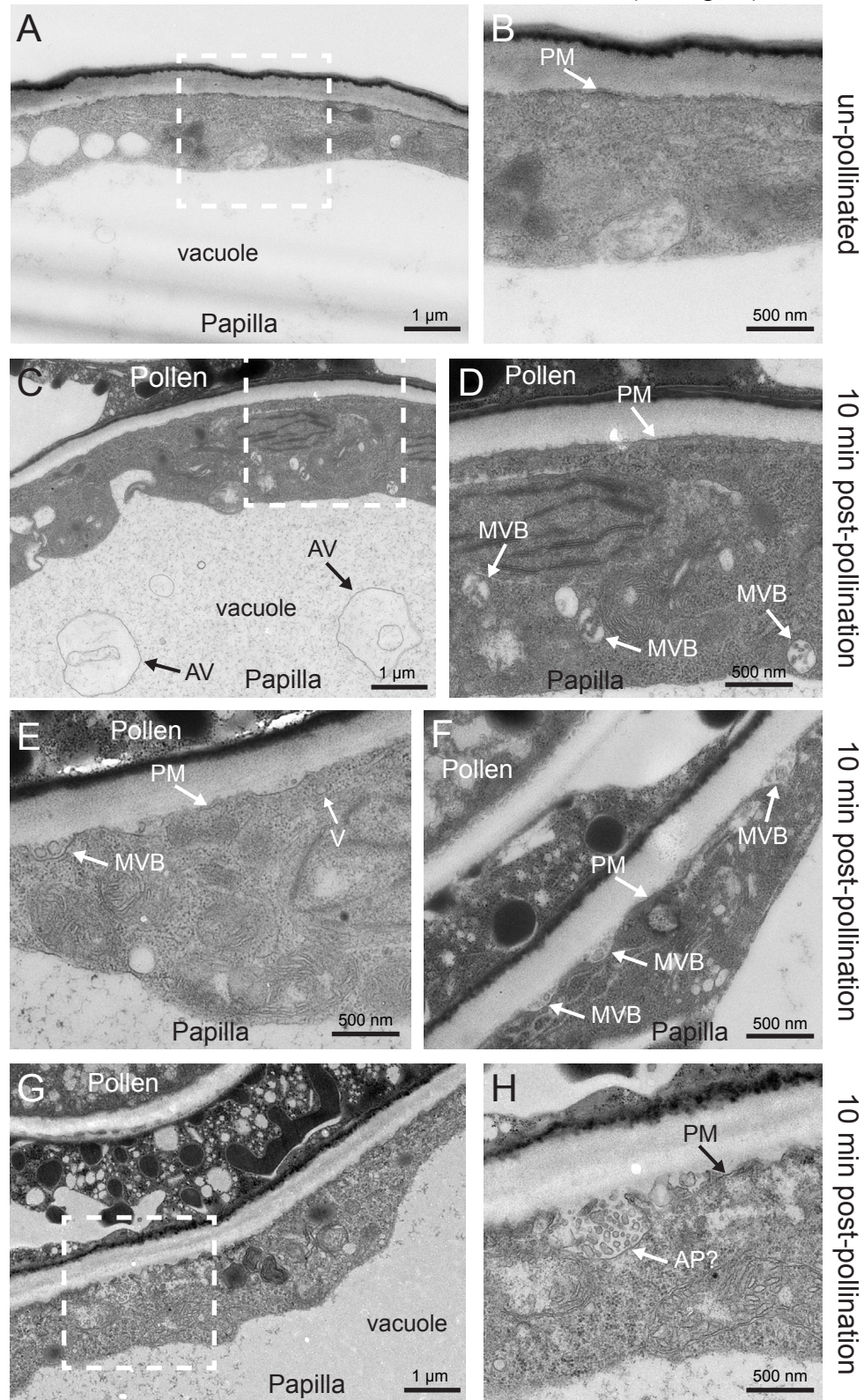
Supplemental Figure 3. The approach herkogamy phenotype arises from increased pistil length in the *A. thaliana* *SCRb-SRKb + ARC1* transgenic flowers.

The mean length of stamens and pistils was determined for each transgene combination. When *SCRb-SRKb + ARC1* are expressed, the stamens lengths are not significantly different from the stamen lengths in the *SCRb-SRKb* transgenic flowers. In contrast, the pistils are significantly longer in the *SCRb-SRKb + ARC1* transgenic flowers when compared to pistil lengths in the *SCRb-SRKb* transgenic flowers.

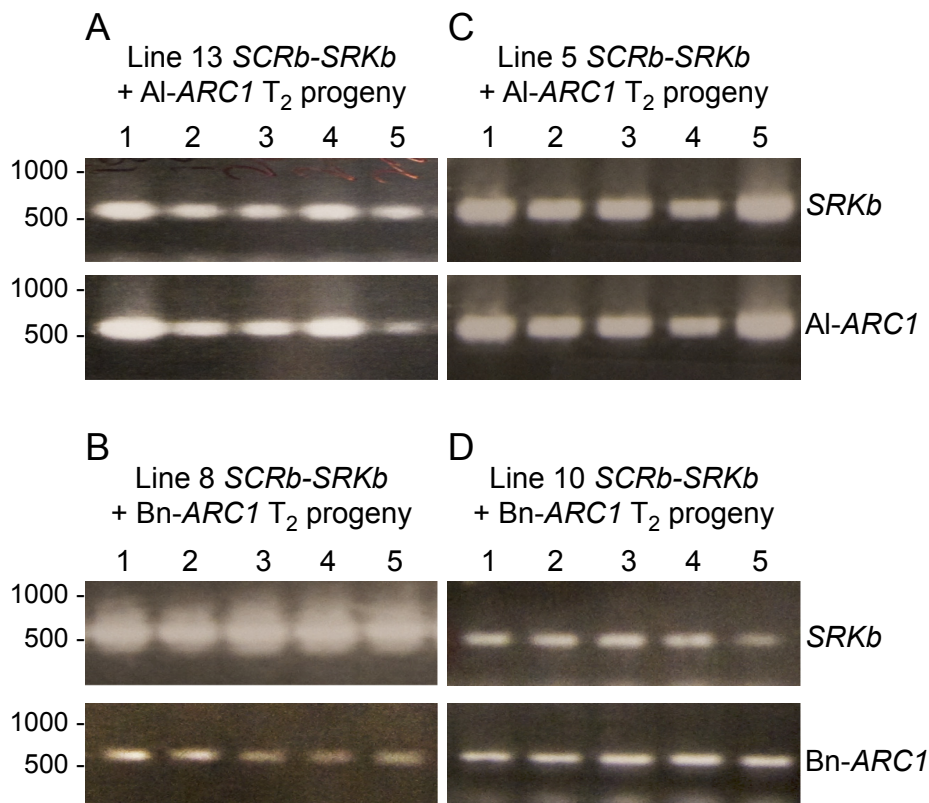
Error bars indicate SE. The different letters represent means that are significantly different at $P < 0.01$ (one-way ANOVA with Tukey-HSD post-hoc tests).

Al-ARC1 n=18 flowers (lines 11 & 16); *SCRb-SRKb* n=27 flowers (lines 10, 9, 14); *SCRb-SRKb+Al-ARC1* n=18 flowers (lines 18, 1); *SCRb-SRKb+Bn-ARC1* n=15 flowers (lines 14, 10).

A. thaliana Col-0 *SCRb-SRKB* + *Bn-ARC1* 8 (strong SI)



Supplemental Figure 4. TEM images of *A. thaliana* Col-0 *SCRb-SRKB* + *Bn-ARC1* line #8 stigmatic papillae in response to self-pollen. **(A)** and **(B)** Unpollinated stigmatic papilla. **(C)** to **(H)** Stigmatic papillae at 10 min post-pollination. Several different events were observed such as autophagic vacuoles (AV) in the vacuole as shown in **(C)**, multivesicular bodies (MVB) in the cytoplasm as shown in **(D)**, vesicles (V) in the cytoplasm as shown in **(E)**, MVBs fusing to the plasma membrane as shown in **(E, F)**, and an autophagosome-like structure (AP?) fusing to the plasma membrane (PM) as shown in **(G, H)**. The white boxed areas in **(A, C, G)** are shown in the **(B, D, H)**, respectively. Scale bars **(A, C, G)** 1 μm; **(B, D, E, F, H)** 500 nm.



Supplemental Figure 5. Genotypes of self-incompatible transgenic T₂ progeny.

(A, B) *A. thaliana* Col-0 transgenic T₂ plants.

(C, D) *A. thaliana* Sha transgenic T₂ plants.

Self-incompatible transgenic T₂ plants were confirmed to carry both the *SCRb-SRKb* and *ARC1* constructs by PCR genotyping. Data are shown for five different self-incompatible transgenic T₂ plants for each line. The product size for each primer pair is as follows: *SRKb*, 567 bp; *AI-ARC1*, 507 bp; and *Bn-ARC1*, 567 bp. As the *SCRb* and *SRKb* are in the same construct, *SRKb* was used for genotyping for the presence of both transgenes. Primers specific to each transgene were used and are listed in Supplemental Table 2.

Supplemental Table 1. Cellular responses in transgenic *A. thaliana* plants at 10 min post-pollination.

Transgenic Line	Vesicles at PM	Compressed cytoplasm	EXPO-like at PM	# of samples*		Autophagosome /MVBs in cytoplasm	Debris in vacuole	Autophagic organelles in vacuole	Vesicles in vacuole
				Autophagosome /MVBs at PM	Vesicles in cytoplasm				
<i>A. thaliana</i> Col-0 <i>SCRb-SRKb</i> + <i>Bn-ARC1-8</i> strong self-incompatible	0	0	0	5	9	4	9	2	6

*n=10 (where 2 stigmatic papillae per stigma for 5 stigmas were examined).

Supplemental Table 2. Primers used for PCR cloning and analyses.

Primer	Sequence
Al-ARC1 cloning 5' 1.12 kb forward	5' -ATGGTCACCGAGGC-3'
Al-ARC1 cloning 5' 1.12 kb reverse	5' -GAAGCAGGAGACTCGTTTGG-3'
Al-ARC1 cloning 3' 1.38 kb forward	5' -TTAACGGGTTTCGTGGCTATC-3'
Al-ARC1 cloning 3' 1.38 kb reverse	5' -CACAAAACAGATACAGGTATAG-3'
Al-ARC1 cloning full length forward	5' -CCCGGGATGGTCACCGAGGC-3'
Al-ARC1 cloning full length reverse	5' -GAATTCTCACAAAACAGATACAGGTATAG-3'
Bn-ARC1 cloning full length forward	5' -CCCGGGATGGCCACTGATTCAGCA-3'
Bn-ARC1 cloning full length reverse	5' -CCCGGGTTATCTCTGTGTGTTCTG-3'
<i>Elf1α</i> qRT-forward	5' -TTCCTCCGTTATCACCAGCG-3'
<i>Elf1α</i> qRT-reverse	5' -GGTCTGCCTCATGTCCCTAA-3'
<i>TUB4</i> qRT-forward	5' -AACGCTGACGAGTGATGGTT-3'
<i>TUB4</i> qRT-reverse	5' -CCAAAGGTAGGATTAGCGAGC-3'
Al-ARC1 qRT-forward	5' -GTGGTGGAGAAGGTGGTGAG-3'
Al-ARC1 qRT- reverse	5' -GCTTAGCCCGATTTGTACCC-3'
Bn-ARC1 qRT-forward	5' -AAAGCCCCTCTCTTTCAAGC-3'
Bn-ARC1 qRT-reverse	5' -TCAACGTAGTGCTGCATGTG-3'
Al-SRKb qRT-forward	5' -GCGTTTGGAGAAGGAAACAG-3'
Al-SRKb qRT-reverse	5' -TTTCCAGGCGATTCATTAGC-3'
Al-SCRb qRT-forward	5' -GGAGAGGAGACGTGCAAAAA-3'
Al-SCRb qRT-reverse	5' -TCGCATAAACGTGCAAAATC-3'
Al-SRK1 qRT-forward	5' -GGTACAGGTTGTGTGATTTGG-3'
Al-SRK1 qRT-reverse	5' -TTCCTCCGTTATCACCAGCG-3'
Al-ARC1 genotyping forward	5' -CAAATGTAGATGGCTGCATCA-3'
Al-ARC1 genotyping reverse	5' -ACATCATCGCTGTGTTTTTCG-3'
Bn-ARC1 genotyping forward	5' -TTCGAGAACGGGAGTGTACC-3'
Bn-ARC1 genotyping reverse	5' -TTCCGTCGAAGCTCTTGTTT-3'
Al-SRKb genotyping forward	5' -GTGGTGGCAGAGCTTCTTTC-3'
Al-SRKb genotyping reverse	5' -ACAAATCGGTGACGTGTTCA-3'