

Patterns of evolution at the gametophytic self-incompatibility *Sorbus aucuparia* (Pyrinae) S-pollen genes support the non-self recognition by multiple factors model

Bruno Aguiar, Jorge Vieira, Ana Estefânia Cunha, Nuno A. Fonseca, David Reboiro-Jato , Miguel Reboiro-Jato, Florentino Fdez-Riverola, Olivier Raspé, and Cristina P. Vieira

Supp. Table S1. Primers used in this work

Forward		Reverse		Annealing temperature (°C)	Size of the expected amplification product (bp)
<i>S-RNase</i>					
SorbusRNaseF	AAGTTGTTTACGGTTCAC	SorbusRNaseR	TATTCTTTTGGCACTTGA	48	variable (larger than 409)
S-RNaseFT-F *	TTTACGCAGCAATATCAG	S-RNase(I/T)W- R *	ACRTTYGGCCAAATARTT	48	variable (larger than 213)
MaCiF1+	ATTWTC AATTTACGCAGCAAT AATCAG	Mac2/3R1+	GBACGTTYGGCCAAATAATTNYC	50	variable (larger than 219)
S2-RNaseF	GGACCTCACCCAGAAAAT	S2-RNaseR	GTTCAATCTTCGCCTTTG	51	450
S10-RNaseF	CTCTAATGGACCTGACCC	S10-RNaseF	GCGTATGGCACTTTCAAT	51	1050
S17-RNaseF	TTCAGTTCACGGTTTG	S17-RNaseF	CGTTTTGTTTCTGGGTTT	50	550
S20-RNaseF	TGGGAAACACAGTGGGAA	S20-RNaseF	TTGGCTTTGGATAGGTAA	47	200
<i>SFBBs</i>					
SFBBgenF	AAGTCYCTGATGMGR TTC	SFBBgenR	GTCCATTACCCAYRTYTC	48	870 to 889
SFBB1F	ATGAACAACAAACTATCG	SFBB1R	TAAATCAAATGAAAGTAA	50	642
SFBB2F	CCACTTCTATCCTTCTCC	SFBB2-1R	CATTACCCATATTTCAAG	49	767

		SFBB2-2R	TAATGAGAGCAAAAAGAA	48	705
SFBB3F	TTCTCAACCGTTCTCAGT	SFBB3R	AATCAAATGAAAGTATGC	52	588
SFBB5F	GTGAAACTCCTGAAGATC	SFBB5R	CCTAGTGTCAATGGATAA	49	1015
SFBB6F	TTCTCATCCTTCACTTGC	SFBB6R	AACCTCGTCACATTTCCA	50	879
SFBB7F	CATCAATAGTCCAAGTTT	SFBB7-1R	AAGCAATAYGAAGTGACA	50	789
		SFBB7-2R	AACCAGAATAGGGAATGC	50	614
SFBB8F	CTCTTATCAATAGTCCAT	SFBB8R	AAGGTCCAGCAGTTAGGT	48	897
SFBB9F	TGTATCCTTCTCAACCGT	SFBB9R	CTCGCCATCGTTTGCAAG	52	579
SFBB11F	CTTCTACCTTCCCCwCCT	SFBB11R	ATAACAAAATCCCTTCAT	52	279
SFBB13F	ATTCAGGCAACTTCCCC	SFBB13R	TTAGAAAACCTCAAATCC	52	417
SFBB14 F	CAGGGAAAACCTGTTATTA	SFBB14R	AAGAAGTGATGGATTCAT	52	489
SFBB15F	AGACCATCATCCTGTAGT	SFBB15R	GACAAGAATCAAGATAGC	52	371
SFBB5Belg6S2F	GTGAAACTCCTGAAGATC	SFBB5Belg6S2R	TAACGAGAGCAATAAGAG	52	893
SFBB5Belg5S20F	TGGACAGCAAACCTCTCAT	SFBB5Belg5S20R	CTCATCACCTAAATCAAT	55	650
SFBB6Belg6S2F	GAGCGTATTCTTCTTCCC	SFBB6Belg6S2R	AACCTCGTCACATTTCCA	56	438
SFBB8Belg6S2F	GTGGACAACAAACTCTCA	SFBB8Belg6S2R	AATAATGTGGAAGGCTCA	49	779
SFBB8Belg5S17F	TGTATCCTTCTCAACTGC	SFBB8Belg5S17R	TCCGTCATCATAGTCCAT	48	783
SFBB8Belg5S20F	GCAACTTCTGATTCATA	SFBB8Belg5S20R	TTAGGTGTTTTGTCCATA	48	560

* according to Kim *et al.* 2009

+ according to Carrera *et al.* 2009

Supp. Table S2. RFLP's used to identify *SFBB1*, *SFBB2*, *SFBB3*, *SFBB5*, *SFBB7*, *SFBB8*, *SFBB9*, *SFBB11*, *SFBB13*, and *SFBB14* genes of the *S22* and *Sa*-haplotypes

Gene	Primers	Enzyme	Haplotype			
			<i>S22</i>	<i>Sa</i>	<i>Sc</i>	
<i>SFBB1</i>	SFBB1F+SFBB1R	<i>S22</i>	<i>Sau</i> 3AI	115;168; <u>359</u>	115;126;168;233	n.a.
		<i>Sa</i>	<i>Hind</i> III	642	86; <u>556</u>	n.a
<i>SFBB2</i>	SFBB2F+SFBB2-1R	<i>S22</i>	<i>Aci</i> I	115; <u>652</u>	767	767
		<i>Sa</i>	<i>Bcc</i> I	767	68; <u>263</u> ; <u>436</u>	732
<i>SFBB3</i>	SFBB3F+SFBB3R	<i>S22</i>	<i>Taq</i> I	<u>78</u> ;217;293	293;295	293;295
		<i>Sa</i>	<i>Rsa</i> I	63;114;160;251	28;63; <u>223</u> ; <u>274</u>	63;114;160;251
<i>SFBB5</i>	SFBB5F+SFBB5R	<i>S22</i>	<i>Ple</i> I	145;870	1015	915
		<i>Sa</i>	<i>Hpy</i> CH4IV	226;789	226; <u>297</u> ; <u>492</u>	226;789
<i>SFBB7</i>	SFBB7F+SFBB7R	<i>S22</i>	<i>Ple</i> I	202; <u>567</u>	789	202;615 (n.a)
		<i>Sa</i>	<i>Bsr</i> I	789	<u>316</u> ; <u>473</u>	789
<i>SFBB8</i>	SFBB8F+SFBB8R	<i>S22</i>	<i>Ava</i> II	6; <u>194</u> ;697	6;52;142;697	6;52;142;697
		<i>Sa</i>	<i>Alu</i> I+ <i>Mbo</i> I	30;334;515	30;204; <u>311</u> ;334	30;54;204;255;334
<i>SFBB9</i>	SFBB9F+SFBB9R	<i>S22</i>	<i>Mse</i> I	122; <u>457</u>	88;122;369	88;122;369

		<i>Sa</i>	<i>AluI+Sau3AI</i>	89;132;358	<u>257</u> ;322	36;221;322
<i>SFBB11</i>	SFBB11F+SFBB11R	<i>S22</i>	<i>DdeI</i>	17;63; <u>93</u> ;106	17;106;156	n.a
		<i>Sa</i>	<i>HpyCH4IV</i>	279	<u>139</u> ; <u>140</u>	279
<i>SFBB13</i>	SFBB13F+SFBB13R	<i>S22</i>	<i>RsaI</i>	49; <u>81</u> ;287	130;287	130;287
		<i>Sa</i>	<i>NlaIII</i>	200; <u>217</u>	24;193;200	24;193;200
<i>SFBB14</i>	SFBB14F+SFBB14R	<i>S22</i>	<i>MwoI</i>	225; <u>264</u>	489	n.a.
		<i>Sa</i>	<i>HindIII</i>	489	<u>110</u> ; <u>379</u>	489

Supp. Table S3. Patterns used on the identification of alleles from *SFBB1*, *SFBB2*, *SFBB3*, *SFBB5*, *SFBB6*, *SFBB7*, *SFBB8*, *SFBB9*, *SFBB11*, *SFBB13*, and *SFBB14* genes in the segregation experiment.

Gene	Characterized alleles with specific primers	Primers	Restriction enzymes	Belgium5S17	Belgium5S20	Belgium6S2	Belgium6S10
<i>SFBB1</i>	<i>S2-SFBB1</i> * <i>S17-SFBB1</i> <i>S20-SFBB1</i>	SFBB1F + SFBB1R	<i>Nla</i> III	52;58;75;129;328	52;58;75;129;328	52;58;129; <u>403</u>	n.a.
<i>SFBB2</i>	<i>S10-SFBB2</i> * <i>S20-SFBB2</i> *	SFBB2F + SFBB2-2R	<i>Dde</i> I	n.a	42;300; <u>363</u>	n.a	<u>159;246;300</u>
<i>SFBB3</i>	<i>S2-SFBB3</i>	SFBB3F + SFBB3R	<i>Bse</i> XI	<u>145;443</u>	588	588	588
	<i>S10-SFBB3</i>		<i>Mae</i> III	588	<u>247;341</u>	588	588
	<i>S17-SFBB3</i> *						
<i>SFBB5</i>	<i>S20-SFBB3</i> *						
	<i>S2-SFBB5</i> * <i>S10-SFBB5</i> <i>S17-SFBB5</i>	SFBB5Belg6S2F+SFBB5Belg6S2F		n.a	n.a	893	n.a
	<i>S20-SFBB5</i> *	SFBB5Belg5S20F+SFBB5Belg5S20R		n.a	650	n.a	n.a
<i>SFBB6</i>	<i>S2-SFBB6</i> * <i>S10-SFBB6</i>	SFBB6Belg6S2F+ SFBB6Belg6S2R		n.a	n.a	438	n.a
	<i>S17-SFBB6</i> *	SFBB6F + SFBBgenR	<i>Ava</i> I	780	<u>304;476</u>	780	780

	<i>S20-SFBB6</i>						
<i>SFBB7</i>	<i>S2-SFBB7</i> ,	SFBB7F + SFBB7-1R	<i>DdeI</i>	<u>264;525</u>	<u>35;264;490</u>	n.a	n.a.
	<i>S10-SFBB7*</i>	SFBB7F + SFBB7-2R	<i>Sau3AI</i>	52;227;335	52;227;335	n.a	<u>52;161;174;227</u>
	<i>S17-SFBB7*</i>						
	<i>S20-SFBB7*</i>						
<i>SFBB8</i>	<i>S2-SFBB8*</i>	SFBB8Belg6S2F+SFBB8Belg6S2R		n.a	n.a	779	n.a
	<i>S10-SFBB8</i>			-	-	-	-
	<i>S17-SFBB8*</i>	SFBB8Belg5S17F+SFBB8Belg5S17R		783	n.a	n.a	n.a
	<i>S20-SFBB8*</i>	SFBB8Belg5S20F+SFBB8Belg5S20R		n-a	560	n.a	n.a
<i>SFBB9</i>	<i>S2-SFBB9*</i>	SFBB9F + SFBB9R	<i>Sau3AI</i>	<u>257;322</u>	<u>579</u>	89; <u>168;322</u>	89; <u>490</u>
	<i>S10-SFBB9*</i>						
	<i>S17-SFBB9*</i>						
	<i>S20-SFBB9*</i>						
<i>SFBB11</i>	<i>S10-SFBB11*</i>	SFBB11F + SFBB11R	<i>AciI</i>	<u>93;186</u>	279	n.a	279
	<i>S17-SFBB11*</i>		<i>RsaI</i>	47;232	47; <u>88;144</u>	n.a	47;232
	<i>S20-SFBB11*</i>						
<i>SFBB13</i>	<i>S2-SFBB13*</i>	SFBB13F + SFBB13R	<i>NcoI</i>	<u>417</u>	n.a	<u>204;213</u>	n.a
	<i>S10-SFBB13*</i>		<i>PvuI</i>	<u>200;217</u>	n.a	<u>417</u>	n.a
<i>SFBB14</i>	<i>S2-SFBB14*</i>	SFBB14F + SFBB14R	<i>ApoI</i>	42;51;57; <u>339</u>	42;93;152; <u>244</u>	42;51;396	42;51;396
	<i>S10-SFBB14*</i>		<i>MfeI</i>	72;99;318	72; <u>417</u>	72;99;318	99; <u>388</u>

*S17-SFBB14**

NlaIII

489

67;422

67;422

67;422

*S20-SFBB14**

* alleles that could be followed in the segregation experiment

Supp. Table S4. Polymorphism at the amino acid sites under positive selection between *S22* and *Sa*-haplotypes

Gene		25	42	47	52	89	91	147	149	179	197	212	220	236	266
<i>SFBB1</i>	<i>S22</i>	M	N	D	P	F	E	I	T	E	T	E	C	T	Q
	<i>Sa</i>
<i>SFBB2</i>	<i>S22</i>	V	M	D	R	H	S	T	Q	M	T	D	N	S	L
	<i>Sa</i>
<i>SFBB3</i>	<i>S22</i>	L	F	D	R	P	Q	T	H	E	T	S	P	T	N
	<i>Sa</i>	E	T	.	.	E
<i>SFBB5</i>	<i>S22</i>	I	V	D	Q	N	E	I	K	E	M	D	P	C	K
	<i>Sa</i>
<i>SFBB6</i>	<i>S22</i>	V	V	D	R	N	K	L	K	K	T	D	P	N	N
	<i>Sa</i>
<i>SFBB7</i>	<i>S22</i>	M	V	D	Q	N	E	T	K	R	P	E	P	Y	E
	<i>Sa</i>	D	.	.	G	T	.	.	.	K
<i>SFBB8</i>	<i>S22</i>	V	A	E	Q	E	E	T	K	G	M	K	P	C	K
	<i>Sa</i>
<i>SFBB9</i>	<i>S22</i>	V	M	D	Y	P	Q	T	E	E	T	K	Q	N	K
	<i>Sa</i>
<i>SFBB11</i>	<i>S22</i>	V	A	D	Q	F	L	S	Q	E	A	T	S	T	R
	<i>Sa</i>	N	.	.	T
<i>SFBB13</i>	<i>S22</i>	M	A	D	P	F	Q	I	G	Q	M	E	H	I	E
	<i>Sa</i>
<i>SFBB14</i>	<i>S22</i>	I	M	D	Y	P	Q	I	G	E	I	K	P	S	K
	<i>Sa</i>	S	.	.	.	E	.	.	.