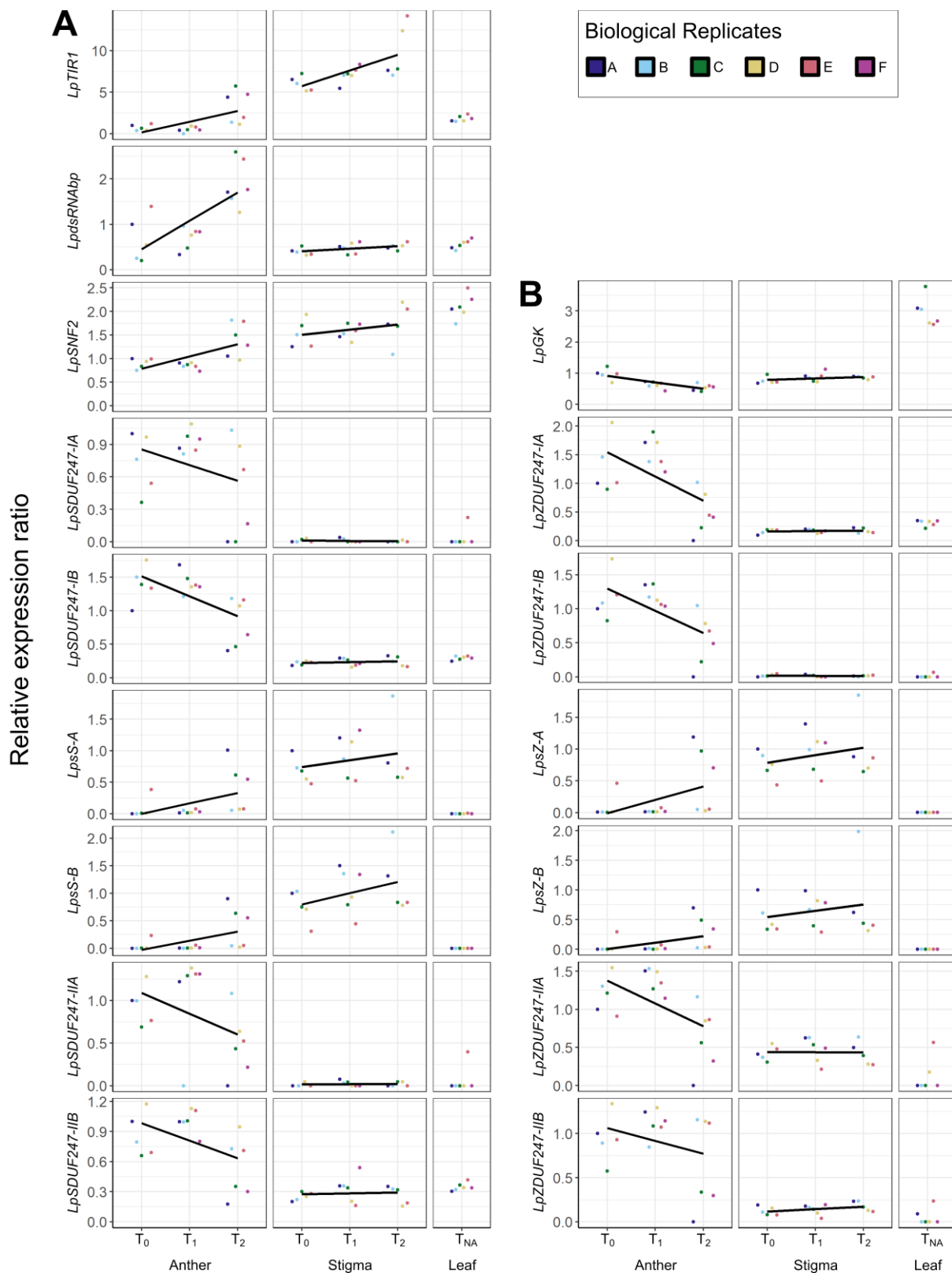


Supplementary material

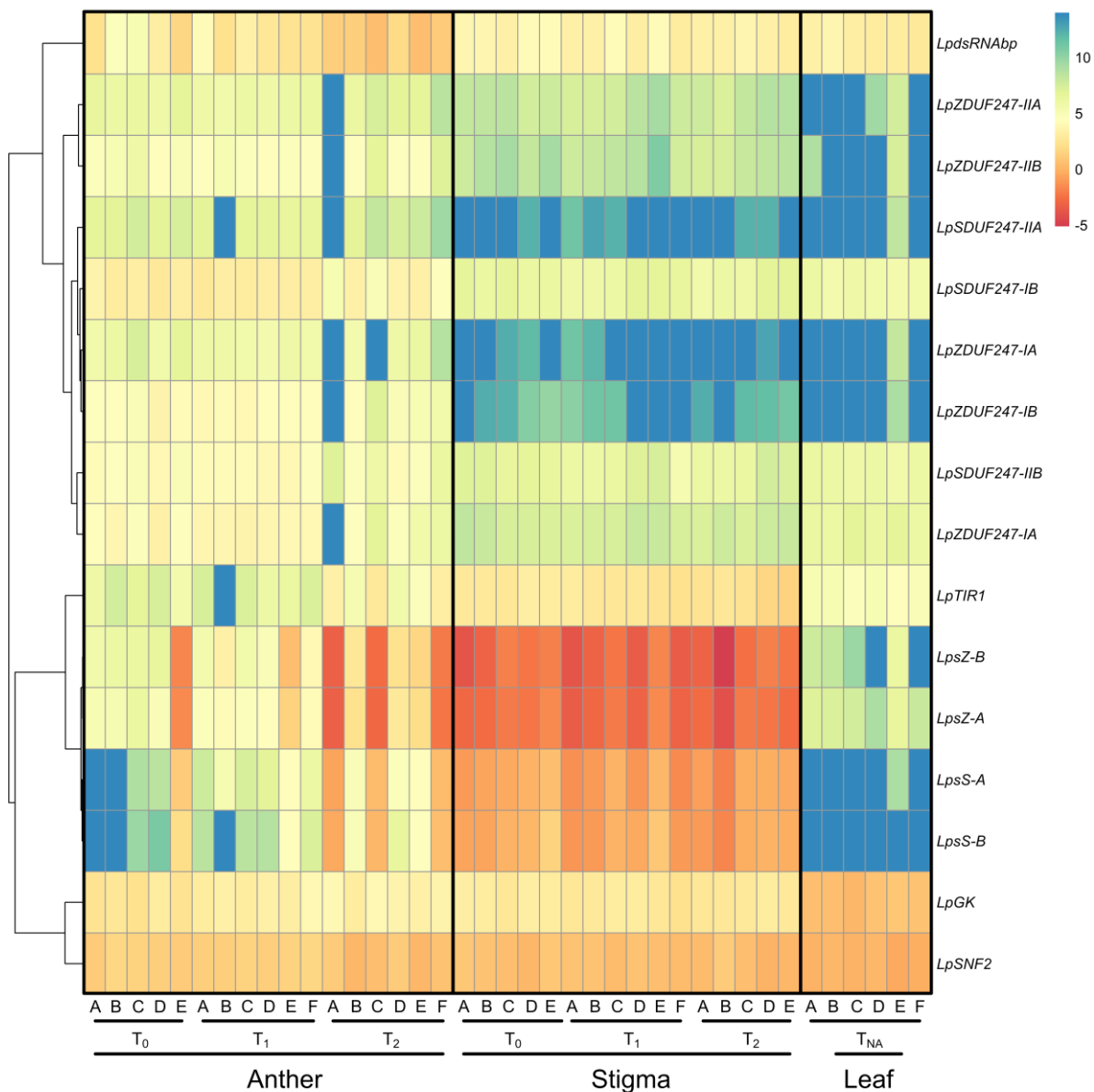
A			SDUF247-I						SDUF247-II						ZDUF247-I						ZDUF247-II							
Species and genotype	Localization	Protein name	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.
1. <i>L. perenne</i> Kyuss	chr1	SDUF247-I	100	80.72	78.81	81.85	78.81	77.36	43.02	42.77	41.01	43.53	41.01	42.26	45.69	43.46	42.88	43.46	42.91	44.38	42.52	41.38	41.84	41.15	41.84	38.96	41.17	40.73
2. <i>L. perenne</i> P226/135/16	chr1		80.72	100	77.23	81.36	77.23	79.85	42.75	42.5	41.31	44.23	41.31	42.94	46.97	43.19	43.57	43.19	42.64	44.29	42.64	40.54	41.57	41.35	41.57	38.12	39.15	40.66
3. <i>L. perenne</i> S23 Z	contig11029		78.81	77.23	100	78.02	100	82.61	43.5	43.43	42.6	43.9	42.6	44.49	43.82	42.38	42.97	42.38	43.4	43.71	42.54	41.78	42.25	41.65	42.25	40.04	41.33	42
4. <i>L. perenne</i> S23 Z	contig12948		81.85	81.36	78.02	100	78.02	79.1	44.1	44.23	42.47	45.4	42.47	44.08	46.77	44.72	44.15	44.72	43.6	44.68	43.8	41.49	42.72	40.96	42.72	38.89	41.28	41.23
5. <i>L. multiflorum</i>	scf1212		78.81	77.23	100	78.02	100	82.61	43.5	43.43	42.6	43.9	42.6	44.49	43.82	42.38	42.97	42.38	43.4	43.71	42.54	41.78	42.25	41.65	42.25	40.04	41.33	42
6. <i>L. multiflorum</i>	scf818		77.36	79.85	82.61	79.1	82.61	100	42.08	42.42	40.85	43.16	40.85	42.48	45.31	43.3	43.49	43.3	41.59	42.47	42.17	39.31	40.15	39.35	40.15	38.05	40.04	39.62
7. <i>L. perenne</i> Kyuss	chr1	SDUF247-II	43.02	42.75	43.5	44.1	43.5	42.08	100	76.18	82.18	75.85	82.18	72.45	46.84	43.41	42.83	43.41	45.42	45.61	46.86	38.54	42.69	42.47	42.69	41.15	40.96	41.36
8. <i>L. perenne</i> P226/135/16	chr1		42.77	42.5	43.43	44.23	43.43	42.42	76.18	100	74.59	76.23	74.59	72.5	47.64	44.51	44.89	44.51	46.71	46.71	46.98	40.04	43.68	42.12	43.68	41.49	41.11	41.31
9. <i>L. perenne</i> S23 Z	contig11029		41.01	41.31	42.6	42.47	42.6	40.85	82.18	74.59	100	74.91	100	71.58	47.04	43.13	42.55	43.13	46.11	45.72	46.97	37.5	41.92	40.73	41.92	42.03	42.03	41.67
10. <i>L. perenne</i> S23 Z	contig11774		43.53	44.23	43.9	45.4	43.9	43.16	75.85	76.23	74.91	100	74.91	72.97	47.52	44.81	42.86	44.81	45.67	46.26	47.14	40.08	43.77	42.38	43.77	42.41	42.02	41.76
11. <i>L. multiflorum</i>	scf1212		41.01	41.31	42.6	42.47	42.6	40.85	82.18	74.59	100	74.91	100	71.58	47.04	43.13	42.55	43.13	46.11	45.72	46.97	37.5	41.92	40.73	41.92	42.03	42.03	41.67
12. <i>L. multiflorum</i>	scf818		42.26	42.94	44.49	44.08	44.49	42.48	72.45	72.5	71.58	72.97	71.58	100	47.08	42.48	42.48	42.48	45.4	46.55	45.66	39.28	42.61	40.87	42.61	40.72	42.45	41.49
13. <i>L. perenne</i> Kyuss	chr2	ZDUF247-I	45.69	46.97	43.82	46.77	43.82	45.31	46.84	47.64	47.04	47.52	47.04	47.08	100	58.22	57.84	58.22	57.25	58.75	59.36	45.16	47.1	48.16	47.1	46.36	45.29	45.02
14. <i>L. perenne</i> F1-30	P205		43.46	43.19	42.38	44.72	42.38	43.3	43.41	44.51	43.13	44.81	43.13	42.48	58.22	100	73.62	100	55.7	56.24	58.61	42.3	43.29	43.73	43.29	43.71	42.42	43.9
15. <i>L. perenne</i> P226/135/16	chr2		42.88	43.57	42.97	44.15	42.97	43.49	42.83	44.89	42.55	42.86	42.55	42.48	57.84	73.62	100	73.62	54.02	54.38	57.68	43.41	43.1	43.54	43.1	41.09	41.65	44.09
16. <i>L. perenne</i> S23 Z	contig55097		43.46	43.19	42.38	44.72	42.38	43.3	43.41	44.51	43.13	44.81	43.13	42.48	58.22	100	73.62	100	55.7	56.24	58.61	42.3	43.29	43.73	43.29	43.71	42.42	43.9
17. <i>L. perenne</i> S23 Z	contig7728		42.91	42.64	43.4	43.6	43.4	41.59	45.42	46.71	46.11	45.67	46.11	45.4	57.25	55.7	54.02	55.7	100	79	57.47	42.29	44.57	44.25	44.57	44.4	43.13	43.07
18. <i>L. multiflorum</i>	scf1905		44.38	44.29	43.71	44.68	43.71	42.47	45.61	46.71	45.72	46.26	45.72	46.55	58.75	56.24	54.38	56.24	79	100	58.76	42.51	45.44	44.36	45.44	43.86	43.71	44.61
19. <i>L. multiflorum</i>	scf3448	42.52	42.64	42.54	43.8	42.54	42.17	46.86	46.98	46.97	47.14	46.97	45.66	59.36	58.61	57.68	58.61	57.47	58.76	100	42.29	42.53	44.32	42.53	42.69	43.5	43.07	
20. <i>L. perenne</i> Kyuss	chr2	ZDUF247-II	41.38	40.54	41.78	41.49	41.78	39.31	38.54	40.04	37.5	40.08	37.5	39.28	45.16	42.3	43.41	42.3	42.29	42.51	42.29	100	49.43	50.47	49.43	50.28	50.47	51.39
21. <i>L. perenne</i> F1-30	P205		41.84	41.57	42.25	42.72	42.25	40.15	42.69	43.68	41.92	43.77	41.92	42.61	47.1	43.29	43.1	43.29	44.57	45.44	42.53	49.43	100	67.04	100	50.28	51.06	50.86
22. <i>L. perenne</i> P226/135/16	chr2		41.15	41.35	41.65	40.96	41.65	39.35	42.47	42.12	40.73	42.38	40.73	40.87	48.16	43.73	43.54	43.73	44.25	44.36	44.32	50.47	67.04	100	67.04	49.81	47.97	50.67
23. <i>L. perenne</i> S23 Z	contig55097		41.84	41.57	42.25	42.72	42.25	40.15	42.69	43.68	41.92	43.77	41.92	42.61	47.1	43.29	43.1	43.29	44.57	45.44	42.53	49.43	100	67.04	100	50.28	51.06	50.86
24. <i>L. perenne</i> S23 Z	contig4538		38.96	38.12	40.04	38.89	40.04	38.05	41.15	41.49	42.03	42.41	42.03	40.72	46.36	43.71	41.09	43.71	44.4	43.86	42.69	50.28	50.28	49.81	50.28	100	73.5	49.44
25. <i>L. multiflorum</i>	scf1905		41.17	39.15	41.33	41.28	41.33	40.04	40.96	41.11	42.03	42.02	42.03	42.45	45.29	42.42	41.65	42.42	43.13	43.71	43.5	50.47	51.06	47.97	51.06	73.5	100	48.76
26. <i>L. multiflorum</i>	scf3448	40.73	40.66	42	41.23	42	39.62	41.36	41.31	41.67	41.76	41.67	41.49	45.02	43.9	44.09	43.9	43.07	44.61	43.07	51.39	50.86	50.67	50.86	49.44	48.76	100	

B			sS						sZ						C	
Species and genotype	Localization	Protein name	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	
1. <i>L. perenne</i> Kyuss	chr1	sS	100	52.44	62.65	48.15	62.65	57.32	30.16	30.16	27.94	20	30.16	22.95	24.62	100
2. <i>L. perenne</i> P226/135/16	chr1		52.44	100	53.57	55	53.57	60.71	35.29	30.88	31.51	21.54	30.88	24.62	24.29	90
3. <i>L. perenne</i> S23 Z	contig11029		62.65	53.57	100	39.51	100	51.19	29.23	30.77	26.76	22.58	30.77	24.19	19.4	80
4. <i>L. perenne</i> S23 Z	contig12948		48.15	55	39.51	100	39.51	53.09	30.65	32.26	37.31	33.9	32.26	33.33	32.81	70
5. <i>L. multiflorum</i>	scf1212		62.65	53.57	100	39.51	100	51.19	29.23	30.77	26.76	22.58	30.77	24.19	19.4	60
6. <i>L. multiflorum</i>	scf818		57.32	60.71	51.19	53.09	51.19	100	27.69	29.23	32.86	25.81	29.23	24.19	26.87	50
7. <i>L. perenne</i> Kyuss	chr2	sZ	30.16	35.29	29.23	30.65	29.23	27.69	100	31.08	34.18	35.62	31.08	32.43	29.73	40
8. <i>L. perenne</i> F1-30	P205		30.16	30.88	30.77	32.26	30.77	29.23	31.08	100	53.26	24.39	100	24.39	28.05	30
9. <i>L. perenne</i> P226/135/16	chr2		27.94	31.51	26.76	37.31	26.76	32.86	34.18	53.26	100	28.41	53.26	21.84	34.52	20
10. <i>L. perenne</i> S23 Z	contig4358		20	21.54	22.58	33.9	22.58	25.81	35.62	24.39	28.41	100	24.39	53.75	30.26	
11. <i>L. perenne</i> S23 Z	contig55097		30.16	30.88	30.77	32.26	30.77	29.23	31.08	100	53.26	24.39	100	24.39	28.05	
12. <i>L. multiflorum</i>	scf1905		22.95	24.62	24.19	33.33	24.19	24.19	32.43	24.39	21.84	53.75	24.39	100	26.32	
13. <i>L. multiflorum</i>	scf3448		24.62	24.29	19.4	32.81	19.4	26.87	29.73	28.05	34.52	30.26	28.05	26.32	100	

Supplementary Figure 1: Pairwise comparison of the protein sequences of the male and female self-incompatibility candidates. (A) Pairwise comparison for the SI-DUF247s. (B) Pairwise comparison for the sS and sZ. (C) Color code indicating the protein sequence identity in percentage.



Supplementary Figure 2: Relative expression ratios of *S*- and *Z*-locus genes in *Lolium perenne* L. genotype S23 Z. The relative expression ratios were calculated according to Pfaffl (2001) and normalized to four reference genes (*EF1-alpha*, *CPB20*, *eIF4A-2*, *eIF4A-1*). For *LpsS* and *LpsZ*, the expression value in stigma tissue at time point 0 (T_0) in the first biological replicate was set as 1. For all the other genes displayed, the expression value in anthers at time point 0 (T_0) in the first biological replicate was set to 1. The color scheme in the top right corner is used to distinguish the biological replicates. The relative expression ratios can be seen on the Y-axis and the different sample tissues at different time points on the X-axis. Furthermore, for the *LpsS*, *LpsZ*, and the *SI-DUF247s*, the relative expression ratio measurement was explicitly performed for each allele present in the genotype. The two different alleles are indicated by A and B after the gene name. Only data points were included, where the C_t difference was below 0.5 and the percent deviation was below 3% between the two technical replicates. Therefore, the standard error is not displayed in the figure. (A) Relative expression ratios of *S*-locus genes. (B) Relative expression ratios of *Z*-locus genes.



Supplementary Figure 3: Expression levels of S- and Z-locus genes relative to four reference genes in *Lolium perenne* L. genotype S23 Z. The ΔC_t values were calculated as the C_t value of the gene of interest minus the geometrical mean C_t value of the four reference genes (*EF1-alpha*, *CPB20*, *eIF4A-2*, *eIF4A-1*). The rows indicate the different genes localized within the S and Z loci. For the *LpsS*, *LpsZ* and the *SI-DUF247s*, the ΔC_t was calculated for both alleles present in the genotype. The two different alleles are indicated by A and B after the gene name. The columns indicate the different sample types at different time points. The biological replicates are indicated with A-F. No scaling was applied, and the data were clustered on the level of genes (rows). A dendrogram is drawn on the left, illustrating the hierarchical relationship between the expression pattern of the genes. Blue corresponds to low expression, whereas red corresponds to high expression.

Supplementary Table 1: Number of the recombination events observed between molecular markers at the Z-locus region in the VrnA-XL and DTZ population.

Marker names	Forward primer sequence	Reverse primer sequence	VrnA-XL	DTZ
CADELP	TGCTCTGAGGTTTGGCTAT	ATTGAGTCTTCAACAGCATTTTT	22	48
37600	AAGTGGGACAAAAGGGAACC	ATGAAGTACAAGCAAGAACAATCA	1	0
19060	CCGATTGGTAAAAACGAGTTG	GGCAAAAGGTCAGCTTGCTA	0	NA ^a
15550	TGAGGTGCCTTCTGAAACTG	CGTTAGCTGGTGTGTGGT	0	NA ^a
12000	CCTGGTACAAGGCGGTTTC	CGGCTAACTGATACACCACCT	0	NA ^a
10280	TGCCATAGTGTGGATGGATG	GCTCACTTCCAGTCTCCTGA	0	0
7800	CGTTGATGGTGGTACTG	AACGTAAAGCTAACCACAAACAGA	0	0
6500	TTTGTTTGACGCCCTTAAC	TTCAAACCAGCATCCACATT	0	NA ^a
5000	CGTGCCGGAGTAGATAGAGG	GAGAGTTATAGATGGATCCGAT	0	NA ^a
BAC_BEG	AAGCGACAATGGAAGATTCG	CAGTGGAGTTCTCCCGTTA	1	7
171R	GCGGGCGTTTCCAACATTA	TGAATTGCACGGTAGTCCTG	7	NA ^a
Lp02_555	GTCAGAGCTCGACCCTTC	CCCTTGGTGATCATTGCAC	67	51

Note: The populations have been screened for recombination using twelve markers, mapping within the Z-locus region. The fine-mapped Z-locus is defined by the region without any recombination events. In addition, the primer sequences of the polymorphic molecular markers used for the fine-mapping of the self-incompatibility locus Z are given.

^a Markers were either monomorphic or did not amplify in DTZ

Supplementary Table 2: Primers used to investigate the expression pattern of S- and Z-locus genes using RT-qPCR.

	Gene name	Primer F	Primer R	Amplicon size	Primer efficiency
S-locus genes of interest	<i>LpTIR1</i>	5'-CAATGTTGCTAGCCTGCTTC-3'	5'-AATGCTGGCAGGTAATCTGG-3'	176	1.79
	<i>LpdsRNA</i>	5'-AGAGTCGAAAAAGCCAGCAG-3'	5'-CTTGGTGGAGTTGCATGTC-3'	180	1.76
	<i>LpSNF2</i>	5'-TGACCATAAACTGTCACG-3'	5'-CCCCATCATCTCATCCTAGTG-3'	184	1.81
	<i>LpSDUF247-IA</i>	5'-GAATGCGGTTCCAGAAAAGG-3'	5'-GCAGTGATGACATCCCAAC-3'	181	1.77
	<i>LpSDUF247-IB</i>	5'-TCTGGGCTTGAGAAAAGC-3'	5'-CCTCTCGTCTACCATGTTTTCC-3'	180	1.82
	<i>LpSDUF247-IIA</i>	5'-AGAAACGAGCAAGGATGTGC-3'	5'-CTCGTGATTTCCGGCATGTG-3'	176	1.91
	<i>LpSDUF247-IIB</i>	5'-TGAGGACCATTCCGGCTATTG-3'	5'-TAGACCCTCCAATCTGTTG-3'	177	1.81
	<i>LpsS-A</i>	5'-CGTCATGGTTCTGATGGTTATG-3'	5'-GATGCCCAAATGGTTTTCC-3'	72	1.78
	<i>LpsS-B</i>	5'-CGGTTTCTCCGAATCTAAGG-3'	5'-CCTGTACATGCAAGGTGGTC-3'	80	1.66
Z-locus genes of interest	<i>LpZDUF247-IA</i>	5'-GGTGGCAATTGAGGAACTTG-3'	5'-CTCAAGAGTGCGCATTTAC-3'	184	1.87
	<i>LpZDUF247-IB</i>	5'-GCGCAGAGTTGCACAATATC-3'	5'-CTCGAACGCAATAAGGTTCC-3'	175	1.79
	<i>LpZDUF247-IIA</i>	5'-GCGAGCAGTAGAATACCATCG-3'	5'-GGATCTGTTTGCTCAAGTGC-3'	183	1.77
	<i>LpZDUF247B-IIB</i>	5'-TGTCAGGCTAACCAAAGACG-3'	5'-GATGACCGCAGCAAATACAG-3'	176	1.80
	<i>LpGK</i>	5'-AACTGGTGCCTTCATCCTTC-3'	5'-TAATGATCCCAAGGCTGTCC-3'	179	1.77
	<i>LpsZ-A</i>	5'-TTACCCCAACTGAACAC-3'	5'-AACCAGGCTGTTGGTATGG-3'	145	1.79
	<i>LpsZ-B</i>	5'-AACAAAGTGGTCGTCCTTCTG-3'	5'-GAGAAAGTGAATCCCCATGC-3'	97	1.78
Reference genes	<i>EF1-alpha</i>	5'-TTGACAAGCGTGATCGAG-3'	5'-TGACCAGGAGCATCAATGAC-3'	184	1.81
	<i>eIF4A-2</i>	5'-ATGCATGTGTTGGAGGAACC-3'	5'-CCTTGAACCACGAGAAAGC-3'	183	1.80
	<i>CPB20</i>	5'-ACGCAGGAGGATTCGAG-3'	5'-GGTCTTGGTGTCTTGTGCGAG-3'	155	1.81
	<i>eIF4A-1</i>	5'-GAAAGACTGCCACCTTCTGC-3'	5'-CGAACAGATGTCCACCAAC-3'	181	1.74

Note: The suffixes A and B in the gene name represent the two alleles of a specific gene. The displayed primer efficiencies were calculated using LinReg PCR 7.5 (Ramakers et al. 2003)

Supplementary Table 3: Quality and quantity assessment of the RNA samples used for RT-qPCR.

Tissue Type	Name	Weight [g]	Conc. Qbit [ng/ μ l]	RIN Value
Anther	Anther T ₀ A	0.048	210	6.9
	Anther T ₀ B	0.05	262	7.5
	Anther T ₀ C	0.072	404	8.4
	Anther T ₀ D	0.05	222	8.6
	Anther T ₀ E	0.056	165	7
	Anther T ₁ A	0.059	250	7.6
	Anther T ₁ B	0.053	160	8.4
	Anther T ₁ C	0.051	256	6
	Anther T ₁ D	0.07	166	7.5
	Anther T ₁ E	0.057	41	8.8
	Anther T ₁ F	0.057	97	8.7
	Anther T ₂ A	0.048	32	6.5
	Anther T ₂ B	0.068	70	NA
	Anther T ₂ C	0.074	50	7
	Anther T ₂ D	0.053	60	8
	Anther T ₂ E	0.066	52	8
	Anther T ₂ F	0.066	54	8.4
	Stigma	Stigma T ₀ A	0.051	80
Stigma T ₀ B		0.055	62	9.3
Stigma T ₀ C		0.05	260	7.6
Stigma T ₀ D		0.055	78	9.1
Stigma T ₀ E		0.072	83	8.9
Stigma T ₁ A		0.04	50	7.6
Stigma T ₁ B		0.043	51	7.8
Stigma T ₁ C		0.045	42	5.6
Stigma T ₁ D		0.048	130	8.7
Stigma T ₁ E		0.044	74	9.5
Stigma T ₁ F		0.029	238	8.8
Stigma T ₂ A		0.047	122	9.1
Stigma T ₂ B		0.048	55	9.1
Stigma T ₂ C		0.065	120	9.1
Stigma T ₂ D		0.046	90	8.8
Stigma T ₂ E	0.051	129	9.1	
Leaf	Leaf T _{NA} A	0.041	47	5.2
	Leaf T _{NA} B	0.054	31	4.5
	Leaf T _{NA} C	0.064	33	5
	Leaf T _{NA} D	0.056	117	5.5
	Leaf T _{NA} E	0.047	60	5.6
	Leaf T _{NA} F	0.046	51	5.3