Supplementary Table 1. QTL analysis for UV-absorbance in corolla limb of *P. axillaris* x *P. exserta* F_2 population.

Chromosome	Marker	LRS	CI (cM)	PVE (%)	*p*	Add	Dom
II	PAL1	308.0	3	79	0.00000	-2.61	2.18
II	FLS	289.6	3	77	0.00000	-2.61	2.23
III	MYB109	17.3	53	8	0.00018	-1.46	0.96
IV	MYB60	8.2	66	4	0.01655	0.57	0.79
VI	3-KAT	9.4	58	5	0.00927	-0.08	1.08

The FLS marker is included to show the association with the QTL on chromosome II. FLS is located 1.1 cM from the PAL1 marker (see our website for genetic map). Marker regression statistics for the F_2 cross of *P. axillaris* x *P. exserta* were calculated by the QTX program[75] using Kosambi mapping corrections and a free regression model with no correction for other QTLs. LRS, likelihood ratio statistic for the association of the trait with the locus; CI, confidence interval is an estimate of the 95% confidence interval for a QTL of this strength, using the estimate of ref. 93; PVE, phenotypic variance explained is the amount of the total trait variance which is explained by the QTL at this locus, expressed as a percentage; *p*, *p* value obtained by comparing the LRS to a χ^2 distribution; Add, additive regression coefficient for the association; Dom, dominant regression coefficient for the association.

Supplementary Table 2. Summary of all sectors obtained in transposon tagging experiment that indicated the presence of a transposon insertion in *MYB-FL*, either by displaying a larger PCR product in the PCR screen and/or by showing positive results for the transposon display method

Name of F_1 plant/sector	Paternal parent	PCR screen: approx. size (kb) of band	Analysed by transposon display	*MYB-FL* sequence detected in transposon display	Sanger sequenced	Transposon	Genbank accession	Reference for transposon
C2-6	IL2-1^{Pax}	2.3	Yes	No	Yes	Unidentified	KT962946	-
C1-C8	IL2-1^{Pax}	1.4	Yes	No	No	-	-	-
C2-26	IL2-1^{Pax}	1.4	Yes	No	No	-	-	-
C1-C7	IL2-1^{Pax}	1.9	Yes	Yes	Unsuccessful	-	-	-
A2-66	*P. axillaris*	1.9	Yes	No	Yes	*dTph12*	KT962945	(MV, unpublished data)
C2-60	IL2-1^{Pax}	1.4	Yes	No	Yes	*dTph1*	KT962947	ref. 94
A2-24	*P. axillaris*	1.4	No	-	No	-	-	-
A2-72	*P. axillaris*	none present	Yes	Yes	Yes	*dTph1*	KT962948	ref. 94
A1-95_1	*P. axillaris*	1.9	No	-	Yes	*dTph4*	KT962941	ref. 95
A1-95_2	*P. axillaris*	1.9	Yes	No	Yes	*dTph4*	KT962942	ref. 95
A1-95_3	*P. axillaris*	1.9	Yes	Yes	Yes	*dTph4*	KT962943	ref. 95
A1-95_4	*P. axillaris*	1.9	No	-	Yes	*dTph4*	KT962944	ref. 95

A1-95_1 to A1-95_4 were sectors sampled from four individual flowers from the same branch of a single F_1 individual, A1-95.

Supplementary Table 3. Results of genotyping individuals from the *P. axillaris* x *P. exserta* F_2 population with markers for the *FLS* gene and for the *MYB-FL* gene

Individual	*FLS* genotype	*MYB-FL* genotype
1	H	H
2	A	A
3	B	B
4	B	B
5	A	A
6	H	H
7	H	H
8	H	H
9	H	H
10	B	B
11	H	H
12	H	H
13	H	H
14	B	B
16	B	B
17	A	A
18	A	A
19	H	H
22	B	B
23	B	B
24	B	B
25	H	H
26	H	H
27	H	H
28	B	B
29	B	B
30	H	H
31	H	H
32	H	H
33	H	H
34	A	A
35	B	B
36	H	H
37	H	H
38	B	B
39	H	H
40	A	A
41	A	A
42	H	H
43	H	H
44	H	H
45	H	H
46	B	B
47	H	H
48	H	H
49	A	A
50	H	H
51	A	A
52	H	H
53	H	H
54	B	B
55	H	H
56	A	A
58	H	H
60	A	A
61	H	H
62	H	H
63	B	B
64	H	H
65	H	H
67	B	B

A, *P. axillaris* homozygous; B, *P. exserta* homozygous; H, heterozygous.

Supplementary Table 4. Bioinformatic prediction of *cis*-regulatory elements in the 977 bp insertion in the promoter of *P. axillaris* and *P. exserta MYB-FL* using PLACE (a Database of Cis-regulatory Elements)[96]

Cis-regulatory element	Location	Motif	Sequence in *MYB-FL* promoter	PLACE Reference
ARR1AT	numerous	NGATT		S000454
CAATBOX1	numerous	CAAT	CAAT	S000028
GATABOX	numerous	GATA	GATA	S000039
WRKY71OS	numerous	TGAC	TGAC	S000447
EBOXBNNAPA/	-1148 to -1143	CANNTG	CATTTG	S000114/S00047
MYCCONSENSUSAT	-1106 to -1101		CATCTG	
	-675 to -670		CAACTG	
NAPINMOTIFBN	-1110 to -1104	TACACAT	TACACAT	S000040
ROOTMOTIFTAPOX1	-1072 to –1068	ATATT	ATATT	S000098
	-557 to -553			
GAREAT	-992 to -986	TAACAAR	TAACAAA	S000439
SEBFCONSSTPR10A	-961 to -955	YTGTCWC	TTGTCTC	S000391
ARFAT	-960 to -955	TGTCTC	TGTCTC	S000270
NODCON2GM/	-957 to -953	CTCTT	CTCTT	S000462/S000468
OSE2ROOTNODULE	-683 to -679		CTCTT	
ERELEE4	-877 to -870	AWTTCAAA	AATTCAAA	S000037
POLLEN1LELAT52	-865 to -861	AGAAA	AGAAA	S000245
	-410 to -406		AGAAA	
GT1CONSENSUS/	-864 to -859	GRWAAW/	GAAAAA	S000198/S000453
GT1GMSCAM4	-516 to -511	GAAAAA	GAAAAA	
	-362 to -357		GAAAAA	
INRNTPSADB	-849 to -842	YTCANTYY	TTCAATTT	S000395
	-623 to -616		TTCAATTT	
SEF4MOTIFGM7S	-845 to -839	RTTTTTR	ATTTTTA	S000103
	-641 to -635			
MYBCORE	-743 to -738	CNGTTR	CTGTTG	S000176
GTGANTG10	-732 to -729	GTGA	GTGA	S000378
	-378 to -375		GTGA	
	-350 to -347		GTGA	
WBOXATNPR1	-718 to -714	TTGAC	TTGAC	S000390
SEF3MOTIFGM	-594 to -589	AACCCA	AACCCA	S000115
-10PEHVPSBD	-572 to -567	TATTCT	TATTCT	S000392
ACGTTBOX	-491 to -486	AACGTT	AACGTT	S000132
ACGTATERD1	-490 to -487	ACGT	ACGT	S000415
NTBBF1ARROLB	-435 to -430	ACTTTA	ACTTTA	S000273
S1FBOXSORPS1L21	-329 to -324	ATGGTA	ATGGTA	S000223
TAAAGSTKST1	-323 to -319	TAAAG	TAAAG	S000387
	-311 to -307			
	-265 to -261			
NODCON1GM/	-310 to -305	AAAGAT	AAAGAT	S000461/S000467
OSE1ROOTNODULE				
ANAERO1CONSENSUS	-193 to -187	AAACAAA	AAACAAA	S000477

Cis-regulatory elements were limited to those on the positive strand and only from dicotyledonous plants.

Supplementary Table 5. *P. exserta* and *P. axillaris* populations sampled and genotyped for *MYB-FL*. Populations represent a subset of those described[49] and correspond to the same classification.

	Sampling places	Genotype of individuals for single base-pair deletion in *MYB-FL*					Geographical coordinates	
		A	H	E	Unknown	TOTAL		
	Hybrid populations							
	Pedra da Cruz	8	5	8	6	27	30º 53' 48''S	53º 25' 16''W
	Ponto 143	NA	NA	NA	NA	NA	30º 50' 14''S	53º 30' 15''W
	Petunia exserta							
1	tower 1/shelter 2 Caçapava do Sul - RS			1		1	30º 50' 18''S	53º 29' 43''W
3	tower 2/shelter 4 Caçapava do Sul - RS			7		7	30º 50' 11''S	53º 30' 17''W
4	tower 2/ shelter 5 Caçapava do Sul - RS			15		15	30º 50' 13''S	53º 30' 19''W
5	tower 2/shelter 3 Caçapava do Sul - RS			6		6	30º 50' 10''S	53º 30' 16''W
8	tower 3/shelter 6 Caçapava do Sul - RS			3		3	30º 49' 54''S	53º 30' 09''W
9	tower 3/shelter 7 Caçapava do Sul - RS			5		5	30º 49' 50''S	53º 30' 08''W
10	tower 3/shelter 8 Caçapava do Sul - RS			4		4	30º 49' 52''S	53º 30' 10''W
13	tower 4/ shelter 9 Caçapava do Sul - RS			4		4	30º 53' 48''S	53º 25' 15''W
14	tower 5/ shelter 15 Caçapava do Sul - RS			9		9	30º 50' 18''S	53º 30' 39''W
16	tower 6/shelter 17 Caçapava do Sul - RS			7		7	30º 50' 24''S	53º 30' 17''W
17	tower 6/ shelter 18 Caçapava do Sul - RS			7		7	30º 50' 22''S	53º 30' 12''W
18	tower 6/shelter 19 Caçapava do Sul - RS			6	1	7	30º 50' 26''S	53º 30' 20''W
19	tower 7/shelter 20 Caçapava do Sul - RS			2		2	30º 50' 40''S	53º 31' 11''W
22	tower 8/shelter 23 Caçapava do Sul - RS			8		8	30º 50' 07''S	53º 30' 34''W
24	tower 9/shelter 25 Caçapava do Sul - RS			9		9	30º 50' 14''S	53º 30' 22''W
26	tower 10/shelter 27 Caçapava do Sul - RS	1				1	30° 49' 56''S	53° 29' 47''W
27	tower 13/shelter 29 Caçapava do Sul - RS			3		3	30º 50' 09''S	53º 30' 24''W
28	tower 14/shelter 30 Caçapava do Sul - RS			12		12	30º 50' 05''S	53º 30' 02''W
38	tower 11/ shelter 14 Pinheiro Machado - RS			3		3	31º 13' 30''S	53º 29' 51''W
39	tower 12/shelter 28 Pinheiro Machado - RS			27		27	31° 13' 39''S	53° 30' 31''W
	TOTAL	**1**	**0**	**138**	**1**	**140**		
	Petunia axillaris							
2	Ruta 1 - Colônia - Uruguay	2				2	34º 20' 09''S	57º 20' 05''W
3	Praia do Rio La Plata - Colonia - Uruguay	3				3	34º 26' 06''S	57º 16' 27''W
4	Ruta 1 - Delta del Tigre - San José - Uruguay	4				4	34º 45' 53''S	56º 24' 25''W
5	Ruta Panoramica - Maldonado - Uruguay	2				2	34º 53' 13''S	55º 11' 57''W
6	Punta Ballena - Maldonado - Uruguay	3				3	34º 54' 48''S	55º 02' 45''W

	Sampling places	Genotype of individuals for single base-pair deletion in *MYB-FL*					Geographical coordinates	
		A	H	E	Unknown	TOTAL		
7	Ruta - Maldonado - Uruguay	2				2	34º 54' 35''S	54º 59' 59''W
8	Ruta1 - Maldonado - Uruguay	2				2	34º 55' 04"S	54º 58' 29''W
9	Ruta 10 - La Barra - Maldonado - Uruguay	2				2	34º 52' 20''S	54º 45' 05''W
10	Ruta 104 - José Ignácio- Maldonado - Uruguay	3				3	34º 46' 35''S	54º 40' 59''W
11	Ruta 9 - Rocha- Uruguay	2				2	34º 30' 57''S	54º 20' 43''W
12	Cabo Polonio - Rocha- Uruguay	4	1			5	34º 24' 09"S	53º 47' 01"W
13	Ruta 13 - Castillos - Rocha - Uruguay	3				3	34º 3' 18"S	53º 53' 27''W
14	Ruta 8 - Lavalleja - Uruguay	3				3	34º 21' 46"S	55º 09' 50"W
17	Ruta 4 - Artigas - Uruguay	3				3	30º 34' 08''S	56º 36' 16''W
18	Ruta 31 - Salto - Uruguay	2				2	31º 18' 45''S	57º 05' 34''W
19	Ruta 31 - Salto - Uruguay	3				3	31º 20' 04''S	57º 19' 34''W
20	Ruta 3 - Salto - Uruguay	4				4	31º 27' 21''S	57º 54' 19''W
21	Ruta 123 - Mercedes - Corrientes - Argentina	1				1	29º 33' 44"S	57º 30' 40"W
22	Ruta 2 - Entre Rios - Argentina	3				3	30º 31' 38''S	58º 32' 59''W
23	Sauce - Corrientes - Argentina	2				2	30º 12' 08''S	58º 47' 21'' W
24	Ruta 126 - Sauce- Corrientes - Argentina	4				4	30º 10' 45''S	59º 00' 46''W
25	Ruta 2 - Entre Rios - Argentina	1				1	30º 23' 24''S	58º 42' 52'' W
26	Pueblo Libertador - Corrientes - Argentina	3				3	30º 19' 50''S	59º 14' 42''W
28	Ruta 126 - Corrientes - Argentina	1				1	30º 13' 1''S	59º 23' 39''W
29	Ruta 12 - Corrientes- Argentina	2				2	30º 12' 45''S	59º 29' 26''W
30	Ruta 12 - Esquina - Corrientes - Argentina	3				3	29º 48' 18''S	59º 23' 42''W
31	Ruta 12 - Esquina - Corrientes - Argentina	5				5	29º 39' 53''S	59º 21' 40''W
33	Ruta 38 - Cordoba - Argentina	3				3	30º 51' 21"S	64º 31' 47"W
36	Ruta 15 - Las Calles - Córdoba - Argentina	2				2	31º 47' 49"S	65º 00' 23"W
37	Santana Do Livramento - RS - Brazil	5				5	30º 36' 56''S	55º 56' 19''W
39	Santana Do Livramento - RS - Brazil	7				7	30º 34' 27''S	56º 03' 50''W
40	Quaraí - RS - Brazil	4			1	5	30º 18' 41''S	56º 28' 42''W
41	Alegrete - RS - Brazil	4				4	29º 51' 35''S	55º 56' 47''W
42	Alegrete - RS - Brazil	5				5	29º 56' 13''S	56º 04' 20''W
43	Alegrete - RS - Brazil	3				3	30º 00' 50''S	56º 13' 07''W
46	Uruguaiana - RS - Brazil	1				1	29º 50' 29''S	56º 56' 28''W
48	Pinheiro Machado - RS - Brazil	3				3	31º 41' 25''S	53° 01' 27''W
49	Hulha Negra - RS - Brazil	2				2	31º 23' 36''S	53º 49' 17'' W
50	Hulha Negra - RS - Brazil	3				3	31º 23' 28''S	53º 50' 08''W
51	Bagé - RS - Brazil	4				4	31º 21' 50''S	53º 55' 36''W

	Sampling places	Genotype of individuals for single base-pair deletion in *MYB-FL*					Geographical coordinates	
		A	**H**	**E**	**Unknown**	**TOTAL**		
52	Bagé - RS - Brazil	1				1	31º 21' 15''S	53º 56' 24''W
53	Bagé - RS - Brazil	6				6	31º 13' 03''S	54º 16' 53''W
54	Bagé - RS - Brazil	1				1	31º 09' 39''S	54 21' 31'' W
58	Pantano Grande - RS - Brazil	3				3	30° 12' 55''S	52° 33' 55''W
59	Pantano Grande - RS - Brazil	2				2	30º 12' 11''S	52º 27' 23''W
61	Casa de Pedra - Bagé-RS – Brazil[a]	2				2	30° 58' 35''S	53° 36' 19''W
62	Casa de Pedra - Bagé-RS – Brazil[a]	5				5	30° 58' 22''S	53° 36' 19''W
63	Casa de Pedra - Bagé-RS – Brazil[a]	2				2	30° 58' 04''S	53° 35' 43''W
64	Casa de Pedra - Bagé-RS – Brazil[a]	3				3	30° 58' 06''S	53°35' 22''W
65	tower 1 - Caçapava do Sul-RS – Brazil[a]	2				2	30º 50' 17''S	53º 29' 42''W
66	tower 2 - Caçapava do Sul-RS – Brazil[a]	1	2			3	30º 50' 12''S	53º 30' 17''W
67	tower 3 - Caçapava do Sul - RS – Brazil[a]	1				1	30º 49' 54''S	53º 30' 09''W
68	tower 4 - Caçapava do Sul - RS – Brazil[a]	6				6	30º 53' 48''S	53º 25' 15''W
71	tower 9 - Caçapava do Sul - RS – Brazil[a]	2				2	30º 50' 14'' S	53º 30' 24''W
	TOTAL	**155**	**3**	**0**	**1**	**159**		

A, *P. axillaris* homozygous; B, *P. exserta* homozygous; H, heterozygous; NA, Not applicable; RS, Rio Grande do Sul; [a]*Petunia axillaris* sympatric to *P. exserta* populations

Supplementary Table 6. Oligonucleotides used in experimental procedures

Purpose	Gene	Primer name	Primer sequence (5' to 3')	Reference
Quantitative RT-PCR, reference gene	*SAND*	SAND-F	CTTACGACGAGTTCAGATGCC	ref. 77
Quantitative RT-PCR, reference gene	*SAND*	SAND-R	TAAGTCCTCAACACGCATGC	ref. 77
Quantitative RT-PCR, reference gene	*RAN1*	RAN1-F	AAGCTCCCACCTGTCTGGAAA	ref. 77
Quantitative RT-PCR, reference gene	*RAN1*	RAN1-R	AACAGATTGCCGGAAGCCA	ref. 77
Quantitative RT-PCR	*MYB-FL*	*MYB-FL*-qPCR-F	TACCACCACCACTACCACAG	
Quantitative RT-PCR	*MYB-FL*	*MYB-FL*-qPCR-R	ACCTATCGCTGATCCTGCAT	
Quantitative RT-PCR	*FLS*	NA265	CCAAGTTGAGATTCTTAGCAATGG	ref. 54
Quantitative RT-PCR	*FLS*	NA266	ACCGGCCATGACATTCTTG	ref. 54
Screening for transposon insertions; amplification of *MYB-FL*	*MYB-FL*	B371	TTCAGATTCAGATCCCCATT	
Screening for transposon insertions; amplification of *MYB-FL*	*MYB-FL*	B369	TTTAGATTCAAAGATTAGTCAAA	
Positive control for wild accessions	*EF1α*	B464	CATTGGCCATGTCGACTCTG	
Positive control for wild accessions	*EF1α*	B465	GGCTTGTCTGAGGGTCTCTT	
Positive control for wild accessions	*MYB-FL*	B466	GAGAGGAAGATGGACAGCTGA	
Positive control for wild accessions	*MYB-FL*	B467	TCCCAAGGAGCATTGCAATT	
Amplifying coding sequence of FLS exon 1	*FLS*	B370	CCTAGAAGCTCGGCGAAAG	
Amplifying coding sequence of FLS exon 1	*FLS*	B374	AATTATCAGCTAAGCGATCTGAAT	
Amplifying coding sequence of FLS exon 2	*FLS*	B47	GTCAGAGTTAGGTCGGCC	
Amplifying coding sequence of FLS exon 2	*FLS*	B375	AGCTGTTTTGTTTTCCCTTTCC	
Amplifying coding sequence of FLS exon 3	*FLS*	B376	TTGTCATGATCATATCTTTTCGGTA	
Amplifying coding sequence of FLS exon 3	*FLS*	B282	GTTTTCCCTATTAACTTGGC	
Amplification of *MYB-FL* gene in Pax and Pex	*MYB-FL*	B402	TGCTCCAAAGATACTCTTCCGT	
Amplification of *MYB-FL* gene in Pax and Pex	*MYB-FL*	B403	ACTTGCCATCAAAGACCACCT	
Amplification of *MYB-FL* gene in Pax and Pex	*MYB-FL*	B404	TTTGGTGCCCTGCTACCAG	
Amplification of *MYB-FL* gene in Pax and Pex	*MYB-FL*	B407	CCCCCTCTTACCCTCTTATACT	
Amplification of *MYB-FL* gene in Pax and Pex	*MYB-FL*	B367	CCCTCCAAAAGCTCACTCTC	
Amplification of *MYB-FL* gene	*MYB-FL*	B419	TCTTTTCCCAGAAGCAGCAG	
Amplification of *MYB-FL* promoter	*MYB-FL*	B461	ACCTCATGTCTTGCTCAGCT	
Amplification of *MYB-FL* promoter	*MYB-FL*	B462	TCAGCTGTCCATCTTCCTCTC	
Transposon display adapter ligation		MfeI-bio-adapter-top	Biotin-TCGTAGACTGCGTACG	
Transposon display adapter ligation		MfeI-bio-adapter-bot	AATTCGTACGCAGTC	
Transposon display adapter ligation		MseI/BfaI-adapter-top	GACGATGAGTCCTGAG	
Transposon display adapter ligation		MseI/BfaI-adapter-bot	TACTCAGGACTCAT	
Transposon display preamplification		MfeI+ACAC primer	AGACTGTGTACGAATTGACAC	

Purpose	Gene	Primer name	Primer sequence (5’ to 3’)	Reference
Transposon display preamplification		MfeI+AACC primer	AGACTGTGTACGAATTGAACC	
Transposon display selective amplification		MseI+0 primer	GACGATGAGTCCTGAGTAA	
Transposon display selective amplification		BfaI+0 primer	GACGATGAGTCCTGAGTAG	
Transposon display selective amplification		MfeI-N7-IRoutw	CATATACAATTGNNNNNNNGTAGCTCCG CCCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-1	CATATACAATTGCGCACGTGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-2	CATATACAATTGCTACTGTGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-3	CATATACAATTGCATGTGTGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-4	CATATACAATTGTCGCTACGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-5	CATATACAATTGCTCATGTGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-6	CATATACAATTGTCGTACTGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-7	CATATACAATTGTGACAGAGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-8	CATATACAATTGCGTAGACGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-9	CATATACAATTGCGTGAGTGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-10	CATATACAATTGTCGCTGAGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-11	CATATACAATTGCGCAGCTGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-12	CATATACAATTGTACAGATGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-13	CATATACAATTGTACTAGAGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-14	CATATACAATTGTCAGACAGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-15	CATATACAATTGTAGACGAGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-16	CATATACAATTGGACATACGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-17	CATATACAATTGCGTCTCAGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-18	CATATACAATTGTACGTGTGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-19	CATATACAATTGCGTATCTGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-20	CATATACAATTGTCAGCTAGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-21	CATATACAATTGCTCAGTCGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-22	CATATACAATTGCGCTCACGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-23	CATATACAATTGCTACGTCGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-24	CATATACAATTGCTAGACAGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-25	CATATACAATTGTGTATGAGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-26	CATATACAATTGCTCATACGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-27	CATATACAATTGTCAGCACGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-28	CATATACAATTGTAGCATCGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-29	CATATACAATTGTATGCTCGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-30	CATATACAATTGTGATGACGTAGCTCCGC CCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-31	CATATACAATTGTCATAGAGTAGCTCCGC CCCTG	

Purpose	Gene	Primer name	Primer sequence (5’ to 3’)	Reference
Transposon display selective amplification		MfeI-N7-IRoutw-32	CATATACAATTGCTCGTGAGTAGCTCCGCCCCTG	
Transposon display selective amplification		MfeI-N7-IRoutw-35	CATATACAATTGCTGCACAGTAGCTCCGCCCCTG	
Transposon display library preparation		MfeI-illumina adapter-MseI-R-top-X	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGCT	
Transposon display library preparation		MfeI-illumina adapter-MseI-R-bot-X	AATTAGCTCAGATCGGAAGAGCGTCGTGTAGGGAAAGA	
Transposon display library preparation		MfeI-illumina adapter-MseI-R-top-Y	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGACG	
Transposon display library preparation		MfeI-illumina adapter-MseI-R-bot-Y	AATTCGTCGAGATCGGAAGAGCGTCGTGTAGGGAAAGA	
Transposon display library preparation		MfeI-illumina adapter-BfaI-R-top-X	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGT	
Transposon display library preparation		MfeI-illumina adapter-BfaI-R-bot-X	AATTACTAGATCGGAAGAGCGTCGTGTAGGGAAAGA	
Transposon display library preparation		MfeI-illumina adapter-BfaI-R-top-Y	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATACG	
Transposon display library preparation		MfeI-illumina adapter-BfaI-R-bot-Y	AATTCGTATAGATCGGAAGAGCGTCGTGTAGGGAAAGA	
Transposon display library preparation		MfeI-illumina adapter-BfaI-L-top-X	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTATCGCG	
Transposon display library preparation		MfeI-illumina adapter-BfaI-L-bot-X	AATTCGCGATAGAGATCGGAAGAGCGTCGTGTAGGGAAAGA	
Transposon display library preparation		MfeI-illumina adapter-BfaI-L-top-Y	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGCTGCG	
Transposon display library preparation		MfeI-illumina adapter-BfaI-L-bot-Y	AATTCGCAGCTGAGATCGGAAGAGCGTCGTGTAGGGAAAGA	
Transposon display library amplification		ill-PCR Primer 1	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	
Transposon display library amplification		ill-PCR Primer 2	CAAGCAGAAGACGGCATACGAGCTCTTCCGATCTGACGATGAGTCCTGAGTA	

Pax, *P. axillaris*; Pex, *P. exserta*

Supplementary Note

Genotyping wild accessions

Wild accessions were first genotyped for the *MYB-FL* single base-pair deletion marker (MYB-FL-3'CAPS; http://www.ips.unibe.ch/deve/caps/index.html), but not all individuals produced a product and some samples gave an ambiguous banding pattern. For these samples, the *MYB-FL* marker was repeated at least one more time. Any samples that did not produce a PCR product that could be clearly scored were amplified using two positive control PCRs: one that amplified the *elongation factor 1α* (*EF1α*) gene, a conserved gene, and one that amplified the first half of *MYB-FL* corresponding to the conserved DNA binding domain of the protein (primers B464/B465 and B466/B467, respectively; Supplementary Table 5). Samples that did not amplify for *EF1α* were concluded to contain DNA of poor quality and/or quantity and were excluded from analysis (52 *P. axillaris* samples; 21 *P. exserta* samples). One *P. axillaris* sample was also excluded because there was no DNA left to test the positive controls. One *P. axillaris* sample (population 40) amplified for the *EF1α* gene, but for neither the *MYB-FL* positive control PCR nor the *MYB-FL*-3'CAPS marker, and one *P. exserta* sample (population 18) amplified for the *EF1α* gene and for the *MYB-FL* positive control but not for the CAPS marker. Six individuals from the hybrid population Pedra da Cruz gave PCR products with the *EF1α* gene and the *MYB-FL* positive control, but could not be accurately scored. These failed PCRs can be attributed to diversity in the primer-binding sites, some kind of structural polymorphism, or to poor DNA quality/quantity.

Detailed protocol for amplification and sequencing of *dTph1* transposon flanking sequences from sectors and surrounding WT tissues

<u>DNA digestion</u>

Each DNA sample (250 ng DNA dissolved in 10 µL) was digested for 1 h at 37 °C after adding 30 µL of the following mix: For *MfeI/MseI* digestion: 0.5 µL *MfeI* (20 U/µL stock), 1 µL *MseI* (10 U/µL stock), 4 µL NEB 4 buffer (New England Biolabs, 10x stock), 0.4 µL BSA (100x stock) and 24.1 µL H_2O. For *MfeI/BfaI* digestion: 0.5 µL *MfeI* (20 U/µL stock), 2 µL *BfaI* (5 U/µL stock), 4 µL NEB 4 buffer (10x stock), 0.4 µL BSA (100x stock) and 23.1 µL H_2O.

<u>Adapter Ligation</u>

All adapters (Supplementary Table 5) mentioned below were prepared prior to use by combining equimolar amounts of top and bottom ("tom") components in a PCR tube, and heated for 5 min at 80 °C in a PCR machine, followed by a programmed gradual cool down step to room temperature over a period of one hour. The resulting products were kept on ice. For the adapter ligation, we added 10 µL of the following mix directly to each of the digestion mixtures for a further incubation of 3 h at 37 °C. Mix: 2 µL *MfeI*-bio-adapter (5 pmol/µL stock); 2 µL *MseI/BfaI*-adapter (50 pmol/µL stock); 1 µL NEB 4 (10x stock); 0.1 µL BSA (100x stock), 1 µL ATP (10 mM stock), 0.5 µL T4 DNA ligase (5 WeissU/µL stock), 3.4 µL H_2O. After ligation, all samples were cleaned up with a Qiagen PCR purification kit according to the instructions in the manual, and eluted from the columns in 50 µL EB buffer.

<u>Preamplification of *dTph1* flanking sequences</u>

The sequences of all primers described below are shown in Supplementary Table 5.

For each sample, we performed three different PCR pre-amplification experiments: one right border amplification for the *MseI* digested samples, and one right and one left border amplification for the *BfaI* digested samples. These amplification reactions were achieved by combining 18 µL of one of the following mixes with 2 µL of template DNA (from the previous step) in a PCR tube:

- *MseI* right border amplification: 0.6 µL *MfeI* + ACAC primer (10 µM); 0.6 µL *MseI* + 0 primer (10 µM); 0.4 µL dNTP (10 mM); 2 µL 10x PCR buffer; 0.6 U Dream Taq DNA polymerase (ThermoScientific); H_2O to 18 µL.
- *BfaI* right border amplification: 0.6 µL *MfeI* + ACAC primer (10 µM); 0.6 µL *BfaI* + 0 primer (10 µM); 0.4 µL dNTP (10 mM); 2 µL 10x PCR buffer; 0.6 U Dream Taq DNA polymerase; H_2O to 18 µL.
- *BfaI* left border amplification: 0.6 µL *MfeI* + AACC primer (10 µM); 0.6 µL *BfaI* + 0 primer (10 µM); 0.4 µL dNTP (10 mM); 2 µL 10x PCR buffer; 0.6 U Dream Taq DNA polymerase; H_2O to 18 µL.

The samples were incubated according to the following PCR profile: 1 x (30” 94 °C); 13 x (15” 94 °C, 30” 65 °C >>56 °C (Δt = -0.7 °C/cycle), 80” 72 °C); 22 x (15” 94 °C, 30” 56 °C, 60” 72 °C).

<u>Selective amplification of *dTph1* flanking sequences</u>

The PCR products from the pre-amplification were diluted 10x in H_20, and 5 µL was used for selective amplification by adding 30 µL of the following mix:

- *MseI* right border amplification mix: (2 µL *MfeI*-N^7-IR_{outw} primer (5 µM)*, 1 µL *MseI* + 0 primer (10 µM), 0.65 µL dNTP (10 mM), 3.5 µL 10x PCR buffer, 1 U Dream Taq DNA polymerase, H_2O to 30 µL).
- *BfaI* left & right border amplification mix (each separately): (2 µL *MfeI*-N^7-IR_{outw} primer (5 µM)*, 1 µL *BfaI* + 0 primer (10 µM), 0.65 µL dNTP (10 mM), 3.5 µL 10xPCR buffer, 1 U Dream Taq DNA polymerase, H_2O to 30 µL)

The samples were incubated according to the following PCR profile: 1 x (30” 94 °C); 13 x (15” 94 °C, 30” 65 °C >>56 °C (Δt = -0.7 °C/cycle), 80” 72 °C); 22 x (15” 94 °C, 30” 56 °C, 60” 72 °C).

*Every sample is amplified with a unique *MfeI*-N^7-IR_{outw} primer containing a 7 bp barcode (N^7), preceeded by a *MfeI* restriction site (CAATTG), and the 5’ addition of 6 nucleotides (CATATA) to later facilitate *MfeI* digestion of DNA ends to allow directional ligation of Illumina adapters (see further). The primer terminates with 15 nucleotides complementary to the Terminal Inverted Repeats of the *dTph1* transposon (Supplementary Table 5). The 32 samples in each of the three series were amplified using barcode variants 1 to 32. Finally, the samples were amplified a second time using barcode variant 35 for all samples. This allows distinguishing the sample series from other unrelated samples in the same sequencing run that were also amplified with barcodes 1-32 (see further). Individual PCR amplification products within each series of 32 PCR reactions (six series in total: *MseI*-right; *BfaI*-right; *BfaI*-left; amplified with either barcodes 1-32 or with barcode 35) were pooled together, resulting in 6 samples.

<u>Library preparation for Illumina sequencing: *MfeI* digestion and directional Illumina adapter ligation</u>

For each of the six samples, 1 µg (in 10 µL) of DNA was digested for 1 h at 37 °C after adding 40 µL of the following mix: 0.5 µL *MfeI* (20 U/µL stock), 5 µL NEB 4 buffer (New England Biolabs, 10x stock),

0.5 µL BSA (100x stock) and 34 µL H_2O. Next, Illumina adapters were ligated by adding 10 µL of the following mix [2 µL *MfeI*-illumina adapter**(50 pmol/µL stock), 1 µL NEB 4 (10x stock), 0.1 µL BSA (100x stock), 1 µL ATP (10 mM stock), 0.5 µL T4 DNA ligase (5 WeissU/µL stock), 5.4 µL H_2O] directly to the digestion mixtures, and incubated further for 3 h at 37 °C. The adapter-ligated samples were cleaned up using Qiagen PCR purification kit according to the instructions in the manual, and eluted in 40 µL EB buffer.

**Each of the six samples was ligated with a differently barcoded Illumina adapter (Supplementary Table 5), to allow retracing of library origin.

PCR amplification of the Illumina libraries

To 2 µL of each of the samples of the last step, we added 23 µL of the following mix: 0.6 µL ill-PCR Primer 1 (10 µM), 0.6 µL ill-PCR Primer 2 (10 µM), 0.5 µL dNTP (10 mM), 2.5 µL 10x PCR buffer (Thermo Scientific), 0.6 U Dream Taq DNA polymerase (Thermo Scientific), H_2O to 23 µL.

The samples were incubated according to the following profile: 30” 94 °C; 10x(15” 94 °C, 30” 56 °C, 60” 72 °C), 5x(15” 94 °C, 30” 65 °C, 60” 72 °C); 120” 72 °C.

The resulting PCR products were cleaned using the Qiagen PCR purification kit and eluted with 40 µL EB buffer. Samples were combined into one sample that was ready to load as a template for sequencing on a High Seq 2000 Sequencing Platform (100 bp single reads, Baseclear, The Netherlands). Transposon flanking sequences were assigned to each of the 32 DNA samples based on the barcodes present in *MfeI*-N^7-IR_{outw} primers and *MfeI*-illumina adapters.

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