

Supplementary Table 1. QTL analysis for UV-absorbance in corolla limb of *P. axillaris* x *P. exserta* F₂ population.

Chromosome	Marker	LRS	CI (cM)	PVE (%)	<i>p</i>	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 F₂ cross of *P. axillaris* x *P. exserta* were calculated by the QTX program⁷⁵ 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	<i>MYB-FL</i> 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	<i>P. axillaris</i>	1.9	Yes	No	Yes	<i>dTph12</i>	KT962945	(MV, unpublished data)
C2-60	IL2-1 ^{Pax}	1.4	Yes	No	Yes	<i>dTph1</i>	KT962947	ref. 94
A2-24	<i>P. axillaris</i>	1.4	No	-	No	-	-	-
A2-72	<i>P. axillaris</i>	none present	Yes	Yes	Yes	<i>dTph1</i>	KT962948	ref. 94
A1-95_1	<i>P. axillaris</i>	1.9	No	-	Yes	<i>dTph4</i>	KT962941	ref. 95
A1-95_2	<i>P. axillaris</i>	1.9	Yes	No	Yes	<i>dTph4</i>	KT962942	ref. 95
A1-95_3	<i>P. axillaris</i>	1.9	Yes	Yes	Yes	<i>dTph4</i>	KT962943	ref. 95
A1-95_4	<i>P. axillaris</i>	1.9	No	-	Yes	<i>dTph4</i>	KT962944	ref. 95

A1-95_1 to A1-95_4 were sectors sampled from four individual flowers from the same branch of a single F₁ individual, A1-95.

Supplementary Table 3. Results of genotyping individuals from the *P. axillaris* x *P. exserta* F₂ population with markers for the *FLS* gene and for the *MYB-FL* gene

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

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

<i>Cis</i> -regulatory element	Location	Motif	Sequence in <i>MYB-FL</i> promoter	PLACE Reference
ARR1AT	numerous	NGATT		S000454
CAATBOX1	numerous	CAAT	CAAT	S000028
GATABOX	numerous	GATA	GATA	S000039
WRKY71OS	numerous	TGAC	TGAC	S000447
EBOXBNNAPA/ MYCCONSENSUSAT	-1148 to -1143 -1106 to -1101 -675 to -670	CANNTG	CATTTG CATCTG CAACTG	S000114/S00047
NAPINMOTIFBN ROOTMOTIFTAPOX1	-1110 to -1104 -1072 to -1068 -557 to -553	TACACAT ATATT	TACACAT ATATT	S000040 S000098
GAREAT SEBFCONSSTPR10A ARFAT	-992 to -986 -961 to -955 -960 to -955	TAACAAR YTGTCWC TGTCTC	TAACAAA TTGTCTC TGTCTC	S000439 S000391 S000270
NODCON2GM/ OSE2ROOTNODULE ERELEE4	-957 to -953 -683 to -679 -877 to -870	CTCTT	CTCTT CTCTT	S000462/S000468
POLLEN1LELAT52	-865 to -861 -410 to -406	AWTTCAAA AGAAA	AATTCAAA AGAAA AGAAA	S000037 S000245
GT1CONSENSUS/ GT1GMSCAM4	-864 to -859 -516 to -511 -362 to -357	GRWAAW/ GAAAAA	GAAAAA GAAAAA GAAAAA	S000198/S000453
INRNTPSADB	-849 to -842 -623 to -616	YTCANTYY	TTCAATTT TTCAATTT	S000395
SEF4MOTIFGM7S	-845 to -839 -641 to -635	RTTTTTR	ATTTTTA	S000103
MYBCORE GTGANTG10	-743 to -738 -732 to -729 -378 to -375 -350 to -347	CNGTTR GTGA	CTGTTG GTGA GTGA GTGA	S000176 S000378
WBOXATNPR1 SEF3MOTIFGM -10PEHVPSBD ACGTTBOX ACGTATERD1 NTBBF1ARROLB S1FBOXSORPS1L21 TAAAGSTKST1	-718 to -714 -594 to -589 -572 to -567 -491 to -486 -490 to -487 -435 to -430 -329 to -324 -323 to -319 -311 to -307 -265 to -261	TTGAC AACCCA TATTCT AACGTT ACGT ACTTTA ATGGTA TAAAG	TTGAC AACCCA TATTCT AACGTT ACGT ACTTTA ATGGTA TAAAG	S000390 S000115 S000392 S000132 S000415 S000273 S000223 S000387
NODCON1GM/ OSE1ROOTNODULE ANAERO1CONSENSUS	-310 to -305 -193 to -187	AAAGAT AAACAAA	AAAGAT AAACAAA	S000461/S000467 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 described⁴⁹ and correspond to the same classification.

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

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

Sampling places		Genotype of individuals for single base-pair deletion in <i>MYB-FL</i>				Geographical coordinates	
		A	H	E	Unknown		
52	Bagé - RS - Brazil	1				1	31° 21' 15"S 53° 56' 24"W
53	Bagé - RS - Brazil	6				6	31° 13' 03"S 54° 16' 53"W
54	Bagé - RS - Brazil	1				1	31° 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	30° 12' 11"S 52° 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	30° 50' 17"S 53° 29' 42"W
66	tower 2 - Caçapava do Sul-RS – Brazil ^a	1	2			3	30° 50' 12"S 53° 30' 17"W
67	tower 3 - Caçapava do Sul - RS – Brazil ^a	1				1	30° 49' 54"S 53° 30' 09"W
68	tower 4 - Caçapava do Sul - RS – Brazil ^a	6				6	30° 53' 48"S 53° 25' 15"W
71	tower 9 - Caçapava do Sul - RS – Brazil ^a	2				2	30° 50' 14" S 53° 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	<i>SAND</i>	SAND-F	CTTACGACGAGTTCAGATGCC	ref. 77
Quantitative RT-PCR, reference gene	<i>SAND</i>	SAND-R	TAAGTCCTCAACACGCATGC	ref. 77
Quantitative RT-PCR, reference gene	<i>RAN1</i>	RAN1-F	AAGCTCCCACCTGTCTGGAAA	ref. 77
Quantitative RT-PCR, reference gene	<i>RAN1</i>	RAN1-R	AACAGATTGCCGGAAGCCA	ref. 77
Quantitative RT-PCR	<i>MYB-FL</i>	<i>MYB-FL</i> -qPCR-F	TACCACCACCACTACCACAG	
Quantitative RT-PCR	<i>MYB-FL</i>	<i>MYB-FL</i> -qPCR-R	ACCTATCGCTGATCCTGCAT	
Quantitative RT-PCR	<i>FLS</i>	NA265	CCAAGTTGAGATTCTTAGCAATGG	ref. 54
Quantitative RT-PCR	<i>FLS</i>	NA266	ACCGCCATGACATTCTTG	ref. 54
Screening for transposon insertions; amplification of <i>MYB-FL</i>	<i>MYB-FL</i>	B371	TTCAGATTCAGATCCCCATT	
Screening for transposon insertions; amplification of <i>MYB-FL</i>	<i>MYB-FL</i>	B369	TTTAGATTCAAAGATTAGTCAAA	
Positive control for wild accessions	<i>EF1α</i>	B464	CATTGGCCATGTCGACTCTG	
Positive control for wild accessions	<i>EF1α</i>	B465	GGCTTGCTGAGGGTCTCTT	
Positive control for wild accessions	<i>MYB-FL</i>	B466	GAGAGGAAGATGGACAGCTGA	
Positive control for wild accessions	<i>MYB-FL</i>	B467	TCCAAGGAGCATTGCAATT	
Amplifying coding sequence of FLS exon 1	<i>FLS</i>	B370	CCTAGAAGCTCGGCGAAAG	
Amplifying coding sequence of FLS exon 1	<i>FLS</i>	B374	AATTATCAGCTAAGCGATCTGAAT	
Amplifying coding sequence of FLS exon 2	<i>FLS</i>	B47	GTCAGAGTTAGGTCGGCC	
Amplifying coding sequence of FLS exon 2	<i>FLS</i>	B375	AGCTGTTTTGTTTTCCCTTCC	
Amplifying coding sequence of FLS exon 3	<i>FLS</i>	B376	TTGTCATGATCATATCTTTTCGGTA	
Amplifying coding sequence of FLS exon 3	<i>FLS</i>	B282	GTTTTCCCTATTAACCTGGC	
Amplification of <i>MYB-FL</i> gene in Pax and Pex	<i>MYB-FL</i>	B402	TGCTCAAAGATACTCTTCCGT	
Amplification of <i>MYB-FL</i> gene in Pax and Pex	<i>MYB-FL</i>	B403	ACTTGCCATCAAAGACCACCT	
Amplification of <i>MYB-FL</i> gene in Pax and Pex	<i>MYB-FL</i>	B404	TTTGGTGCCCTGCTACCAG	
Amplification of <i>MYB-FL</i> gene in Pax and Pex	<i>MYB-FL</i>	B407	CCCCCTCTTACCCTCTTATACT	
Amplification of <i>MYB-FL</i> gene in Pax and Pex	<i>MYB-FL</i>	B367	CCCTCAAAGCTCACTCTC	
Amplification of <i>MYB-FL</i> gene	<i>MYB-FL</i>	B419	TCTTTTCCAGAAGCAGCAG	
Amplification of <i>MYB-FL</i> promoter	<i>MYB-FL</i>	B461	ACCTCATGTCTTGCTCAGCT	
Amplification of <i>MYB-FL</i> promoter	<i>MYB-FL</i>	B462	TCAGCTGTCCATCTTCTCTC	
Transposon display adapter ligation		MfeI-bio-adapter-top	Biotin-TCGTAGACTGCGTACG	
Transposon display adapter ligation		MfeI-bio-adapter-bot	AATTCGTACGCAGTC	
Transposon display adapter ligation		MseI/Bfal-adapter-top	GACGATGAGTCCTGAG	
Transposon display adapter ligation		MseI/Bfal-adapter-bot	TACTCAGGACTCAT	
Transposon display preamplification		MfeI+ACAC primer	AGACTGTGTACGAATTGACAC	

Purpose	Gene	Primer name	Primer sequence (5' to 3')	Reference
Transposon display preamplification		Mfel+AACC primer	AGACTGTGTACGAATTGAACC	
Transposon display selective amplification		Msel+0 primer	GACGATGAGTCCTGAGTAA	
Transposon display selective amplification		Bfal+0 primer	GACGATGAGTCCTGAGTAG	
Transposon display selective amplification		Mfel-N7-IRoutw	CATATACAATTGNNNNNNNGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-1	CATATACAATTGCGCACGTGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-2	CATATACAATTGCTACTGTGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-3	CATATACAATTGCATGTGTGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-4	CATATACAATTGCTCGCTACGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-5	CATATACAATTGCTCATGTGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-6	CATATACAATTGCTGCTACTGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-7	CATATACAATTGTGACAGAGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-8	CATATACAATTGCGTAGACGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-9	CATATACAATTGCGTGAGGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-10	CATATACAATTGCTGCTGAGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-11	CATATACAATTGCGCAGCTGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-12	CATATACAATTGTACAGATGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-13	CATATACAATTGTACTAGAGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-14	CATATACAATTGTCAGACAGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-15	CATATACAATTGTAGACGAGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-16	CATATACAATTGGACATACGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-17	CATATACAATTGCGTCTCAGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-18	CATATACAATTGTACGTGTGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-19	CATATACAATTGCGTATCTGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-20	CATATACAATTGTCAGTAGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-21	CATATACAATTGCTCAGTCGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-22	CATATACAATTGCGCTCACGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-23	CATATACAATTGCTACGTCGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-24	CATATACAATTGCTAGACAGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-25	CATATACAATTGTGTATGAGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-26	CATATACAATTGCTCATACTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-27	CATATACAATTGTCAGCACGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-28	CATATACAATTGTAGCATCGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-29	CATATACAATTGTATGCTCGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-30	CATATACAATTGTGATGACGTAGCTCCG CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-31	CATATACAATTGTCATAGAGTAGCTCCG CCCTG	

Purpose	Gene	Primer name	Primer sequence (5' to 3')	Reference
Transposon display selective amplification		Mfel-N7-IRoutw-32	CATATACAATTGCTCGTGAGTAGCTCCGC CCCTG	
Transposon display selective amplification		Mfel-N7-IRoutw-35	CATATACAATTGCTGCACAGTAGCTCCGC CCCTG	
Transposon display library preparation		Mfel-illumina adapter-Msel-R-top-X	ACACTCTTCCCTACACGACGCTCTCCGA TCTGAGCT	
Transposon display library preparation		Mfel-illumina adapter-Msel-R-bot-X	AATTAGCTCAGATCGGAAGAGCGTCGTG TAGGGAAAGA	
Transposon display library preparation		Mfel-illumina adapter-Msel-R-top-Y	ACACTCTTCCCTACACGACGCTCTCCGA TCTCGACG	
Transposon display library preparation		Mfel-illumina adapter-Msel-R-bot-Y	AATTCGTGAGATCGGAAGAGCGTCGTG TAGGGAAAGA	
Transposon display library preparation		Mfel-illumina adapter-Bfal-R-top-X	ACACTCTTCCCTACACGACGCTCTCCGA TCTAGT	
Transposon display library preparation		Mfel-illumina adapter-Bfal-R-bot-X	AATTACTAGATCGGAAGAGCGTCGTGTA GGGAAAGA	
Transposon display library preparation		Mfel-illumina adapter-Bfal-R-top-Y	ACACTCTTCCCTACACGACGCTCTCCGA TCTATACG	
Transposon display library preparation		Mfel-illumina adapter-Bfal-R-bot-Y	AATTCGTATAGATCGGAAGAGCGTCGTGT AGGGAAAGA	
Transposon display library preparation		Mfel-illumina adapter-Bfal-L-top-X	ACACTCTTCCCTACACGACGCTCTCCGA TCTCTATCGCG	
Transposon display library preparation		Mfel-illumina adapter-Bfal-L-bot-X	AATTCGCGATAGAGATCGGAAGAGCGTC GTGTAGGGAAAGA	
Transposon display library preparation		Mfel-illumina adapter-Bfal-L-top-Y	ACACTCTTCCCTACACGACGCTCTCCGA TCTCAGCTGCG	
Transposon display library preparation		Mfel-illumina adapter-Bfal-L-bot-Y	AATTCGAGCTGAGATCGGAAGAGCGTC GTGTAGGGAAAGA	
Transposon display library amplification		ill-PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC TCTTCCCTACACGACGCTCTCCGATCT	
Transposon display library amplification		ill-PCR Primer 2	CAAGCAGAAGACGGCATAACGAGCTCTTC CGATCTGACGATGAGTCCTGAGTA	

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

DNA digestion

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 H₂O. 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 H₂O.

Adapter Ligation

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 H₂O. 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.

Preamplification of *dTph1* flanking sequences

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 *Bfal* 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 .
- *Bfal* right border amplification: 0.6 μL *MfeI* + ACAC primer (10 μM); 0.6 μL *Bfal* + 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 .
- *Bfal* left border amplification: 0.6 μL *MfeI* + AACC primer (10 μM); 0.6 μL *Bfal* + 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 ($\Delta t = -0.7$ °C/cycle), 80" 72 °C); 22 x (15" 94 °C, 30" 56 °C, 60" 72 °C).

Selective amplification of *dTph1* flanking sequences

The PCR products from the pre-amplification were diluted 10x in H_2O , and 5 μL was used for selective amplification by adding 30 μL of the following mix:

- *MseI* right border amplification mix: (2 μL *MfeI*-N⁷-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).
- *Bfal* left & right border amplification mix (each separately): (2 μL *MfeI*-N⁷-IR_{outw} primer (5 μM)*, 1 μL *Bfal* + 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 ($\Delta t = -0.7$ °C/cycle), 80" 72 °C); 22 x (15" 94 °C, 30" 56 °C, 60" 72 °C).

*Every sample is amplified with a unique *MfeI*-N⁷-IR_{outw} primer containing a 7 bp barcode (N⁷), preceded 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; *Bfal*-right; *Bfal*-left; amplified with either barcodes 1-32 or with barcode 35) were pooled together, resulting in 6 samples.

Library preparation for Illumina sequencing: *MfeI* digestion and directional Illumina adapter ligation

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 μ L BSA (100x stock) and 34 μ L H₂O. Next, Illumina adapters were ligated by adding 10 μ L of the following mix [2 μ L *MfeI*-illumina adapter** (50 pmol/ μ L stock), 1 μ L NEB 4 (10x stock), 0.1 μ L BSA (100x stock), 1 μ L ATP (10 mM stock), 0.5 μ L T4 DNA ligase (5 WeissU/ μ L stock), 5.4 μ L H₂O] 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 μ L 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 μ L of each of the samples of the last step, we added 23 μ L of the following mix: 0.6 μ L ill-PCR Primer 1 (10 μ M), 0.6 μ L ill-PCR Primer 2 (10 μ M), 0.5 μ L dNTP (10 mM), 2.5 μ L 10x PCR buffer (Thermo Scientific), 0.6 U Dream Taq DNA polymerase (Thermo Scientific), H₂O to 23 μ L.

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 μ L 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*-N⁷-IR_{outw} primers and *MfeI*-illumina adapters.

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