

Phenotypic, genetic and molecular characterization of *7B-1*, a conditional male-sterile mutant in tomato

Theoretical and Applied Genetics

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Online resource 1 List of primers and PCR conditions used for markers analysis, sequencing and qRT-PCR

Use made in the research	Marker/gene/ primer pair name	SGN or Solyc ID	Marker type/Gene region amplified	Primer sequence (5'-3')	Ta (°C)	No. of cycles	Product length (bp) ^a
Markers for mapping	T0360	Solyc04g081570	In/del	F: AAACCTCTCCGAGCTAGTGCGG R: ACCCAACACCAAATTGTCCAA	56	30	649 _E 700 _p
	C2_At4g33350	Solyc04g079000	CAPS (<i>Hinf</i> I)	F: TATCATCTATCTCTTCCGTTTCGCTTC R: ATGCGATTCTTCAACCTTCAAC	57	35	1112+651 _E 800+651+312 _p
	SSR356	Solyc00g005020	In/del	F: ACCATCGAGGCTGCATAAAG R: AACCATCCACTGCCTCAATC	57	32	259 _E 200 _p
	<i>TM6</i>	Solyc02g084630	CAPS (<i>Ase</i> I)	F: TCGACAAACAGGCAGGTCAC R: CGTTCACGAATCTCAGCAAC	60	35	629+225 _E 404+225 _p
	SSR38	Solyc08g077480	In/del	F: GTTCTATAGCTGAAACTCAACCTG R: GGGTTCATCAAATCTACCATCA	57	32	237 _E 190 _p
	<i>LePI</i>	Solyc08g067230	In/del	F: GGATTGTAAACAGAAGCTTGGAC R: GTTGTCTCTGTCATTAATTTGCC	57	35	1991 _E 1850 _p
	T0834	Solyc06g053980	In/del	F: TAATTGGGACCCCATCAGAA R: CCCTTTAGGAGCACAAGCAG	60-50	15-25	838 _E 940 _p
	<i>LeGLO2</i>	Solyc06g059970	CAPS (<i>Taq</i> αI)	F: GTTTTCTTCGAGTGATAGTGACAAA R: ACCATGGGAAGAGCCCATATA	60	36	310+100 _E 320+90 _p
	C2_At1g77470	Solyc06g060150	In/del	F: TGCCCTACAATCACGATGTACACG R: AAACCACCCTCAGGGACATCAAG	60	40	2465 _E 2000 _p
	C2_At1g52200	Solyc06g066590	In/del	F: ACATTTGGACAAATAGCAGAAGTC R: TGAGAGCAGACAGCAGGCATCATC	55	35	150 _E 100 _p
<i>SIGLO2</i> sequencing	<i>LeGLO2 1</i>	Solyc06g059970	Promoter -1 st exon	F: TCACCACAGCCCAATACTCA R: CTTGCCTGTTGTTTGTGTTTC	55	36	580
	<i>LeGLO2 2</i>		1 st exon – 2 nd intron	F: GTGATCATCATGGGGAGAGG R: TTCTGCTCCTTAAATAAGCATTC	58	36	505
	<i>LeGLO2 3</i>		2 nd intron	F: GGGAGGAGACTATGGGATG R: CGAGATCGAGTGATAATTTTCG	60	36	820
	<i>LeGLO2 4</i>		2 nd intron—4 th exon	F: TTCGAAAATTATCACTCGATCTC R: GTCCATTTTGTAAGGCTTC	55	36	480
	<i>LeGLO2 5</i>		4 th exon - 3'UTR	F: TGTATGTGTACAGGCACCTCAA R: TTTTGTGTCCATTTTCTTTGC	62-56	12-24	1750

	<i>LeGLO2-6</i>		6 th exon - 3'UTR	F: GGTTTTCTCGGGGATTAGTATG R: GTTTTCAGAATCCATCTAGACC	60	36	610
	<i>LeGLO2_UTR</i>		3'UTR- downstream	F: TGATTTTGGTCTAGATGGATTCTG R: CCTTTGTCTTTTCGCCATTG	55	36	568
	<i>LeGLO2 p1</i>		Promoter region	F: GAATAGTCCAAAACGCCCCT R: GTCTTGTCTAGGTGCCTTGA	56	35	932
	<i>LeGLO2 p2</i>		Promoter region	F: GTCCACAAGGCAAATGATTGAG R: GCTTACCAAGGGGTGAATGAC	58	35	990
	<i>LeGLO2 p3</i>		Promoter region	F: TGAAACGGAGGAAGTACTCGT R: TGTGAGTATTGGGCTGTGGT	68	35	760
	<i>LeGLO2 cDNA 1</i>		5' portion of the cDNA	F: GTGATCATCATGGGGAGAGG R: TCCTCCTCCAGAATTTGATCC	57	36	477
	<i>LeGLO2 cDNA 2</i>		3' portion of the cDNA	F: TTCTAGTATCAGTGCCAAGCAGG R: ACCATGGGAAGAGCCCATA	57	36	418
Gene expression analysis	<i>qLeGLO2</i>	Solyc06g059970	qRT-PCR	F: TTCTAGTATCAGTGCCAAGCAGG R: CCTCTCATATTTCCACTTCCACCA	58	40	144
	<i>qSlDEF</i>	Solyc04g081000	qRT-PCR	F: TAAGTCCCTCTATCACGACCAAC R: TCCTATTACATCCTTTAGCTTCC	58	40	150
	<i>qTM6</i>	Solyc02g084630	qRT-PCR	F: CAAGCCCAAACACTACGACA R: CTTCCCCTGTTCTCTGCCTT	58	40	162
	<i>qLeGLO1</i>	Solyc08g067230	qRT-PCR	F: GGAGCAGGAGCAAGATCAAC R: GTTGGACTCTGAAAGCAAAAGGC	58	40	149
	<i>qCRC</i>	Solyc05g012150	qRT-PCR	F: GCACCTTTTGTGTGTAACCTCCT R: AGCTTCTCTATGTGGTATCTCTGG	58	40	150
	<i>SlPRE1</i>	Solyc02g087860	qRT-PCR	F: CCTTACCACAACCTCCTCTCCT R: CGACCTTCTGCTCGACATCC	58	40	208
	<i>SlPRE5</i>	Solyc02g067380	qRT-PCR	F: TGTCTGGGAGAAGGTCAAGG R: CTTGTTGGAGCGACGATTACG	58	40	131
	<i>qtubulin</i>	Solyc04g077020	qRT-PCR	F: TGAGGTCTTCTCACGCATTGACCA R: AATCCTTCTCGAGGGCAGCAAGAT	58	40	100

^a Subscripts indicate product length in *S. lycopersicum* (E) or *S. pennellii* (P) for in/del markers or the banding pattern for CAPS markers. Values are PCR product or digested fragment lengths in base pairs (bp); values for the *S. pennellii* alleles are approximated by comparison with molecular weight ladders.

Online Resource 2 Morphometric traits of flowers of the tomato *sl* mutant and of the near isogenic WT₂ in the original seed stock and in F₁ plants from the cross with the *7B-1* mutant. The phenotype, recorded under LD growth conditions, includes details on the frequency of normal stamens (NO), stamens with carpelloid structures on the adaxial surface (CS), stamens with carpelloid structures and external ovules (EO) and stamens completely transformed in carpels, free or adnate to the legitimate pistil (TC).

Each value represents the average of 16 flowers per genotype

Floral organ	Trait	Phenotype in the original seed stock			Phenotype in F ₁ hybrids		
		WT ₂ ^a	<i>sl</i>	<i>P</i> ^b	WT	“mutant”	<i>P</i> ^b
Sepals	No.	6.2	6.0	ns	6.1	6.6	*
	Length	11.8	12.2	ns	11.5	12.5	ns
	Width	1.2	1.1	ns	1.5	1.1	***
Petals	No.	6.4	6.3	ns	6.2	6.5	ns
	Length	13.3	12.4	ns	12.6	11.5	ns
	Width	2.6	1.8	**	2.8	2.5	ns
Stamens	No.	6.2	6.2	ns	6.3	6.8	*
	NO (%)	91.9	0	-	100	14.6	-
	CS (%)	6.8	0	-	0	29.3	-
	EO (%)	0	0	-	0	24.4	-
	TC (%)	1.4	100	-	0	31.7	-
Ovary	Length	1.5	1.8	*	1.6	1.5	ns
	Width	1.4	2.6	***	1.6	1.5	ns

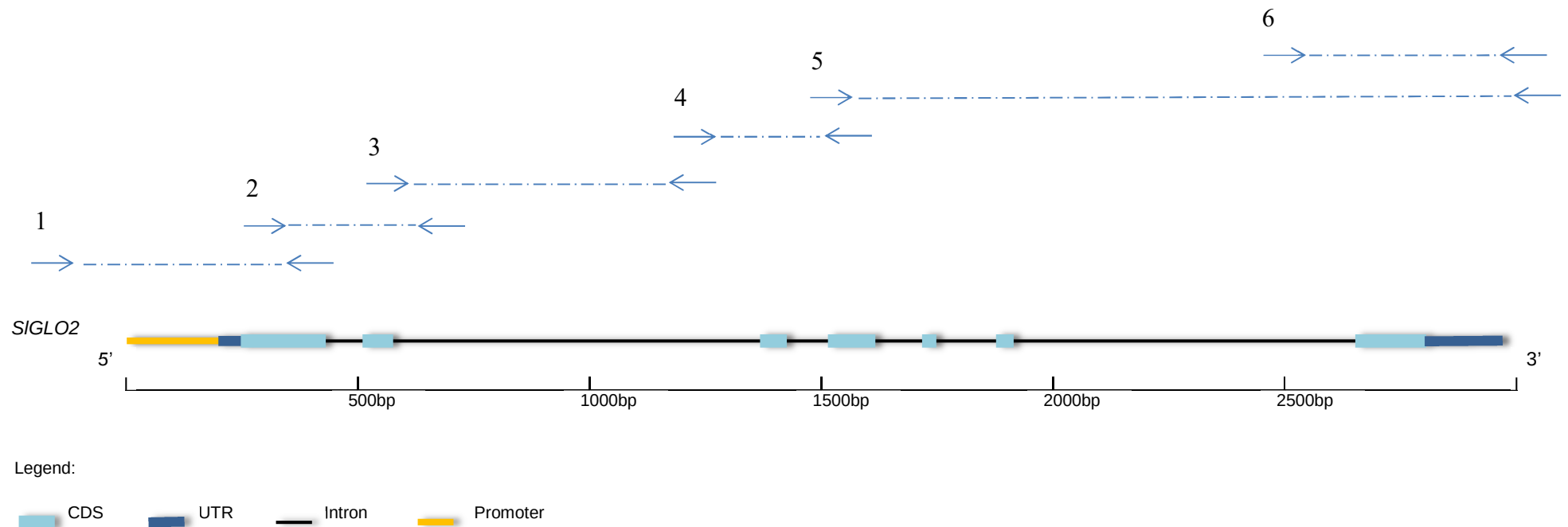
^a Segregating population, includes heterozygotes

^b Student's *t* test statistics: -, not determined; ns, not significant; *, ** and ***, significant for $P \leq 0.05$, 0.01 and 0.001 respectively

Online Resource 3 Morphometric traits in flowers of the *7B-1* and *sl-2* mutants compared with the respective WTs as varied according to the Genotype (G), the growth season in LD or SD (E) and their interaction (G*E)

Floral organ	Trait	<i>7B-1</i> system							<i>sl-2</i> system						
		LD		SD		<i>P</i> ^a			LD		SD		<i>P</i> ^a		
		WT ₁	<i>7B-1</i>	WT ₁	<i>7B-1</i>	G	E	G*E	WT ₄	<i>sl-2</i>	WT ₄	<i>sl-2</i>	G	E	G*E
Sepals	No.	6.0	6.9	6.1	6.7	***	-	-	6.2	6.0	6.3	5.8	ns	ns	ns
	Length	13.9	14.8	11.7	11.4	-	***	-	11.4	13.4	9.5	9.6	*	***	ns
	Width	1.7	1.6	1.7	1.3	**	-	-	1.3	1.3	1.6	1.0	-	-	**
Petals	No.	5.9	6.8	6.1	6.7	***	-	-	6.1	6.1	6.5	5.9	ns	ns	ns
	Length	15.1	12.4	13.7	12.6	**	-	-	9.1	10.5	15.7	12.3	-	-	**
	Width	3.8	3.1	3.6	3.2	*	-	-	2.2	2.6	4.6	3.8	ns	***	ns
Stamens	No.	5.9	6.5	7.0	7.6	***	***	-	6.2	6.1	6.9	6.0	*	ns	ns
	Length	11.7	9.5	9.9	8.0	***	***	-	10.7	7.8	10.9	8.0	***	ns	ns
Pistil	Length	8.3	9.7	6.2	6.4	-	**	-	8.3	8.7	7.0	5.8	-	-	**
Ovary	Length	1.9	1.8	2.2	1.9	-	*	-	1.5	1.4	1.9	1.3	-	-	***
	Width	1.7	2.0	2.2	2.0	-	*	*	1.2	1.4	2.2	1.1	-	-	***

^a ANOVA statistics: ns, not significant; *, ** and ***, significant for $P \leq 0.05$, 0.01 and 0.001 respectively



Online Resource 4 Schematic representation of the *SIGLO2* gene structure and position of the primer pairs designed for amplification and sequencing. Numbers indicate the position of the Forward primer for the combinations *SIGLO2-1* to *SIGLO2-6* described in Online Resource 1. Primers used for sequencing the promoter and the region downstream to the polyadenylation site are not depicted here, but reported in Online Resource 1

Online resource 5

Alignment of the published *SIGLO2* genomic (upper line) and *SIGLO2* mRNA (lower line) sequence (<http://www.sgn.cornell.edu>). Start and stop codons are highlighted in red; the beginning of exons are marked on the right; the two repeated regions within the 2nd intron are highlighted in yellow and the MITE-related sequences in light blue; the sequence that could not be obtained in the present material is underlined

1	GGGTAAGATGTTTTTTAATGTATAATTTTAAACAATCCAATAAAAGGTAGTATCAAAAGGA	
1		
61	CAAGATATTAATCAGTGGTTTGGCATTGTCAAATGCCCATAAAAAACCTACTTTGCTGA	
1		
121	GAAGATATAAAGTTTGTGGTCTACCAAACCTCTAATAGAAAATACCATTCAATTATTTTCC	
1		
181	TCTATAAAAAAATCTTCAAACCTTATTCAATTTCAAGAAGAAAAATTACTAGTAAGTGA	
1	AACTTATTCAATTTCAAGAAGAAAAATTACTAGTAAGTGA	
241	TCATC ATG GGGAGAGGTAAAATAGAGATAAAGAGAATAGAAAACACAAACAACAGGCAAG	1 st exon
43	TCATC ATG GGGAGAGGTAAAATAGAGATAAAGAGAATAGAAAACACAAACAACAGGCAAG	
301	TAACTTATTCAAAAAGAAGAAATGGTATAATAAAGAAAGCTAAAGAAATTACTGTTCTTT	
103	TAACTTATTCAAAAAGAAGAAATGGTATAATAAAGAAAGCTAAAGAAATTACTGTTCTTT	
361	GTGAAGCTAAGGTTTCACTTATAATCTTTGCTAGTTCTGGAAAGATGCATGAATATTGTA	
163	GTGAAGCTAAGGTTTCACTTATAATCTTTGCTAGTTCTGGAAAGATGCATGAATATTGTA	
421	GCCCTTCTACTACGTAAGTCAATTTTTTATTTTATTTAACTTTTTAATGATTTGTTAGTT	
223	GCCCTTCTACTACG.....	
481	TGTTGATTTCTATTTTCTTTAAATAATATTAAGGATAAGTGATATGTTGGATGGTTATC	2 nd exon
236	ATAAGTGATATGTTGGATGGTTATC	
541	AAAAAGCTTCTGGGAGGAGACTATGGGATGCTAAGCATGAGGTATAATTTTTTATTTAT	
262	AAAAAGCTTCTGGGAGGAGACTATGGGATGCTAAGCATGAG.....	
601	TTTATTTTATTTTTTTTGCTAAAATTATGTTAAATGAGTTGAAAAATGAAATATAGGAGT	
302		
661	AGATCTACAAATAGGATGTTTATGTAGCTTTGTAGATCCCTATTTTTACTGAATATTATG	
302		
721	<u>AATGCTTATTTAAGGAGCAGAAATTCTGTATGAAATGTTACATCTATCAATAGATCTGGA</u>	

302

781 AAAAAATAACACAAAATCATATGATCTGTCTTTTTCTTTTTCTTTTTTCATGCAATTC

302

841 TTTGTACTGTTAATTTTTTTGGTAAAAATATTTAGT AAAAAAGAACAAATTTTGATTGGA

302

901 TAATTTGTTTCATGTGTTTTTTGGAACTATCACTCGATCTCGACTAGTTAGGAATAAGAT

302

961 CTGTGTATCTAAAATAACTAATCATCGAGAAATTTATTCTCAACTAAACGAGTTGTGATA

302

1021 TTACTATAATTAATTTGCATATAACAATTTAAGCTTGTGC TTTTCTTCTTTTTTCTTT

302

1081 TTCATGCAATTTCTTTGTACTGTTAATTCTTTTGGTAAATATTTAGTAAA AAAAAAGGAC

302

1141 AAATTTTGATTGGATAATTTGTTCAAGTGTTTTTCGAAAATTATCACTCGATCTCGATTA

302

1201 GTAAAAATAAAATCTGTGTATCTAAAATAACTAATCATCAAGTAATTTATTCTCAACTA

302

1261 AATGAGTTGCGATATTACTATAATTAATTTGCATATAACAATTTACAATTGTGC TTTTTT

302

1321 CTTCTTTTTTTTTTTGAATAAAATGGAATGCTTTAATAATACAGAATTTGAGTAATGAAAT

302AATTTGAGTAATGAAAT

1381 TGATAGAATCAAGAAAGAGAATGACAGTATGCAGGTTAAGCTCAGGTAATTAATTTCACT

320 |||||
TGATAGAATCAAGAAAGAGAATGACAGTATGCAGGTTAAGCTC.....

1441 ATTTTAAATTAATTAATTAATTTTTCTCCTTTTATATTATTTTATTGATGTACAAGATT

362

1501 ATGTATGTGTACAGGCACCTCAAAGGAGAAGATATCAATCAACTTACCCATAAAGAGCTT

362AGGCACCTCAAAGGAGAAGATATCAATCAACTTACCCATAAAGAGCTT

1561 ATAATTATGGAAGAAGCCTTACAAAATGGACTTTCTAGTATCAGTGCCAAGCAGGTAAAC

411 |||||
ATAATTATGGAAGAAGCCTTACAAAATGGACTTTCTAGTATCAGTGCCAAG.....

1621 TAATTAATTAATTTTCCCTAATTTTTATCATATCTTAATTAATTATACTTTGATTTTGAT

3rd exon

4th exon

462		
1681	ATCCTTTTTTTTGTTCATATTTACTCTTGGACAGTCTGAAATCTTGAGGATGGTCAGG	5 th exon
462	CAGTCTGAAATCTTGAGGATGGTCAGG	
1741	AAAAATGTAAGTGTGCAAATAATAAGTAATATATTTTTGAATAATTCAACATGAATGCGA	
489	AAAAAT.....	
1801	CATCGAAAATGAGAAATTTGCGGGATAAATTACTAATATCTATTTATTTATTTGTGGGAA	
495		
1861	ATAATTCAGTTTTGTGTCCATTTTCTTTGCGATCAAATTCTGGAGGAGGAAAATAAGCAA	6 th exon
495	GATCAAATTCTGGAGGAGGAAAATAAGCAA	
1921	CTTCAATATGCTTTGGTAAGTCATAATTATTTTATACTTAATTAATTTGCCACAATTAC	
525	CTTCAATATGCTTTG.....	
1981	AAACATTAGAACTATCAACAATAATATTATCACTATAATATATAATTCTTGTGCATTTA	
539		
2041	TTCTCTCTTTCTTAATTTTAATTAATTGTGAGAACTCTTTCACCATATGCCAATATATAT	
539		
2101	ATTATATGTTATGTACACGCATGTGAAGAATACAAGGTTTTCAATTTTCACACTGCTTAT	
539		
2161	ATAGTAAAAACAATTATACAATGTTTTTATATGTTACTCGAGAAAAAAAATAAATCAC	
539		
2221	CAAACACCAGTCTATTATCGAATTTCTAACTACACACAAATACTTTACGGAAGTTCTATT	
539		
2281	ACCCCTAAACCTTTAAAAGTGGACTATATATAAACCCCTCAAGTACTAAGTTGTGTGTAG	
539		
2341	TTAAAAATTGGAATATTTTATGTATGTTACTCCCTTTGTCTCATATTAGTTGTCAAGTCT	
539		
2401	ATTAAACTCTAAAAGTACTTGCTCCAGTTTACTTGTTATCTGAATGTACATACACTATC	
539		
2461	AGAAAAGTGTGAATTGCTTGGAGAGTTTTCTCGGGGATTAGTATGAAAATTTACAGGAAA	
539		
2521	ATAAGTTTCATGCGAATTTTCATACTAATTTCTACGAAAATCTCCATATTATTCATAGTT	

539		
2581	TTCTTCGAGTGATAGTGACAAAATTAAAAATAGATGTTTGGTTAACTATTTTGTCTCTTC	
539		
2641	AAATTGTATGTTTTTGGATTCTAAAGCACCAAAAGGAGATGGGAGCCATTGGTGGAAGT	7 th exon
539	CACCAAAAGGAGATGGGAGCCATTGGTGGAAGT	
2701	GGAAATATGAGAGGAATTCATGAAGAAGTGTATCATCAAAGAGAAAGGGATTATGAGTAC	
573	GGAAATATGAGAGGAATTCATGAAGAAGTGTATCATCAAAGAGAAAGGGATTATGAGTAC	
2761	CAAATGCCATTTGGCCTACGAGTTCAGCCAATGCAGCCAAATCTACATGAAAGAATGTAA	Stop codon
633	CAAATGCCATTTGGCCTACGAGTTCAGCCAATGCAGCCAAATCTACATGAAAGAATGTAA	
2821	AATTATATTAATTCCCACTTTAAATTAAGAACTCTAATAATTTTAATTTGGGTTTCTACT	
693	AATTATATTAATTCCCACTTTAAATTAAGAACTCTAATAATTTTAATTTGGGTTTCTACT	
2881	ATAGCTAGAAACACTAATTAATCAATTCTAGTTATATGTAATGAGTATTAGCTAAGACTT	
753	ATAGCTAGAAACACTAATTAATCAATTCTAGTTATATGTAATGAGTATTAGCTAAGACTC	
2941	TGTTTCATGTTGGAATTATTACCTAATTTTAATGGGCTCTTCCCATGGTTTTAATTAAGT	3'UTR end
813	TGTTTCATGTTGGAATTATTACCTAATTTTAATGGGCT.....	
3001	ACTGAAATGTAGTGACATATTTATATGAATTGTTCTACTTACCTAGCTACTACAT	
851		