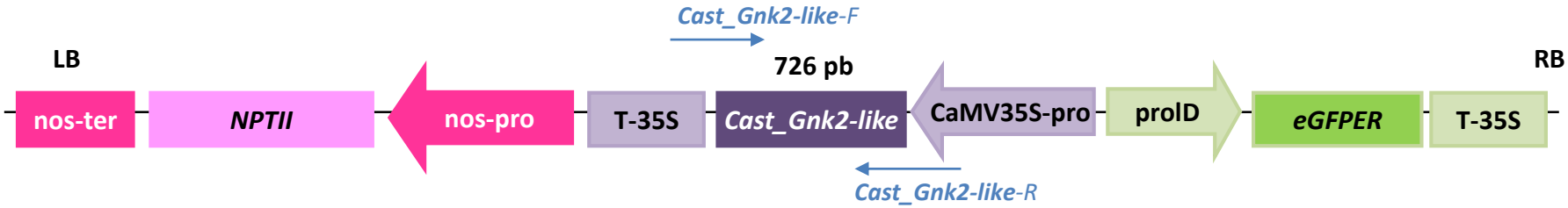


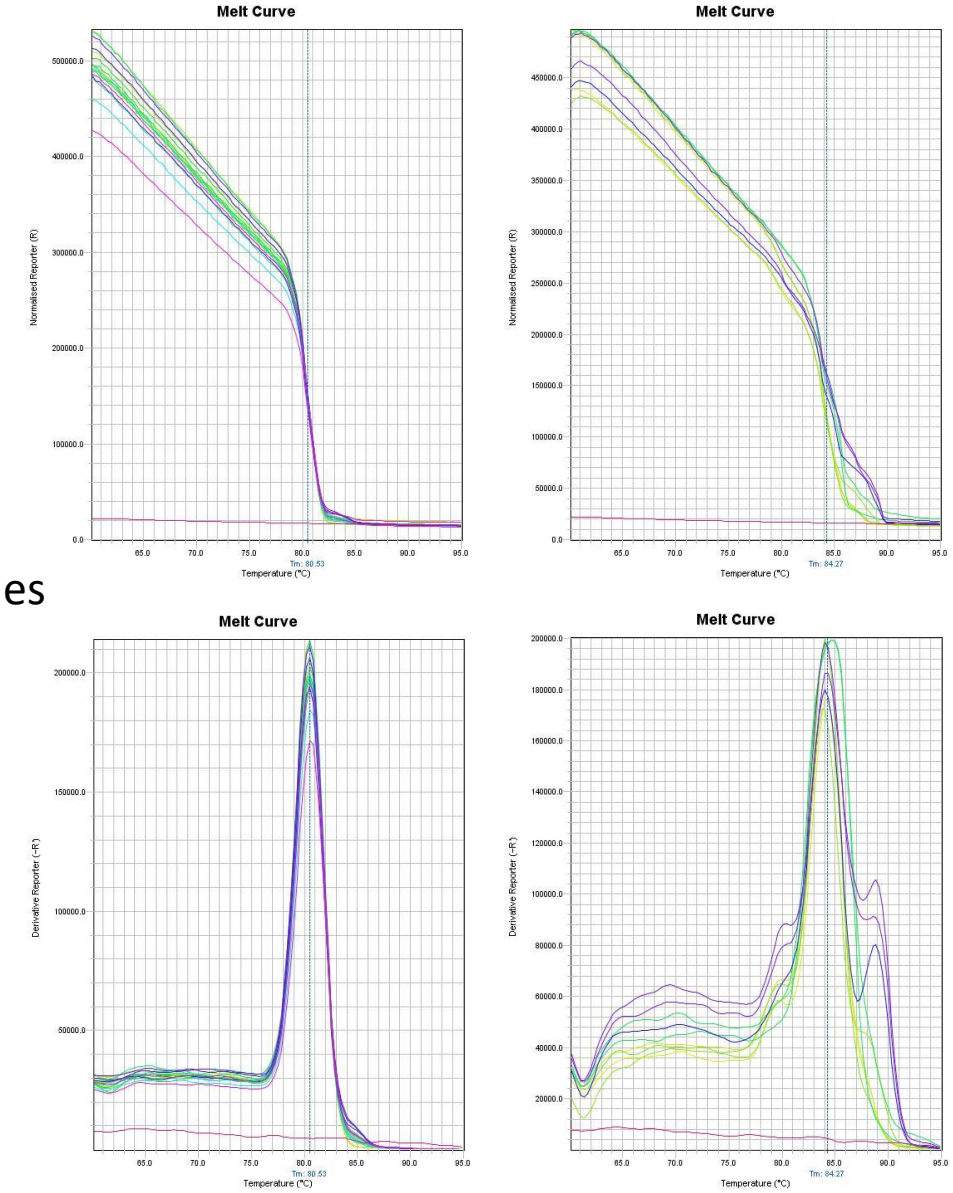
Online resource 1. A schematic representation of T-DNA region of the plasmid pK7WG2D-Gnk2. *nos-ter*, *nos-pro* terminator and promoter of nopaline synthase gene, respectively; *NPTII* neomycin phosphotransferase marker gene; CaMV*35S-pro, T-35S* promoter and terminator of Cauliflower mosaic virus gene, respectively; *eGFPER* green fluorescence protein gene*; Cast_Gnk2-like* gene encoding a Ginkbilobin-2 protein from *C. crenata*; *prolD* rol root loci D promoter; *RB* right border; *RB* left border.

Online resource 2. Primers and amplification programs utilized in the present report.

GENE or PROMOTER	PRIMER NAME	PRIMER SEQUENCE (5′-3′)	PCR CONDITIONS	FRAGMENT AMPLIFIED (bp)	PURPOSE	qPCR Efficiency (%) and R^2
NPTII	NPTII-F	GTCATCTCACCTTGCTCCTGCC	35 cycles: 94ºC x 30s 60ºC x 30s 72ºC x 42s	472	PCR analysis	-
	NPTII-R	AAGAAGGCGATAGAAGCGA				
GFP	EGFP-F	CACCGGGGTGGTGCCCAT	40 cycles: 94ºC x 15s 56ºC x 30s 72ºC x 1min	740	PCR analysis	-
	EGFP-R	CTAGTGGATCCCCCGGGC				
Cast_Gnk2-like-F[1]	T35S-R	AGGTCACTGGATTTTGGT	35 cycles: 98ºC x 10s 56ºC x 30s 72ºC x 1min	890	PCR analysis	-
	GIN-D	CTGCCACTAGCCGTTATGGT				
Cast_Gnk2-like-R[2]	p35S-D	GATCTAACAGAACTCGCC	35 cycles: 98ºC x 10s 56ºC x 30s 72ºC x 1min	1227	PCR analysis	-
	GIN-R	CTGGTGCATTGAGCCAAACC				
CaMV35S	P35S-F	GGACGATTCAAGGCTTGCT	Tm 58ºC	137	qPCR copy number	See Results
	P35S-R	AGTCTTCACGGCGAGTTCT				
Cast_Gnk2-like	Cc_Gnk2-F	GGGGACCTAAAGCTTGACTCA	Tm 62ºC	129	Transgene expression for qPCR	94; 0.991
	Cc_Gnk2-R	CATCGCAACAGTTGGGAAGTT				
EF1α	EF1α-F	GTGCCGTCCTCATTATTGAC	Tm 60ºC	72	Reference gene for qPCR	91; 0.959
	EF1α-R	CACGGGTCTGACCATCCTT				
β-Tubulin	Tub-F	CTGCGGTCGCTATGTTCCT	Tm 60ºC	147	Reference gene for qPCR	90; 0.995
	Tub-R	CCCTTGGCCCAGTTGTTTC				

The presence of *Cast_Gnk2-like* gene was verified by PCR in both transcriptional senses employing the specific primers Cast_Gnk2-like-F and Cast_Gnk2-like-R. [1]This fragment includes T-35S region (See Online resource 1). [2] This fragment includes CaMV35S region (See Online resource 1). F: forward; R: reverse.

Transformed lines



WT

Online resource 3. Melt curve analysis of *Q. ilex* transformed lines (left) and WT genotypes (right) upon qPCR reactions with CAMV35S promoter primers. Normalised reporter (up) shows the SYBR green fluorescence variation with the increment of temperature starting from the Tm of the primers, and the abrupt decrease in fluorescence correspondent to the Tm of the amplicon (indicated in the x-axis in blue). Derivative reporter (down) highlights the Tm of the amplicon in the form of a peak. *Q. ilex* transformed lines show a unique peak that corresponds to a unique amplicon. WT genotypes show a Tm of the amplicon distinct from the transformed lines. The low fluorescence pink in all images corresponds to the control without DNA. Images from the melt curve report on the StepOne Software v2.3.

Q8-WT

Q8-GIN1

Q8-GIN2

Q8-GIN3

E2-WT

E2-GIN1

Online resource 4. Morphological appearance of non-transformed plantlets and transformed plantlets obtained after embryo germination.