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Article

Efficient Transformation of Somatic Embryos and Regeneration of Cork Oak Plantlets with A Gene (*CsTL1*) Encoding a Chestnut Thaumatin-Like Protein

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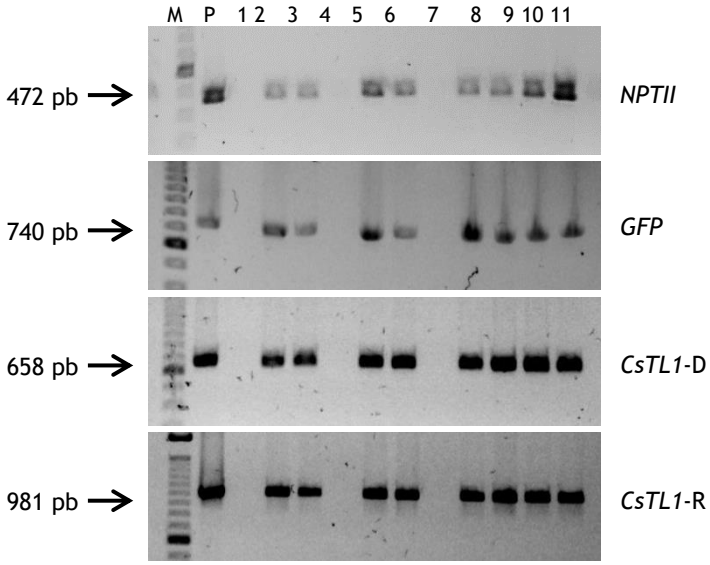
Supplementary material:

Online Resource 1. Cryopreservation of transgenic and non-transgenic (wt) cork oak lines.

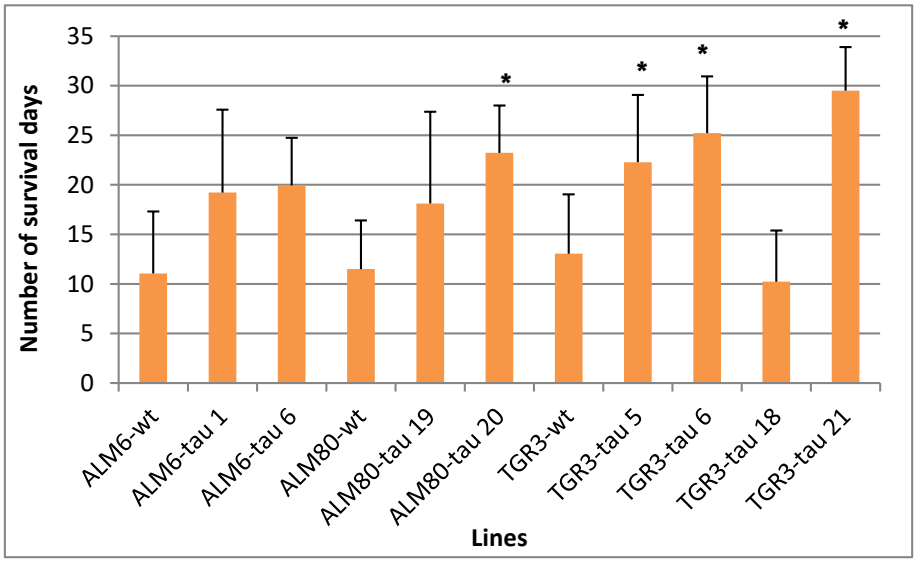
Online Resource 1A. A. Embryo survival and embryo recovery rates in cryopreserved cork oak somatic embryos of transgenic and non-transgenic (wt) lines after preculture on sucrose medium, exposure to PVS2 solution for 60 min at 0ºC and subsequent immersion in liquid nitrogen.

EMBRYOGENIC LINE	SURVIVAL (%)	EMBRYO RECOVERY (%)
ALM6-wt	63.3 ± 7.0	60.0 ± 5.8
ALM6-tau 1	86.7 ± 1.9	80.0 ± 0.0
ALM6-tau 6	96.7± 1.9	90.0 ± 0.0
ALM6-tau 12	93.3 ± 1.9	86.7 ± 5.1
ALM80-wt	90.0 ± 5.8	83.3 ± 3.9
ALM80-tau 13	96.7± 1.9	76.7 ± 1.9
ALM80-tau 19	96.7 ± 1.9	93.3 ± 1.9
ALM80-tau 20	80.0 ± 0.0	76.7 ± 1,9
TGR3-wt	66.7 ± 5.1	66.7 ± 5.1
TGR3-tau 2	83.3 ±4.7	80.0 ± 8.2
TGR3-tau 4	63.3 ± 18.9	53.3 ± 12.5
TGR3-tau 5	90.0 ± 3.4	90.0 ± 3.4
TGR3-tau 6	100.0 ± 0.0	100.0 ± 0.0
TGR3-tau 9	90.0 ± 8.2	80.0 ± 0.0
TGR3-tau 18	100.0 ± 0.0	100.0 ± 0.0
TGR3-tau 21	93.3 ± 1.9	93.3 ± 1.9
TGR3-tau 23	93.3 ± 4.7	83.3 ± 4.7
TGR3-tau 34	76.7 ± 17.0	66.7 ± 9.4
TGR3-tau 36	90.0 ± 10.0	75.0 ± 25.0
TGR3-tau 42	90.0 ± 14.1	86.7 ± 18.9
TGR3-tau 45	96.7 ± 4.7	70.0 ± 8.2

Each value represents the mean ± standard error (SE) of 3 replicates (10 explants each).



Online Resource 1B. PCR analysis of transgenic and non-transgenic (wt) lines after the cryopreservation process. M: molecular weight marker; P: plasmid (positive control); lanes 1-3: ALM6 non-transgenic (1) and transformed lines (2, 3); lanes 4-6: ALM80 non-transgenic (4) and transformed lines (5, 6); lanes 7-11: TGR3 non-transgenic (7) and transformed lines (8-11).



Online Resource 2. Number of days of survival of the transgenic and non-transgenic (wt) cork oak lines infected with *P. cinnamomi* and incubated for a period of 31 days. A total of 18 plants were evaluated for each line. Data analyzed by Kruskal-Wallis ($p \leq 0.05$). The error bars represent the standard errors. Asterisks indicate statistical significance ($p \leq 0.05$) of the lines with more days of survival.

Online Resource 3. Primers and PCR conditions used in the study.

Gene	Name	Primer sequence (5′-3′)	PCR conditions	Fragment amplified (bp)	Purpose
NPTII	NPTII-F	GTCATCTCACCTTGCTCCTGCC	35 cycles: 94ºC x 30s 60ºC x 30s 72ºC x 42s	472	PCR analysis
	NPTII-R	AAGAAGGCGATAGAAGCGA			
EGFP	EGFP-F	CACCGGGGTGGTGCCCAT	40 cycles:	740	PCR analysis

	EGFP-R	CTAGTGGATCCCCCGGGC	94ºC x 15s 60ºC x 30s 72ºC x 1min		
CsTL1-Forward[1]	CsTL1-F-F	AGGTCACTGGATTTTGGT	40 cycles: 94ºC x 15s 60ºC x 30s 72ºC x 1min	981	PCR analysis
	CsTL1-F-R	CACCATGATGAAAACCCTG			
CsTL1-Reverse[2]	CsTL1-R-F	GGTAAGGCCGTAGAGT	40 cycles: 94ºC x 15s 60ºC x 30s 72ºC x 1min	658	PCR analysis
	CsTL1-R-R	GATCTAACAGAACTCGCC			
CsTL1	CsTL1-Fq	GTTCAAGCTCCATGGAAAGG	40 cycles: 95ºC x15s 60ºC x 1min	-	qPCR analysis of *CsTL1*
	CsTL1-Rq	ACCTGACCGGTGCTACAATC			
TUB	QpTUBqF	CTCGTGCTGTTCTCATGGATCT	40 cycles: 95ºC x15s 60ºC x 1min	-	Reference gene for qPCR
	QpTUBqR	TGGCCGAAAACGAAGTTGTC			
ACT	QsACT-F	GCCCCACGAGCTGTGTTC	40 cycles: 95ºC x15s 60ºC x 1min	-	Reference gene for qPCR
	QsACT-R	TCTGGCCCATTCCAACCA			
EF	QsEF-F	TTGTGCCGTCCTCATTATTGACT	40 cycles: 95ºC x15s 60ºC x 1min	-	Reference gene for qPCR
	QsEF-R	TCACGGGTCTGACCATCCTT			

CsTL1 gene was confirmed by PCR in both transcriptional senses using the specific primers CsTL1-Forward and CsTL1-Reverse. [1]This fragment includes T-35S region. [2] This fragment includes p35S region. F: forward; R: reverse;*TUB*: tubulin; *ACT*: actin; *EF*: elongation factor.