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Effect of different factors on regeneration and transformation efficiency of tomato (*Lycopersicum esculentum*) hybrids

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Electronic Supplementary Material (ESM)

Table S1. Sequence of the primers used for PCR and RT-qPCR

Primer name	Sequence (5'–3')
GST4F5	TGGCCAAGTCCATTTGGGATGAGGG
GST4R5	TGGGTTTGCCATTGTGGATGAG
nptIIF	CTTTACCTATTTCCGCCCGG
nptIIR	GTGACAACGTCGAGAGCTGCG
GST4F	GCAGGATGAGGTAGTGTTATTAGATTTCTG
GST4R	CCAAACCTCCTCAATGTACTGAACAGC
ACTF	GTGCTGGACTCTGGAGATGGTGTC
ACTR	CATTGATGGTTGGAACAGCACTTCTGG

Table S2. Effect of acetosyringone (As) in the co-culture medium and thiamine (T) in the selection medium supplemented with kanamycine (100 mg/l) and cefotaxime (250 mg/l) on the genetic transformation efficiency of tomato cultivar Felina; *In vitro* shoot regeneration and % of transformation efficiency of putative transformed cotyledons grown in selection media supplemented with 0, 100 and 200 μM As and 0.1 and 0.4 mg/l T; the experiments were performed in triplicate with 9 explants each

Parameters	Regeneration medium (C2)					
As (μM)	0		100		200	
T (mg/l)	0.1	0.4	0.1	0.4	0.1	0.4
Regeneration efficiency	1.67 ± 0.33	2 ± 0	1.33 ± 0.33	2.66 ± 0.33	2.66 ± 0.33	3.33 ± 0.33
% transformation efficiency	18.52	22.22	14.81	29.63	29.63	37.04

Data are mean ± SE; n = 9; *significant differences of each treatment at $P \leq 0.01$

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Table S3. Micropropagation, rooting and acclimatization of tomato hybrids

Hybrids	Felina	Siena	Don Jose
Micropropagation index	4.67 ± 0.11^{a}	4.66 ± 0.12^{a}	4.59 ± 0.09^{a}
In vitro rooting	36.6 ± 0.88^{a}	32 ± 0.57^{b}	31 ± 1.15^{b}
In vivo acclimatization	10 ± 0.57^{a}	9 ± 1.52^{b}	8.33 ± 0.88^{b}
Acclimatized plants (%)	83.33	75.00	69.44

Data are mean ± SE; significant differences of each treatment at $P \leq 0.01$; values followed by the same letter are not statistically different; micropropagation index: n = 3 with 9 plants each; *in vitro* rooting for a total of 41, 41 and 44 plants from Don Jose, Siena and Felina hybrids; *in vitro* acclimatization: n = 3 with 12 plants each

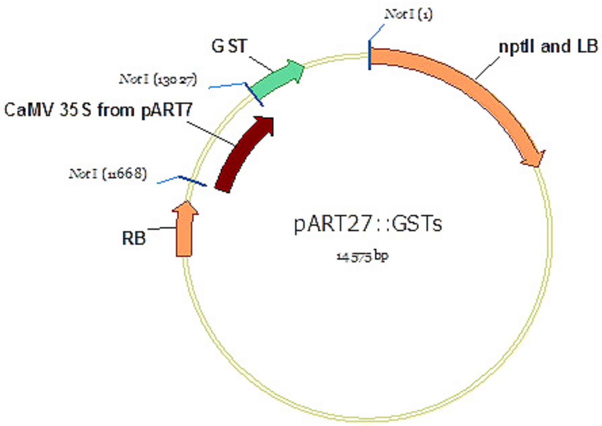

Figure S1. Map of the binary plasmid vector pART27::GSTs; according to Benekos *et al.* (2010), the transgene *GmGSTU4* was ligated into the pART7 EcoRI/HindIII restriction sites creating the primary cloning vector pART7-*GSTU4*, containing the open reading frame of the *GmGSTU4* gene under the control of CaMV 35S promoter; the expression cassette was then ligated in the T-DNA region into the *Not*I restriction sites of the pART27 binary vector pART27-GSTU4 with the 5'–3' oriented transgene being transferred into *Agrobacterium tumefaciens* octopine type strain LBA4404 by direct transformation

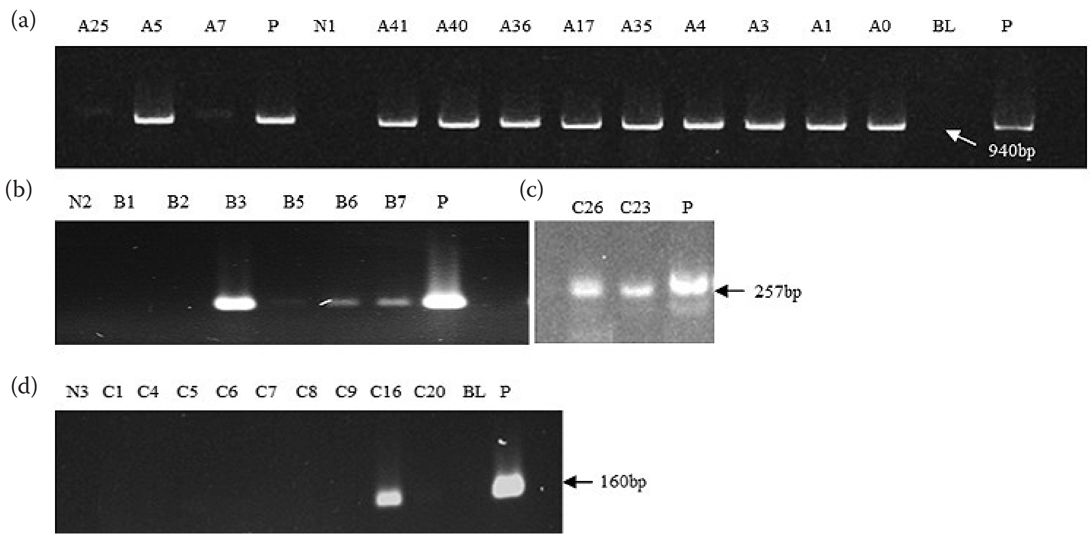

Figure S2. PCR verification of putative transformed tomato plants of the cultivars: (a) Felina: amplification of the region between the 35S promoter and the *GmGSTU4* gene (940 bp), (b) Siena, (c) Don Jose: amplification of a region within the *NPTII* gene (257 bp) and (d) Don Jose: amplification within the *GmGSTU4* gene (160 bp)

Lanes: N1 – wild type Felina; N2 – wild type Siena; N3 – wild type Don Jose; P – positive transgenic tobacco plant overexpressing the *GmGSTU4* gene; BL – blank sample (H_2O); A25, A5, A7, A41, A40, A36, A17, A35, A4, A3, A1, A0, B1, B2, B3, B5, B6, B7, C1, C4–9, C16 and C20 – putative transformed plants regenerated from individual events; the PCR conditions were: 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 20 s., annealing at 53°C for 20 s and elongation at 72°C for 30 s, with a final cycle of 1 min at 72°C

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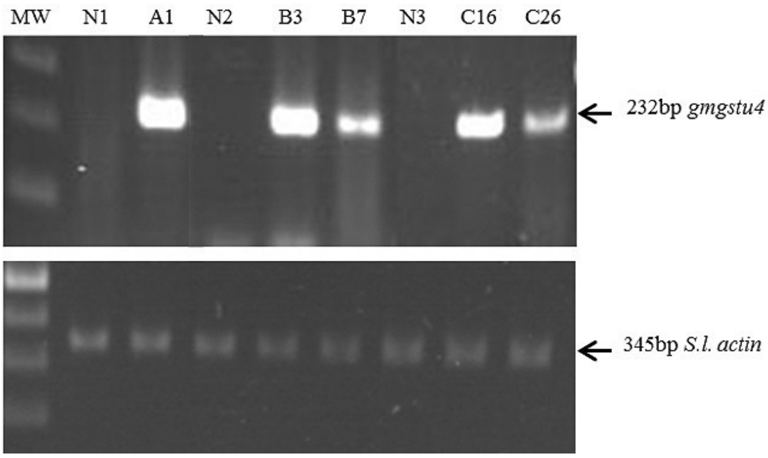

Figure S3. RT-PCR analysis of the overexpression of the *35S:GmGSTU4* transgene and the native *S. lycopersicum* actin gene RNA from the T_0 transgenic tomato plants was used

Lanes: MW – 2log ladder; N 1–3 – DNA samples from the wild-type plants of the N1(Felina), N2 (Siena) and N3 (Don Jose) cultivars; A1, B3, B7, C16, C26 – DNA samples from the transformed plants; the conditions of the RT-qPCR were: 95°C for 1 min, followed by 30 cycles of denaturation at 95°C for 5 s, annealing at 62°C for 20 s and elongation at 72°C for 3 s, with a final cycle of 10 min at 72°C; for the quantitative expression of the *GmGSTU4*, the critical values (C_T values) for the transgene and the actin reference gene were determined; each sample was run in triplicate and mean C_T values and standard deviations were used in the $\Delta\Delta C_T$ calculations; the fold difference in *gmgstu*4 relative to the endogenous control was calculated as $2^{-\Delta\Delta CT}$ with a range of $\Delta\Delta C_T$ + s and $\Delta\Delta C_T$ – s, where s is the standard deviation of the $\Delta\Delta C_T$ value