

Article

Heat Stress in *Pinus halepensis* Somatic Embryogenesis Induction: Effect in DNA Methylation and Differential Expression of Stress-Related Genes

Cátia Pereira ^{1,2}, Ander Castander-Olarieta ², Ester Sales ³, Itziar A. Montalbán ², Jorge Canhoto ^{1,*} and Paloma Moncaleán ^{2,*}

Supplementary Materials

Table S1. One-way analysis of variance for methylation rates (%) detected in *P. halepensis* embryonal masses (EMs) and needles from *in vitro* somatic plants induced under different temperature treatments (23 °C, 9 weeks; 40 °C, 4 h; 50 °C, 30 min; 60 °C, 5 min).

Kruskal-Wallis	df	X ² test	p value
EMs	3	5.960	n.s.
Needles	3	5.359	n.s.

¹ not statistically significant.

Table S2. Total methylation rates (%) detected in *P. halepensis* proliferating embryonal masses (EMs) and needles from *in vitro* somatic plants induced under different temperature treatments (23 °C (control); 40 °C, 4 h; 50 °C, 30 min; 60 °C, 5 min). Data are presented as mean values ± SE and significant differences at *p* < 0.05 are indicated by different letters.

Methylation (%)	23 °C (Control)	40 °C (4 h)	50 °C (30 min)	60 °C (5 min)
EMs	38.01 ± 0.72 ^a	39.48 ± 1.94 ^a	40.82 ± 0.84 ^a	37.52 ± 0.45 ^a
Needles	40.00 ± 0.21 ^a	40.25 ± 1.21 ^a	41.47 ± 0.43 ^a	41.56 ± 0.41 ^a

Table S3. One-way analysis of variance for expression of different genes detected in *P. halepensis* embryonal masses (EMs) and needles from *in vitro* somatic plants induced under different temperature treatments (23 °C, 9 weeks; 40 °C, 4 h; 50 °C, 30 min; 60 °C, 5 min).

Kruskal-Wallis	df	X ² Test	p Value
EMs			
<i>P439</i>	3	5.286	n.s. ¹
<i>P444</i>	3	6.795	n.s.
<i>DI19</i>	3	35.23	< 0.0001
<i>SOD</i>	3	7.057	n.s.
Needles			
<i>P439</i>	3	3.983	n.s.
<i>P444</i>	3	15.75	0.0013
<i>DI19</i>	3	3.297	n.s.
<i>SOD</i>	3	15.42	0.0015

¹ not statistically significant.