

Supplementary Material

Temperature and water availability during maturation affect the cytokinins and auxins profile of radiata pine somatic embryos

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1 Supplementary Tables

Table 1. Analysis of variance of the water availability (mg) in nine maturation media for *Pinus radiata*, containing different gellan gum concentrations (8, 9 and 10 gL⁻¹) and stored at different temperatures (18, 23 and 28°C).

Source	df	F value	p value
Temperature (T)	2	34.781	<0.001
Gellan gum (G)	2	9.298	<0.001
T x G	4	0.770	0.554

Table 2. Analysis of deviance of the logistic regression for the number of *Pinus radiata* somatic embryos per gram of embryonal mass in nine maturation media, containing different gellan gum concentrations (8, 9 and 10 gL⁻¹) and stored at different temperatures (18, 23 and 28°C).

Source	df	χ^2 test	p value
Temperature (T)	2	121.633	<0.001
Gellan gum (G)	2	70.068	<0.001
T x G	4	5.039	0.283

Table 3. Analysis of deviance of the logistic regression for germination rates of *Pinus radiata* somatic embryos from nine maturation media, containing different gellan gum concentrations (8, 9 and 10 gL⁻¹) and stored at different temperatures (18, 23 and 28°C).

Source	df	χ^2 test	<i>p</i> value
Temperature (T)	2	108.649	<0.001
Gellan gum (G)	2	7.939	<0.05
T x G	4	14.560	<0.01

Table 4. Analysis of variance of endogenous cytokinin bases [*cis*-zeatin (cZ), dihydrozeatin (DHZ), N6-isopentenyladenine (iP) (pmol g⁻¹ DW)] in *Pinus radiata* somatic embryos from nine different maturation treatments (matured at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ gellan gum).

Source	Variable	df	F value	<i>p</i> value
Temperature (T)	cZ	2	7.922	<0.01
	DHZ	2	16.907	<0.001
	iP	2	19.509	<0.001
Gellan gum (G)	cZ	2	1.902	0.178
	DHZ	2	57.568	<0.001
	iP	2	6.064	<0.05
T x G	cZ	4	7.438	<0.01
	DHZ	4	15.509	<0.001
	iP	4	16.612	<0.001

Table 5. Analysis of variance of endogenous cytokinin ribosides [*cis*-zeatin riboside (cZR), dihydrozeatin riboside (DHZR), N6-isopentenyladenosine (iPR) and *trans*-zeatin riboside (tZR) (pmol g⁻¹ DW)] in *Pinus radiata* somatic embryos from nine different maturation treatments (maturated at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ gellan gum).

Source	Variable	df	F value	<i>p</i> value
Temperature (T)	tZR	2	3.143	0.069
	cZR	2	0.623	0.548
	DHZR	2	1.547	0.241
	iPR	2	18.946	<0.001
Gellan gum (G)	tZR	2	6.280	<0.01
	cZR	2	4.567	<0.05
	DHZR	2	4.154	<0.05
	iPR	2	13.976	<0.001
T x G	tZR	4	13.698	<0.001
	cZR	4	3.632	<0.05
	DHZR	4	4.219	<0.05
	iPR	4	23.416	<0.001

Table 6. Analysis of variance of endogenous cytokinin *O*-glucosides [cis-zeatin *O*-glucoside (cZOG), dihydrozeatin *O*-glucoside (DHZOG) (pmol g⁻¹ DW)] in *Pinus radiata* somatic embryos from nine different maturation treatments (matured at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ gellan gum).

Source	Variable	df	F value	<i>p</i> value
Temperature (T)	cZOG	2	22.143	<0.001
	DHZOG	2	2.471	0.116
Gellan gum (G)	cZOG	2	13.583	<0.001
	DHZOG	2	14.490	<0.001
T x G	cZOG	4	13.584	<0.001
	DHZOG	4	10.846	<0.001

Table 7. Analysis of variance of endogenous isoprenoid cytokinin pools [cis-zeatin pool (cZ-type), dihydrozeatin pool (DHZ-type), N6-isopentenyladenine pool (iP-type) and trans-zeatin pool (tZ-type) (pmol g⁻¹ DW)] in *Pinus radiata* somatic embryos from nine different maturation treatments (maturated at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ gellan gum).

Source	Variable	df	F value	p value
Temperature (T)	tZ-type	2	3.747	<0.05
	cZ-type	2	0.612	0.553
	DHZ-type	2	15.042	<0.001
	iP-type	2	14.363	<0.001
Gellan gum (G)	tZ-type	2	6.459	<0.01
	cZ-type	2	4.259	<0.05
	DHZ-type	2	50.588	<0.001
	iP-type	2	17.502	<0.001
T x G	tZ-type	4	14.549	<0.001
	cZ-type	4	3.546	<0.05
	DHZ-type	4	14.299	<0.001
	iP-type	4	17.243	<0.001

Table 7. Analysis of variance of endogenous cytokinin pools based on structural and functional forms [bases, ribosides and *O*-glucosides (pmol g⁻¹ DW)] in *Pinus radiata* somatic embryos from nine different maturation treatments (matured at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ gellan gum).

Source	Variable	df	F value	<i>p</i> value
Temperature (T)	Bases	2	9.450	<0.01
	Ribosides	2	4.206	<0.05
	<i>O</i> -glucosides	2	23.805	<0.001
Gellan gum (G)	Bases	2	52.845	<0.001
	Ribosides	2	3.908	<0.05
	<i>O</i> -glucosides	2	13.428	<0.001
T x G	Bases	4	20.951	<0.001
	Ribosides	4	9.132	<0.001
	<i>O</i> -glucosides	4	14.362	<0.001

Table 8. Endogenous levels (pmol g⁻¹ DW) of cytokinin bases in somatic embryos from nine different treatments (maturated at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ Gelrite®). Mean ± standard error; BA, N6-benzyladenine; cZ, *cis*-zeatin; DHZ, dihydrozeatin; iP, N6-isopentenyladenine; mT, *meta*-topolin; oT, *ortho*-topolin; pT, *para*-topolin; tZ, *trans*-zeatin.

Treatment	tZ	cZ	DHZ	iP	BA	oT	mT	pT
18°C 8 g/L	<LOD	1.15 ± 0.21	144.34 ± 20.25	9.04 ± 1.47	<LOD	<LOD	<LOD	<LOD
18°C 9 g/L	<LOD	0.40 ± 0.05	5.83 ± 1.509	4.47 ± 0.52	<LOD	<LOD	<LOD	<LOD
18°C 10 g/L	<LOD	0.96 ± 0.05	8.68 ± 0.89	2.43 ± 0.34	<LOD	<LOD	<LOD	<LOD
23°C 8 g/L	<LOD	0.99 ± 0.12	151.67 ± 27.91	13.38 ± 2.65	<LOD	<LOD	<LOD	<LOD
23°C 9 g/L	<LOD	0.63 ± 0.09	23.90 ± 4.79	5.87 ± 0.80	<LOD	<LOD	<LOD	<LOD
23°C 10 g/L	<LOD	0.93 ± 0.16	14.80 ± 2.50	5.92 ± 1.28	<LOD	<LOD	<LOD	<LOD
28°C 8 g/L	<LOD	1.34 ± 0.14	8.85 ± 0.87	6.32 ± 0.66	<LOD	<LOD	<LOD	<LOD
28°C 9 g/L	<LOD	1.91 ± 0.36	12.49 ± 2.33	12.53 ± 2.28	<LOD	<LOD	<LOD	<LOD
28°C 10 g/L	<LOD	0.76 ± 0.16	11.54 ± 2.32	36.97 ± 7.04	<LOD	<LOD	<LOD	<LOD

Table 9. Endogenous levels (pmol g⁻¹ DW) of cytokinin ribosides in somatic embryos from nine different treatments (maturated at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ Gelrite®). Mean ± standard error; BAR, N6-benzyladenosine; cZR, *cis*-zeatin riboside; DHZR, dihydrozeatin riboside; iPR, N6-isopentenyladenosine; mTR, *meta*-topolin riboside; oTR, *ortho*-topolin riboside; pTR, *para*-topolin riboside; tZR, *trans*-zeatin riboside.

Treatment	tZR	cZR	DHZR	iPR	BAR	oTR	mTR	pTR
18°C 8 g/L	6.69 ± 0.45	101.11 ± 18.86	25.18 ± 9.98	70.41 ± 11.09	<LOD	<LOD	<LOD	<LOD
18°C 9 g/L	5.04 ± 0.49	45.26 ± 7.52	0.67 ± 0.34	23.43 ± 2.88	<LOD	<LOD	<LOD	<LOD
18°C 10 g/L	15.49 ± 2.42	60.41 ± 6.24	3.50 ± 0.87	51.87 ± 7.82	<LOD	<LOD	<LOD	<LOD
23°C 8 g/L	7.93 ± 1.34	100.13 ± 13.68	10.75 ± 0.92	45.82 ± 6.43	<LOD	<LOD	<LOD	<LOD
23°C 9 g/L	4.45 ± 0.94	45.36 ± 7.32	10.22 ± 0.85	45.94 ± 7.80	<LOD	<LOD	<LOD	<LOD
23°C 10 g/L	12.25 ± 1.85	82.37 ± 15.03	8.93 ± 2.36	182.11 ± 23.86	<LOD	<LOD	<LOD	<LOD
28°C 8 g/L	3.57 ± 0.69	76.08 ± 15.25	4.38 ± 1.89	38.09 ± 7.52	<LOD	<LOD	<LOD	<LOD
28°C 9 g/L	11.81 ± 2.29	98.11 ± 14.54	5.92 ± 0.59	58.38 ± 4.76	<LOD	<LOD	<LOD	<LOD
28°C 10 g/L	2.56 ± 0.49	62.34 ± 11.25	5.66 ± 1.61	27.46 ± 4.54	<LOD	<LOD	<LOD	<LOD

Table 10. Endogenous levels (pmol g⁻¹ DW) of the cytokinin nucleotides in somatic embryos from nine different treatments (matured at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ Gelrite®). BARMP, N6-benzyladenosine-5'-monophosphate; cZRMP, *cis*-zeatin riboside-5'-monophosphate; DHZRMP, dihydrozeatin riboside-5'-monophosphate; iPRMP, N6-isopentenyladenosine-5'-monophosphate; tZRMP, *trans*-zeatin riboside-5'-monophosphate.

Treatment	tZRMP	cZRMP	DHZRMP	iPRMP	BARMP
18°C 8 g/L	<LOD	<LOD	<LOD	<LOD	<LOD
18°C 9 g/L	<LOD	<LOD	<LOD	<LOD	<LOD
18°C 10 g/L	<LOD	<LOD	<LOD	<LOD	<LOD
23°C 8 g/L	<LOD	<LOD	<LOD	<LOD	<LOD
23°C 9 g/L	<LOD	<LOD	<LOD	<LOD	<LOD
23°C 10 g/L	<LOD	<LOD	<LOD	<LOD	<LOD
28°C 8 g/L	<LOD	<LOD	<LOD	<LOD	<LOD
28°C 9 g/L	<LOD	<LOD	<LOD	<LOD	<LOD
28°C 10 g/L	<LOD	<LOD	<LOD	<LOD	<LOD

Table 11. Endogenous levels (pmol g⁻¹ DW) of cytokinin *O*-glucosides in somatic embryos from nine different treatments (maturated at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ Gelrite®). Mean ± standard error; cZOG, *cis*-zeatin *O*-glucoside; cZROG, *cis*-zeatin riboside *O*-glucoside; DHZOG, dihydrozeatin *O*-glucoside; DHZROG, dihydrozeatin riboside *O*-glucoside; tZOG, *trans*-zeatin *O*-glucoside; tZROG, *trans*-zeatin riboside *O*-glucoside.

Treatment	tZOG	tZROG	cZOG	cZROG	DHZOG	DHZROG
18°C 8 g/L	<LOD	<LOD	0.28 ± 0.04	<LOD	0.38 ± 0.07	<LOD
18°C 9 g/L	<LOD	<LOD	0.17 ± 0.09	<LOD	0.05 ± 0.01	<LOD
18°C 10 g/L	<LOD	<LOD	0.32 ± 0.06	<LOD	0.15 ± 0.02	<LOD
23°C 8 g/L	<LOD	<LOD	0.20 ± 0.10	<LOD	0.27 ± 0.01	<LOD
23°C 9 g/L	<LOD	<LOD	0.12 ± 0.01	<LOD	0.07 ± 0.04	<LOD
23°C 10 g/L	<LOD	<LOD	0.20 ± 0.04	<LOD	0.07 ± 0.01	<LOD
28°C 8 g/L	<LOD	<LOD	0.42 ± 0.10	<LOD	0.09 ± 0.02	<LOD
28°C 9 g/L	<LOD	<LOD	0.69 ± 0.03	<LOD	0.22 ± 0.03	<LOD
28°C 10 g/L	<LOD	<LOD	3.72 ± 0.79	<LOD	0.10 ± 0.02	<LOD

Table 12. Endogenous levels (pmol g⁻¹ DW) of CK N-glucosides in somatic embryos from nine different treatments (matured at 18, 23 and 28°C and at 8, 9 and 10g/L-1 Gelrite®). BA9G, N6-benzyladenine-9-glucoside; cZ9G, *cis*-zeatin-9-glucoside; DHZ7G, dihydrozeatin-7-glucoside; DHZ9G, dihydrozeatin-9-glucoside; iP7G, N6-isopentenyladenine-7-glucoside; iP9G, N6-isopentenyladenine-9-glucoside; mT9G, *meta*-topolin-9-glucoside; oT9G, *ortho*-topolin-9-glucoside; tZ7G, *trans*-zeatin-7-glucoside; tZ9G, *trans*-zeatin-9-glucoside.

Treatment	tZ7G	tZ9G	cZ9G	DHZ7G	DHZ9G	iP7G	iP9G	BA9G	oT9G	mT9G
18°C 8 g/L	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
18°C 9 g/L	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
18°C 10 g/L	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
23°C 8 g/L	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
23°C 9 g/L	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
23°C 10 g/L	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
28°C 8 g/L	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
28°C 9 g/L	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
28°C 10 g/L	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table 13. Endogenous isoprenoid cytokinin pools (pmol g⁻¹ DW) in somatic embryos from nine different treatments (matured at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ Gelrite®) mean ± standard error. Endogenous aromatic cytokinins were under the limit of detection.

Treatment	tZ-type	cZ-type	DHZ-type	iP-type
18°C 8 g/L	6.69 ± 0.45	102.54 ± 19.06	169.90 ± 27.46	79.44 ± 9.64
18°C 9 g/L	5.04 ± 0.49	45.83 ± 7.66	6.55 ± 0.77	27.90 ± 3.23
18°C 10 g/L	15.49 ± 2.42	61.69 ± 6.31	12.33 ± 0.98	54.30 ± 7.98
23°C 8 g/L	7.93 ± 1.34	101.32 ± 13.85	162.69 ± 28.77	59.19 ± 7.79
23°C 9 g/L	4.45 ± 0.94	46.11 ± 7.41	34.19 ± 5.48	51.81 ± 8.15
23°C 10 g/L	12.25 ± 1.85	83.50 ± 15.21	23.80 ± 4.15	188.03 ± 25.14
28°C 8 g/L	3.57 ± 0.69	77.84 ± 15.46	13.33 ± 2.76	44.41 ± 8.04
28°C 9 g/L	11.81 ± 2.29	100.71 ± 14.87	18.63 ± 2.92	70.90 ± 6.86
28°C 10 g/L	2.56 ± 0.49	66.82 ± 12.03	17.30 ± 3.24	64.43 ± 11.23

Table 14. Endogenous total cytokinin pools based on structural and functional forms (pmol g⁻¹ DW) in somatic embryos from nine different treatments (matured at 18, 23 and 28°C and at 8, 9 and 10gL⁻¹ Gelrite®) mean ± standard error. Nucleotides and N-glucosides were under the limit of detection.

Treatment	Bases	Ribosides	O-glucosides
18°C 8 g/L	154.52 ± 19.03	203.38 ± 39.03	0.67 ± 0.11
18°C 9 g/L	10.70 ± 0.58	74.39 ± 8.92	0.22 ± 0.09
18°C 10 g/L	12.06 ± 1.07	131.27 ± 16.74	0.47 ± 0.07
23°C 8 g/L	166.03 ± 28.93	164.63 ± 20.84	0.47 ± 0.14
23°C 9 g/L	30.39 ± 4.20	105.98 ± 16.24	0.19 ± 0.04
23°C 10 g/L	21.65 ± 3.89	285.66 ± 42.27	0.27 ± 0.06
28°C 8 g/L	16.52 ± 1.64	122.12 ± 25.30	0.51 ± 0.12
28°C 9 g/L	26.93 ± 4.93	174.21 ± 20.81	0.91 ± 0.02
28°C 10 g/L	49.27 ± 6.11	98.02 ± 16.70	3.82 ± 0.81

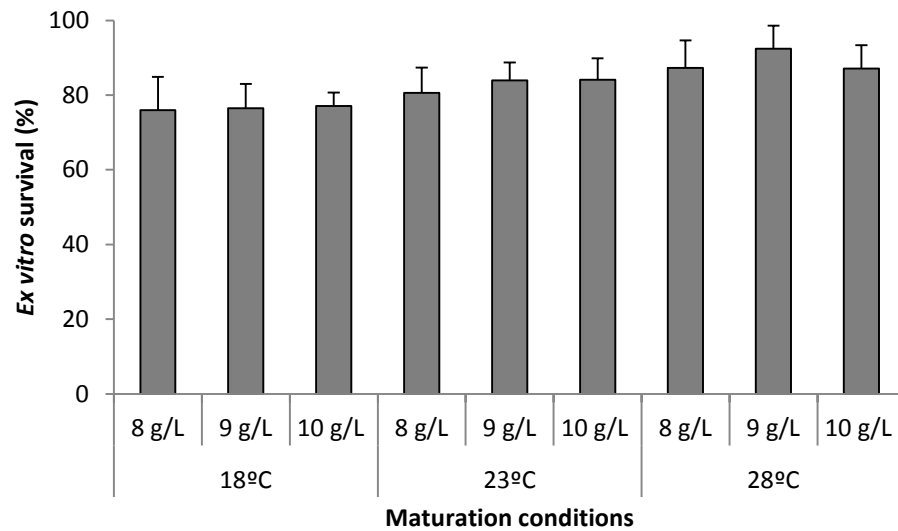


Fig. 1. *Ex vitro* survival (%) in *Pinus radiata* plantlets coming from embryogenic cell lines matured at three different temperatures (18, 23 and 28°C) and three gellan gum concentrations (8, 9, 10 g L⁻¹).

