

Supplementary Materials

(Tables S1, S2 and S3)

Somaclonal variation during *Picea abies* and *P. omorika* somatic embryogenesis and cryopreservation

Acta Biologica Cracoviensia Series Botanica

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TABLE S1. The set of microsatellite loci used to monitor genetic integrity of plant material maintained *in vitro* and in liquid nitrogen.

Locus	Primer sequence	Repeat motif	Dye label	Annealing temperature (°C)
SpAGC1	F: TTCACCTTAGCCGAGAACC R: CACTGGAGATCTTCGTTCTGA	(TC) _n TT(TC) _n	6-FAM	57
SpAGC2	F: TACCATTCAACGCAAGGG R: GTGTATGGTTTTCTTTTCGCA	(TA) _n (GA) _n	NED	57
SpAGG3	F: CTCCAACATTTCCATGTAGC R: AGCATGTTGACCCATATAGACC	(GA) _n	HEX	57
SpAC1F7	F: TTCCTCCACTGCATTCTAGC R: TGTTGGCCTTGCAAGTTATAG	(AC) _n	HEX	57-60
SpAC1H8	F: CCCAAGAAAAAAGTCATGGAT R: CCATTGGGATATGTGATACTTCC	(GT) _n	6-FAM	57-60
EATC1E03	F: CCCCTTATTCTAACGTCAAA R: TACCAGTGGTGACAACGATG	(CAT) _n CGT(CAT) _n	6-FAM	57°C
EATC1D02	F: TGGCATGAGATTTATGTGGTT R: GTGTGCCACTCAACCTCAC	(CAT) _n CGT(CAT) _n	HEX	57°C
EATC1B02	F: TTGTCATCGTCGTCATTGTC R: TTTTAGCCTCTGTTTTCTAGC	(TCC) _n N ₁₅ (TCA) _n	6-FAM	60°C
EATC2B02	F: GATGGATCTATGTTGGTTCACC R: TTGGTCTCAAGGGAAGTTAATC	(CAT) _n N ₅₂ (ATC) _n (AAC)	NED	57°C

WS0023.B03	F: AGCAGCTGGGGTCAAAGTT	(AT) _n	6-FAM	53°C
	R: AAAGAAAGCATGCATATGACTCAG			
WS0019.M09	F: TTTCCAATCGGAGTGCATTG	(AT) _n	VIC	53°C
	R: GGAGATCGTGGTAACCCAA			
WS0022.B15	F: TTTGTAGGTGCTGCAGAGATG	(AG) _n	NED	53°C
	R: TGGCTTTTTATTCCAGCAAGA			
WS00716.F13	F: TCAAGTAATGGACAAACGATACA	(GA) _n	PET	53°C
	R: TTTCCAATAGAATGGTGGATTT			

TABLE S2. Genetic stability of embryogenic cultures of *Picea abies* and *P. omorika* maintained *in vitro* (Experiment 1). Detected mutation are in bold type.

Species	ET Line	Material	SpAGC1	SpAGG3	SpAGC2	SpAC1F7	SpAC1H8	EATC1E03	EATC1D02	EATC1B02	
<i>Picea abies</i>		Mother Tree	100/100	85/85	124/128	96/96	126/126	190/193	193/208	192/192/0	
	K1	ETv	76/96	85/87	120/124	96/96	126/126	190/190	211/211	198/198	
		CSE	76/96	85/87	120/124	96/96	126/126	190/190	211/211	198/198	
		GSE	76/96	85/87	120/124	96/96	126/126	190/190	211/211	198/198	
	K2	ETv	100/100	85/97	126/126	96/106	156/156	190/193	193/193	198/198	
		CSE	100/100	85/97	126/126	96/96	124/156	190/193	208/208	198/198	
		GSE	100/100	85/97	126/126	96/96	124/156	190/193	193/193	198/198	
	K3	ETv	100/102	85/87	118/124	96/96	94/126	190/193	208/211	198/198	
		CSE	100/102	85/87	118/124	106/114	94/126	190/193	208/211	198/198	
		GSE	100/102	85/87	118/124	96/96	94/126	190/193	208/211	198/198	
			Mother Tree	100/100/0	89/89/0	128/146	96/106	92/96	193/193	205/205	198/198
	S1	ETv	76/100	89/89	108/146	96/106	96/96	172/193	205/205	198/198	
		CSE	76/100	89/89	108/146	96/106	96/96	172/193	205/205	198/198	
		SS	76/100	89/89	108/146	96/106	96/96	172/193	205/205	198/198	
	S2	ETv	96/96	89/89	128/128	96/106	92/122	172/193	205/205	198/198	
		CSE	96/96	89/89	128/128	96/106	92/122	172/193	205/205	198/198	
		GSE	96/96	89/89	128/128	96/106	92/122	172/193	205/205	198/198	
	S3	ETv	96/118	89/89	132/148	106/106	96/102	172/193	205/205	198/198	
		CSE	96/118	89/89	132/148	106/106	96/102	172/193	205/205	198/198	
		GSE	96/118	89/89	132/148	106/106	96/102	172/193	205/205	198/198	
	S4	ETv	96/118	89/123	122/124	96/108	98/122	172/193	193/214	198/198	
		CSE	96/118	89/123	122/124	96/108	98/122	172/193	193/214	198/198	
		GSE	96/118	89/123	122/124	96/108	98/122	172/193	193/214	198/198	
	S5	ETv	86/118	85/85	128/136	96/108	98/98	172/193	196/196	198/198	
		CSE	86/118	85/85	128/136	96/108	98/98	172/193	196/214	198/198	
		GSE	86/118	85/85	128/136	96/108	98/98	172/193	196/196	198/198	
	S6	ETv	100/100	83/83	126/128	96/108	92/92	172/193	196/214	198/198	
		CSE	100/100	83/83	126/128	96/108	92/92	172/193	196/214	198/198	
		GSE	100/100	83/83	126/128	96/108	92/92	172/193	196/214	198/198	
	S7	ETv	70/70	83/83	128/128	98/98	92/120	172/193	196/208	198/198	
		CSE	70/70	83/83	128/128	96/96	92/120	172/193	196/208	198/198	
		GSE	70/70	83/83	128/128	96/96	92/120	172/193	196/208	198/198	
	<i>P. omorika</i>	AK1.1	Mother Tree	120/120	179/179	140/140	96/96	110/110	181/181	199/212	186/186

	ETV	120/120	179/179	140/140	82/82	110/110	181/184	199/199	186/186
	CSE	120/120	179/179	140/140	92/92	110/110	181/184	199/199	186/186
	SS	120/120	179/179	140/140	92/92	110/110	181/184	199/199	186/186
AK1.2	ETV	120/120	179/179	140/140	96/96	110/110	181/184	199/199	186/186
	CSE	120/120	179/179	140/140	96/96	110/110	181/184	199/199	186/186
	GSE	120/120	179/179	140/140	96/96	110/110	181/184	199/199	186/186
AK1.3	ETV	120/120	179/179	132/140	96/96	110/110	181/184	199/199	186/186
	CSE	120/120	179/179	132/140	96/96	110/110	181/184	199/199	186/186
	SS	120/120	179/179	132/140	96/96	110/110	181/184	199/199	186/186
AK2.1	Mother								
	Tree	108/116	155/175	128/134	96/96	82/112	181/181	196/196	186/186
	ETV	106/108	175/175	134/134	96/96	112/112	181/184	-	186/186
	CSE	106/108	175/175	134/134	96/96	112/112	181/184	-	186/186
	SS	106/108	175/175	134/134	96/96	112/112	181/184	-	186/186
AK2.2	ETV	106/116	155/175	128/128	96/96	112/112	181/184	-	186/186
	CSE	106/116	155/175	128/128	96/96	112/112	181/184	-	186/186
	SS	106/116	155/175	128/128	96/96	112/112	181/184	-	186/186
AK2.3	ETV	106/116	155/175	134/134	96/96	112/112	181/184	-	186/186
	CSE	106/116	155/175	134/134	94/94	112/112	181/184	-	186/186
	SS	106/116	155/175	134/134	96/96	112/112	181/184	-	186/186
AK2.4	ETV	102/102	157/157	116/132	96/96	82/112	181/205	196/196	186/206
	CSE	102/102	157/157	116/132	96/96	82/112	181/205	196/196	186/206
	GSE	102/102	157/157	116/132	96/96	82/112	181/205	196/196	186/206
AK2.5	ETV	106/116	167/167	128/138	96/96	82/112	181/187	196/196	186/206
	CSE	106/116	167/167	128/138	96/96	82/112	181/187	196/196	186/206
	GSE	106/116	167/167	128/138	96/96	82/112	181/187	196/196	186/206
AK2.6	ETV	106/108	167/189	128/138	96/96	82/110	181/187	196/196	186/206
	CSE	106/108	167/189	128/138	96/96	82/110	181/187	196/196	186/206
AK2.7	ETV	106/116	155/155	128/134	96/96	82/112	181/187	196/196	186/206
	CSE	106/114	155/155	128/134	96/96	82/112	181/187	196/196	-
	GSE	106/114	155/155	128/134	96/96	82/112	181/187	196/196	186/206
AK2.8	ETV	108/116	155/155	130/134	96/96	82/110	181/187	196/196	186/206
	CSE	108/112	155/155	130/134	96/96	82/110	181/187	196/196	186/206
	GSE	112/128	155/155	130/134	96/96	82/110	181/187	196/196	186/206
AK2.9	ETV	116/116	151/165	128/128	96/96	82/110	181/187	196/196	186/206
	CSE	116/116	151/165	128/128	96/96	82/110	181/187	196/196	186/206
	GSE	112/128	151/165	128/128	96/96	82/110	181/187	196/196	186/206
AK2.10	ETV	106/116	153/175	134/138	96/96	82/110	181/187	196/196	186/206
	CSE	106/116	153/175	134/138	96/96	82/110	181/187	196/196	186/206
	GSE	106/116	153/175	134/138	96/96	82/110	181/187	196/196	-
DK1.1	Mother								
	Tree	108/108	165/171	124/130	96/96	82/112	181/181	196/196	186/186
	ETV	108/108	171/171	130/142	96/96	82/112	181/187	196/196	186/206
	CSE	108/108	171/171	130/142	96/96	82/112	181/187	196/196	186/206

	GSE	108/108	171/171	130/142	96/96	82/112	181/187	196/196	186/206
DK1.2	ETv	106/108	165/175	124/136	96/96	82/112	181/187	196/196	186/206
	CSE	106/108	165/175	124/136	96/96	82/112	181/187	196/196	186/206
	GSE	106/108	165/175	124/136	96/96	82/112	181/187	196/196	186/206
DK2.1	Mother								
	Tree	108/108/0	161/171	124/134	96/96	82/110	181/181	196/196	186/186
	ETv	-	161/161	128/134	96/96	82/110	181/187	196/196	186/206
	CSE	-	171/175	124/136	96/96	82/110	181/187	196/196	186/206
DK2.2	ETv	106/108	171/171	124/142	96/96	82/110	181/187	196/196	186/206
	CSE	106/108	171/171	124/142	96/96	82/110	181/187	196/196	186/206
DK2.3	ETv	120/120	171/171	124/136	96/96	82/110	181/187	196/196	186/206
	CSE	120/120	171/171	124/136	96/96	82/110	181/187	196/196	186/206
DK2.4	ETv	120/120	171/171	124/142	96/96	82/110	181/187	196/196	186/206
	CSE	120/120	167/167	124/142	96/96	82/110	181/187	196/196	186/206

K, S, AK1, AK2, DK1, DK2 – origin of ET lines; successive numbers –line number of the specific origin; ETv – embryogenic tissue maintained in vitro (3-27 months; See Tab.1, main text; CSE – 5-week-old somatic embryos at the cotyledonary stage; GSE – 1-month-old germinating somatic embryos or SS – 6-month-old somatic seedlings (needles). The mother’s genotypes with null alleles are shaded and mutations are in italics.

TABLE S3. Genetic stability of embryogenic cultures of *Picea abies* and *P. omorika* after cryopreservation using the pregrowth-dehydration method (Experiment 2).

Species	ET line	Material	SpAGC1	SpAGC2	SpAGG3	SpAC1F7	SpAC1H8	EATC1E03	EATC1D02	EATC1B02	EATC2B02	WS0023.B03	WS0019.M09	WS0022.B15	WS00716.F13
<i>P. abies</i>	K4	Mother													
		Tree	100/100	124/128	85/85	96/96/0	126/126	190/193	193/208	192/192	182/182	193/207	219/219	192/192	228/228
		ET v	100/100	124/128	85/85	116/116	126/166	172/187	206/208	198/202	182/182	193/193	219/219	192/194	228/238
		ET c	100/100	124/128	85/85	116/116	126/166	172/187	206/208	198/202	182/182	193/193	219/219	192/194	228/238
		SE 3 ET c	100/100	124/128	85/85	116/116	126/166	172/187	206/208	198/202	182/182	193/193	219/219	192/194	228/238
		SE 5 ET c	100/100	124/128	85/85	116/116	126/166	172/187	206/208	198/202	182/182	193/193	219/219	192/194	228/238
<i>P. omorika</i>	AK2.6*	Mother													
		Tree	108/116	128/134	155/175	96/96/0	82/112	181/181/0	196/196/0	186/186/0	185/185	191/213	255/255	190/198	236/242
		ET v	114/114	124/128	153/153	94/94	112/132	178/178	203/203	172/172	185/185	191/213	255/255	190/200	236/242
		ET c	106/106	126/132	153/153	94/94	112/132	178/178	203/203	172/172	185/185	191/213	255/255	190/200	236/236
		SE 3 ET c	114/114	124/126	153/153	94/94	112/132	178/178	203/203	172/172	185/185	191/213	255/255	190/200	236/242
		SE 5 ET c	114/114	124/126	153/153	94/94	112/132	178/178	203/203	172/172	185/185	191/213	255/255	190/200	236/242
	DK1.3	Mother													
		Tree	108/108	124/130	165/171	96/96/0	96/96/0	181/181/0	196/196/0	186/186/0	185/185	217/217	257/257	190/202	236/240
		ET v	106/108	130/132	167/167	94/94	114/114	178/178	203/203	172/172	185/185	199/217	257/257	190/200	232/240
		ET c	106/108	130/132	167/167	94/94	114/114	178/178	203/203	172/172	185/185	199/217	257/257	190/200	232/240
		SE 3 ET c	106/108	130/132	167/167	94/94	114/114	178/178	203/203	172/172	185/185	199/217	257/257	190/200	232/240
		SE 5 ET c	106/108	130/132	167/167	94/94	114/114	178/178	203/203	172/172	185/185	199/217	257/257	190/200	232/240

K, S, AK1, AK2, DK1, DK2 – origin of ET lines; successive numbers –line number of the specific origin; ETv - embryogenic tissue maintained in vitro (18 to 44 months; See Tab.1, main text) ; ETc – embryogenic tissue recovered after cryopreservation; SE 3 ET c and SE 5 ET c– somatic embryos at 3 and 5 weeks on the maturation medium obtained from ET stored in liquid nitrogen; * this cell line was also analyzed previously, after maintenance in vitro only (See Tab.1 and 3, Supplementary material). The mother’s genotypes with null alleles are shaded and mutations are in italics.