Somatic embryogenesis as an effective regeneration support for reverse genetics in maritime pine: the Sustainpine collaborative project as an illustration

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Somatic embryogenesis as an effective regeneration support for reverse genetics in maritime pine: the Sustainpine collaborative project as an illustration

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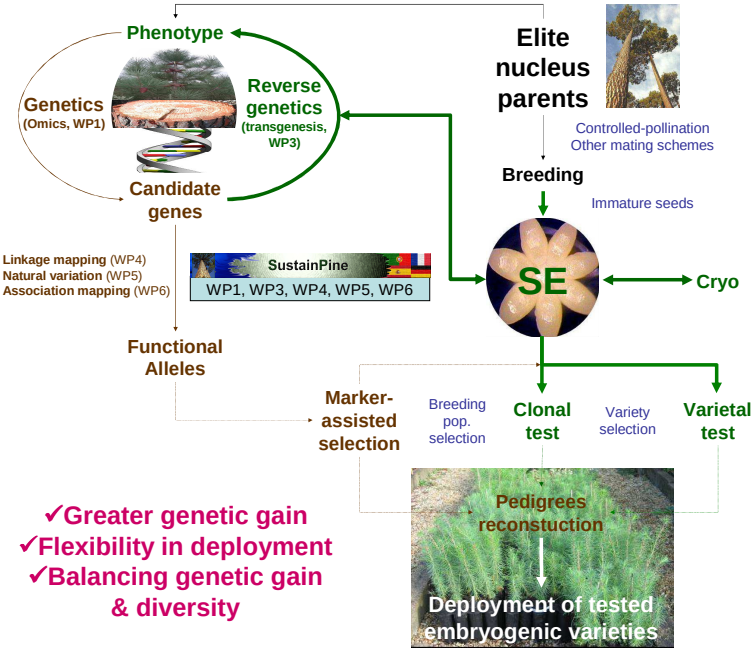
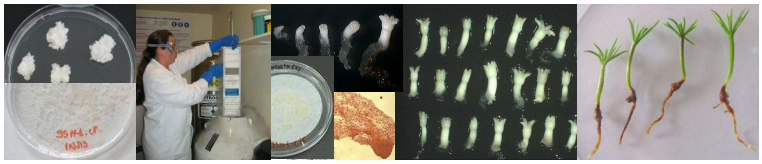
1- SE: a key technology in maritime pine breeding towards multivarietal forestry (MVF, Park Y.S., see Klimaszewska *et al.* 2007)



✓Greater genetic gain
✓Flexibility in deployment
✓Balancing genetic gain & diversity

2- SE in model genotype (PN519) for reverse genetics: effective transfer to Sustainpine partners

Published protocols P5, P7, P8 (reviewed in Klimaszewska *et al.* 2007)



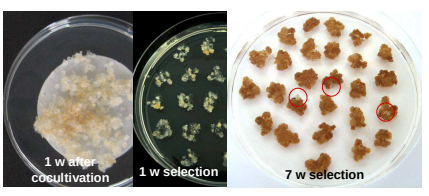
Multiplication	Cryo/react.	Maturation	Conversion
Callus/plating method	Slow cooling	Plating method	Petri dish/Magenta box
mLV, sucrose 0.09M	mLV, sucrose 0.09-0.5M	mLV, sucrose 0.2M	mLV, sucrose 0.09M
2,4D/BA	2,4D/BA, DMSO	ABA 80 µM, Gelrite 9 g.l^{-1}	No PGR
✓High rate	✓100% recovery	✓ Minor adaptations	✓Correct
All parters	All parters		
Increase in f.m. (%)		**P3** 72 SE g^{-1} f.m.	**P3** 35%
P8 1582±121 (ref.)		**P4** 18 (quite low)	**P5** 31-46
		P5 103-221	**P7/P8** 35-45 (ref.)
		P7/P8 85±16 (ref.)	**P10** 50-60
		P10 60-120 (modified prot.)	

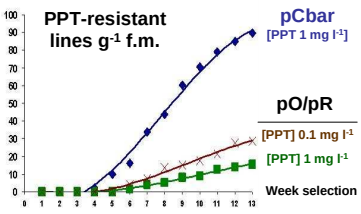
3- PN519 transformation rate (TR) with ref. vector

Published protocols P5, P7, P8 (reviewed in Trontin *et al.* 2007) + common protocol P7/P8

4- TR with selected overexpression (OE) & ihpRNA (RNAi) vectors (Karimi *et al.* 2002): optimization

Selection	P3 Modified	P4 Standard	P5 Modified	P8 Reduced [PPT] = 0.1 mg l^{-1}	P10 Reduced [PPT] = 0.1 mg l^{-1}
pMBb7Fm21GW-UBIL **(OE, pO, *bar*)**	26.4	nd	67.2	8.0	4.0
pBb7GW-I-WG-UBIL **(RNAi, pR, *bar*)**	9.1	3.0	nd	6.0	8.7





✓Lower selection efficiency compared with ref. vector pCbar
✓Modified selection with improved yield in development
✓Reduced [PPT] improved yield & growth of transgenic lines

5- Ongoing transgenesis in the frame of Sustainpine (2010-2013): 63 constructs from 39 genes

	Planned	Achieved*	In prog.^{ip}
U. Alcalá (P3)	9		6
U. València (P4)	12		12
IBET (P5)	14		13
FCBA/INRA (P8/P7)	4^{pp}	4^{pp}	
FCBA (P8)	14	12	
HUB (P10)	10		9
Total	63	16	40

^{pp}Previous project

Wood formation OE	Wood formation RNAi	C/N metabolism Ammonium regulation OE	C/N metabolism Ammonium regulation RNAi	Stress resistance (drought, nutrition) OE	Stress resistance (drought, nutrition) RNAi	Development Embryogenesis OE	Development Embryogenesis RNAi
PAL	PAL*	PII^{ip}	PII^{ip}			UPK	
CAD^{ip}	CAD^{*pp}	IDH^{ip}				Rab1^{ip}	Rab1^{ip}
	COMT*	Susy^{ip}	Susy^{ip}			LPT1^{ip}	LPT1^{ip}
	LAC*	GS1a*	GS1a^{ip}			LEC^{ip}	
LAC		GS1b	GS1b^{ip}			SERK^{ip}	
CesA2		GS1b (gfp)^{ip}				BBM	
XETG^{ip}	XETG*	GS2^{ip}				WOX2^{ip}	
MYB8^{ip}	MYB8*	Dof5^{ip}	Dof5				
LIM^{ip}	LIM^{ip}		Fd-GOGAT^{ip}				
	KOR^{*pp}		NADH-GOGAT^{ip}				
	GRP^{*pp}	ASN1^{ip}	ASN1^{ip}				
	MYB14^{*pp}	ASPG^{ip}	ASPG^{ip}				
		AAT^{ip}					
		GDC^{ip}	GDC^{ip}	NOD^{ip}	NOD^{ip}		
		ARS20^{ip}	ARS20^{ip}	AP2^{ip}	AP2^{ip}		
		dOAT^{ip}	dOAT^{ip}	DH2^{ip}	DH2^{ip}		
MYB5^{ip}		AMP1^{ip}		DH2 (gfp)^{ip}			
MYB1^{ip}	MYB1*						

6- Transgenic plants already available for reverse genetics studies

GS1a (OE)

CAD, KOR, GRP, MYB14 (RNAi)

7- Concluding remarks

Ongoing method transfer for maritime pine SE and transgenesis
Improvement of the transformation toolbox during Sustainpine
One of the greatest efforts for gene functional analysis in conifers

References: **Christensen & Quail (1996).** Transgenic Res. 5: 213-218. **Roberts *et al.* (1998).** Vector release man. v3.05, CAMBIA, Canberra. **Klimaszewska *et al.* (2007).** Tree & For. Sci. & Biotech. 1: 11-25. **Karimi *et al.* (2002).** Trends in Plant Sci. 7: 193-195. **Litvay *et al.* (1985).** Plant Cell Rep. 4, 325-328. **Trontin *et al.* (2007).** Transgenic Plant J. 1: 314-329.

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