



HAL
open science

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

Jean-François Trontin, Sandrine Debille, Francis Canlet, Luc Harvengt, Marie-Anne Lelu-Walter, Philippe Label, Caroline Teyssier, Célia Miguel, José de Vega-Bartol, Mariagrazia Tonelli, et al.

► To cite this version:

Jean-François Trontin, Sandrine Debille, Francis Canlet, Luc Harvengt, Marie-Anne Lelu-Walter, et al.. Somatic embryogenesis as an effective regeneration support for reverse genetics in maritime pine: the Sustainpine collaborative project as an illustration. 2. International Conference of the IUFRO Working Party 2.09.02, "Somatic Embryogenesis and Other Vegetative Propagation Technologies", Jun 2012, Brno, Czech Republic. 2012, Integrating vegetative propagation, biotechnologies and genetic improvement for tree production and sustainable forest management. Book of abstracts. hal-01268053

HAL Id: hal-01268053

<https://hal.science/hal-01268053>

Submitted on 3 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

SustainPine

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

Trontin J.-F.¹, Debille S.¹, Canlet F.¹, Harvengt L.¹, Lelu-Walter M.-A.², Label P.², Teyssier C.², Miguel C.³, De Vega-Bartol J.³, Tonelli M.³, Santos R.³, Rupps A.⁴, Hassani S.B.⁴, Zoglauer K.⁴, Carneros E.⁵, Díaz-Sala C.⁵, Abarca D.⁵, Arrillaga I.⁶, Mendoza-Poudereux I.⁶, Segura J.⁶, Avila Saez C.⁷, Rueda M.⁷, Canales J.⁷, Cánovas F.M.⁷



FCBA
INSTITUT TECHNOLOGIQUE
Biotech. & advanced Forestry Dpt,
Genetics & Biotech group, 71, Route
d'Arcachon, Plerrot, 33610 Cestas
FRANCE (Partner **P8**)
jean-francois.trontin@fcba.fr

INRA
INRA d'Orléans, UR 588
Breeding, Genetic & Physiol. of Forest trees
2163 Av. de la Pomme de pin
CS 4001 Ardon, 45075 Orléans Cedex 2
FRANCE (Partner **P7**)
Marie-anne.Lelu-Walter@orleans.inra.fr

ITQB/IBET-UNL
Forest Biotechnology Lab
Av. República Qta. Do Marques (EAN)
Apartado 12, 2781-901 Oeiras
PORTUGAL (Partner **P5**)
cmiguel@itqb.unl.pt

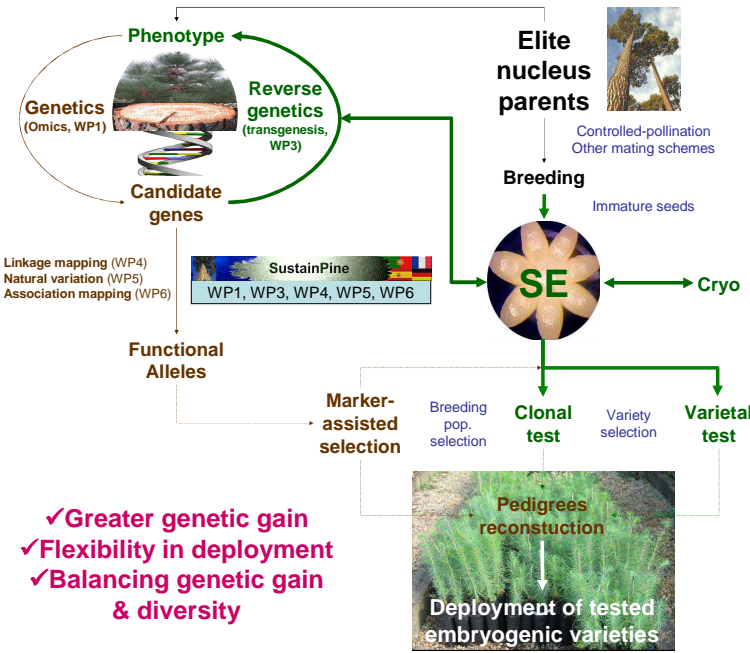
Humboldt University of Berlin
Institute of Biology
D-10115
Berlin, Invalidenstr. 42
GERMANY (Partner **P10**)
kurt.zoglauer@biologie.hu-berlin.de

University of Alcalá
Department of Plant Biology
28871,
Alcalá de Henares, Madrid
SPAIN (Partner **P3**)
carmen.diazsala@uah.es

University of Valencia
Dpto. Biología Vegetal,
Facultad de Farmacia
46100 Buñassot, Valencia
SPAIN (Partner **P4**)
isabel.arrillaga@uv.es

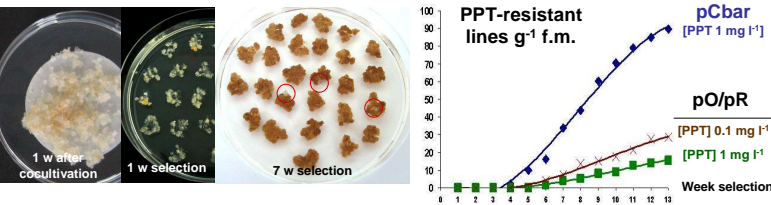
University of Málaga
Molecular Biology Lab.
Campus de Teatinos,
29071 Málaga
SPAIN (Partner **P1**)
canovas@uma.es

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



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

	P3	P4	P5	P8	P10
Selection	Modified	Standard	Modified	Reduced [PPT] = 0.1 mg l ⁻¹	
pMb7Fm21GW-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

6- Transgenic plants already available for reverse genetics studies



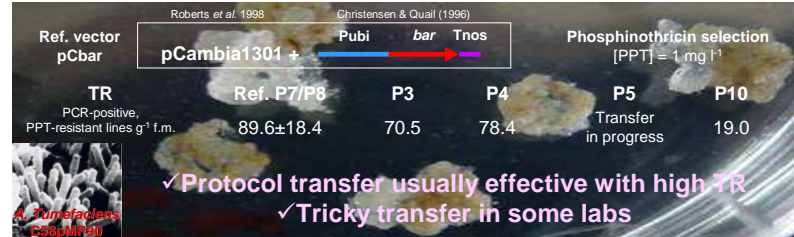
2- SE in model genotype (PN519) for reverse genetics: effective transfer to Sustainpine partners

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

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

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

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



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

	Planned	Achieved*	In prog.**
U. Alcalá (P3)	9		6
U. Valencia (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

**Previous project

OE	RNAi	OE	RNAi	OE	RNAi	OE	RNAi
PAL	CAD	LAC	CesA2	XETG	MYB8 ^{pp}	MYB5 ^{pp}	MYB1 ^{pp}
PAL*	CAD ^{pp}	COMT*	LAC*	XETG*	MYB8*	MYB5*	MYB1*
GS1b	GS1b (gfp)	GS2 ^{pp}	Dof5 ^{pp}	ASN1 ^{pp}	ASPG ^{pp}	ARS20 ^{pp}	dOAT ^{pp}
GS1b	GS1b	GS2	Dof5	ASN1	ASPG	ARS20	dOAT
Fd-GOGAT ^{pp}	NADH-GOGAT ^{pp}						
NOD ^{pp}	AP2 ^{pp}	DH2 ^{pp}	DH2 (gfp)	UPK	Rab1 ^{pp}	LEC1 ^{pp}	LEP1 ^{pp}
NOD	AP2	DH2					

Wood formation | C/N metabolism Ammonium regulation | Stress resistance (drought, nutrition) | Development Embryogenesis

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.



June 25-28, 2012
Brno, Czech Republic
Poster S1-17P