Towards the integration of somatic embryogenesis in Larix breeding program : from understanding the developmental pathway to clonal propagation

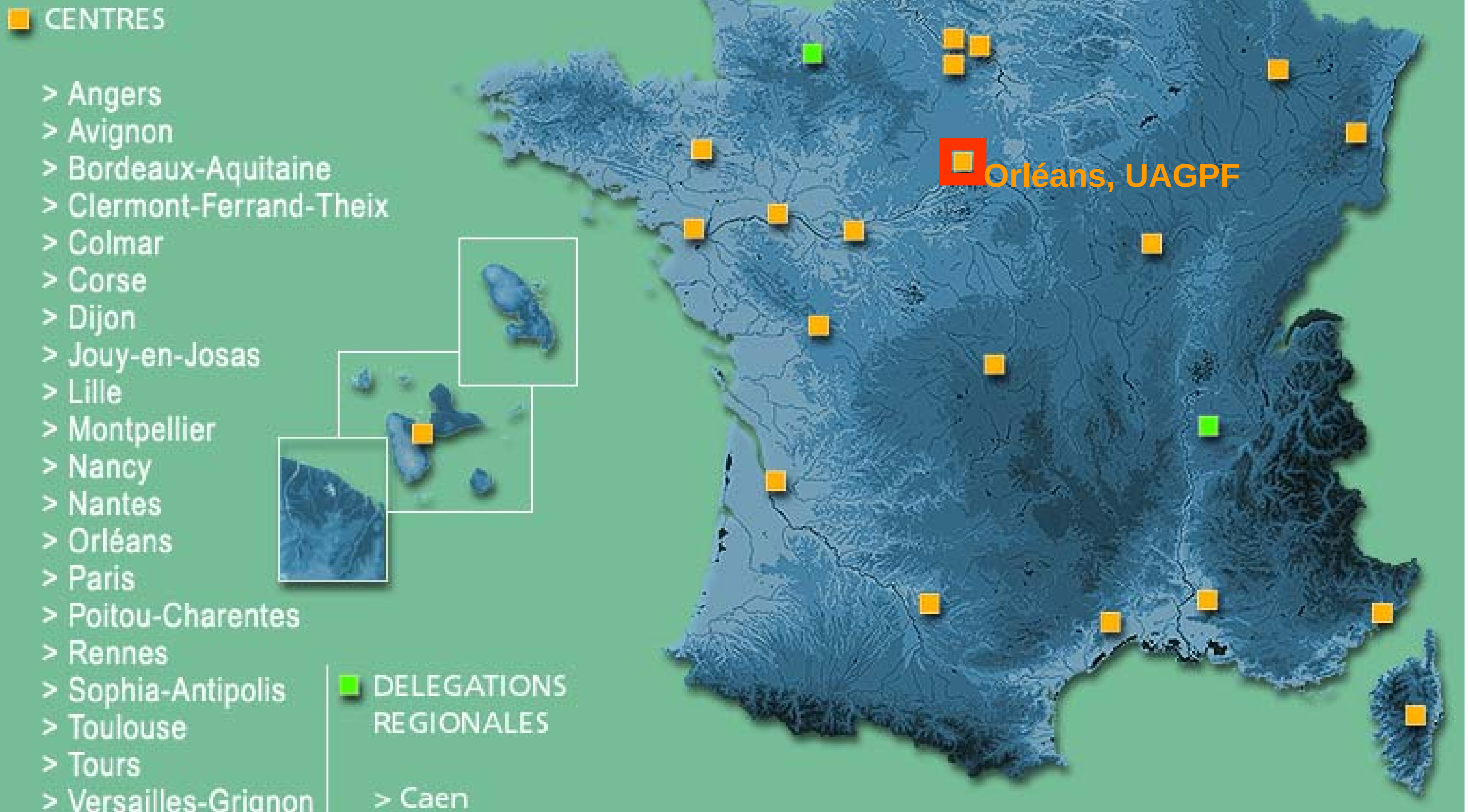
Marie-Anne Lelu-Walter

Towards the integration of somatic embryogenesis in *Larix* breeding program : from understanding the developmental pathway to clonal propagation

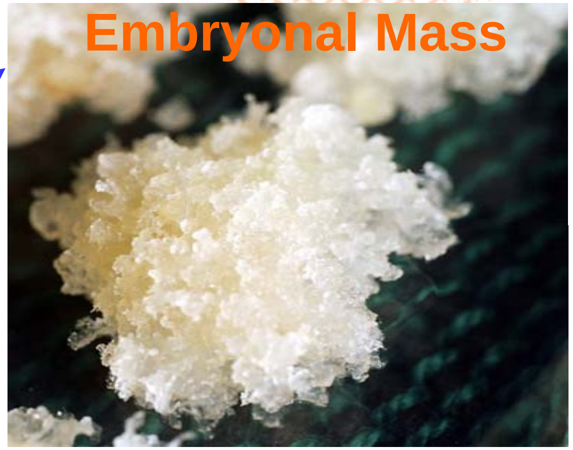
Marie-Anne Lelu-Walter
I.N.R.A. Research Unit on Breeding, Genetic and Physiology of Forest Trees , Orléans, France

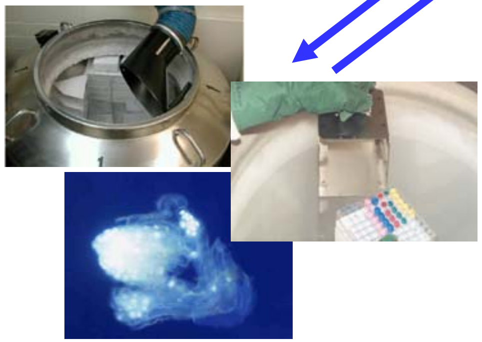
National establishment

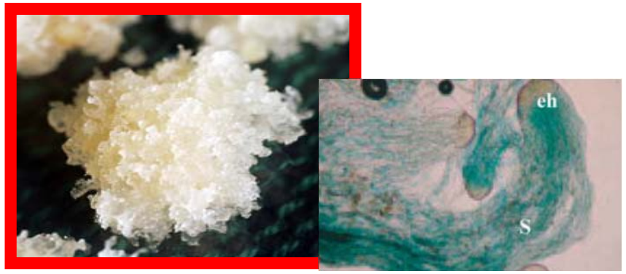
- 21 regional research centres
- 468 units



Somatic embryogenesis: for fundamental and applied researches







Cryopreservation

To keep material:

- in juvenile and safe state
- at the long term
- at low cost

→ ***Conservation of genetic resources***

Genetic transformation

To validate candidate gene function in Gymnosperms (abiotic stress, wood formation)

Plant regeneration

- Physiological researches
- Breeding program
- Deployment, commercialisation

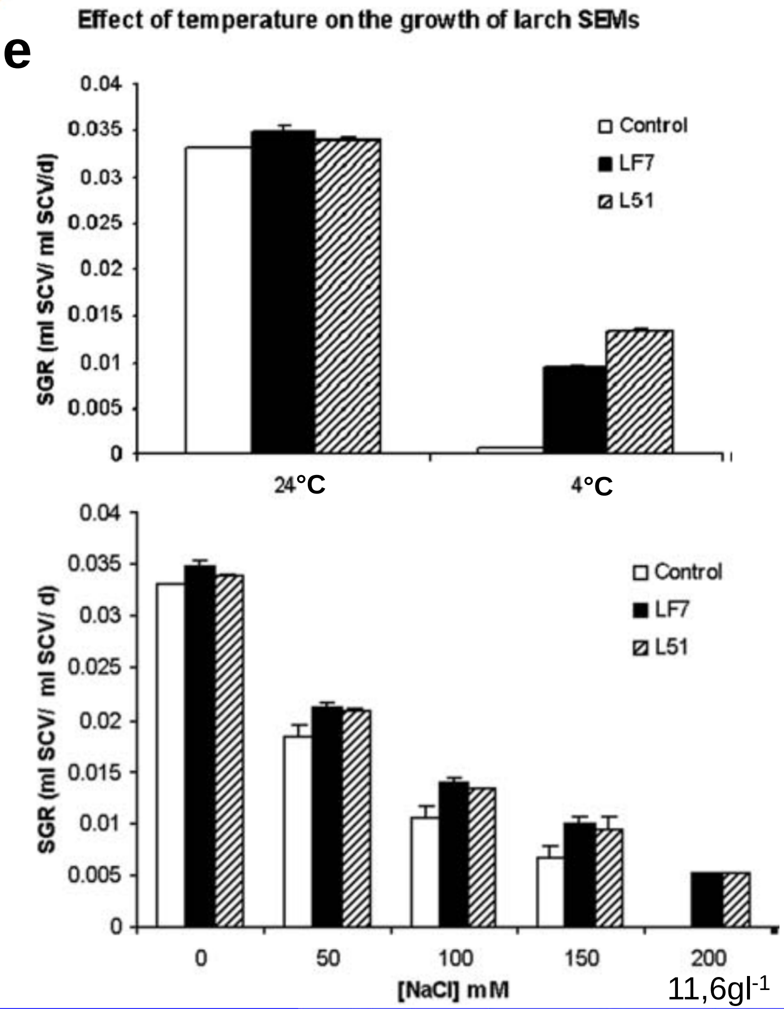
Genetic transformation in hybrid larch: abiotic stress (salt, frost)

Over expression gene coding for pyrroline 5-carboxylate synthase (P5CS):

Cell line	Intracellular proline (µmoles per gFW)	
	EM	Needles of regenerated plants
Control	0.042 ± 0.006	0.021 ± 0.001
L51	1.361 ± 0.004	1.028 ± 0.016
LF7	1.161 ± 0.004	0.970 ± 0.008

- endogenous proline x 30 x46
- improvement frost tolerance
- improvement salt tolerance

Gleeson et al. Mol Breed 2005



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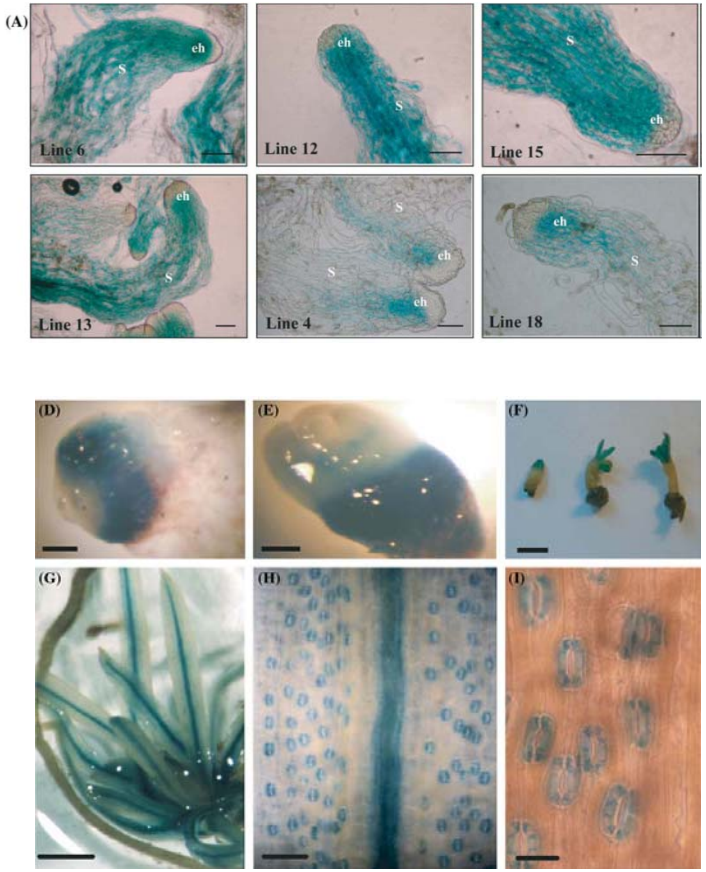
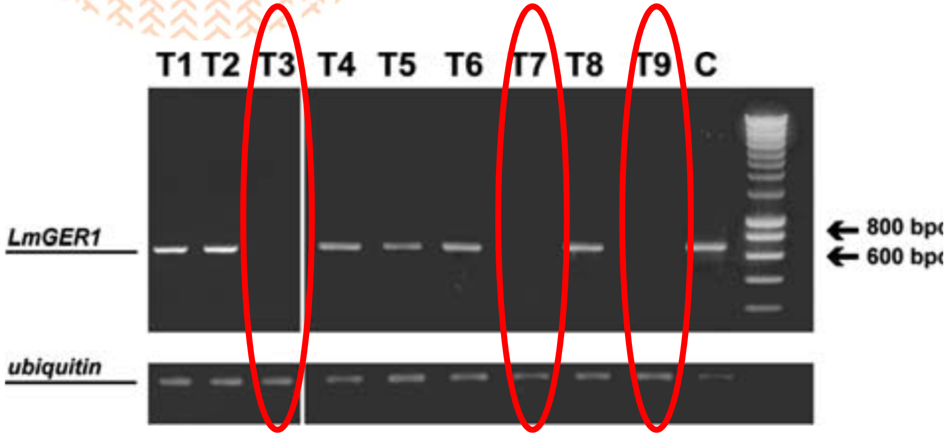
Genetic transformation in hybrid larch: Somatic embryo development



LmGER1-GUS expression during SE maturation
Mathieu et al. Plant Mol Biol 2006

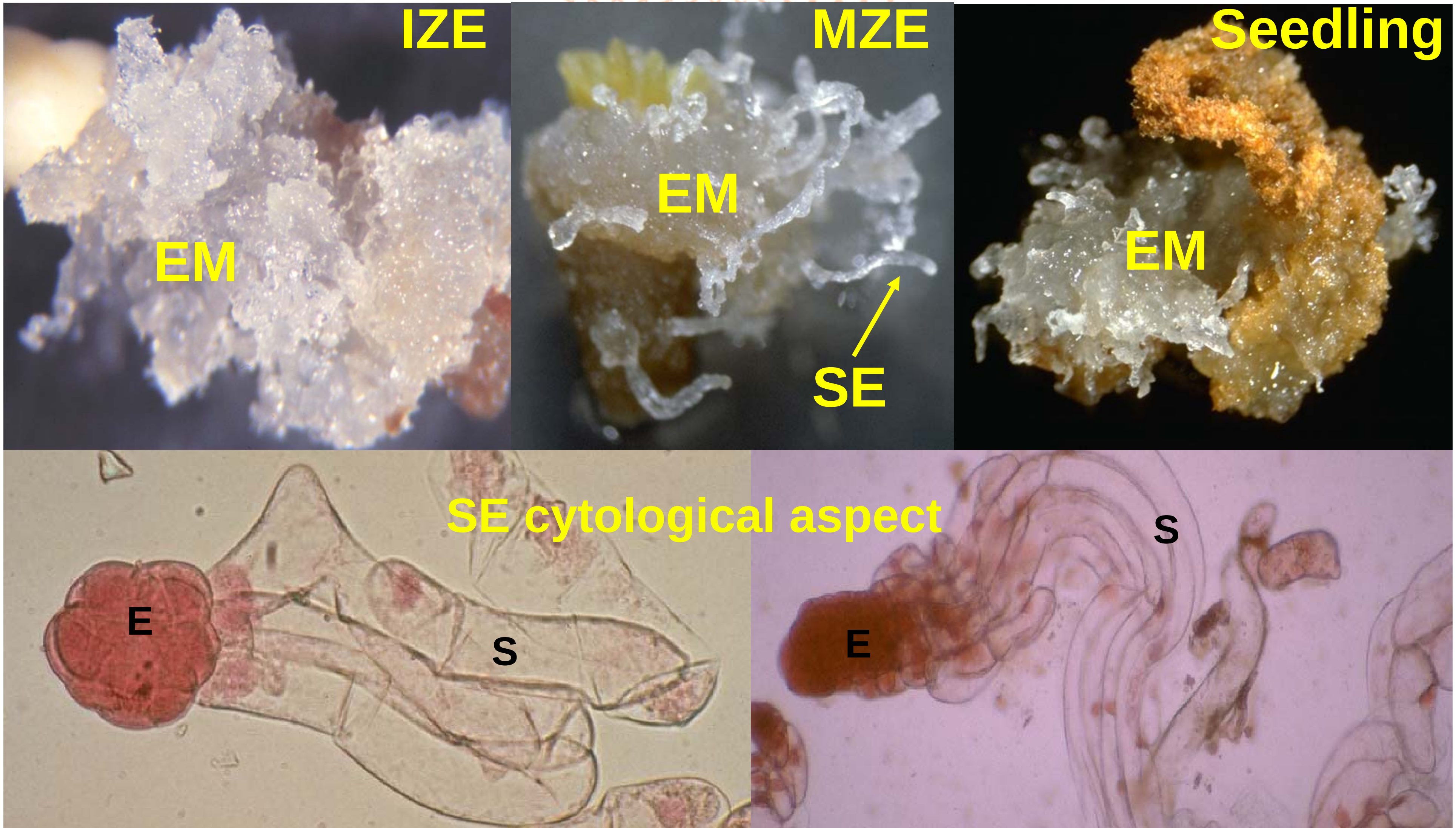


LmGER1-GUS expression in IR-PTGS transformed embryonal masses (9 independant lines T1-T9)

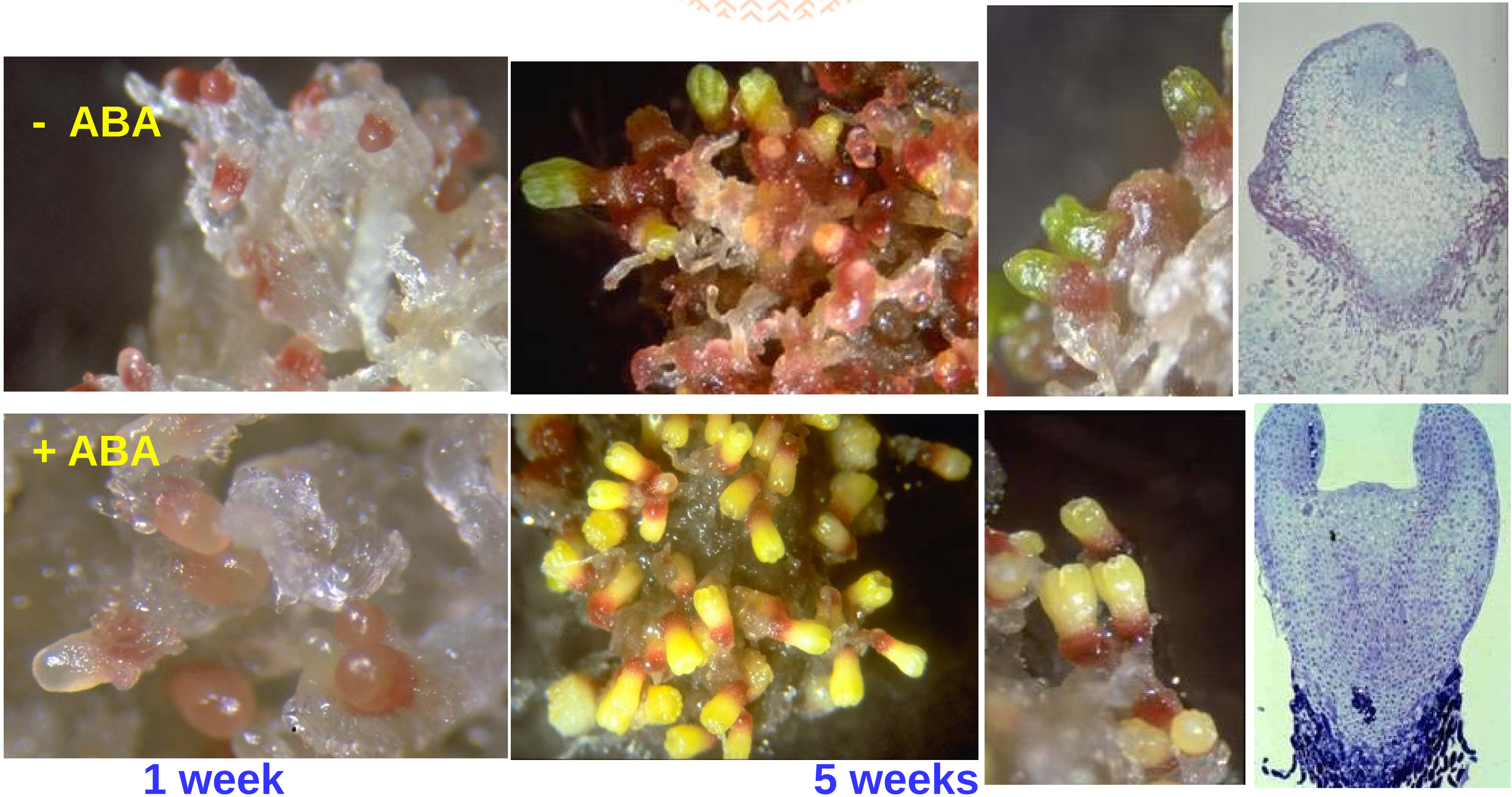
- Germin Like Protein
- ihp RNA early stage of SE

Somatic embryogenesis of hybrid larch

Somatic embryogenesis : initiation



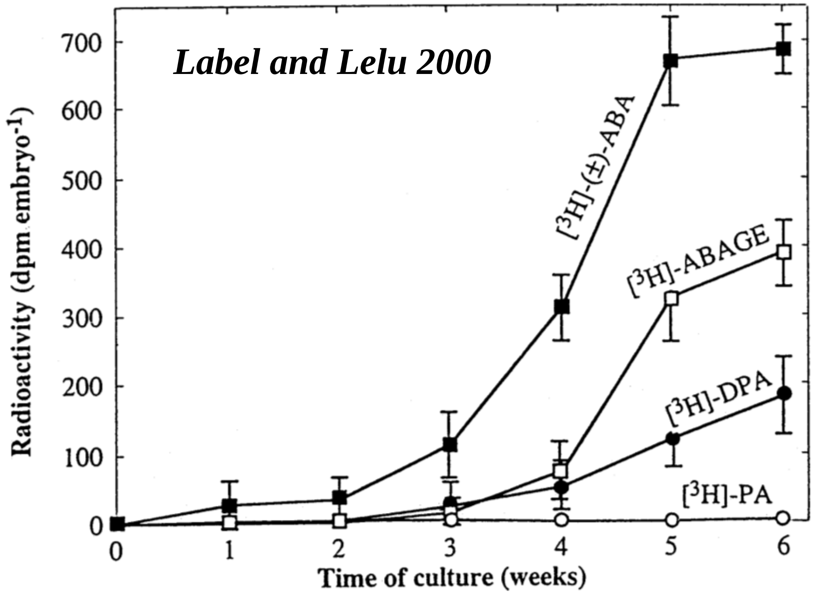
Somatic embryo maturation : hormonal control abscisic acid (ABA)



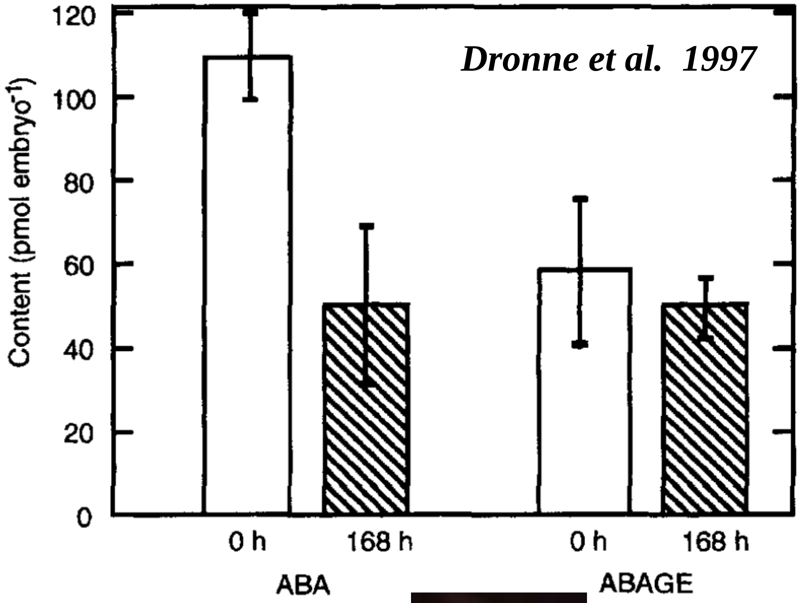
1 week 5 weeks

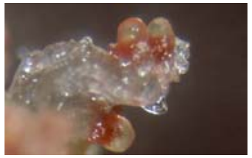
Somatic embryo maturation : hormonal control abscisic acid (ABA)

Maturation

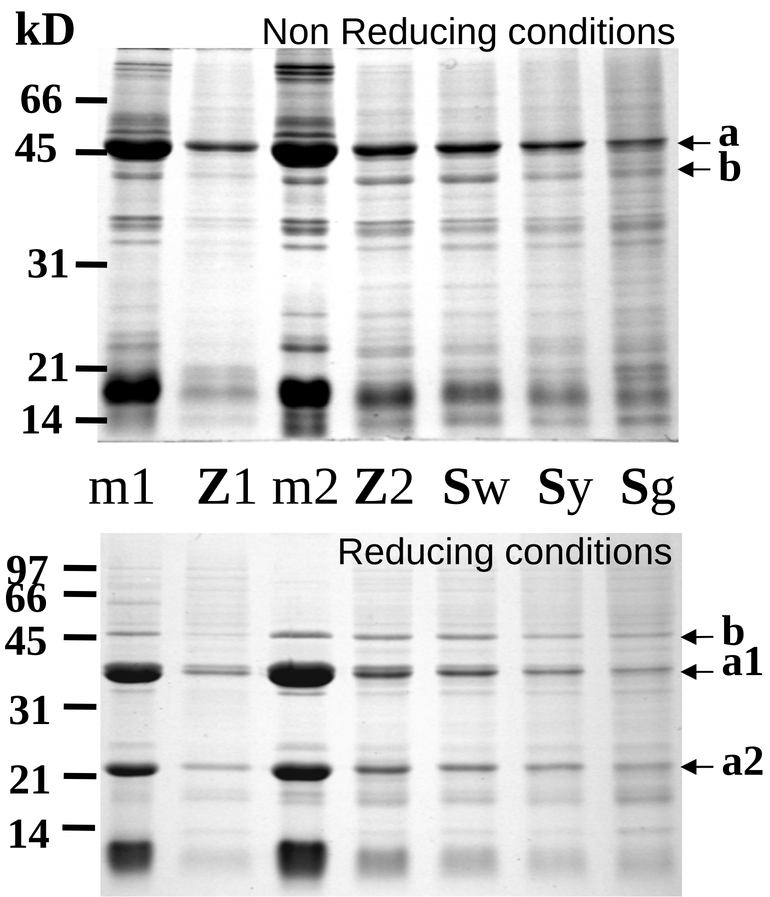


Desiccation

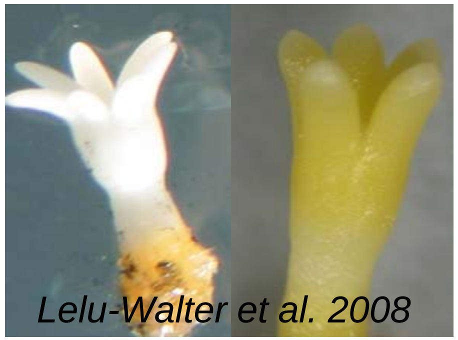




Somatic embryo maturation : proteomic analysis
Comparison somatic embryo vs zygotic embryo



kD/	45 (b)	36 (a1)	22 (a2)	55 (a)	11.5
Zygote	100	100	100	100	100
SE white (8weeks)	**96**	**78**	**78**	**81**	**74**
SE yellow (10weeks)	53	48	54	64	48
SE green (12weeks)	63	34	48	45	47


Lelu-Walter et al. 2008

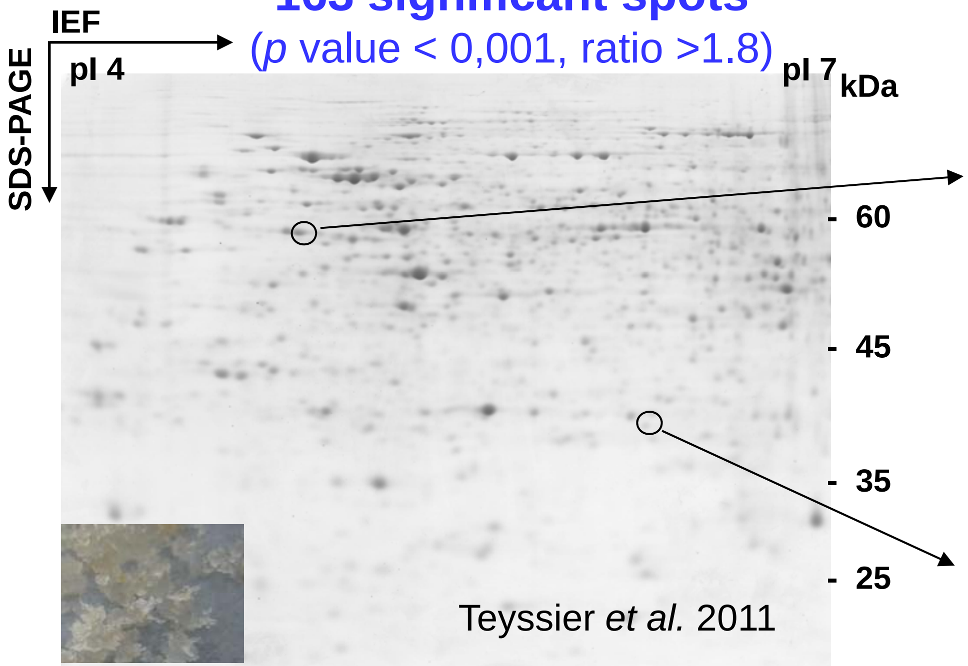
Somatic embryo maturation : 2D proteomic analysis

Immature vs mature somatic embryo

Soluble protein content (µg/mg fresh weight)	2,47 ± 0,20	21,46 ± 1,92

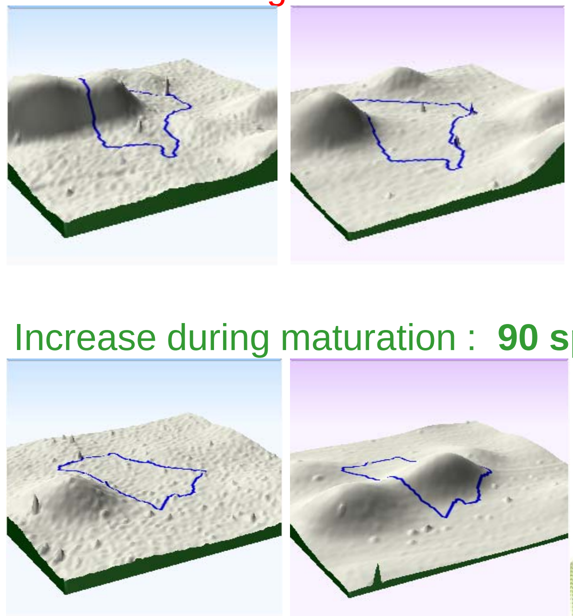
163 significant spots

(p value < 0,001, ratio >1.8)



Decrease during maturation : **73 spots**

Increase during maturation : **90 spots**



Somatic embryo maturation : 2D proteomic analysis

Immature vs mature somatic embryo

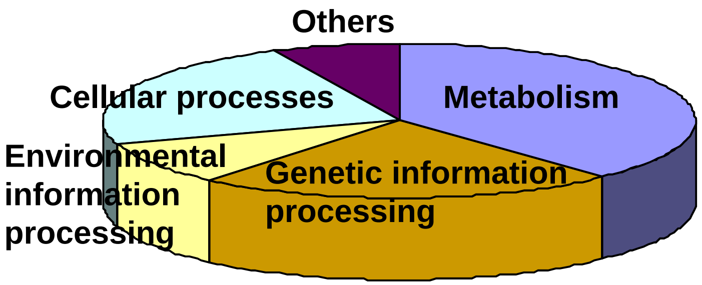
Spot no.	Assignement	TC	# pep.	% cov.	Accession no.	Species
eID0107	Pyruvate decarboxylase isozyme 2	TC81547	4	4,8	Q9FVF0	*Fragaria x ananassa*
eID0113	Glucose6phosphate isomerase cytosolic A	TC81691	4	6,9	Q8H8M6	*Vitis vinifera*
eID0165	Pyruvate decarboxylase	TC81547	5	3,5	Q9FVF0	*Fragaria ananassa*
eID0352	GRP94	TC101142	3	9,0	A7YAU9	*Pinus taeda*
eID0588	Alcohol dehydrogenase	TC81108	3	10,0	Q43023	*Pinus banksiana*
eID0649	flavanone 3-hydroxylase	TC81214	3	11,9	Q5XPX2	*Ginkgo biloba*
eID0657	NAD-dependent sorbitol dehydrogenase	TC94525	4	14,0	Q9MBD7	*Prunus persica*
eID0712	Chalcone synthase	TC93000	6	16,4	Q2ENB1	*Abies alba*
eID0773	1-aminocyclopropane-1-carboxylic acid oxidase	TC81819	2		Q84L58	*Cicer arietinum*
eID0791	type IIIa membrane protein cp-wap13	TC106627	3	8,0	O24548	*Vigna unguiculata*
eID0793	Heat shock protein 81-3	TC108803	3	5,0	P51818	*Arabidopsis thaliana*
eID0801	Heat shock protein 101	TC96791	3	4,6	Q6F2Y7	*Oryza sativa subsp. japonica*
eID0825	Adenosine kinase isoform 2T	TC108369	2	12,0	Q5DKU6	*Nicotiana tabacum*
eID0996	Thiosulfate sulfurtransferase	TC93574	4	9,2	Q9ZPK0	*Datisca glomerata*
eID1018	GRP94	TC110737	3	9,3	A7YAU9	*Pinus taeda*
eID1633	Aspartate aminotransferase, chloroplast precursor	TC97755	4	8,2	P46248	*Arabidopsis thaliana*
eID1763	Enolase 1	TC80765	2	5,9	Q9LEJ0	*Hevea brasiliensis*
eID1785	Fructose-bisphosphate aldolase	TC114376	2	9,9	A7P3F7	*Vitis vinifera*
eID2151	Heat shock protein 81-3	TC108803	5	8,0	P51818	*Arabidopsis thaliana*
eID2461	3-isopropylmalate dehydrogenase	TC83730	7	19,7	A2XK82	*Oryza sativa*
eID2486	Heat shock 70 kDa protein mitochondrial	TC98916	3	8,7	Q08276	*Solanum tuberosum*
eID2535	Phosphoglucomutase	TC102152	4	12,1	A5HSI1	*Bambusa oldhamii*
ID0347	Oligopeptidase A	TC100165	2	3,7	A9YWR9	*Medicago truncatula*
ID2050	Argininosuccinate synthase	TC95974	3	5,2	A9TX31	*Physcomitrella patens sub*
ID2138	RPT2A; ATPase	TC80721	4	10,0	Q2HTA3	*Medicago truncatula*
ID2430	dihydrolipoyl dehydrogenase 1 mitochondrial	TC99823	2	3,8	Q9LNF3	*Arabidopsis thaliana*
ID2574	Chromosome chr8 scaffold_29	TC93736	4	4,1	A7PT66	*Vitis vinifera*
ID2578	Heat shock protein 81-3	TC108803	6	9,1	P51818	*Arabidopsis thaliana*
ID3118	Endoplasmin homolog	TC110737	3	9,3	P35016	*Catharanthus roseus*
ID4294	Enolase 1	TC80765	5	5,4	Q9LEJ0	*Hevea brasiliensis*
ID4972	Superoxide dismutase	TC83788	2	5,7	A5JVZ3	*Ginkgo biloba*

Somatic embryo maturation : 2D proteomic analysis

Immature vs mature somatic embryo

Functional classification

	Stage 1	Stage12	%
Metabolism	**7**	**19**	**38%**
carbohydrate metabolism	5	11	41,0%
energy metabolism	0	2	5,1%
lipid metabolism	2	1	7,7%
nucleotide metabolism	0	1	2,6%
amino acid metabolism	1	5	15,4%
metabolism of other amino acids	1	0	2,6%
metabolism of cofactors and vitamins	0	1	2,6%
biosynthesis of secondary metabolites	3	4	17,9%
xenobiotics biodegradation and metabolism	1	1	5,1%
Genetic information processing	**5**	**11**	**23%**
Environmental information processing	**3**	**3**	**9%**
Cellular processes	**6**	**10**	**23%**
Others	**2**	**3**	**7%**



Interpretation of the biologic function of identified proteins in function of maturation stage

Identification proteins markers

Perspectives for breeding

- High efficiency in plant regeneration
- Maintenance of juvenility *via* cryopreservation

→ To maximize breeding efficiency of forest trees
For accelerating deployment of improved varieties

Larch breeding programme in France : major steps
Dr Luc Pâques

- 1957: first provenance trials for European and Japanese larches
- 1970: establishment of European (350 clones) and Japanese (300 clones) larch breeding populations
- 1979: first hybrids produced by control crosses
- 1978-1985: plantation of pure species + hybridisation seed orchards
- 1981-1989: development of ‘bulk’ vegetative propagation by cutting
- **1990- 1999**: development of somatic embryogenesis
- 2005: certification of a new hybrid variety *Larix eurolepis* (*Larix decidua* x *L. marschlinsii*) INRA H1, REVE VERT

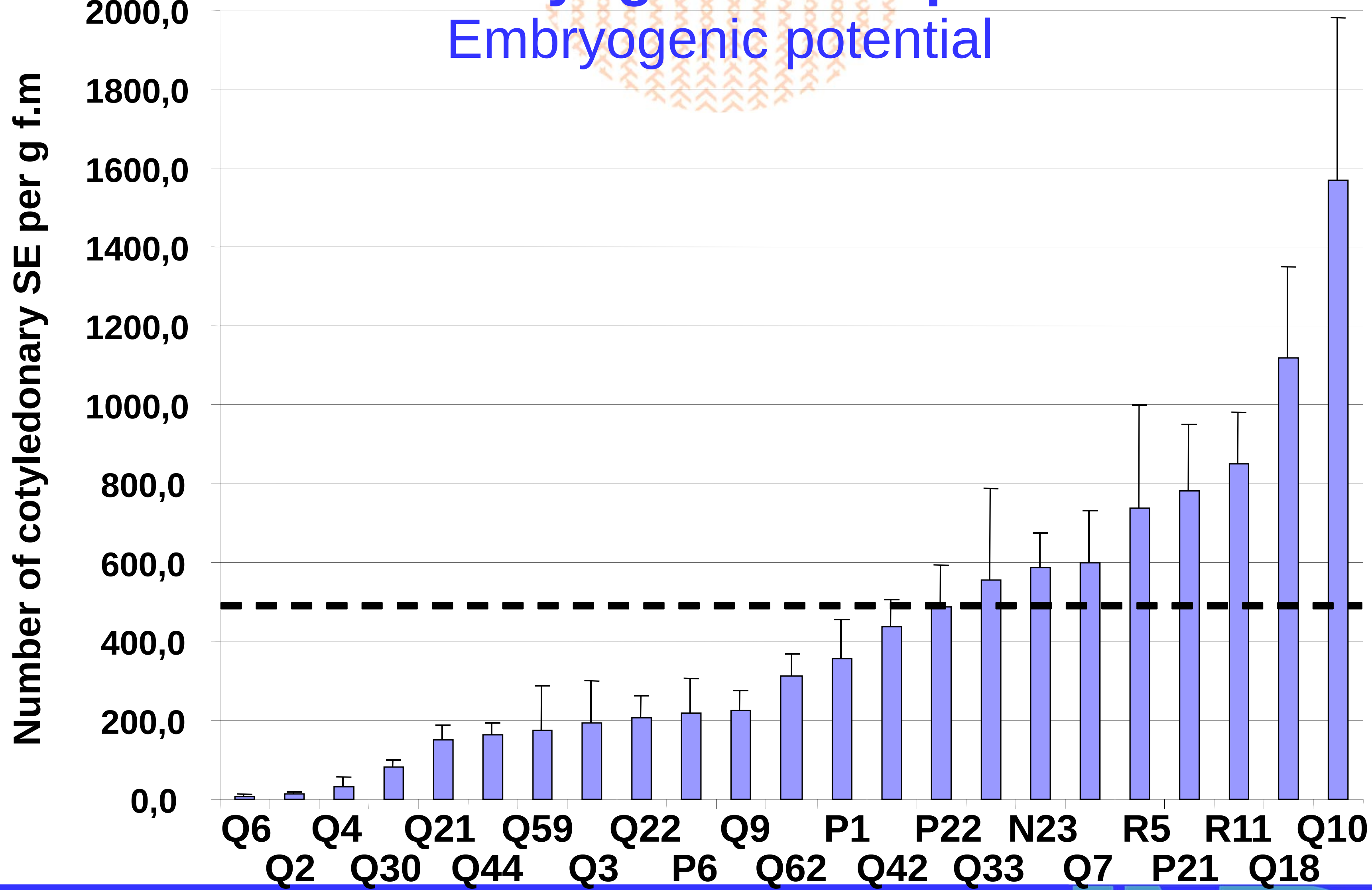
Somatic embryogenesis improvements:
Initiation frequency

	2003	2004	2005	2006
Initiation	12%	53%	60%	75%
		↓	↓	↓
EMs Proliferation		66%	66%	78%

100 introduced zygotic embryos will give rise to 60 established lines

Somatic embryogenesis improvements:

Embryogenic potential



Somatic embryogenesis improvements:
Embryogenic potential

	Responding	Non Responding
Hybrid larch 51 lines tested	**94%** (*48*)	6% (*3*)
European larch 16 lines tested	**94%** (*15*)	6% (*1*)

For hybrid larch, variety REVE VERT
100 introduced zygotic embryos will give rise to
54 maturing lines

Questions arising from the technique:

Age effect of the line on embryogenic potential?

Origine	Line	Age	N° SE g FW
Larix decidua x	N23	7 months	750
Larix marschlinsii	N23	2 years	680
(REVE VERT)	N23	3 years	707
	N23	4 years	580
Larix marschlinsii	69-18	1 year	930
x *Larix decidua*	69-18	5 years	2430
	69-18	9 years	1832

The embryogenic capacity is maintained over time
There is no decrease as in pine species.

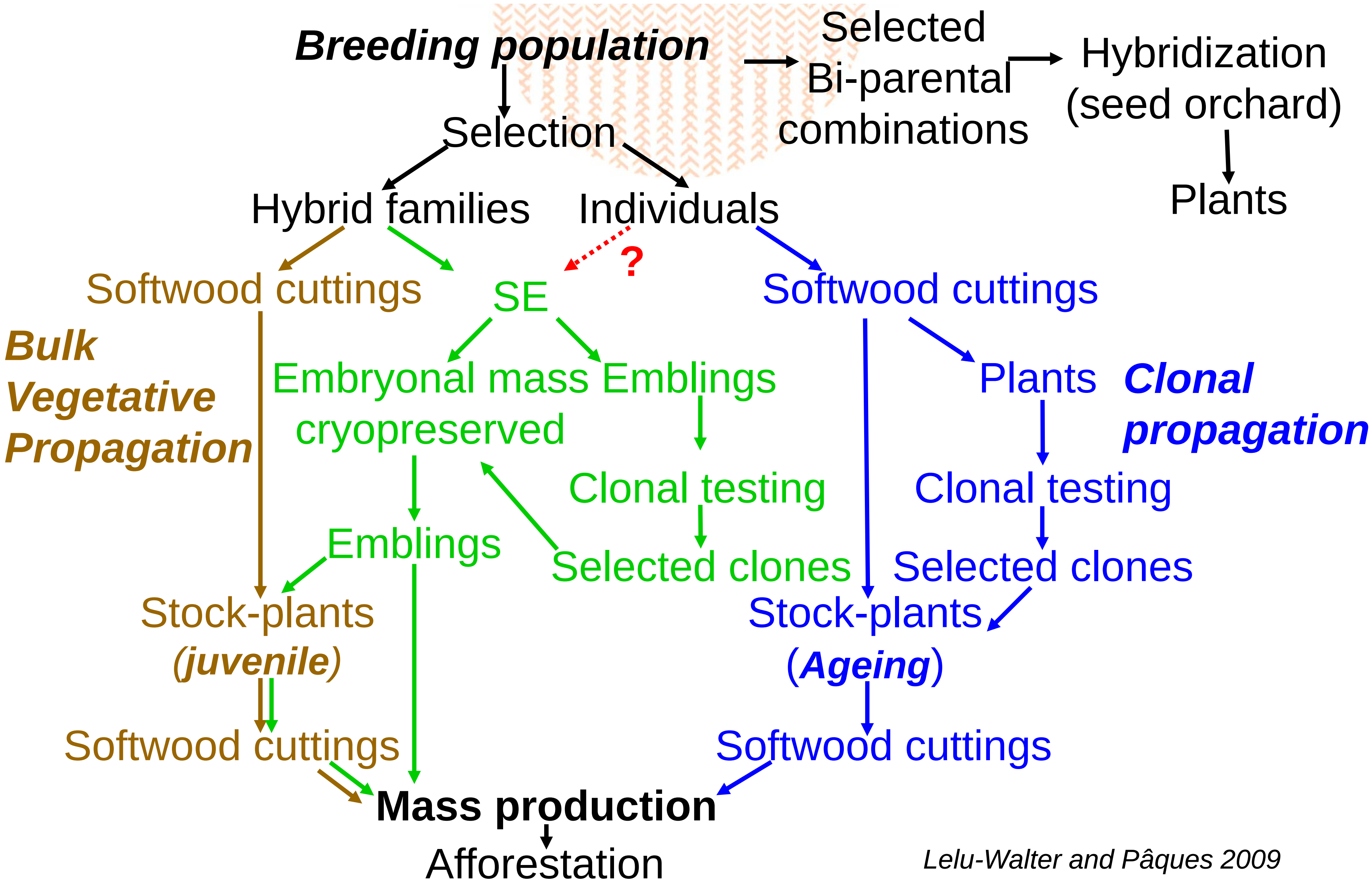
Questions arising from the technique:
Cryopreservation effect on embryogenic potential?

Storage duration in N2	N° SE g FW
Original (69-18)	930
1 year (69-18c)	1106
3 years (69-18c2)	744

Cryopreservation did not change the embryogenic potential

Performances of each step of hybrid larch SE

Step	Performance
	100 Zygotic Embryos INRA REVE-VERT
Initiation	↓ *65%*
	65 embryonal masses
Proliferation/ Cryopreservation	↓ *78%*
	51 established lines → (*100%*) 51 cryopreserved lines
Maturation: SE recovery	↓ *94%*
	48 maturing lines
	↓ *100%*
Germination/ Conversion	48 lines giving rise to plants



Lelu-Walter and Pâques 2009

Needs for the future :

- Check behaviour of the emblings in the field demonstration plots 30-40 lines from REVE VERT
- Enlarge the cryobank
- SE propagation from mature material
- Genetic diversity ?

hybrid larch emblings REVE VERT

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Thank you for your attention

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