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

Marie-Anne Lelu-Walter, Luc E. Pâques

임업인에게 희망을
국민에게 행복을

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KOREA FOREST RESEARCH INSTITUTE

Current Biotechnology Strategies
for Forest Productivity Improvement

KFRI Tree Biotechnology Symposium 2015

- Date : May 12 (Tuesday), 2015
- Location : Department of Forest Genetic Resources, KFRI (Suwon)

PROCEEDINGS

Program

Topic : Current Biotechnology Strategies for Forest Productivity Improvement

Time	Program
08:00~09:00	Registration
	Opening Ceremony
Moderator : Dr. Hyo-Shin Lee (KFRI, Korea)	
09:00~09:20	**Opening Address** * *Dr. Seong-Hyeon Nam (President, Korea Forest Research Institute)* **Congratulatory Address** * *Dr. Jae-Seon Yi (Professor, Kangwon National University)*
09:20~09:30	Photo Time
	Session Ⅰ. Somatic Embryogenesis and Clonal Propagation
Moderator : Dr. Heung-Kyu Moon (KFRI, Korea)	
09:30~10:00	Integration of conifer somatic embryogenesis and genomic selection into multi-varietal forestry **** Dr. Yill-Sung Park (Canadian Forest Service, Canada)***
10:00~10:30	Towards the integration of somatic embryogenesis in Larix breeding program: from understanding the developmental pathway to clonal propagation **** Dr. Marie-Anne Lelu Walter (INRA, France)***
10:30~11:00	Clonal propagation through somatic embryogenesis in Japanese larch (Larix kaempferi) **** Dr. Young-Wook Kim (KFRI, Korea)***
11:00~11:30	Progress in somatic embryogenesis of seven Japanese conifer species **** Dr. Tsuyoshi Maruyama (FFPRI, Japan)***
11:30~13:00	**Lunch & Poster session**
13:00~13:30	In vitro multiplication of plus trees of Gmelina arborea and Cedrela odorata **** Dr. Aguilar Maria Elena (CATIE, Costa Rica)***
	Session Ⅱ. Recent Trends in Genome and GMO Research
Moderator : Dr. Byeong-Wook Yoon (Kyungpook National University, Korea)	
13:30~14:00	Ac/Ds-based transposon activation tagging in poplar: a powerful tool for gene discovery **** Dr. Matthias Fladung (Thünen Institute, Germany)***
14:00~14:30	Cellular cytokinin level differentially regulates ckx genes and cytokinin-induced auxin biosynthesis in transgenic poplars **** Dr. Young-Im Choi (KFRI, Korea)***
14:30~15:00	Discovery of genes affecting environmentally and economically traits in trees via activation tagging **** Dr. Victor B. Busov (Michigan Technological University, USA)***
15:00~15:30	Coffee break
Moderator : Dr. Yong-Eui Choi (Kangwon National University, Korea)	
15:30~16:00	Genetic variation in metabolites, growth and biotic interactions in aspen (Populus tremula) **** Dr. Kathryn Robinson (UPSC, Sweden)***
16:00~16:30	Forest Resources Genome Project: the first step on tree genome research in Korea **** Dr. Eung-Jun Park (KFRI, Korea)***
16:30~17:00	Comparative genomics of aspen species **** Dr. Nathaniel R. Street (Umeå University, Sweden)***

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

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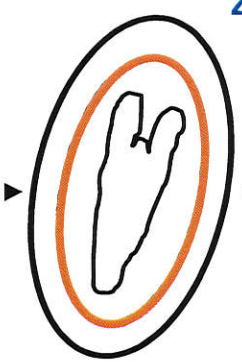
Embryogenesis in Gymnosperms

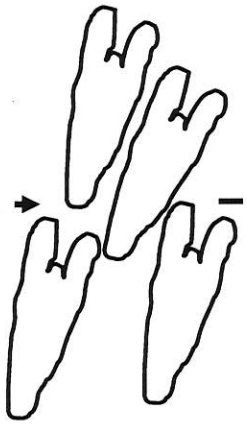
Zygotic embryogenesis: 1 seed = 1 tree

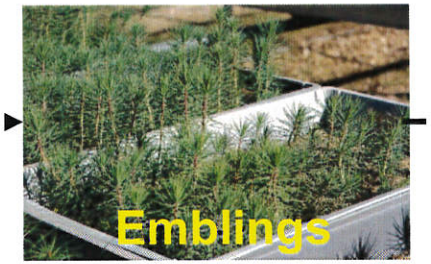
Zygote → Immature ZE



in vitro Culture

Embryonal Mass = Proliferation of somatic embryos





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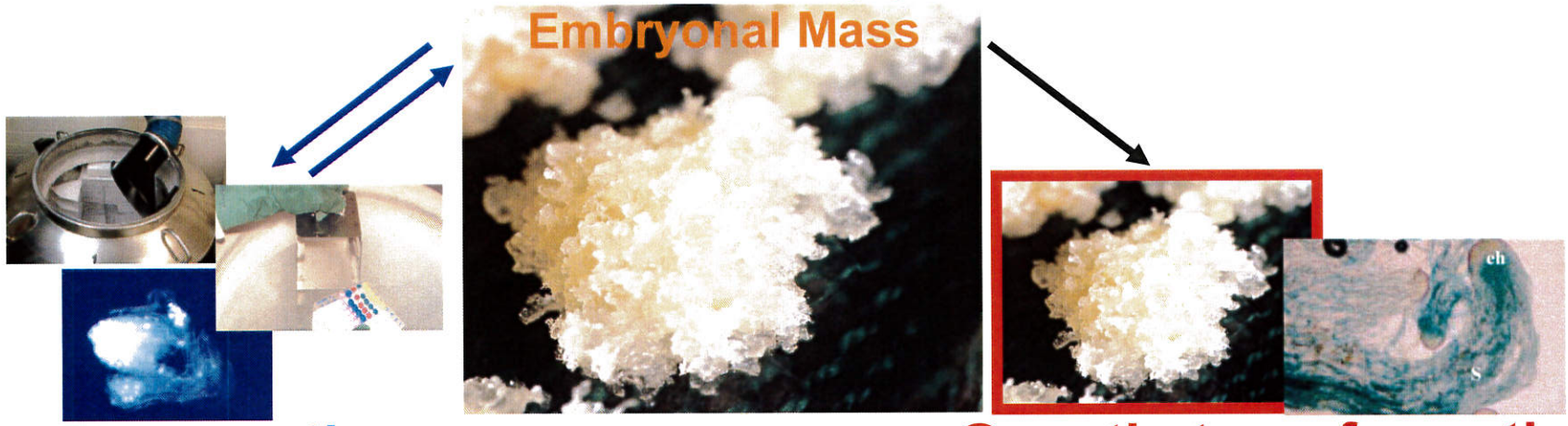
Somatic embryogenesis: 1 seed = X trees

Differences between embryogenesis

Somatic embryogenesis: for fundamental and applied researches



Cryopreservation

To keep material:
- in **juvenile** and safe state
- on the long run
- at low cost

→ ***Conservation of genetic resources***

Genetic transformation

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

Agrobacterium tumefaciens
hybrid larch, Levée *et al.* 1997

Plant regeneration

- Physiological researches
- Breeding program
- Deployment, commercialisation

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Importance of larch species

1st conifer in Northern hemisphere

In Europe = EL + JL + (HL)
600 000 ha in native range
& **900 000 ha** outside in lowland plantation

Yield in plantation: at least 3 times that in native stands
with low improved FRM (1st generation SO)
Nearly doubled with hybrids (EL x JL)

Extension of HL in Europe: impeded by reproduction
- generative: seed yield & hybrid rate
- vegetative: ageing & cost

Somatic embryogenesis

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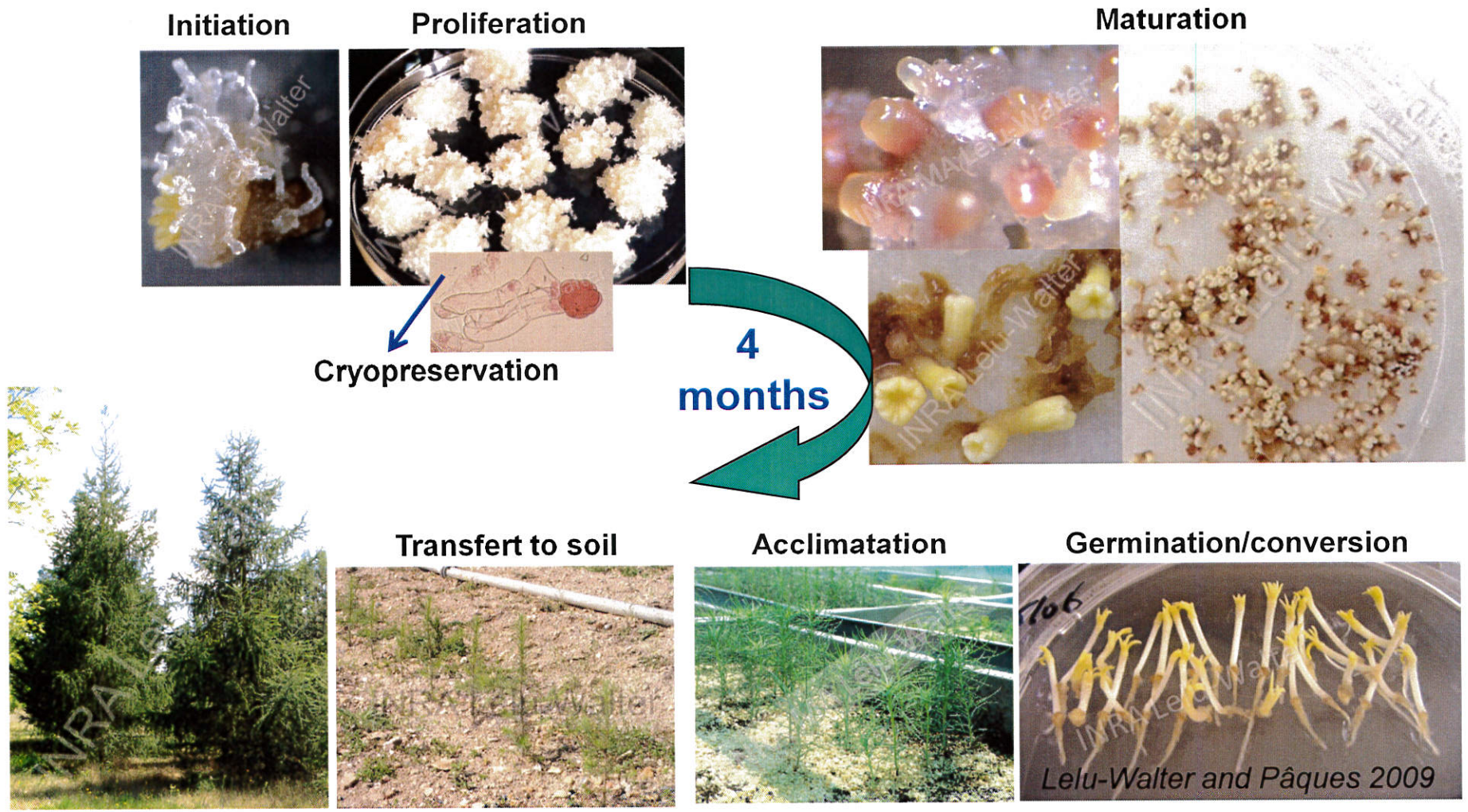
Somatic embryogenesis in larch species

1989	*Larix* x *eurolepis*, *Larix* x *marschlinsii*	Klimaszewska
90s-	*Larix decidua*	von Aderkas *et al.* 1990, Lelu *et al.* 1994; Szczygieł K 2005
	Larix kaempferi	von Aderkas *et al.* 1990, Kim *et al.* 1999
	Larix occidentalis	von Aderkas and Thompson 1992
	Larix laricina	Klimaszewska *et al.* 1997
	Larix sibirica	Tretiakova *et al.* 2012

Significant improvements

Larix kaempferi	Kim *et al.* 1999; Kim and Moon 2007
Larix x *eurolepis*, *Larix* x *marschlinsii*	Lelu-Walter and Pâques 2009

Somatic embryogenesis of hybrid larch



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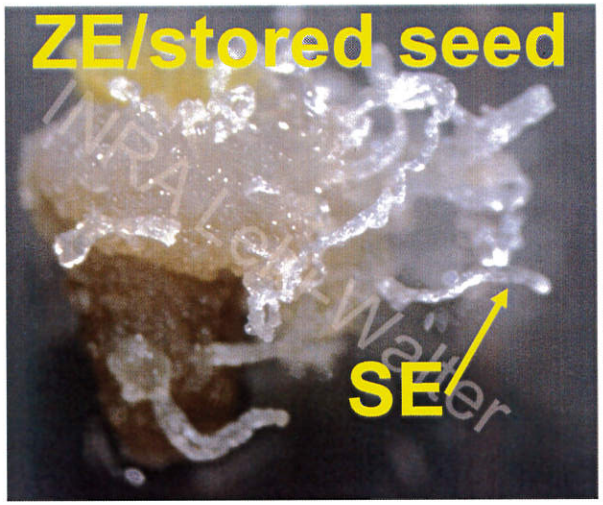
Somatic embryogenesis : initiation

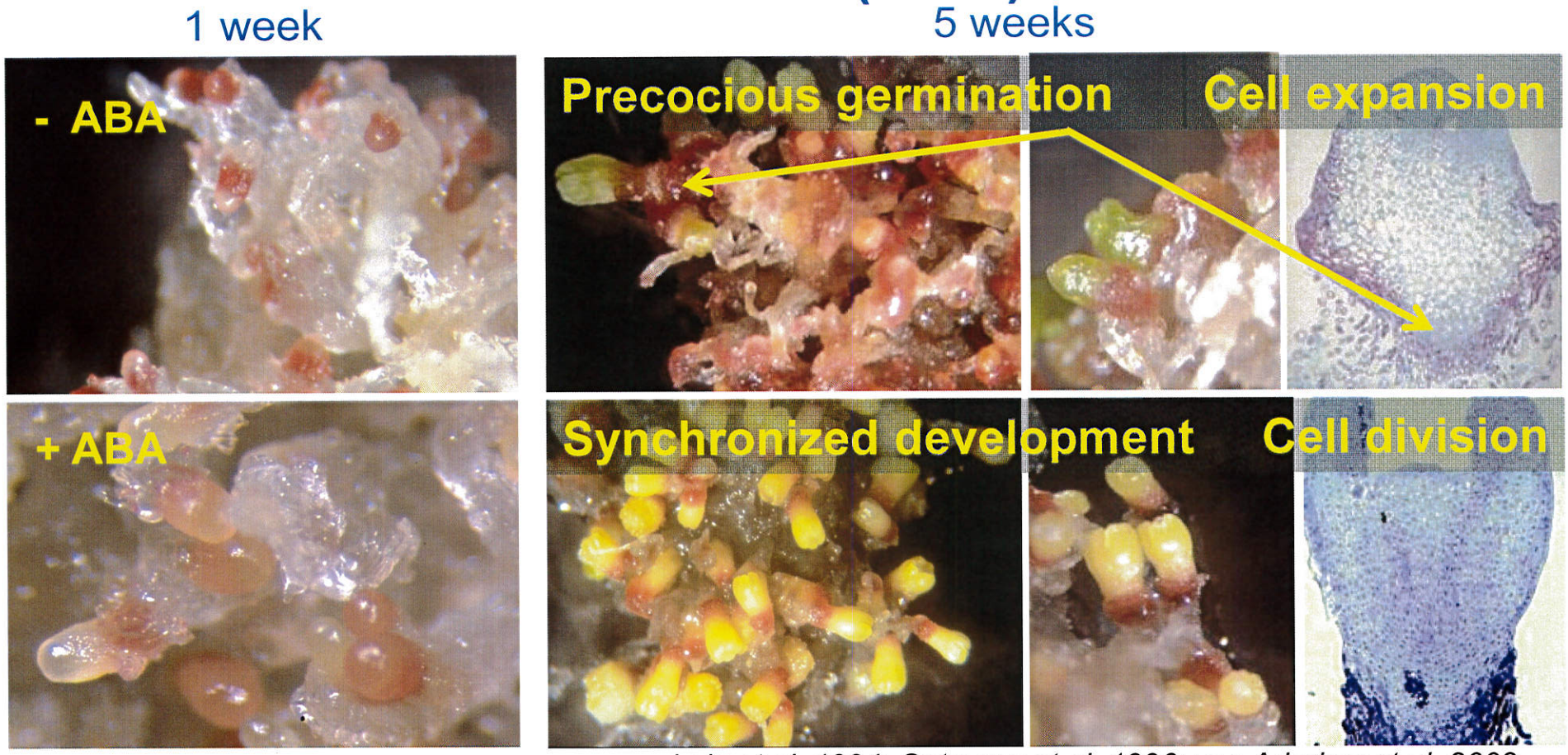


Larix decidua (36%)
Larix kaempferi (**67**%)
Larix laricina (44%)
Larix occidentalis (**93**%-3% prol.)
Larix sibirica (18%)
Larix x *eurolepis* (**78**%)
Larix x *marschlinsii* (**62**%)

Larix decidua (5%)

L x *marschlinsii* (3%)

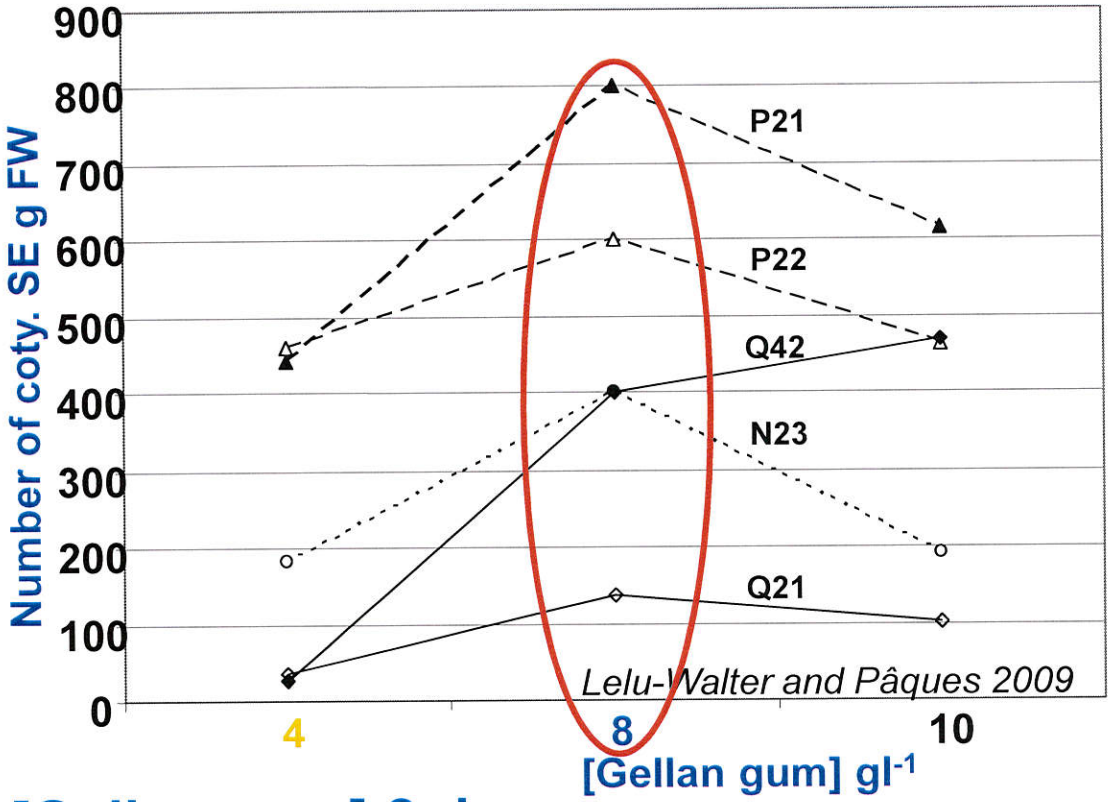
Somatic embryogenesis : maturation abscisic acid (ABA)

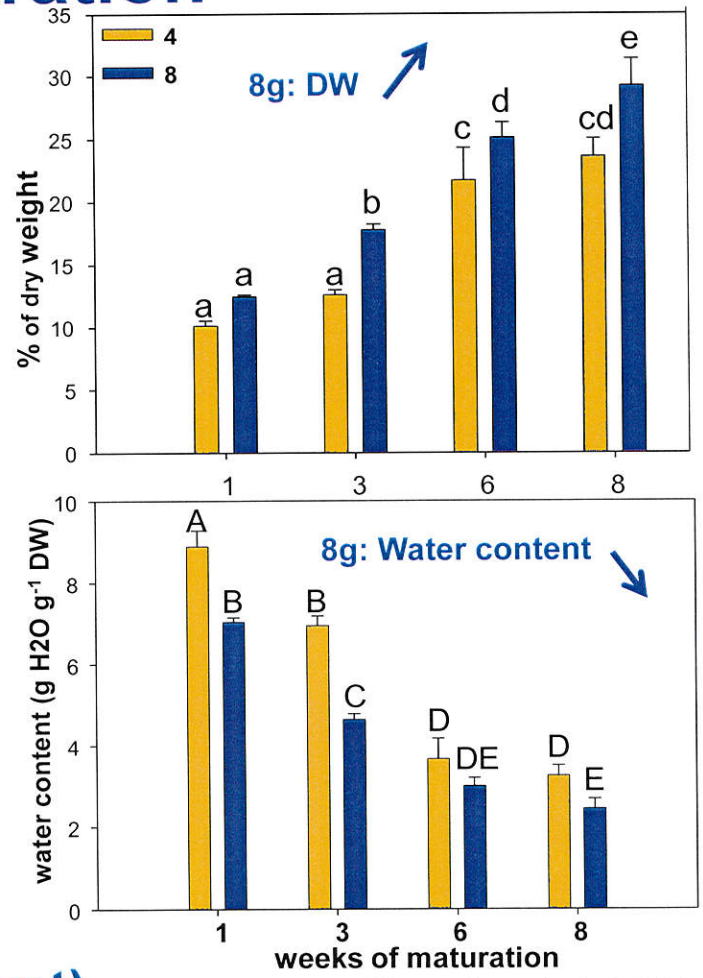


Lelu et al. 1994; Gutmann et al. 1996; von Aderkas et al. 2002

→ ABA 60µM; 0.2M sucrose or maltose (Kim and Moon 2007)

Somatic embryogenesis : maturation
Gellan gum concentration





Teyssier et al. 2011

[Gellan gum] 8gl:

- **Improve number SE** (Kim and Moon 2007)
- **improve SE quality (↗DW, ↘water content)**

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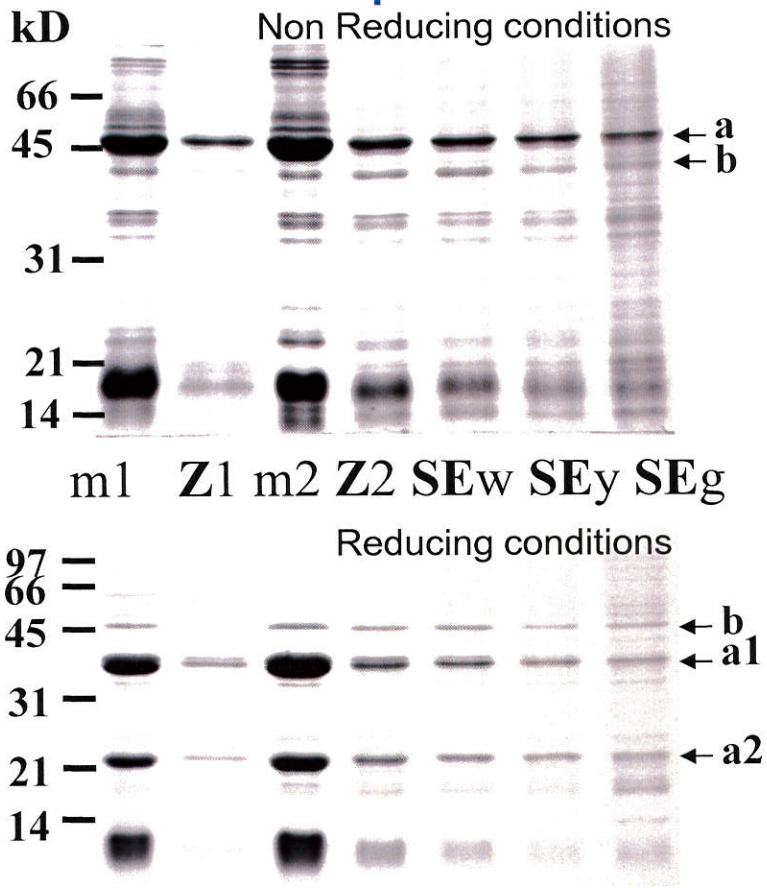
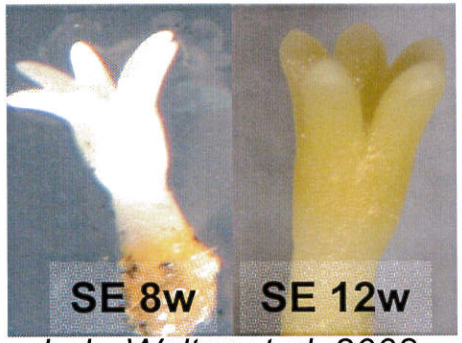
Somatic embryo maturation : when harvesting SE?

Scots pine 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

Maturation 8 to 12 w: ↘ protein content

Cotyledonary SE 8w old similar to ZE

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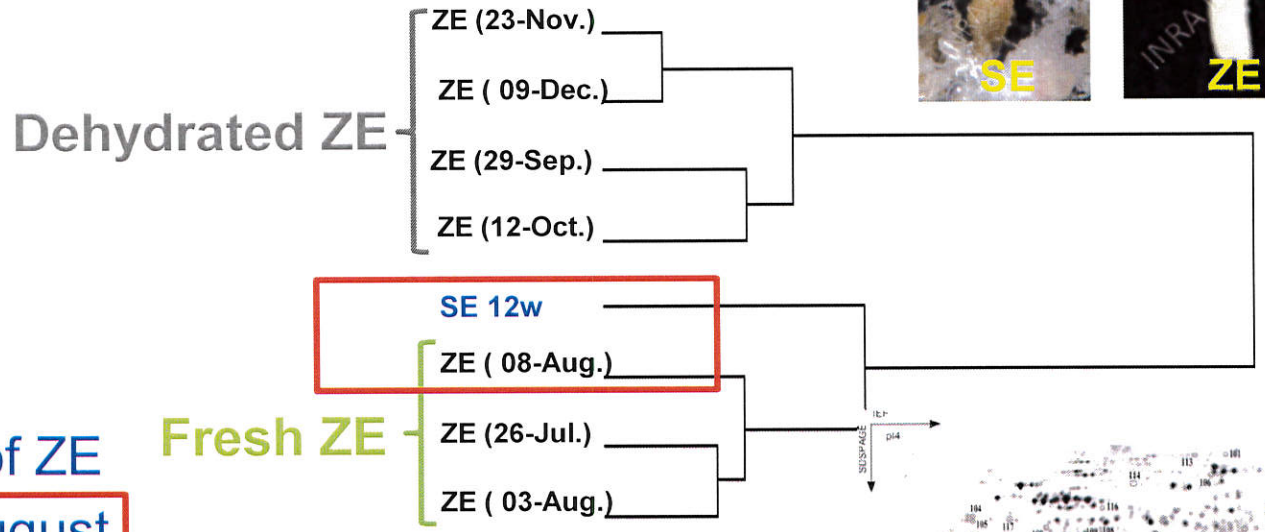
Somatic embryo maturation : when harvesting SE?

Maritime pine somatic embryo *vs* zygotic embryo

Coty ZE from July-December *vs* coty SE (10, 12, 14 weeks)

Hierarchical ascendant classification (HAC), 9 explicative variables

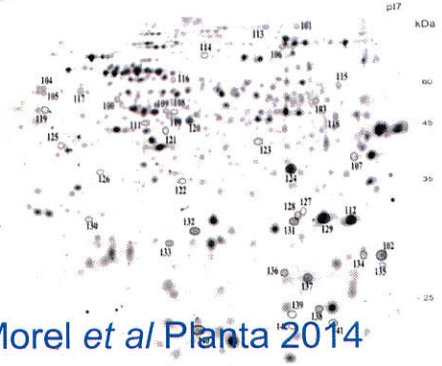
- Water content
- Fructose
- Galactose
- Glucose
- Maltose
- Sucrose
- Stachyose
- Raffinose
- Total proteins



2 groups of ZE

12w SE ~ ZE 08 August

Proteome profiling 95% similarity



Morel *et al* Planta 2014

Maturation conditions are satisfactory

Harvesting SE at 12 weeks is correct

12w SE closed to fresh cotyledonary ZE

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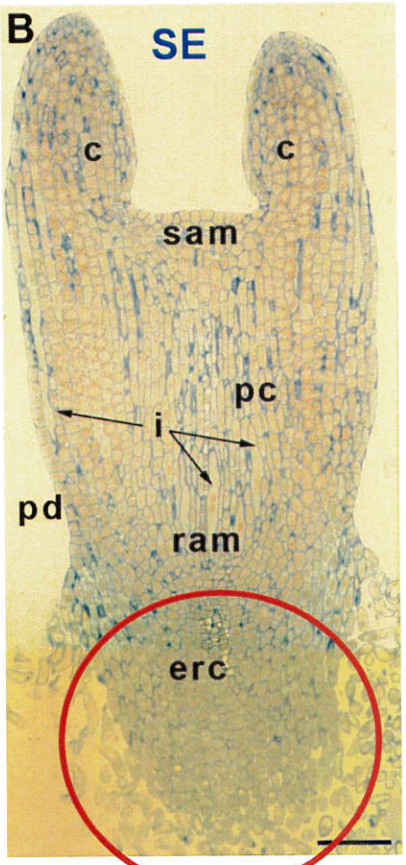
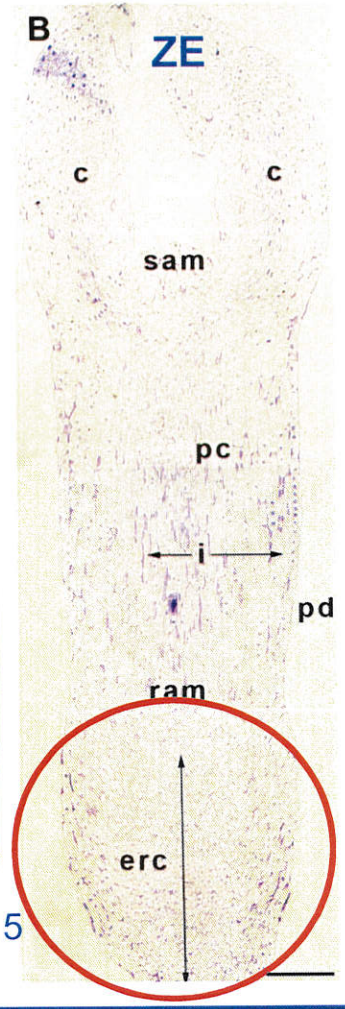
Somatic embryo maturation : when harvesting SE?

Hybrid larch somatic embryo *vs* zygotic embryo





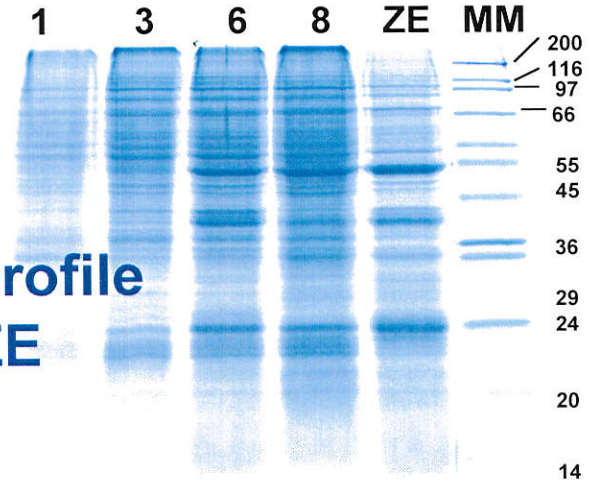
Von Aderkas *et al.* 2015

Total proteins in somatic and zygotic embryo of hybrid larch

Embryo	Maturation time (week)	Protein quantity µg.mg^{-1} FW	Protein quantity µg. unit^{-1}
Somatic	1	10.45 ±2.5^{a}	n.d.*
Somatic	3	31.69 ±1.7^{b}	10.47 ±0.9$^{\alpha}$
Somatic	6	63.36 ±8.0^{c}	61.16 ±4.0$^{\beta}$
Somatic	8	59.06 ±16.0^{c}	78.63±17.2$^{\gamma}$
Zygotic		n.d.	98.34 ±11.09$^{\gamma}$

* n.d.: not determined

Cytology,
Protein content/ profile
SE 8w similar to ZE



Teyssier *et al.* 2014

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Perspectives for breeding

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

→ To maximize breeding efficiency of forest trees

- Better precision in breeding value evaluation
  - More copies/genotype
  - More sites
- Access to phenotypic plasticity
- Access to repeatable and stable genetic references
  - Less subjective scoring (scoring scale)
  - Estimation of gains

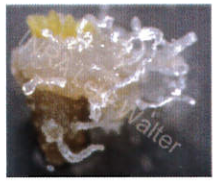
→ For accelerating deployment of improved varieties

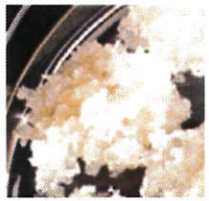
- Faster implementation
- Higher flexibility (better fit to selection progress)
- More gain

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: >1000 progenies tested
- 1978-1985: plantation of 1st generation 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 *Larix marschlinsii*) INRA H1, REVE VERT

Somatic embryogenesis improvements:
Initiation frequency





	2003	2004	2005	2006
	100 ZE	100 ZE	100 ZE	100 ZE
Initiation	20%	**53%**	**60%**	**75%**
Proliferation		35 **66%**	40 **66%**	60 **78%**

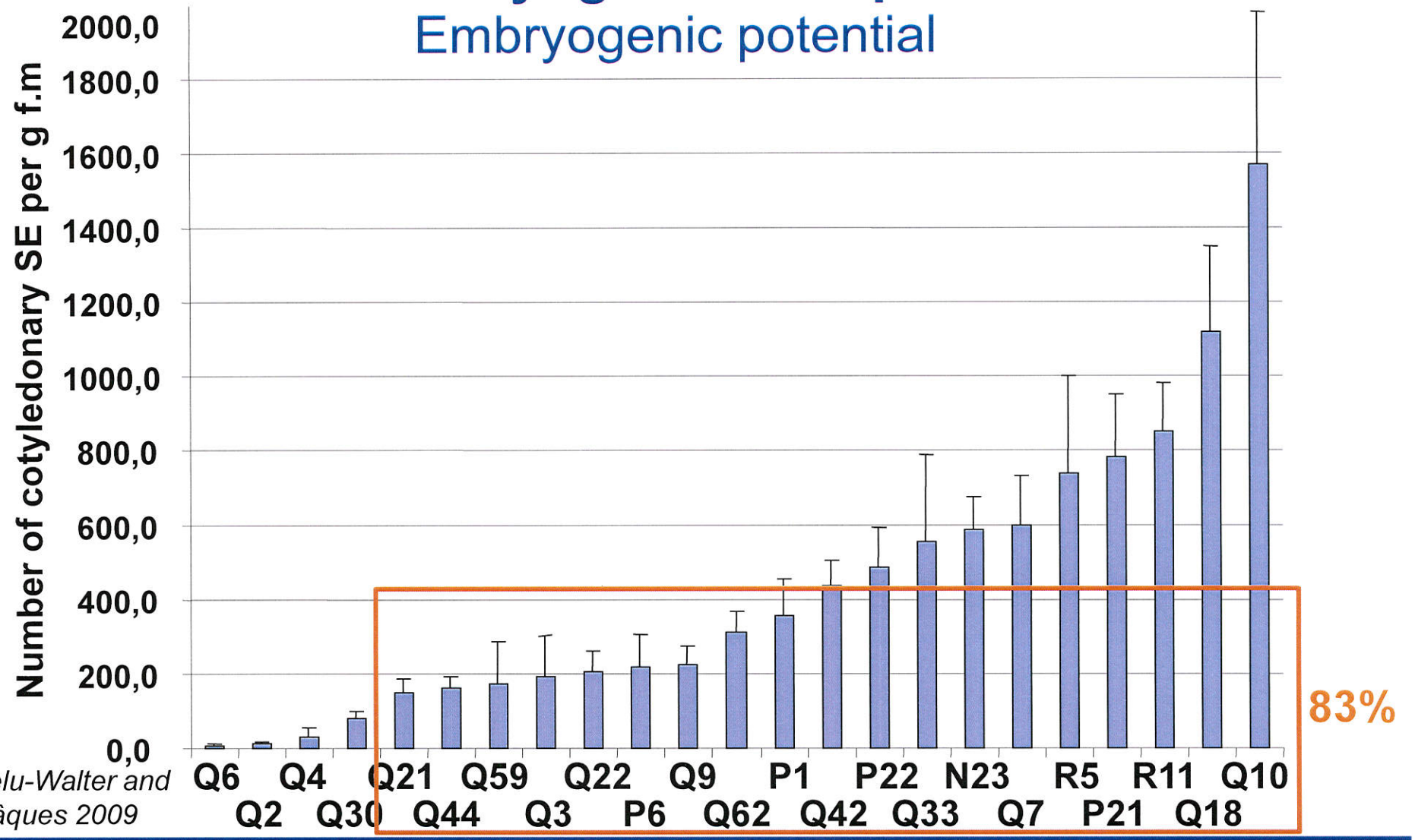
High initiation frequency
Proliferation frequency/ 60 established lines

Somatic embryogenesis improvements:
Embryogenic potential

	Responding	Non Responding
Hybrid larch 53 lines tested	**94%** (50)	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
50 maturing lines

Somatic embryogenesis improvements:



Lelu-Walter and Pâques 2009

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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?

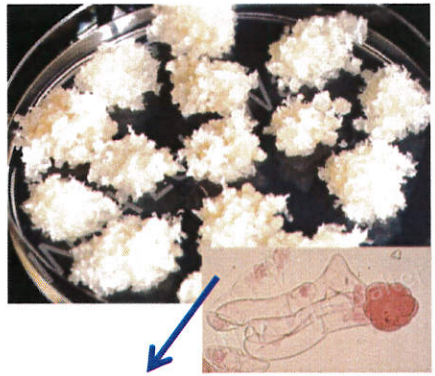
Storage duration in N2	N° SE g FW
Original (69-18)	930
1 year (69-18c)	1106
3 years (69-18c2)	744

Cryopreservation did not change the embryogenic potential

Performances of each step of hybrid larch SE

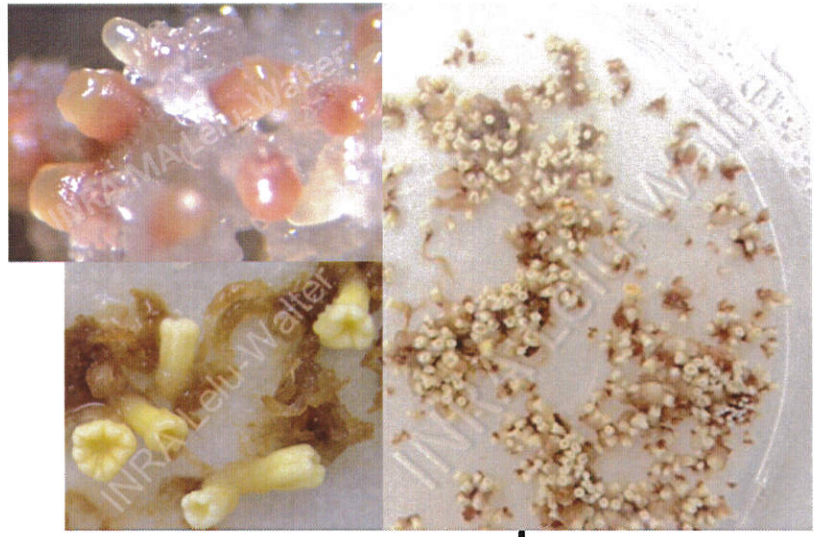
100 ZE → **Initiation: 68%**
68 EMs

Proliferation: 78%
53 established lines

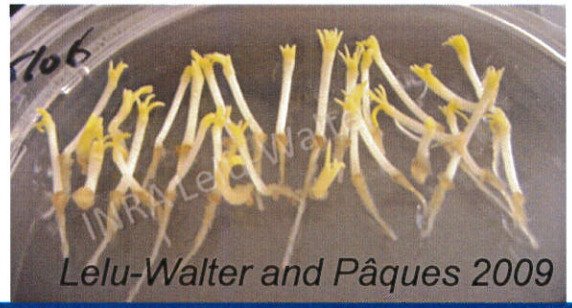


Cryopreservation: 100%

Maturation: 94%
50 maturing lines



Germination/conversion: 100%
50 lines giving rise to plants



Acclimatation: 100% ← **Transfert to soil**

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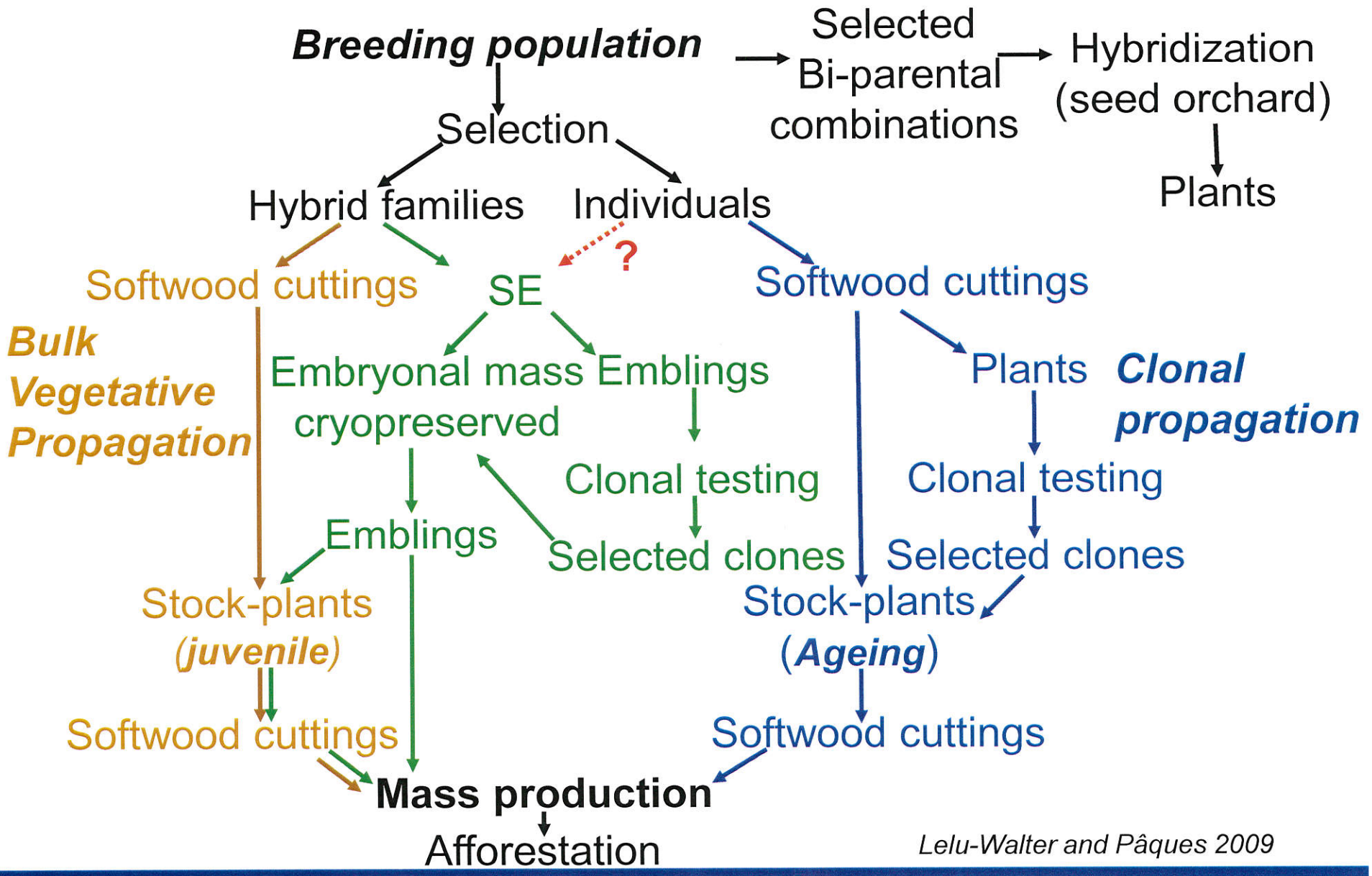

Lelu-Walter and Pâques 2009

Needs for the future :

- Check behaviour of the emblings in the field demonstration plots 30-40 lines from REVE VERT
- Enlarge the cryobank
- Build up a range of reference lines for subjective scoring traits (as straightness)

hybrid larch emblings REVE VERT

Needs for the future :

Middle term:

- Test SE feasibility on other combinations (ExJ, JxE)
- Test SE + Cryo + rootstock + bulk
- Simplify technology, estimate production cost
- Transfer

Long term:

- SE propagation from mature material

INRA protocol ready to transfer for cryopreservation of reference lines and propagate them at needs

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