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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
<b>Opening Ceremony</b>	
Moderator : Dr. Hyo-Shin Lee (KFRI, Korea)	
09:00~09:20	<b>Opening Address</b> * Dr. Seong-Hyeon Nam (President, Korea Forest Research Institute) <b>Congratulatory Address</b> * Dr. Jae-Seon Yi (Professor, Kangwon National University)
09:20~09:30	Photo Time
<b>Session I . Somatic Embryogenesis and Clonal Propagation</b>	
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	<b>Lunch &amp; Poster session</b>
13:00~13:30	In vitro multiplication of plus trees of Gmelina arborea and Cedrela odorata * Dr. Aguilar Maria Elena (CATIE, Costa Rica)
<b>Session II . Recent Trends in Genome and GMO Research</b>	
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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### Summary

Advances in plant biotechnology offer new opportunities in the field of plant propagation. Development of clonal propagation method, such as somatic embryogenesis has potentially numerous applications such as the production of a large number of genetically improved plants and the amenability of embryogenic cultures to cryogenic storage. Since the 90's, INRA engaged researches on somatic embryogenesis in hybrid larch species (*Larix x eurolepis*, *Larix x marschlinsii*). Indeed, compared to the parental species, the hybrids are known for their remarkable vigour as well as for their superior stem form and site adaptation (Pâques 1992). Several improvements and simplifications of somatic embryogenesis protocol were developed at all steps including initiation, cryopreservation, maturation and plant recovery. The improved procedure for somatic embryogenesis of *Larix* species leads to well-synchronised somatic embryo development. Mature cotyledonary SE most closely resemble zygotic embryo counterpart (anatomy, proteins content).

Among conifer species, somatic embryogenesis of *Larix* is becoming a model; its integration in a breeding programme is now undertaken for clonal propagation of improved material of hybrid larch *Larix x eurolepis* (Lelu-Walter and Pâques 2009). Indeed, we believe that somatic embryogenesis should influence breeding strategies by offering an alternative tool for accelerated production of plants for genotype evaluation through clonal testing; in addition, cryoconservation allows keeping juvenility of material and its production at any time. Requirements for the effective integration of somatic embryogenesis into the larch breeding programme are presented.

**Keywords:** Breeding, hybrid larch, maturation, somatic embryogenesis, zygotic embryogenesis

## Introduction

The genus *Larix* is the most abundant conifer in the Northern Hemisphere. In Europe, it is naturally limited to one species: *Larix decidua* which occupies a small territory. Foresters and breeders are also interested by other species like Japanese larch and Siberian larch. However the most promising remains the interspecific hybrid between European larch and Japanese larch which combines favorable properties from its parents.

Advances in plant biotechnology offer new opportunities in the field of plant propagation and genetic engineering. Somatic embryogenesis is recognised as the most efficient *in vitro* culture technique for the clonal propagation of forest tree due to its high multiplication rate and it constitutes a tool for rapid propagation of material from breeding programmes (Lelu-Walter *et al.* 2013). Somatic embryogenesis of larch species has been first reported in 1989 by Klimaszewska for hybrid larches *Larix x eurolepis* Henry (*Larix decidua* x *L. kaempferi*) and *Larix x marschlinsii* Coaz (*Larix kaempferi* x *L. decidua*). Since it has been obtained for European larch (*Larix decidua* Mill.), Japanese larch (*Larix kaempferi*), western larch (*L. occidentalis*), eastern larch (*L. laricina*), and more recently for Siberian larch (*L. sibirica*, Table 1). Improvement in somatic embryogenesis process (especially maturation step) has been obtained for Japanese larch (Kim and Moon 2007) and hybrid larches (Lelu-Walter and Pâques 2009). Current maturation protocols lead to a development of "mature" somatic embryos that morphologically resemble zygotic embryos and are kept in maturation during an arbitrary period before subsequent germination. Such an empirical approach does not give any information concerning the quality of somatic embryos to achieve maximal plant conversion rates. Therefore, we need to develop markers to assess the quality of somatic embryos. An approach was to follow the evolution of storage reserve accumulation such as proteins in zygotic embryos and to compare it with the accumulation pattern in maturing and mature somatic embryos (Teyssier *et al.* 2014).

Since the 90's, INRA has engaged research on somatic embryogenesis in hybrid larch; its integration into the breeding programme is now undertaken for clonal propagation of improved material of hybrid larch *Larix x eurolepis*, variety REVEVERT registered in 2005. Indeed, we believe that somatic embryogenesis should influence breeding strategies by offering an alternative tool for accelerated production of plants for genotype evaluation through clonal testing. Requirements for the effective integration of somatic embryogenesis into the breeding programme are presented.

## Results and conclusions

Recovery of embryogenic cultures depended on the developmental stage of the zygotic embryo. Research carried out over the last two decades has proven that somatic embryogenesis is initiated most efficiently from immature zygotic embryos (Table 1). Indeed, there has been limited progress toward initiating somatic embryogenesis from mature seeds. For the hybrid, *Larix x marschlinii*, needles from somatic plantlet (embling) yielded embryonal masses at a lower frequency (3%) than mature somatic embryos of the same genotype (83%, Lelu *et al.* 1994c). This decrease in embryogenic ability observed among the explants could be attributed to the differentiation related to the maturity of the explant. So far, somatic embryogenesis from mature trees of larch species has not been obtained and remains a challenge.

Once obtained, embryogenic cultures are transferred onto maintenance medium and sub-cultured every 2 weeks onto fresh medium in order to sustain their proliferation. Embryogenic cultures can be stored in liquid nitrogen at -196°C indefinitely without loss of juvenility. Cryopreservation offers real new perspectives for long-term conservation and reactivation of lines at any time (Park *et al.* 1998). In hybrid larch, a simplified cryopreservation method (ie without the use of programmable freezer) has been successfully developed. Until now, this cryopreservation technique has resulted in the recovery of all tested lines. The cryopreservation per se and its duration (at least up to 18 years) had no apparent effect on the yield of somatic embryos (Lelu-Walter and Pâques 2009).

Subsequent development of cotyledonary somatic embryos has been improved:

- when abscisic acid is added to the culture medium (Gutmann *et al.* 1996) up to 60  $\mu\text{M}$  (Lelu *et al.* 1994a; Kim and Moon 2007),
- when osmotic potential is increased to 0.2M with either sucrose (Lelu *et al.* 1994a) or maltose (Kim and Moon 2007),
- and under reduced water availability i.e. high gellam gum concentration up to 0.8% for both Japanese (Kim and Moon 2007) and hybrid larches (Lelu-Walter and Pâques 2009). For hybrid larches, compared to low gellam gum concentration (4 g l<sup>-1</sup>) maturation realised on 8g l<sup>-1</sup> resulted in an improved physiological status of the somatic embryos considering their dry weight, water content (Teyssier *et al.* 2011). Compared to zygotic embryos, somatic embryos matured 8 weeks in dark had a full set of organs (cotyledons, hypocotyl, embryonal root cap, von Aderkas *et al.* 2015) and had similar protein level (Teyssier *et al.* 2014).

For hybrid larches, number of SE produced among the embryogenic lines is ranging from 8 to over 1500 per g FW (Figure 1), a phenomenon commonly observed in conifer species. However, using the improved protocol for hybrid larch, 94% of the lines produced mature somatic embryos (Figure 2). Recovery of high quality somatic embryos resulted in high germination and plant frequencies (96 and 65% respectively).

All these progress have contributed to the development of an improved procedure leading to the routine production of emblings. The new protocol was applied for the propagation of the new hybrid variety REVE-VERT (Lelu-Walter and Pâques 2009, Figure 2). Among conifer species, somatic embryogenesis of *Larix* is becoming a model and its integration in a breeding programme is now undertaken. Perspectives offered by cryoconservation have boosted further its interest as it allows keeping juvenility of material and its production at any time. We believe that somatic embryogenesis should influence breeding strategies by offering an alternative tool for accelerated mass-production of plants from improved genotypes (FS hybrid family). Another use could be to use somatic embryogenesis as an alternative to rooted cuttings to increase precision in progeny testing: vegetative propagating of hybrid full-sib families would allow testing the same genotypes in contrasting environments and evaluating their plasticity but also testing them over a significantly greater number of test sites. Indeed, due to low reproductive success in larch, the number of sibs per FS-family is usually much reduced, especially when one has to achieve factorial/diallel mating designs.

However, additional questions need still to be addressed. As with any propagation system, breeders are concerned by the integrity of the propagated improved variety both in terms of its mean performance and of its genetic diversity. As it has been demonstrated for rooted cuttings, trees produced by somatic embryogenesis must show no detrimental abnormalities and prove to behave like or even better than seedlings in terms of growth, architecture, stability, maturation. Practical questions related to logistic aspects (e.g. how many families and individuals per family can be practically managed over the different steps of the technique?) and cost of propagation by somatic embryogenesis should be properly addressed.

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## References

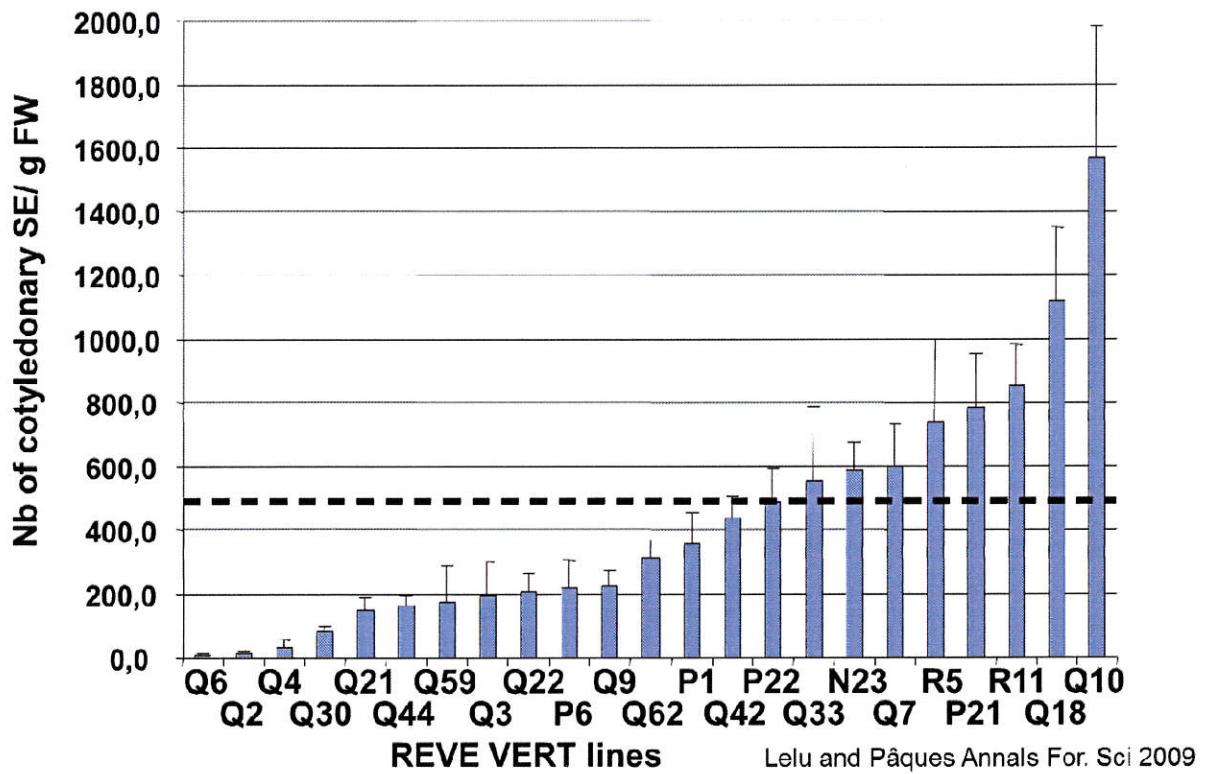
- Gutmann M, von Aderkas P, Label P, Lelu MA: Effects of abscisic acid on somatic embryo maturation of hybrid larch. *J Exp Bot* 1996, 47: 1905-1917.
- Kim YW, Young Y, Noh ER, Kim JC: Somatic embryogenesis and plant regeneration from immature zygotic embryos of Japanese larch (*Larix leptolepis*). *Plant Cell Tissue Organ Cult* 1999, 55: 95-101.
- Kim YW, Moon HK: Enhancement of somatic embryogenesis and plant regeneration in Japanese larch (*Larix leptolepis*). *Plant Cell Tissue Organ Cult* 2007, 88: 241-245.
- Klimaszewska K: Plantlet development from immature zygotic embryos of hybrid larch through somatic embryogenesis. *Plant Science* 1989, 63: 95-103.
- Klimaszewska K, Devantier Y, Lachance D, Lelu MA, Charest P: *Larix laricina* (tamarack) : somatic embryogenesis and genetic transformation. *Can J For Res* 1997, 27: 538-550.
- Lelu-Walter MA, Pâques L: Simplified and improved somatic embryogenesis of hybrid larches (*Larix x eurolepis* and *Larix x marschlinsii*). *Perspectives for breeding. Annals of For Sci* 2009, 66, 104p1- 104p10.
- Lelu MA, Bastien C, Ward C, Klimaszewska K, Charest P: An improved method for somatic plantlet production in hybrid larch (*Larix x leptoeuropaea*): Part 1. Somatic embryo maturation. *Plant Cell Tissue Organ Culture* 1994a, 36: 107-115.
- Lelu MA, Bastien C, Klimaszewska K, Charest P: An improved method for somatic plantlet production in hybrid larch (*Larix x leptoeuropaea*): Part 2. Control of germination and plantlet development. *Plant Cell Tissue Organ Culture* 1994b, 36: 117-127.
- Lelu MA, Klimaszewska K, Charest P: Somatic embryogenesis from immature and mature zygotic embryos and from cotyledons and needles of somatic plantlets of *Larix*. *Can J For Res* 1994c, 24: 100-106.
- Lelu-Walter MA, Thompson D, Harvengt L, Sanchez L, Toribio M, Pâques LE: Somatic embryogenesis in forestry with a focus on Europe: state-of-the-art, benefits, challenges and future direction. *Tree Genet Genomes* 2013, 9: 883-899.
- Pâques LE: Performance of vegetatively propagated *Larix decidua*, *L. kaempferi* and *L. laricina* hybrids. *Ann Sci For* 1992, 49: 63-74.
- Park YS, Barrett JD, Bonga JM: Application of somatic embryogenesis in high-value clonal forestry: deployment, genetic control, and stability of cryopreserved clones. *In Vitro Cell Dev Biol Plant* 1998, 34: 231-239.
- Saly S, Joseph C, Corbineau F, Lelu MA, Côme D: Induction of secondary somatic embryogenesis in hybrid larch (*Larix x leptoeuropaea*) as related to ethylene. *Plant Growth Regulation* 2002, 37: 287-294.

- Szczygieł K: Mikrorozmnażanie modrzewia europejskiego (*Larix decidua* Mill.) metodą somatycznej embriogenezy. (Micropropagation of European larch (*Larix decidua* Mill.) via somatic embryogenesis). Leśne Prace Badawcze 2005, 4: 47-76.
- Teyssier C, Grondin C, Bonhomme L, Lomenech A-M, Vallance M, Morabito D, Label P, Lelu-Walter MA: Increased gelling agent concentration promotes somatic embryo maturation in hybrid larch (*Larix x eurolepis*): a 2- DE proteomic analysis. *Physiol Plant* 2011, 141: 152-165.
- Teyssier C, Maury S, Beaufour M, Grondin C, Delaunay A, Le Metté C, Ader K, Cadene M, Label P, Lelu-Walter MA: In search of markers for somatic embryo maturation in hybrid larch (*Larix x eurolepis*): global DNA methylation and proteomic analyses. *Physiol Plant* 2014, 150: 271-291.
- Thompson RG, von Aderkas P: Somatic embryogenesis and plant regeneration from immature embryos of western larch. *Plant Cell Rep* 1992, 11: 379-385.
- Tretiakova I, Voroshilova E, Ivanitskya A, Shuvaev D, Park M: The embryogenic lines and somatic embryogenesis of coniferous species in Siberia. In: Park, Y.S. and Bonga, J.M. (eds). Proceedings of the IUFRO Working Party 2.09.02 conference on "Integrating vegetative propagation, biotechnologies and genetic improvement for tree production and sustainable forest management" 2012, June 25-28, 2012, Brno Czech Republic. Published online: <http://www.iufro20902.org/>, p71-79.
- von Aderkas P, Klimaszezewska K, Bonga JM: Diploid and haploid embryogenesis in *Larix leptolepis*, *L. decidua* and their reciprocal hybrids. *Can J For Res* 1990, 20: 9-14.
- von Aderkas P, Teyssier C, Charpentier JP, Gutmann M, Pâques L, Le Metté C, Ader K, Label P, Kong L, Lelu-Walter MA: Effect of light conditions on anatomical and biochemical aspects of somatic and zygotic embryos of hybrid larch (*Larix x marschlinsii*). *Annals of Botany* 2015, 115, 605-615.

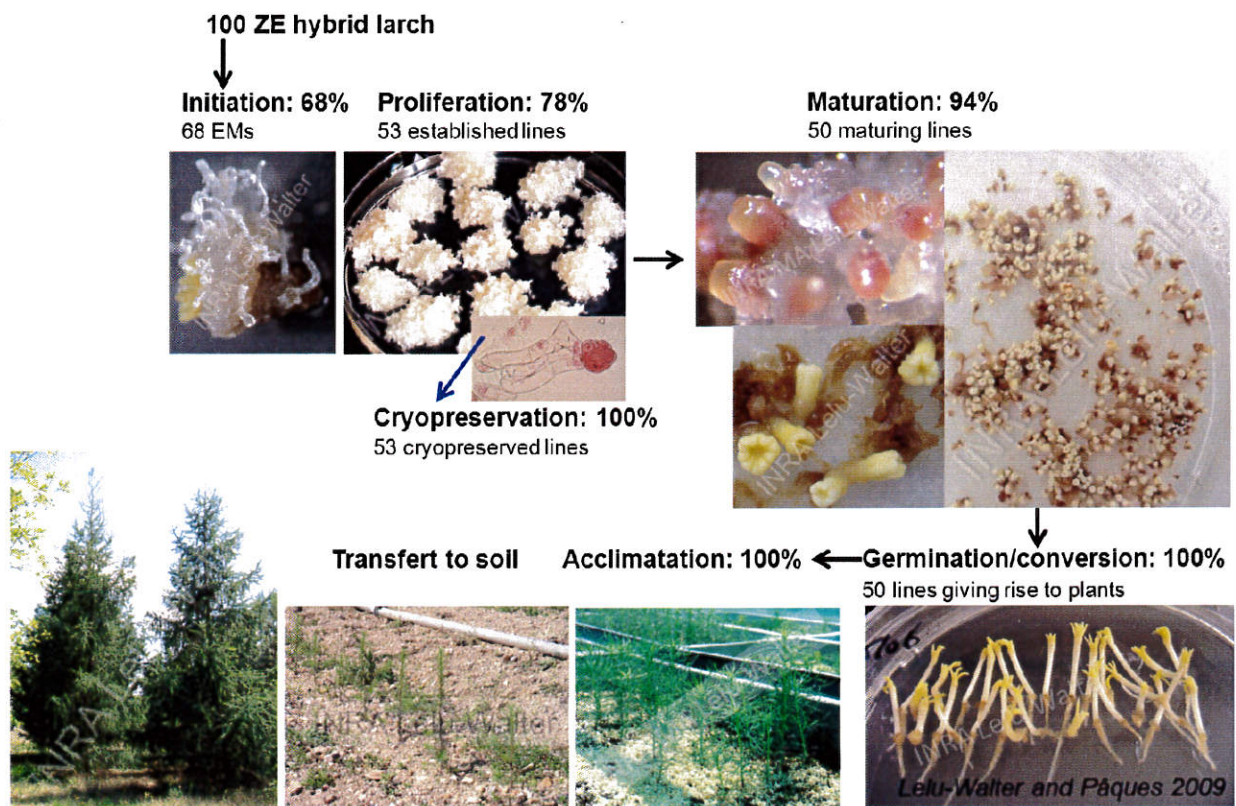
**Table 1.** Reports on somatic embryogenesis in the genus *Larix*.

<b>Species</b>	<b>Explant type</b>	<b>Initiation response (max %)</b>	<b>References</b>
<i>Larix decidua</i>	Precotyledonay ZE	21	von Aderkas <i>et al.</i> 1990
	Precotyledonay ZE	36	Szczygieł K 2005
	Immature coty. ZE	9	Szczygieł K 2005
	Mature ZE (stored seed)	5	Lelu <i>et al.</i> 1994c
<i>Larix kaempferi</i>	Precotyledonay ZE	17	von Aderkas <i>et al.</i> 1990
		67	Kim <i>et al.</i> 1999
<i>Larix laricina</i>	Precotyledonay ZE	44	Klimaszewska <i>et al.</i> 1997
<i>Larix occidentalis</i>	Immature coty. ZE	93	Thompson and von Aderkas 1992
<i>Larix sibirica</i>	Immature coty. ZE	18	Tretiakova <i>et al.</i> 2012
<i>Larix x eurolepis</i> ( <i>L. decidua</i> x <i>L. kaempferi</i> )	Precotyledonay ZE	15	Klimaszewska 1989
		78	Lelu-Walter and Pâques 2009
<i>Larix x marschlinsii</i> <i>L. kaempferi</i> x <i>L. decidua</i> )	Precotyledonay ZE	25	Klimaszewska 1989
		62	Lelu <i>et al.</i> 1994c
	Immature coty. ZE	26	Lelu <i>et al.</i> 1994c
	Cotyledonary SE	98	Saly <i>et al.</i> 2002
	Needle (embling)	3	Lelu <i>et al.</i> 1994c

SE: somatic embryo; ZE: zygotic embryo ; emblings: plant regenerated from a somatic embryo.



**Figure 1:** Mean number of cotyledonary somatic embryos produced per *Larix x eurolepis* line (variety REVE-VERT).



**Figure 2.** Hybrid larch somatic embryogenesis: performances of each step.