Towards a better understanding of conifer somatic embryo development via proteomic analysis

Caroline Teyssier, Ludovic Bonhomme, Domenico Morabito, Michel Vallance, Philippe Label, Marie-Anne Lelu-Walter

Towards a better understanding of conifer somatic embryo development by proteomic analysis

Caroline TEYSSIER[1], Cécile GRONDIN[1], Ludovic BONHOMME[2], Domenico MORABITO[2], Michel VALLANCE[1], Philippe LABEL[1], Marie-Anne LELU-WALTER[1]

[1] INRA, UR 588 Research Unit: Breeding, Genetic and Physiology of Forest Trees, 2163 Avenue de la Pomme de pin, F-45166 Olivet Cedex, France
[2] University of Orléans, LBLGC, UPRES EA 1207. Rue de Chartres, BP 6759 F-45067 Orléans Cedex 2, France

INTRODUCTION

Advances in plant biotechnology offer new opportunities in the field of plant propagation and genetic engineering. 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. It is evident from empirical knowledge that there are critical factors controlling the various stages of somatic embryogenesis in conifers. 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 quality of somatic embryos to achieve maximal plant conversion rates. Therefore, we need to **develop markers to assess the quality of somatic embryos**. Our approach is to follow the evolution of storage reserve accumulation such as proteins in zygotic embryos and compare it to the accumulation pattern in maturing and mature somatic embryos.

MATERIAL & METH.

Experiments were conducted with highly embryogenic lines of hybrid larch *Larix eurolepis* (*Larix decidua* x *Larix kaempferi*). Basal medium was MSG (Becwar *et al*. 1990) containing glutamine. Embryonal tissue was spread over filter paper according to the method developed for *Pinus pinaster* (Lelu-Walter *et al*. 2006). Maturation protocol was adapted from Label and Lelu (2000). Samples were collected from a maturation medium at different times.

For 1D-electrophoresis, total proteins were extracted (50 mg of embryogenic mass and 25 mg of somatic embryos) in presence of Tris buffer with glycerol, SDS, PVPP and beta-ME. The protein pattern was revealed by silver-staining.

For 2D-electrophoresis, the total proteins were extracted by acetone-TCA precipitation, from 4 and 7 weeks-old SE, according to Damerval *et al*. (1986). First dimension was realized on a linear pH gradient 4-7, whereas second dimension was conducted on 11% SDS-PAGE. Polypeptides were revealed using colloïdal Coomassie blue staining, digitalized with ImageScanner II and analyzed with Image Master 2D Platinum software.

RESULTS and DISCUSSION

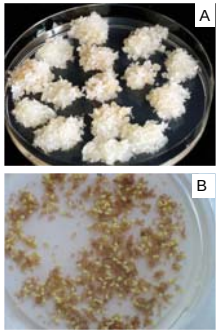

Figure 1: Examples of somatic embryo developmental stages.
A: embryonal mass in multiplication.
B: cotyledonary somatic embryos after 7 weeks on maturation medium.

A

	MEx	MECa	SE7	SE13
µg protein / mg fresh sample	2.18 ± 0.1	2.24 ± 0.1	5.50 ± 0.4	4.52 ± 0.1

Figure 2A: Comparison of the extracted quantity of protein per mg of fresh material. Values are means of 2 samples.

B

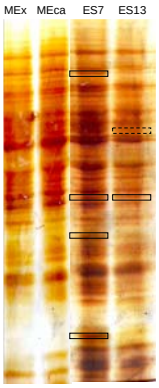

Figure 2B :SDS-PAGE of somatic embryo proteins harvest during multiplication (MEx), after 1 week on charcoal medium (MEca), 7 (SE7) or 13 weeks (SE13) respectively after development.
▭ : increase of the band intensity.
▭ : decrease of the band intensity.

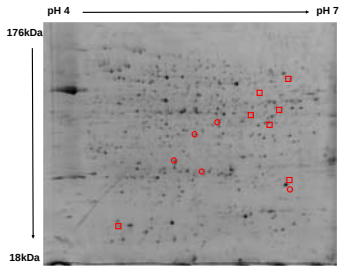

Figure 3 : Protein expression pattern of somatic embryos of 7 weeks. Circles show spots where the normalized volume was at least 8 times superior to spots from 4-weeks somatic embryos. Squares show spots where the normalized volume was at least 8 times inferior to normalized volume spots from 4 weeks old somatic embryos.

We compared the content of proteins in embryos of different ages to evaluate the potential of this parameter as marker of the development of embryos.
The quantity of total proteins showed a clear evolution during that development (Figure 2A) with a peak at 7 weeks. This could be correlated with the highest capacity of embryo to germinate at 7 weeks rather than at 13 weeks (Label and Lelu 1994). At this stage, mature conifer zygotic embryos contain predominant storage protein reserves (Flinn *et al*., 1989) and a global quantification could be used as a first indication of quality as exemplified in Figure 2A. The profile of total protein extract was analyzed by 1D and 2D-gel electrophoresis. Both exhibit some differences with increases and decreases of bands or spots (Figures 2B and 3). The analysis by 2D-gel electrophoresis (Figure 3) presents this evolution for each protein.
In conclusion: in hybrid larch the amount of total proteins could be a rapid and simple method to evaluate the maturation of somatic embryos and their capacity to germinate. 1D and 2D gels reveal the proteins that could be involved in the maturation process. The next step should be their identification by mass-spectrometry.

REF.

Becwar M.R., Nagmani R. and Wann S.R. 1990, Can. J. For. Res. 20 : 810-817 ; Damerval C., D. de Vienne, Zivy M. and Thiellement, H. 1986, Electrophoresis. 7:52-54. ; Flinn B.S., Webb D.T. and Newcomb W. 1989, Can. J. Bot. 67:779-789 ; Label P. and Lelu M-A. 1994, Plant Growth Regul. 15: 175-182 ; Label P. and Lelu M-A. 2000, Physiol. Plant. 109: 456-462 ; Lelu-Walter M-A., Bernier-Cardou M. and Klimaszewska K. 2006, Plant Cell Reports, 25, 767-776 ; Percy R., Klimaszewska K., Cyr D. R. 2000, Can. J. For. Res. 30: 1867-1876.