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Other Vegetative Propagation Technologies

BOOK OF ABSTRACTS

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Are cotyledonary somatic embryos of *Pinus pinaster* Ait. equivalent to the zygotic counterpart?

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In maritime pine, improved protocols are now available for the whole somatic embryogenesis process i.e. from initiation of embryogenic tissue to somatic plant regeneration. However, field trials established in France from somatic plant material have consistently revealed that somatic embryos (SEs) developed at a lower initial growth rate compared to control seedlings. A better understanding of SEs maturation is therefore required in order to produce high-quality, vigorous somatic plants. SEs are currently matured for 12 weeks to reach the cotyledonary stage before being germinated and converted to plantlets. Although regeneration success is highly dependent on SEs quality, the harvesting date is still determined from morphological features. This empirical method does not provide any accurate information about embryo quality with respect to storage compounds (proteins, carbohydrates). We first analyzed SEs matured for 10, 12 and 14 weeks by carrying out biological (dry weight, water content) and biochemical measurements (total protein and carbohydrate contents). No significant difference could be found between collection dates, suggesting that SE harvesting after 12 weeks maturation is appropriate. Cotyledonary SEs were then compared to various stages, from fresh to fully desiccated, in the development of cotyledonary zygotic embryos (ZEs). Using hierarchical ascendant cluster analysis, we demonstrated that cotyledonary SEs matured for 12 weeks are most similar to fresh cotyledonary ZEs sampled from late July to early August (as indicated by dry weight, water content, sucrose, RFOs content, RFO/Sucrose ratio) or at any time up to October (with respect to total protein). Both types of embryo exhibited similar carbohydrate and protein content and signatures. This high level of similarity (94.5%) was further supported by proteome profiling. Highly expressed proteins included storage, stress-related, late embryogenesis abundant (LEA) and energy metabolism proteins. By comparing overexpressed proteins in developing and cotyledonary SEs or ZEs, some (23 proteins) could be identified as candidate biomarkers for the late, cotyledonary stage. Of these, 18 belonged to five large families of proteins including five HSPs, four LEAs and two other stress-related proteins (aldose reductase, 6-phosphogluconate dehydrogenase), five storage proteins and two proteins involved in purine metabolism (adenosine kinase 2, SAM synthase).

This is the first report of useful generic protein markers for monitoring embryo development in maritime pine. Our results also suggest that improvements of SEs quality may be achieved if the current maturation conditions are refined.

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Keywords: carbohydrates, embryo quality, maturation, proteomic, somatic embryogenesis, zygotic embryogenesis.

