Multi-scale analysis of early molecular events during Pinus pinaster Ait. somatic embryo development under reduced water availability

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Multi-scale analysis of early molecular events during *Pinus pinaster* Ait. somatic embryo development under reduced water availability

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Significant progress has been made in the development of maritime pine somatic embryogenesis but there are still technical issues precluding full integration of this powerful vegetative propagation system into the French breeding programme. Maritime pine somatic embryos (SEs) require a reduction in water availability (high gellan gum concentration in the maturation medium) to reach the cotyledonary stage. This key switch, reported specifically for pine species, is not yet well understood. To facilitate the use of somatic embryogenesis for mass propagation of conifers, we need a better understanding of embryo development.

Comparison of the transcriptome (Illumina RNA sequencing) and proteome (2D-SDS-PAGE with MS identification) of immature SEs, cultured on either high (9 gl^{-1}, 9G) or low (4 gl^{-1}, 4G) gellan gum concentration, was performed, together with analysis of water content, fresh and dry mass, endogenous ABA (GC-MS), soluble sugars (HPLC), starch, and confocal laser microscope observations. This multi-scale, integrated analysis was used to unravel early molecular and physiological events involved in SE development. Under conditions unfavorable for SE maturation (4G) both transcriptomic and proteomic profiling indicate enhanced glycolysis leading to proliferation of embryonal masses (EMs) which may be antagonistic to SE maturation. Under favorable conditions (9G), we observed adaptive, ABA-mediated molecular and physiological responses to reduced water availability resulting in early transition of EMs from proliferation to the SE developmental pathway (indicated by active protein synthesis, and overexpression of proteins involved in cell division, embryogenesis and starch synthesis). Specific pathways (synthesis of protective secondary metabolites, regulation of oxidative stress) are also activated, apparently to overcome constraints due to culture conditions.

This is the first study on the early molecular mechanisms involved in somatic embryogenesis of pine following an increase in gellan gum concentration in the maturation medium, and it is also the first report combining transcriptomic and proteomic data analysis during somatic embryogenesis in conifers. We have found novel candidate predictive markers for conifer SE development (germin-like protein and ubiquitin-protein ligase) as well as for adaptive responses (protein phosphatase 2C), which may facilitate practical application of the knowledge gained in this study to monitor early responses of embryogenic tissue to maturation conditions.

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Keywords: somatic embryo development, germin-like protein, glycolysis, transcriptomics, proteomics.