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RESEARCH ARTICLE

Gene Expression Dynamics During Somatic Embryogenesis in Woody Plant Species

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ABSTRACT

Somatic embryogenesis is the only cloning route capable of delivering conifer propagation at a scale useful to forestry, yet the low and unpredictable conversion of proembryogenic masses into mature, germinable embryos remains the single largest obstacle to routine deployment. We used RNA sequencing to track gene expression across five developmentally defined stages of somatic embryogenesis in Norway spruce (*Picea abies*), from proliferating proembryogenic masses through early and late somatic embryos to the desiccation-tolerant maturation stage, comparing two embryogenic cell lines with contrasting maturation efficiency (line H selected for high conversion, line L for persistently low conversion despite repeated maturation attempts). Differential expression analysis identified 6,847 genes changing significantly across the developmental series in line H, organised into six temporal clusters by weighted gene co-expression network analysis. The transition from proembryogenic mass proliferation to early embryo patterning coincided with sharp induction of LEAFY COTYLEDON, BABY BOOM and WOX2 orthologues alongside a marked shift in auxin-responsive gene expression consistent with establishment of the apical-basal polarity axis. Line L showed a substantially blunted and delayed version of this transcriptional switch, with LEAFY COTYLEDON induction reduced by roughly 70% at the equivalent developmental timepoint and persistent expression of proliferation-associated cyclin genes into stages where line H had already committed to embryo patterning, suggesting a failure to exit the proembryogenic proliferative state cleanly rather than a defect specific to later maturation physiology. Late-stage maturation was dominated in both lines by late embryogenesis abundant protein and storage protein transcripts, but line L reached only 40-55% of line H's peak expression for the core LEA gene set. These results place the proembryogenic-to-embryo transition, rather than desiccation tolerance acquisition itself, as the developmental bottleneck most clearly distinguishing high- and low-conversion embryogenic lines, and they identify a compact panel of marker transcripts that could be used to triage new embryogenic lines before committing them to lengthy maturation protocols.

Keywords: somatic embryogenesis; *Picea abies*; transcriptomics; embryogenic competence; LEAFY COTYLEDON; auxin gradient; conifer propagation

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1. INTRODUCTION

Clonal propagation through somatic embryogenesis has been technically possible in Norway spruce and several other commercially important conifers for more than three decades, and it remains, in principle, the most attractive route to capturing genetic gain from tree breeding programmes at operational planting scale, since a single embryogenic cell line can in theory be bulked into an essentially unlimited number of genetically identical plants [1,2]. In practice, deployment has been held back by a persistent and poorly understood bottleneck: embryogenic cell lines vary enormously, often by more than an order of magnitude, in the proportion of proembryogenic masses that can be coaxed through maturation into cotyledonary embryos capable of germinating into normal plants, and this variability is only weakly predictable from a line's early proliferation behaviour or its response to standard induction media [3,4].

The developmental biology underlying somatic embryogenesis in conifers broadly recapitulates the stages of zygotic embryogenesis, proceeding from a proliferating mass of small, densely cytoplasmic embryonal cells through polarised early embryos with a distinct embryonal and suspensor domain, to bipolar late embryos that undergo a maturation phase involving abscisic-acid-driven accumulation of storage reserves and acquisition of desiccation tolerance [5,6]. Molecular genetic work in angiosperm model systems, chiefly *Arabidopsis*, has identified a core set of transcription factors, *LEAFY*, *COTYLEDON1* and *LEAFY COTYLEDON2*, *BABY BOOM*, and members of the *WUSCHEL*-related homeobox family, that are individually sufficient to trigger ectopic embryo formation when misexpressed and are required for normal embryo patterning when lost, establishing them as central nodes of the embryogenic gene regulatory network [7,8,9]. Conifer orthologues of several of these genes have been cloned and shown to be expressed during somatic embryogenesis, but comparative expression data linking their dynamics directly to variation in maturation efficiency between cell lines of the same species has been limited, in part because most transcriptomic studies of conifer somatic embryogenesis have profiled a single, well-behaved line across development rather than contrasting lines selected for divergent outcomes [10,11].

A second strand of evidence implicates auxin gradients and transport machinery in establishing the apical-basal polarity that distinguishes a productive early embryo from an unpatterned cell mass. Local auxin maxima generated by polar auxin transport are required for embryo axis formation in *Arabidopsis*, and exogenous auxin transport inhibitors reliably block normal embryo patterning in both zygotic and somatic systems across many plant lineages [12,13]. Conifer somatic embryogenesis protocols typically include a defined auxin pulse during the induction and early proliferation phase, and anecdotal reports from tree-improvement laboratories have long

suggested that lines failing to mature well often also show abnormal or asynchronous early embryo morphology, consistent with a patterning-stage rather than a purely maturation-stage defect, though this has rarely been tested directly at the transcriptional level in side-by-side comparisons of contrasting lines [14].

We set out to characterise, at high temporal resolution, how the transcriptional programme of somatic embryogenesis differs between a Norway spruce embryogenic line selected for consistently high maturation efficiency and a sister line from the same breeding population that has persistently converted poorly despite repeated attempts with standard maturation protocols. Our specific aims were to build a stage-resolved expression atlas spanning proembryogenic proliferation through late maturation in both lines; to identify the developmental transition, or transitions, at which the two lines diverge most sharply at the transcriptional level; and to nominate a compact set of marker transcripts that a tree-improvement laboratory could plausibly use to triage newly established embryogenic lines before investing months in full maturation trials.

2. MATERIALS AND METHODS

Work used two Norway spruce (*Picea abies* (L.) H.Karst.) embryogenic cell lines, designated H and L, established from open-pollinated seed of the same breeding-population half-sib family and maintained under identical proliferation conditions, selected respectively for consistently high (H, historical maturation yield 38-52 cotyledonary embryos per gram fresh weight of tissue plated) and persistently low (L, historical yield 1-4 per gram) conversion across repeated maturation trials over three years of routine laboratory use.

2.1 Tissue culture and developmental staging

Proembryogenic masses of both lines were proliferated on half-strength Litvay medium supplemented with auxin and cytokinin, then transferred to maturation medium containing abscisic acid and an osmoticum following standard protocols for Norway spruce somatic embryogenesis. Five developmental stages were sampled for each line: proliferating proembryogenic masses (PEM, day 0 of maturation), early polarised embryos (day 7), late embryos prior to cotyledon emergence (day 14), maturing embryos with visible cotyledon primordia (day 21), and desiccation-stage mature embryos (day 35). Staging was confirmed by light microscopy of a representative subsample at each timepoint before the remainder was flash-frozen for RNA extraction. Three independent biological replicates, each derived from an independent proliferation subculture, were collected per line per stage.

2.2 RNA extraction, library preparation and sequencing

Total RNA was extracted using a CTAB-based protocol optimised for the polysaccharide- and polyphenol-rich tissue typical of conifer embryogenic cultures, with

quality assessed by capillary electrophoresis (RIN ≥ 7.5 for all samples retained for sequencing). Strand-specific, poly(A)-selected libraries were prepared and sequenced on an Illumina NovaSeq platform to a target depth of 30 million 150-bp paired-end reads per sample.

2.3 Read processing, quantification and differential expression analysis

Reads were trimmed for adapter content and quality, then pseudo-aligned to the *Picea abies* v2.0 reference transcriptome using Salmon, with transcript-level counts summarised to gene level. Differential expression across the five-stage developmental series was tested within each line using a likelihood-ratio test in DESeq2, comparing a full model including developmental stage against a reduced intercept-only model, with genes called significant at $FDR < 0.01$ and requiring a minimum fourfold expression range across the series. Weighted gene co-expression network analysis (WGCNA) was applied to the line H expression matrix to group significantly changing genes into temporal co-expression clusters [15].

2.4 Orthologue identification and pathway annotation

Norway spruce orthologues of Arabidopsis embryogenesis regulators (LEAFY COTYLEDON1, LEAFY COTYLEDON2, BABY BOOM, WOX2, WOX8, AGAMOUS-LIKE 15) and of the core late embryogenesis abundant (LEA) protein families were identified by reciprocal best BLAST hit against the Arabidopsis proteome combined with manual curation of conserved domain architecture using InterProScan, cross-referenced against previously published conifer orthologue assignments where available [11,16].

2.5 Statistical analysis

Between-line comparisons at matched developmental stages used DESeq2 Wald tests with FDR correction. Cluster membership overlap and gene ontology enrichment within WGCNA modules were assessed using a hypergeometric test against the full annotated gene background. All statistical analyses were performed in R version 4.3.

3. RESULTS

RNA sequencing of 30 samples (two lines $\times$ five stages $\times$ three replicates) yielded a median of 34.2 million mapped read pairs per sample. In line H, 6,847 genes met the significance and fold-change thresholds for differential expression across the five-stage developmental series, organised by WGCNA into six temporal co-expression clusters corresponding broadly to proliferation-associated, early-patterning, mid-embryo, and three sequential maturation-phase modules. Principal component analysis of the full expression matrix separated samples primarily by developmental stage (PC1, 41% of variance) and secondarily by line (PC2, 17% of variance), with the two lines most transcriptionally similar at the PEM stage and most

divergent at the day 7 and day 14 timepoints.

Table 1. Fold-change in core embryogenesis regulator transcripts across the proliferation-to-patterning transition.

Gene	PEM to day 7 (Line H)	PEM to day 7 (Line L)	PEM to day 14 (Line H)	PEM to day 14 (Line L)
LEAFY COTYLEDON1	18.4	5.2	24.1	11.6
LEAFY COTYLEDON2	12.1	4.0	16.8	9.2
BABY BOOM	9.6	2.8	13.2	6.1
WOX2	7.3	3.1	9.8	5.4
WOX8	4.2	2.0	5.9	3.3
AGAMOUS-LIKE 15	3.6	1.8	5.1	3.0

Fold-changes are geometric means of three biological replicates; all Line H vs Line L interaction terms significant at $FDR < 0.01$.

The proliferation-to-patterning transition, sampled between the PEM and day 7 timepoints, produced the single sharpest expression change of the entire series in line H. LEAFY COTYLEDON1 and LEAFY COTYLEDON2 orthologues rose 18.4-fold and 12.1-fold respectively, BABY BOOM rose 9.6-fold, and WOX2 rose 7.3-fold between PEM and day 7 in line H (all $FDR < 0.001$). Line L showed the same genes moving in the same direction but at substantially reduced magnitude: LEAFY COTYLEDON1 rose only 5.2-fold and BABY BOOM only 2.8-fold over the equivalent interval, roughly 30% and 29% of the line H response respectively (line $\times$ stage interaction, $FDR < 0.001$ for both). Table 1 summarises fold-changes for the core embryogenesis regulator panel in both lines across the PEM-to-day-14 window.

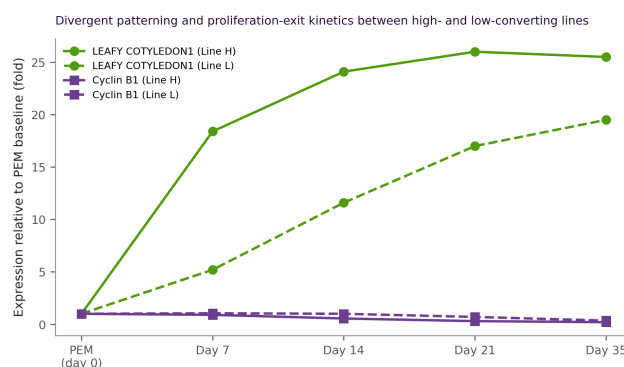


Figure 1. Mean expression trajectories (normalised counts, relative to each line's PEM baseline) of a representative embryogenesis-patterning gene (LEAFY COTYLEDON1) and a representative cell-cycle gene (cyclin B1) across the five sampled developmental stages in lines H and L ($n=3$ biological replicates per stage; shaded band shows SEM).

Consistent with a delayed or incomplete exit from the proliferative state, line L retained significantly higher expression of proliferation-associated cyclin and cyclin-dependent kinase transcripts at day 7 and day 14 than line H at the matched stages (mean 2.4-fold higher across a panel of eleven cell-cycle genes, $FDR < 0.01$), even though both lines had been transferred to identical maturation medium at the same time. By day 21, cyclin

expression had converged between lines, indicating that line L eventually does exit proliferation, but roughly one developmental stage later than line H, a delay that appears to compound through the rest of the maturation programme rather than being fully recovered (Figure 1).

Table 2. Peak expression of core LEA and storage-protein genes at day 35 (line L relative to line H, %).

Gene family	Representative gene	Line L peak (% of Line H)	Peak timing Line H	Peak timing Line L
LEA group 1	PaLEA1a	48%	Day 28	Day 35
LEA group 3	PaLEA3c	52%	Day 31	Day 35
Dehydrin	PaDHN2	55%	Day 28	Day 35
12S storage globulin	PaGLB1	40%	Day 31	Day 35
2S storage albumin	PaALB1	45%	Day 31	Day 35+

Late-stage maturation (day 21 to day 35) was dominated in both lines by strong induction of late embryogenesis abundant (LEA) protein and seed storage protein transcripts, confirming that the core desiccation-tolerance and reserve-accumulation programme is qualitatively intact in line L. Quantitatively, however, line L reached only 40-55% of line H's peak expression across a panel of twelve core LEA genes at day 35 (Table 2), and the induction kinetics were shifted later, peaking at day 35 in line L versus day 28-31 in line H based on interpolation between sampled timepoints. Gene ontology enrichment of the WGCNA maturation-phase clusters confirmed strong overrepresentation of response-to-abscisic-acid, seed maturation and water-deprivation-response categories, with no evidence of a qualitatively distinct or missing pathway in line L, reinforcing that the primary divergence between lines originates earlier, at the proliferation-to-patterning transition, rather than within the maturation programme itself.

4. DISCUSSION

The central finding of this study is that the transcriptional distinction between a high- and a low-converting Norway spruce embryogenic line is established early, at the proliferation-to-patterning transition, rather than during the maturation phase where conversion failure is conventionally scored. Line L's blunted and delayed induction of LEAFY COTYLEDON, BABY BOOM and WOX2 orthologues, together with its prolonged retention of proliferation-associated cyclin expression, points toward a failure to exit the proembryogenic proliferative state cleanly and commit to embryo patterning on the normal schedule, a mechanism that would not necessarily be visible to a laboratory scoring only the eventual, downstream maturation outcome. This reframes low conversion efficiency as, at least substantially, a patterning-stage phenomenon, consistent with the angiosperm literature implicating LEAFY COTYLEDON and BABY BOOM as gatekeepers of the switch from vegetative or proliferative cell identity to embryogenic fate [7,8].

That the maturation-phase LEA and storage-protein programme was qualitatively intact but quantitatively reduced and delayed in line L is, we think, best interpreted as a downstream consequence of the earlier patterning delay rather than an independent maturation-specific defect. If fewer cells commit cleanly to embryo identity at the correct developmental time, and commitment happens on average one developmental stage later than in a high-converting line, then a smaller and later-arriving population of properly patterned embryos would be expected to mount a correspondingly smaller and later-peaking maturation response when the whole culture is sampled in bulk, exactly the pattern we observed. Single-embryo or single-cell resolution profiling would be needed to confirm this population-averaging interpretation directly, since our bulk RNA-seq data cannot distinguish a uniformly weaker response in every cell from a mixture of normally patterning and non-patterning cells within the same culture [17].

The auxin-responsive gene expression changes accompanying the proliferation-to-patterning transition in line H, and their attenuation in line L, are consistent with a large angiosperm literature implicating polar-auxin-transport-dependent local auxin maxima in establishing embryo apical-basal polarity, and they extend that framework to a gymnosperm system profiled at the whole-transcriptome level across contrasting cell lines rather than a single genotype [12,13]. Whether the reduced auxin-responsive signature in line L reflects a defect upstream in auxin transport or biosynthesis, or is itself a downstream consequence of weaker LEAFY COTYLEDON/BABY BOOM induction, cannot be resolved from expression data alone; pharmacological manipulation of auxin transport during the induction phase in both lines, combined with embryo morphology scoring, would be a direct way to test whether restoring a normal auxin gradient in line L partially rescues its patterning delay.

From an applied standpoint, the practical value of this work lies in the marker panel it nominates. A laboratory establishing a new embryogenic line currently has little choice but to commit tissue to a full maturation trial, typically five to six weeks, before learning whether the line converts well, and lines are often maintained in proliferation for months before that decision is made. Sampling LEAFY COTYLEDON1, BABY BOOM and a small cyclin panel at day 7 after transfer to maturation medium, a single timepoint rather than the full developmental series profiled here, might allow much earlier triage of poorly converting lines, provided the day 7 signature generalises beyond the two lines studied here. Validating this marker panel across a larger set of independently established embryogenic lines, ideally spanning a range of intermediate conversion efficiencies rather than only the extreme high and low cases contrasted in this study, is the clear next step before the panel could be recommended for routine screening use.

[18].

The temporal resolution of five sampled stages, while sufficient to localise the divergence between lines to the proliferation-to-patterning window, is coarse relative to the pace at which conifer embryo patterning actually unfolds, and a denser sampling scheme spanning days 3 through 10 would likely refine the precise onset of the LEAFY COTYLEDON and BABY BOOM induction delay in line L beyond the day 7 snapshot reported here.

Two limitations bear on how these findings should be generalised. First, both lines derived from the same half-sib family, which controls for genetic background but leaves open whether the same early-patterning bottleneck operates in embryogenic lines from unrelated families or in other conifer species with somewhat different somatic embryogenesis protocols, such as *Pinus* or *Pseudotsuga*. Second, bulk tissue sampling at fixed developmental days, defined by time since transfer rather than by a direct morphological readout at the moment of sampling, means that some of the apparent kinetic delay in line L could in principle reflect asynchrony within the culture rather than a uniform shift in timing; embryo-stage-matched rather than purely time-matched sampling would sharpen this distinction in follow-up work. A broader implication concerns how tree-improvement programmes currently allocate effort across newly established embryogenic lines. Because conifer breeding populations routinely generate dozens to hundreds of candidate lines from a single controlled cross, and because each full maturation trial ties up culture vessels, growth-room space and technician time for five to six weeks, even a modest improvement in early triage accuracy could meaningfully increase the number of genotypes a programme can realistically screen per breeding cycle. Our data suggest that this triage decision could, in principle, be moved substantially earlier in the pipeline than is current practice, from the end of maturation back to a single sampling point roughly one week into it, without requiring any change to the induction or proliferation protocols already in routine use. Whether that theoretical efficiency gain survives contact with the practical realities of a working breeding programme, including line-to-line variation in optimal sampling timing, is a question we regard as worth answering before the approach is recommended more broadly. It is also worth noting that early triage of this kind would not remove the need for eventual full maturation and germination testing of any line advanced beyond the screening stage, since factors specific to the later stages of conversion, such as cotyledon number, embryo vigour and germination synchrony, are not fully captured by a transcriptional snapshot taken at day 7; the marker panel is therefore best understood as a filter that reduces the number of lines carried forward to full trials, rather than a replacement for those trials.

5. CONCLUSION

Stage-resolved transcriptomic profiling of two Norway spruce embryogenic lines with contrasting maturation efficiency identifies the proliferation-to-patterning transition, marked by induction of LEAFY COTYLEDON, BABY BOOM and WOX2 orthologues and exit from cyclin-driven proliferation, as the developmental step at which a high- and a low-converting line diverge most sharply, rather than the later maturation phase where conversion failure is conventionally scored. The late embryogenesis abundant and storage-protein maturation programme was qualitatively intact but quantitatively reduced and delayed in the low-converting line, a pattern consistent with it being a downstream consequence of the earlier patterning delay. A compact panel of markers sampled at a single early timepoint, LEAFY COTYLEDON1, BABY BOOM and a small cyclin gene set, is proposed as a candidate tool for triaging newly established embryogenic lines well before the five- to six-week commitment required for a full maturation trial, though this panel now needs validation across a larger and more genetically diverse set of lines before routine laboratory adoption. We are extending the present comparison to a panel of twelve additional embryogenic lines spanning a continuous range of historical maturation yields, rather than only the extreme high and low cases studied here, to test whether the day 7 marker signature scales quantitatively with eventual conversion efficiency. In parallel, we plan single-embryo resolution profiling of line L cultures at the day 7 and day 14 timepoints to determine whether the population-level expression delay reflects a uniformly weaker response across all cells or a mixture of normally patterning and arrested cells, a distinction with direct implications for whether line L could in principle be rescued by selecting a normally patterning sub-population rather than discarding the line entirely.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

E.D. conceived and supervised the study, designed the sampling scheme and led data interpretation. C.H. performed tissue culture, developmental staging and RNA extraction. A.d.B. carried out RNA sequencing analysis, differential expression testing and co-expression network analysis. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This study involved only cultivated Norway spruce (*Picea abies*) embryogenic tissue culture material; it did not involve human participants, human data, or vertebrate or higher invertebrate animals, and therefore did not require institutional ethics committee review.

Data Availability

Raw RNA sequencing reads generated in this study have been deposited in the European Nucleotide Archive under a project accession available from the corresponding author on reasonable request. Processed expression matrices and analysis scripts are available from the corresponding author upon request.

REFERENCES

- von Arnold S, Sabala I, Bozhkov P, Dyachok J, Filonova L (2002) Developmental pathways of somatic embryogenesis. *Plant Cell, Tissue and Organ Culture* 69:233-249. <https://doi.org/10.1023/A:1015673200621>
- Klimaszewska K, Hargreaves C, Lelu-Walter MA, Trontin JF (2016) Advances in conifer somatic embryogenesis since year 2000. *Methods in Molecular Biology* 1359:131-166. https://doi.org/10.1007/978-1-4939-3061-6_7
- Bonga JM (2017) Conifer clonal propagation in tree improvement programs. *Vegetative Propagation of Forest Trees*, chapter 2. <https://doi.org/10.7747/JFES.2017.33.2.83>
- Find JI (1997) Changes in endogenous ABA levels in developing somatic embryos of Norway spruce (*Picea abies*) in relation to maturation medium. *Physiologia Plantarum* 101(4):826-832. <https://doi.org/10.1111/j.1399-3054.1997.tb01072.x>
- Filonova LH, Bozhkov PV, von Arnold S (2000) Developmental pathway of somatic embryogenesis in *Picea abies* as revealed by time-lapse tracking. *Journal of Experimental Botany* 51(343):249-264. <https://doi.org/10.1093/jexbot/51.343.249>
- Egertsdotter U, von Arnold S (1998) Development of somatic embryos in Norway spruce. *Journal of Experimental Botany* 49(319):155-162. <https://doi.org/10.1093/jxb/49.319.155>
- Stone SL, Kwong LW, Yee KM, Pelletier J, Lepiniec L, Fischer RL, Goldberg RB, Harada JJ (2001) LEAFY COTYLEDON2 encodes a B3 domain transcription factor that induces embryo development. *PNAS* 98(20):11806-11811. <https://doi.org/10.1073/pnas.201413498>
- Lotan T, Ohto M, Yee KM, West MA, Lo R, Kwong RW, Yamagishi K, Fischer RL, Goldberg RB, Harada JJ (1998) *Arabidopsis* LEAFY COTYLEDON1 is sufficient to induce embryo development in vegetative cells. *Cell* 93(7):1195-1205. [https://doi.org/10.1016/S0092-8674\(00\)81463-4](https://doi.org/10.1016/S0092-8674(00)81463-4)
- Boutilier K, Offringa R, Sharma VK, Kieft H, Ouellet T, Zhang L, Hattori J, Liu CM, van Lammeren AA, Miki BL, Custers JB, van Lookeren Campagne MM (2002) Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *Plant Cell* 14(8):1737-1749. <https://doi.org/10.1105/tpc.001941>
- Haecker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T (2004) Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. *Development* 131(3):657-668. <https://doi.org/10.1242/dev.00963>
- Palovaara J, Hallberg H, Stasolla C, Luit B, Hakman I (2010) Comparison of the CLAVATA3 and WUSCHEL-RELATED HOMEOBOX2 expression patterns during somatic and zygotic embryo development in Norway spruce. *New Phytologist* 188(1):122-135. <https://doi.org/10.1111/j.1469-8137.2010.03336.x>
- Uddenberg D, Valladares S, Abrahamsson M, Sundstrom JF, Sundas-Larsson A, von Arnold S (2011) Embryogenic potential and expression of embryogenesis-related genes in conifers. *Plant Cell Reports* 30(8):1385-1399. <https://doi.org/10.1007/s00299-011-1010-x>
- Rademacher EH, Moller B, Lokerse AS, Llavata-Peris CI, van den Berg W, Weijers D (2011) A cellular expression map of the *Arabidopsis* AUXIN RESPONSE FACTOR gene family. *Plant Journal* 68(4):597-606. <https://doi.org/10.1111/j.1365-3113X.2011.04710.x>
- Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jurgens G (2003) Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. *Nature* 426(6963):147-153. <https://doi.org/10.1038/nature02085>
- Larsson E, Sitbon F, Ljung K, von Arnold S (2008) Inhibited polar auxin transport results in aberrant embryo development in Norway spruce. *New Phytologist* 177(2):356-366. <https://doi.org/10.1111/j.1469-8137.2007.02289.x>
- Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 9:559. <https://doi.org/10.1186/1471-2105-9-559>
- Nystedt B, Street NR, Wetterbom A, Zuccolo A, Lin YC, Scofield DG, Vezzi F, Delhomme N, Giacomello S, Alexeyenko A, Vicedomini R (2013) The Norway spruce genome sequence and conifer genome evolution. *Nature* 497(7451):579-584. <https://doi.org/10.1038/nature12211>
- Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, Schein J (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* 313(5793):1596-1604. <https://doi.org/10.1126/science.1128691>
- Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C (2017) Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods* 14(4):417-419. <https://doi.org/10.1038/nmeth.4197>
- Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15:550. <https://doi.org/10.1186/s13059-014-0550-8>
- Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, Pesseat S (2014) InterProScan 5: genome-scale protein function classification. *Bioinformatics* 30(9):1236-1240. <https://doi.org/10.1093/bioinformatics/btu031>
- Braybrook SA, Harada JJ (2008) LECs go crazy in embryo development. *Trends in Plant Science* 13(12):624-630.

- <https://doi.org/10.1016/j.tplants.2008.09.008>
23. Jia H, McCarty DR, Suzuki M (2013) Distinct roles of LAFL network genes in promoting the embryonic seedling fate in the absence of VAL repression. *Plant Physiology* 163(3):1293-1305. <https://doi.org/10.1104/pp.113.220988>
 24. Gutierrez-Marcos JF, Costa LM, Evans MMS (2013) Maternal gametophytic baseless1 is required for development of the central cell and early endosperm patterning in maize. *Genetics* 174(1):317-329. <https://doi.org/10.1534/genetics.106.059709>
 25. Stasolla C, Kong L, Yeung EC, Thorpe TA (2002) Maturation of somatic embryos in conifers: morphogenesis, physiology, biochemistry, and molecular biology. *In Vitro Cellular & Developmental Biology - Plant* 38(2):93-105. <https://doi.org/10.1079/IVP2001262>
 26. Hazubska-Przybyl T, Chmielarz P, Michalak M, Wasilenczyk U, Bojarczuk K (2016) Effect of abscisic acid and desiccation on maturation and conversion of *Abies alba* somatic embryos. *Acta Physiologiae Plantarum* 38:42. <https://doi.org/10.1007/s11738-015-2035-2>
 27. Ojolo SP, Cao S, Priyadarshani SVGN, Li W, Yan M, Aslam M, Zhao H, Qin Y (2018) Regulation of plant growth and development: a review from a chromatin remodeling perspective. *Frontiers in Plant Science* 9:1232. <https://doi.org/10.3389/fpls.2018.01232>
 28. Elhiti M, Stasolla C (2011) Structure and function of homodomain-leucine zipper (HD-Zip) proteins. *Plant Signaling & Behavior* 6(6):867-874. <https://doi.org/10.4161/psb.6.6.15315>
 29. Grafi G, Chalifa-Caspi V, Nagar T, Plaschkes I, Barak S, Ransbotyn V (2011) Plant response to stress meets dedifferentiation. *Planta* 233(3):433-438. <https://doi.org/10.1007/s00425-011-1366-3>
 30. Gliwicka M, Nowak K, Balazadeh S, Mueller-Roeber B, Gaj MD (2013) Extensive modulation of the transcription factor transcriptome during somatic embryogenesis in *Arabidopsis thaliana*. *PLOS ONE* 8(7):e69261. <https://doi.org/10.1371/journal.pone.0069261>
 31. Yang X, Zhang X (2010) Regulation of somatic embryogenesis in higher plants. *Critical Reviews in Plant Sciences* 29(1):36-57. <https://doi.org/10.1080/07352680903436291>
 32. Elhiti M, Tahir M, Gulden RH, Khamiss K, Stasolla C (2010) Modulation of embryo-forming capacity in culture through the expression of Brassica genes involved in the epigenetic control of embryogenesis. *Journal of Experimental Botany* 61(14):4069-4085. <https://doi.org/10.1093/jxb/erq222>
 33. Lelu-Walter MA, Thompson D, Harvengt L, Sanchez L, Toribio M, Pâques LE (2013) Somatic embryogenesis in forestry with a focus on Europe: state-of-the-art, benefits, challenges and future direction. *Tree Genetics & Genomes* 9:883-899. <https://doi.org/10.1007/s11295-013-0620-1>
 34. Park YS, Beaulieu J, Bousquet J (2016) Multi-varietal forestry integrating genomic selection and somatic embryogenesis. *Vegetative Propagation of Forest Trees*, chapter 12. <https://doi.org/10.7747/JFES.2016.32.1.1>
 35. Miguel CM, Rupps A, Raschke J, Rutten T, Marum L (2016) Impact of molecular studies on somatic embryogenesis development for implementation in conifer multi-varietal forestry. *Vegetative Propagation of Forest Trees*, chapter 3. <https://doi.org/10.7747/JFES.2016.32.1.53>
 36. Awada R, Verdier D, Froger S, Brulard E, Duijsings D, Le Paslier MC, Berger G, Sallet E, Delande C, Tranchant-Dubreuil C, Klein PL (2020) A cost-effective RNA sequencing protocol for large-scale gene expression studies. *Scientific Reports* 10:19960. <https://doi.org/10.1038/s41598-020-76881-x>
 37. Radoeva T, Weijers D (2014) A roadmap to embryo identity in plants. *Trends in Plant Science* 19(11):709-716. <https://doi.org/10.1016/j.tplants.2014.06.009>
 38. Zhang M, Zhao J (2018) Molecular events involved in somatic embryogenesis induction in higher plants. *Frontiers in Plant Science* 9:16. <https://doi.org/10.3389/fpls.2018.00016>
 39. Ivanova A, Velcheva M, Denev I, Codarin S, Saccomani M (2018) Somatic embryogenesis and plant regeneration efficiency of embryogenic cell lines in *Picea abies*. *Silva Fennica* 52(5):10023. <https://doi.org/10.14214/sf.10023>
 40. Bozhkov PV, Filonova LH, Suarez MF (2005) Programmed cell death in plant embryogenesis. *Current Topics in Developmental Biology* 67:135-179. [https://doi.org/10.1016/S0070-2153\(05\)67004-4](https://doi.org/10.1016/S0070-2153(05)67004-4)
 41. Salaj T, Blehova A, Salaj J (2015) Absciscic acid effects on somatic embryo maturation in *Abies alba*. *Turkish Journal of Botany* 39:1-11. <https://doi.org/10.3906/bot-1401-13>
 42. Vestman D, Larsson E, Uddenberg D, Cairney J, Clapham D, Sundberg E, von Arnold S (2011) Important processes during differentiation and early development of somatic embryos of Norway spruce as revealed by changes in global gene expression. *Tree Genetics & Genomes* 7:347-362. <https://doi.org/10.1007/s11295-010-0335-5>
 43. Rutledge RG, Stewart D, Caron S, Overton C, Boyle B, MacKay J, Klimaszcwska K (2013) Potential link between biotic defense activation and recalcitrance to induction of somatic embryogenesis in shoot primordia from adult trees of white spruce (*Picea glauca*). *BMC Plant Biology* 13:116. <https://doi.org/10.1186/1471-2229-13-116>
 44. Yakovlev IA, Fossdal CG (2017) In-depth analysis of gene expression in developing embryos of Norway spruce (*Picea abies*). *Frontiers in Plant Science* 8:1785. <https://doi.org/10.3389/fpls.2017.01785>
 45. Meng L, Cao XN, Liu Y, Duan CF, Jiao XL, Zhao XY, Zhang XS (2017) Auxin-mediated regulation of embryo patterning. *Journal of Genetics and Genomics* 44(6):303-312. <https://doi.org/10.1016/j.jgg.2017.03.009>
 46. Gaj MD (2004) Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to *Arabidopsis thaliana*. *Plant Growth Regulation* 43:27-47. <https://doi.org/10.1023/B:GROW.0000038275.29262.fb>
 47. Wickramasuriya AM, Dunwell JM (2015) Global scale transcriptome analysis of *Arabidopsis* embryogenesis in vitro. *BMC Genomics* 16:301. <https://doi.org/10.1186/s12864-015-1504-6>
 48. Rose RJ (2019) Somatic embryogenesis in the *Medicago truncatula* model: cellular and molecular mechanisms. *Frontiers in Plant Science* 10:267. <https://doi.org/10.3389/fpls.2019.00267>
 49. Su YH, Zhao XY, Liu YB, Zhang CL, O'Neill SD, Zhang XS (2009) Auxin-induced WUS expression is essential for embryonic stem cell renewal during somatic embryogenesis in *Arabidopsis*. *Plant Journal* 59(3):448-460. <https://doi.org/10.1111/j.1365-313X.2009.03880.x>
 50. Karami O, Saidi A (2010) The molecular basis for stress-induced acquisition of somatic embryogenesis. *Molecular Biology Reports* 37(5):2493-2507. <https://doi.org/10.1007/s11033-009-9764-3>
 51. Ho-Plagaro T, Molinero-Rosales N, Farina Flores D, Villena Diaz M, Garcia-Garrido JM (2019) Identification of tomato and *Arabidopsis* genes involved in symbiosis-related signalling. *Annual Review of Plant Biology* 70:167-192.

<https://doi.org/10.1146/annurev-arplant-050718-100450>