Multi-scale characterizations of various desiccation treatments to produce somatic embryos closer to zygotic embryos of hybrid larch.

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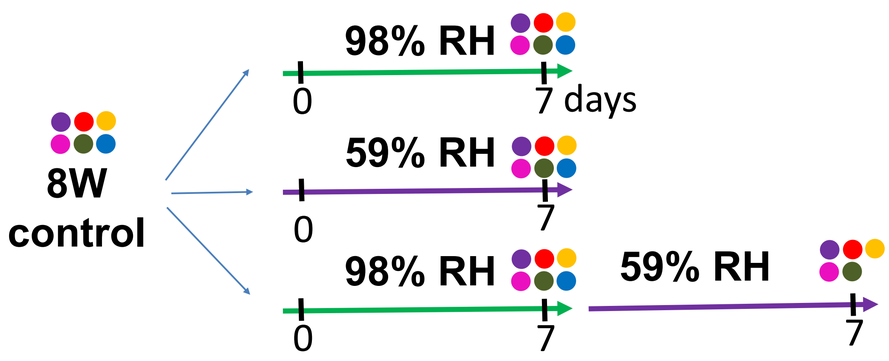
Somatic embryogenesis is a useful method for propagating selected plant material. However, before using this technique for forest tree deployment, it is important to ensure high quality somatic embryos (SEs). This quality depends on how closely SEs resemble zygotic embryos (ZEs). At the end of their maturation, SEs usually resemble ZEs from fresh seeds due to their high water content and biochemical composition. For this reason, an effective desiccation method is needed to lower the SEs' water content to levels similar to fully mature dry ZEs, while ensuring a high percentage of germination and successful conversion into plants. To establish an efficient desiccation protocol, we investigated the biological, histological, biochemical and molecular changes in the SEs of hybrid larch *Larix eurolepis* exposed to desiccation at high relative humidity (RH; 98%), reduced RH (59%), or a combination of both.

Material: Hybrid larch **SEs** from **2 lines** (Q10, N23) and **ZEs** from **stored seeds.**

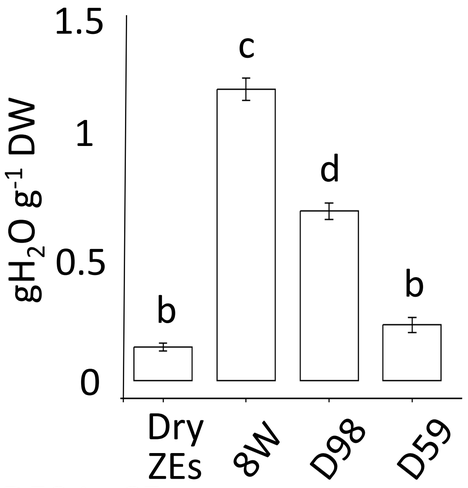
Mature 8-weeks-old SEs (8W, Savane et al. 2023 Physiol Pant**)** were submitted to the following desiccation treatments at 4°C. Both lines gave similar results.



Analysis :

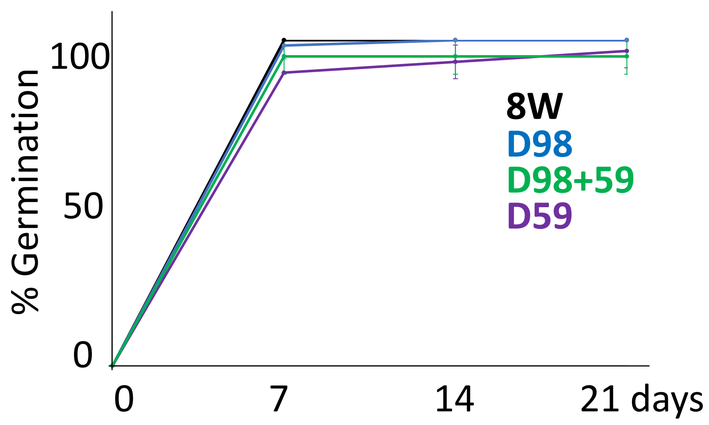
- Water content (Dronne et al. 1997, Physiol Plant)
- Germination & plants (Savane et al. 2025, PPB)
- Histology (Eliášová et al. 2022, Front Plant Sci)
- Carbohydrates (Savane et al. 2025, PPB)
- Abscisic acid (Prerostova et al. 2021, Int J Mol Sci)
- Proteomic (Savane et al. 2025, PPB)

Water content



D59 ↘ SE water content to a level similar to dry ZEs.

Germination & plants

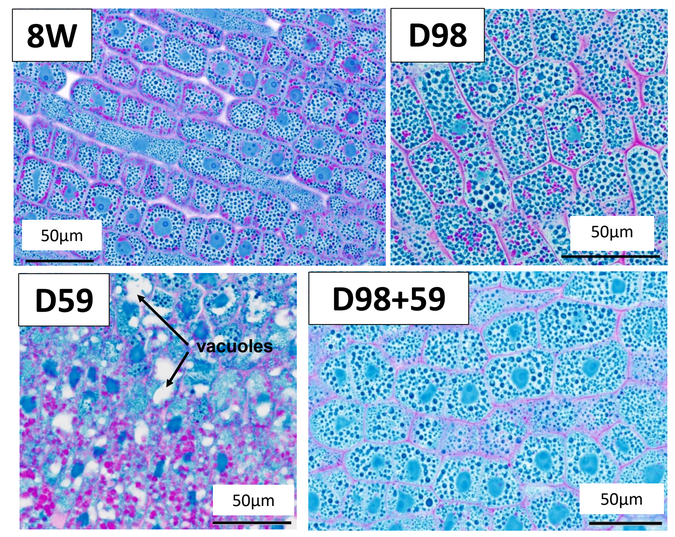


No. of plants %	
8W	78 ± 10^{ab}
D98	68 ± 16^{ab}
D59	54 ± 12^{a}
D98+59	82 ± 20 ^{b}

D98+59 has no effect on the germination rate and plant recovery.

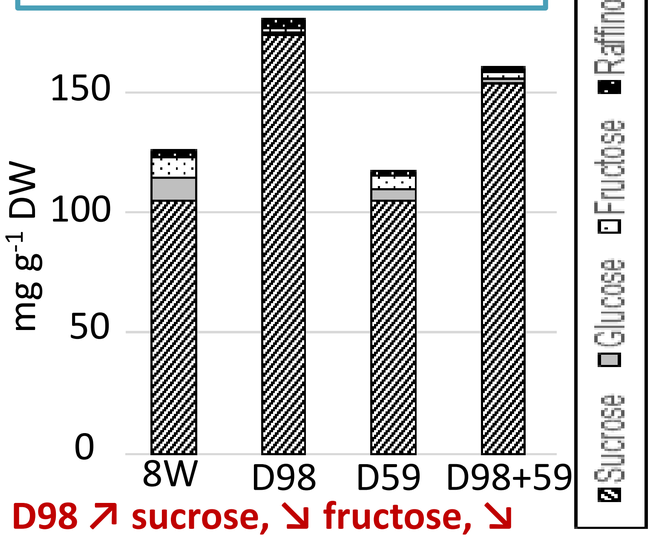
Histology

Details of cells in the hypocotyl



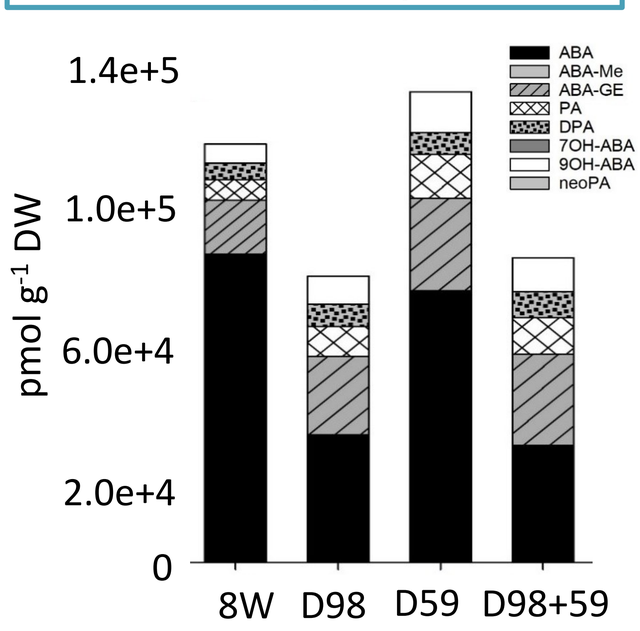
D98 ↘ the number of starch grains while D59 did not significantly ↘ them. In D59 marked vacuolisation in the cells.

Carbohydrates content



D98 ↗ sucrose, ↘ fructose, ↘ glucose, and helps SE to overcome severe water loss.

Abscisic acid (ABA) content



D98+59 ABA ↘ in comparison to D59 : SEs were acclimated to D59 during the first week of D98, and no stress response was observed at the level of ABA-types.

Proteomic analysis

D98 and D98+59

Induced :
- Desiccation tolerance acquisition
- Preparation for germination

D59

Induced :
- Defence mechanism
- Parietal damage

Functional reprogramming of SE metabolism with beneficial effect for D98 and D98+59, and detrimental effect for D59.

For the first time in conifers, we have demonstrated the possibility to obtain desiccated SEs with reduced water content similar to dry ZEs while maintaining a high rate of germination and conversion into plants. This treatment could also be considered as a potential method for SE storage. This multi-scale study contributes significantly to the knowledge on desiccation of conifer SEs.

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