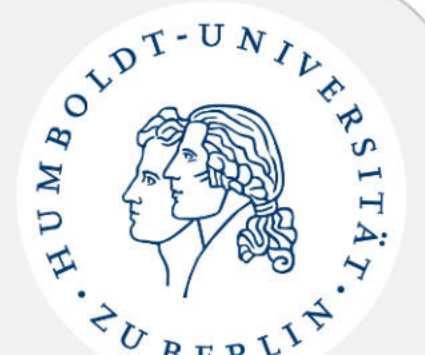




Enhancing Forest Resilience through Hybrid Larch Clonal Production ("LarchForFlexibility")



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Our project

Climate change poses significant challenges to forestry and forest management, necessitating the development of resilient, mixed-species forests. Our objective is to improve silvicultural flexibility by providing characterized and certified propagation material - independent of seed availability. Our species of interest is hybrid larch (*Larix x eurolepis*), which is becoming increasingly attractive for forestry due to its advantages in establishing pioneer forests, rapid juvenile growth, robustness, and drought resistance. Our approach combines traditional breeding methods with modern in vitro culture technologies, specifically somatic embryogenesis (SE), to select and produce high-quality hybrid larch genotypes. We have successfully generated and cryopreserved 256 hybrid larch genotypes through SE, with 70 showing promising characteristics *in vitro* as well as in the field suitable for commercial or scalable applications, which we are aiming to expand further.

Based on this collection, we have established a productive system for cultivating *in vitro* propagated plant material and integrated it as a routine procedure into nursery operations. At this point, our partners from industry were able to offer our optimized strategy and propagation material to the market. The launch goes along with further economic tasks, including streamlining the SE process, particularly the maturation phase, and automating labor-intensive steps to ensure high production volumes and competitive pricing.

Concurrently, we are establishing clone trials at diverse plots in Eastern Germany, which are necessary to secure approval for the propagation material. As a prerequisite for the complex and long approval procedure, we are working on the genetic characterization of our material and developing a molecular marker system for reliable genotype identification. These markers will be stored in a database, ensuring that the entire clone spectrum is documented and can be expanded and supplemented with phenotypic characteristics obtained during the *in vitro* phase as well as subsequent growth characteristics from the field. In this regard, we are placing special emphasis on drought tolerance of the respective genotypes. To this end, we are conducting *in vitro* and *ex vitro* drought tests to categorize the reactions and resistance to water withdrawal.

Selection of high-value genotypes and controlled crossings



Fig. 1: Factors with relevance for controlled crossings:
The preferred trees need to bloom (1) viable pollen must be available (2,3), controlled pollination (4,5) must take place at the correct brief time window; no severe late frosts or pests occur during embryo development; fructification and embryo development must be successful (see ratio seeds to explants in Fig. 6).

Drawing on high value starting material is key to maximize the benefits of a time and resource intensive process such as SE.

Breeding programs started in Saxony 75 years ago. Since then, about 880 plus trees of European and Japanese Larch were selected. Subsequently, about 500 Larch-combinations were crossed and their progenies were tested in trial plots.

During this time, objectives for the larch breeding programme focused on:

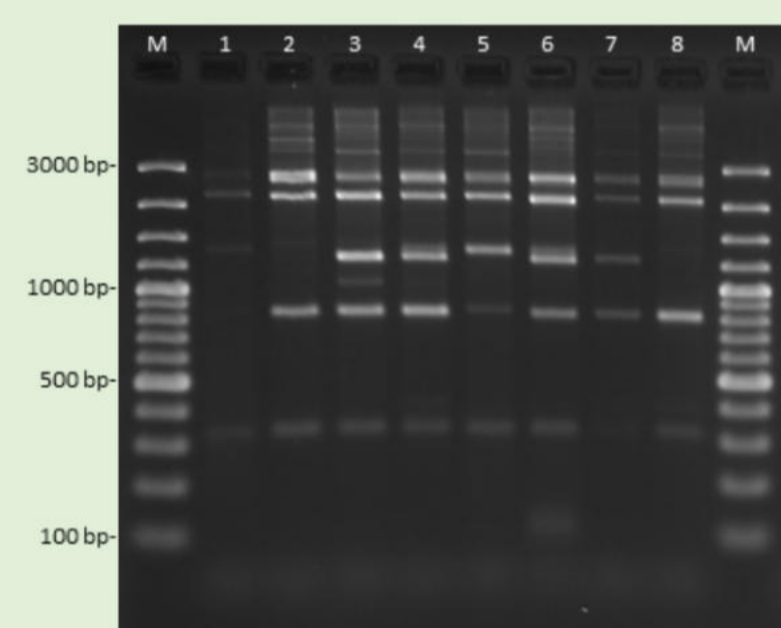
- The expansion of the vertical plantation limit in the mountainous regions in East-Germany
- Resistance against abiotic and biotic damages
- Vigour on disturbed soils, such as lignite follow-up areas

As a result of these efforts, nine hybrid larch crossing-combinations could be approved as suitable. Our partner Staatsbetrieb Sachsenforst initiated controlled crossings amongst these approved parental trees, with European larch (*Larix decidua*) as mother trees and Japanese Larch (*Larix kaempferi*) as fathers.

Preservation of the clones and quality control

To ensure that the genotypes produced via somatic embryogenesis (see orange box below) are preserved, they are stored in the cryobank. The quality of the clones is determined through phenotypic and genotypic characterization of the plant material.

Genotypic characterization and identification



ISAP-PCR Lar-S01_rev/Lar-S06_rev (53°C), lanes are corresponding to different hybrid larch genotypes, M= Marker 100 bp+

Fig. 2: Preparation of genotype-specific fingerprints for the identification and verification of hybrid larch clones.

Reaction towards drought stress

- *in and ex vitro*



Fig. 3: Vegetatively propagated plants are analysed in regard to their reaction to osmotic stress / drought in *in vitro*, greenhouse and field tests.

Phenotyping on test sides



Fig. 4: Recording of adaptive, quantitative and qualitative characteristics in the field.

The *in vitro* process of somatic embryogenesis in hybrid larch and its yield

The induction of SE in conifers is currently limited to juvenile tissue, most frequently zygotic embryos are used as explants. The formation of somatic embryos (Fig. 5, white arrow) in hybrid larch is triggered by exogenous stress in form of high concentrations of 2,4-D and BAP. Emerging somatic embryos are characterized by a round embryo head (Fig. 5, black arrow h) and a suspensor (Fig. 5, black arrow s).

After induction, somatic embryos are transferred to a maintenance medium containing auxin and cytokinin. Long-term storage via cryopreservation is possible at that stage, which also helps avoid soma-clonal variation.

Maturation is induced by changing the medium composition, removing auxin and cytokinin while adding ABA and PEG. Embryos develop through stages similar to zygotic embryos.

For germination, mature embryos are transferred to a regulator-free medium with reduced sugar. Activated charcoal is added to adsorb unwanted substances and promote root growth.

Initiation of somatic embryogenesis on zygotic embryos

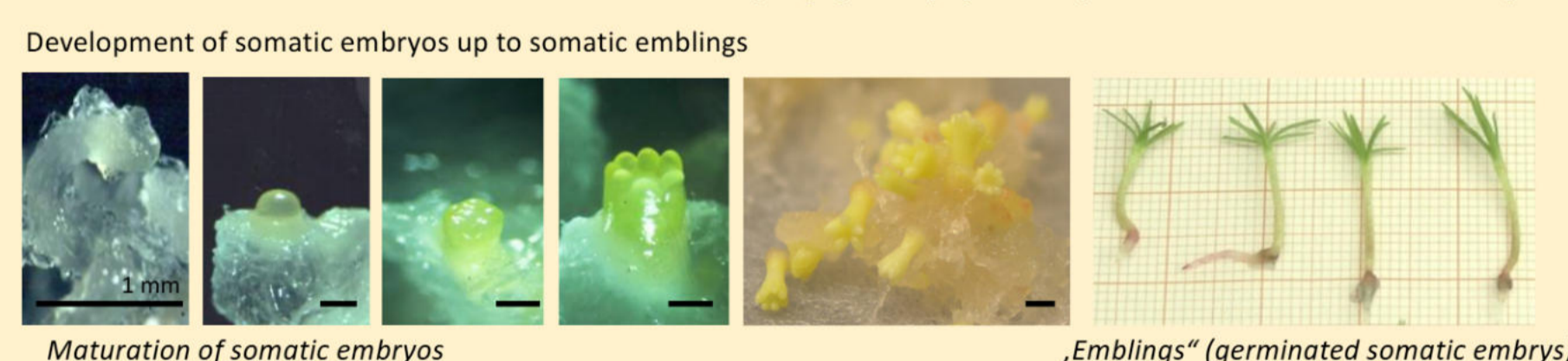
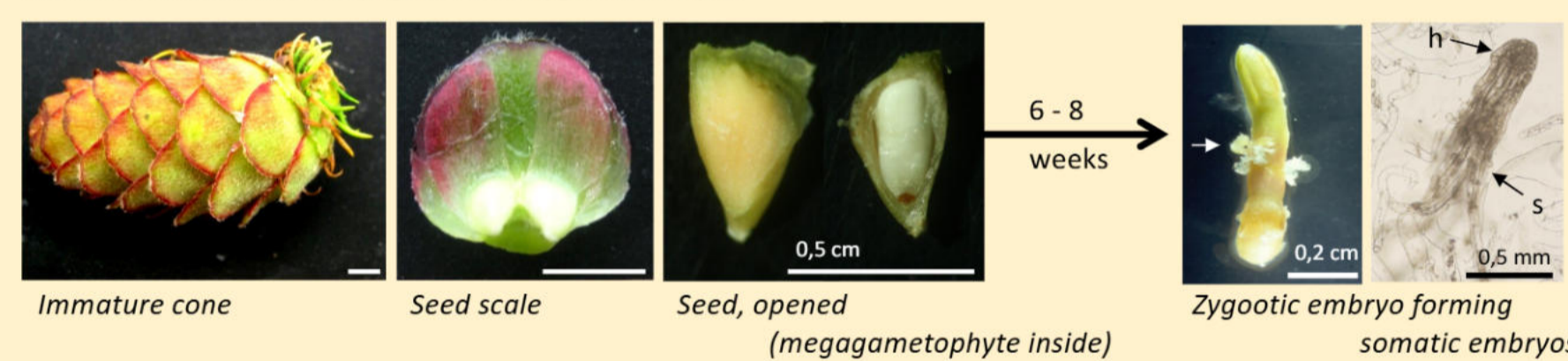


Fig. 5: Initiation and development of somatic embryos of hybrid larch

Throughout the SE process chain (from initiation to germination), losses of explants and cultures must be taken into account (Fig. 6). By quantifying these losses, we aim to understand the efficiency of our protocols and identify key points for improving the overall success rate of SE in hybrid larch.

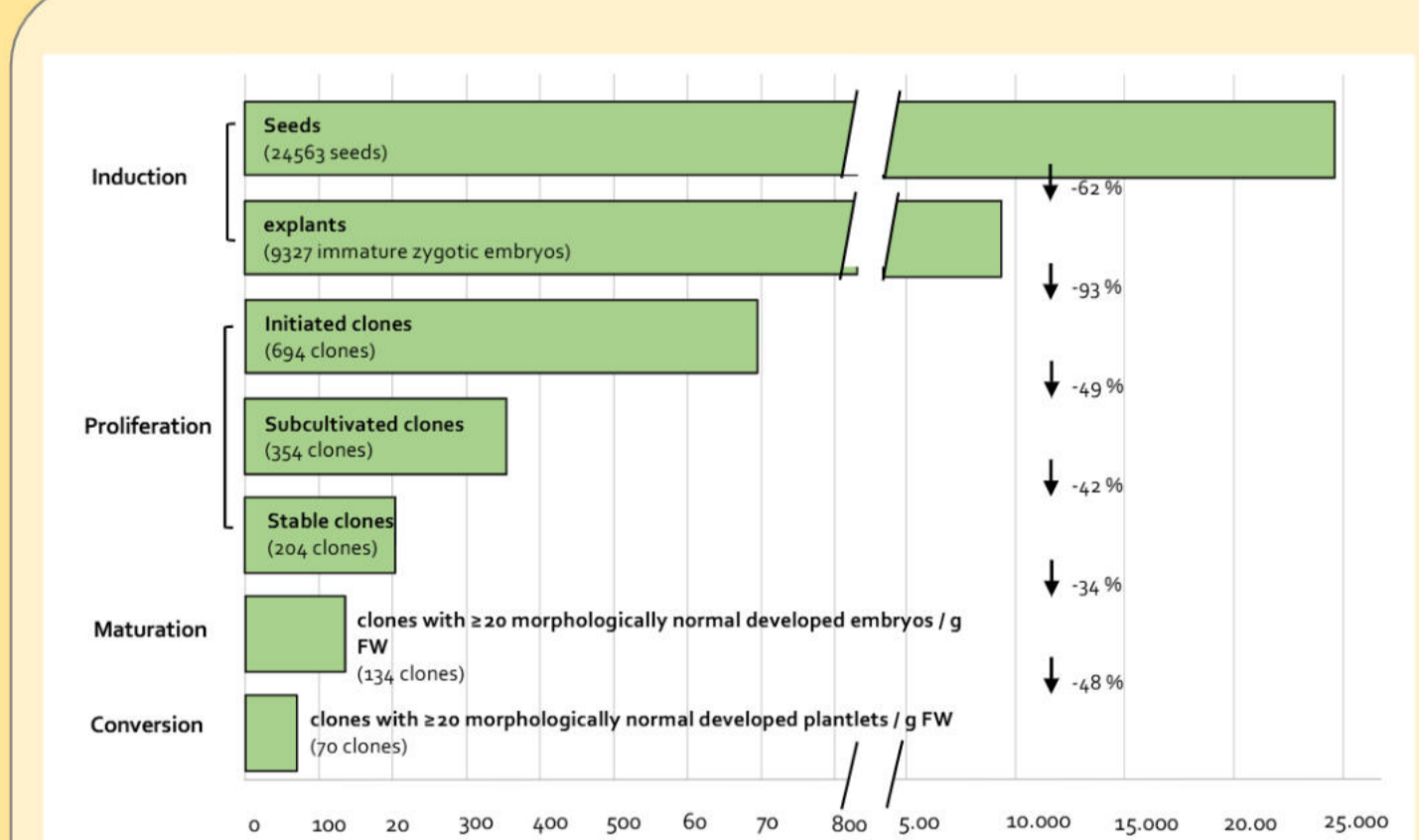


Fig. 6: Somatic embryogenesis line losses during the *in vitro* process

The main bottlenecks concern the following steps:

- Initiation: Not all genotypes are „competent“ (recalcitrant)
- Maintenance: Transfer in stable culture: losses, callus formation
- Maturation: Instable and low maturation results
- Conversion: labour intensive

TITAN-LÄRCHE ready for the market and the forests



Fig. 7: Ready-to be pricked (top) and ready to be planted (below) somatic hybrid larch plants

After several decades of research in larch somatic embryogenesis at Humboldt-Universität zu Berlin, the project partner Baumschulen Oberdorla has just launched the brand TITAN-LÄRCHE (Fig. 8).

Apart from the brand name, their local advertising agency established a website, flyer and designed the brand logo.

Initially, 14 tested clones are available, but the spectrum is expected to grow.

TITAN-LÄRCHE – Die Zukunft des Waldes beginnt hier.



<https://www.titan-laerche.de/>



Fig. 8: Project partner Christin Müller and Hardy Demby (Baumschulen Oberdorla GmbH) present their new brand TITAN-LÄRCHE at a trade fair.

German law on approval of new varieties: Field testing in Saxony

§ The Federal Law on Forest Reproductive Material

Clone testing is an essential process to ensure the quality and performance of forest reproductive material. In Germany, this process for the "Tested" category follows strict guidelines to support sustainable forest management. It takes usually 15 yrs; shortened: 8 yrs, if the family parents of the seeds used for induction of SE have already been approved.

Evaluation criteria are e.g. adaptability, vigor, quality and resistance to relevant biotic and abiotic factors the reproductive material must have at least the same characteristic values as the source material or the same resistance to pathogens of economic importance.



Fig. 9: Somatic larches after 4 growing seasons

Field trials for clone tests in Germany:

First trial areas with somatic hybrid larch plants were initiated in 2015 (70 clones out of 6 crosses à 18 plants each) for preliminary assessment.

14 clones were already tested on two sites according to the legal regulations.

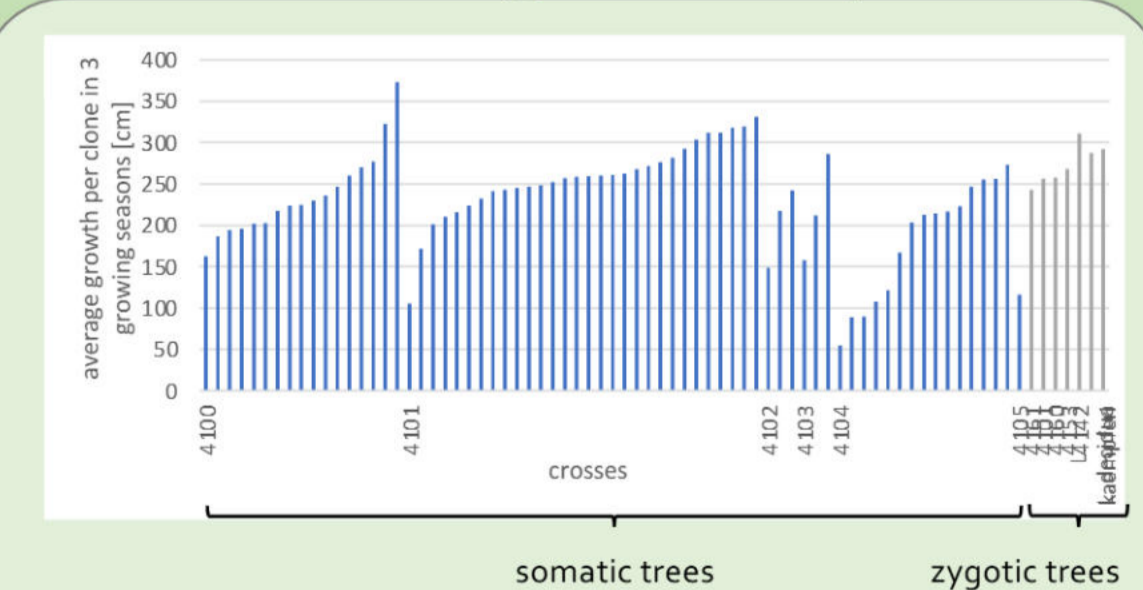


Fig. 10: Comparison of somatic and standard trees: Somatic trees exhibited a similar growth rate and high quality when compared with zygotic trees. Within the crosses, deviations in growth increase were observed. This suggests that there is variability in growth patterns among different genetic combinations, which is an important factor to consider in breeding programs and clone selection.

Outlook:

This research contributes to sustainable forestry by enabling the production of climate-resilient planting material, supporting rapid reforestation of calamity areas, and enhancing forest productivity and resilience against the impacts of climate change. The market launch of the TITAN-LÄRCHE taps into the potential somatic embryogenesis has for high value tree species.

Nevertheless, in order to ensure biodiversity and usability to various habitats, it is essential to continue the process of breeding and building up the clone collection with a broad spectrum of genotypes.

Similarly, other species such as *Abies* (fir) or *Pseudotsuga menziesii* (Douglas fir) are also highly valuable tree species desirable for the costly somatic embryogenesis procedure. Yet, they still have some bottlenecks in their transfer protocols, particularly for acclimatisation and plantlet production, they are progressing more slowly to follow the more straightforward hybrid larch.

For questions or recommendations feel free to contact us: andrea.rupps@hu-berlin.de

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