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Paving the way for commercial somatic embryogenesis: Norway spruce in Finland

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Abstract
Somatic embryogenesis (SE) has been recognised to have vast potential to produce improved reforestation material. By applying SE, genetic gains achieved by breeding can be utilised fully and faster than by seed orchard approach. Yet, there are only a few commercial mass-propagation applications in conifers. Challenges include biological, economical, and regulative issues together with stakeholder and public acceptance, and these are discussed in the case of Norway spruce (*Picea abies* (L.) H. Karst.) SE propagation in Finland.

In Norway spruce, biological issues related to SE have been mostly solved, as highlighted by recent developments such as high initiations rates, high recovery from cryopreservation, and short in vitro germination, all applicable to genetically wide materials. The current SE stock at Natural Resources Institute Finland (LUKE) exceeds 5600 genotypes, derived from the top trees of the Finnish tree breeding program. Also, development of automation to improve cost-efficiency has proceeded successfully; a robot prototype has been developed for in vitro germination phase in collaboration with South-Eastern Finland University of Applied Sciences (XAMK).

LUKE registered the first Norway spruce SE basic material for forestry in 2017, as the first one in Europe, and propagation pilots have been carried out since 2018. The whole SE propagation pipeline has been developed to fit into regulative framework, being compatible with international (European Union, Organisation for Economic Co-operation and Development) and national regulations for the production and marketing of forest reproductive material. Views of forest owners and professionals on spruce SE propagation have been studied, and their wishes for desired/value-added traits in the improved material taken into account. Further, concerns related to loss of genetic diversity and lack of information are being addressed by genetically wide stock of SE materials, development of robust methodology, and information openly available. However, more effort is still needed for public discussion.

Key words
Automation, cost-efficiency, forest reproductive material, genetic diversity, *Picea abies*, public acceptance

Introduction

Somatic embryogenesis (SE) is considered as the most efficient vegetative propagation method, when considering the number of plants produced from a single explant and it has thus a vast potential to produce material also for forestry applications. Propagation using SE could benefit forestry, e.g., in the following ways: if there is a shortage of seeds, that can be compensated by SE; secondly, selected materials with known characteristics cannot always be propagated via seed but their propagation succeeds using SE. Moreover, the application of SE can result in more efficient utilisation of genetic gains achieved by classical breeding or available by marker-assisted or genomic selection (Lelu-Walter et al. 2013; Grattapaglia 2022). In some cases, SE as propagation method may per se have a beneficial effect on the characteristics of the produced plants, such as improved pest resistance (Berggren et al.

2023; Puentes et al. 2018) or provide an opportunity to utilise epigenetic effects in adaptive traits (Carneros et al. 2017; Nascimento et al. 2020; Sales et al. 2022; Viejo et al. 2023). In order to achieve these benefits, of course, high-quality material and well-working, cost-efficient propagation technique are required.

Somatic embryogenesis has been developed for numerous tree species in different genera, but in many species the technique requires further development. Currently, there are only a few practical large-scale applications. One example on SE being applied in commercial forestry is radiata pine in New Zealand. There are several nurseries that produce SE-derived pine plants, the annual propagation numbers being several millions per nursery. The produced plants are either planted on the company-hold land or sold to customers. In New Zealand, SE plants are not used directly for forest regeneration, but as stock plants that are further propagated using organogenesis and cutting propagation (Lelu-Walter et al. 2016). On the contrary, in Canada, J. D. Irving Ltd. is propagating spruces (*Picea glauca, P. abies*) for direct planting on company freehold land. The current annual propagation numbers are around 2.5 million SE-plants (A. McCartney pers. comm.). In Sweden, SweTree Technologies AB has been for many years developing automation for SE aiming to production volumes of tens of millions of plants. Their pilot facility has been constructed and is currently being tested (Herzberg & Abrahamsson 2024).

Overall, the challenges for applying SE in forestry scale include biological factors and economical concerns as well as societal issues such as regulatory aspects and public acceptance (Lelu-Walter et al. 2013). All of these need to be solved for mass-propagation of forest reproductive material (FRM). Smaller scale propagation is easier to achieve, e.g., production of ornamentals or Christmas trees having lower production numbers but higher unit price, and no regulatory or acceptance barriers. The circumstances and most difficult issues for forestry applications vary from one species and country to another.

In Finland, tree breeding efforts are focused on a few economically important tree species such as Norway spruce (*Picea abies* (L.) Karst.). Genetic gains achieved by classical breeding are transferred to silviculture via open-pollinated seed orchards. Unfortunately, in the case of Norway spruce the orchards are either at the end of their operational lifespan or still juvenile and do not produce seed with their full potential yet (Haapanen et al. 2017). Furthermore, Norway spruce is a species having naturally irregular flowering abundance, and consequently, also seed yields vary enormously from year to year. As the periods of several consecutive years without proper flowering are not unusual, also the availability of improved forest regeneration material can change rapidly (Jansson et al. 2013, Haapanen et al. 2017). Seed pest and disease problems, together with proceeding banning of pesticides make the situation more difficult. To ease the intermittent shortage of improved spruce material and to implement breeding results more effectively, an effort to build a SE-pipeline for Norway spruce in Finland was initiated in 2011 (Tikkinen et al. 2019).

The aim of the present paper is to discuss the development of Norway spruce SE propagation in Finland, within the context of the recognised challenges for SE mass-propagation (biological, economical and societal). What has been achieved since the in 2012–24, what are the remaining challenges in the Finnish context, and how these could be managed.

Materials and Methods

Materials

For building the SE pipeline, Norway spruce material derived from the Finnish tree breeding programme has been used. Natural Resources Institute Finland (LUKE) is responsible for national tree breeding programme and its realisation as a statutory task. Both operational tree breeding and SE development are carried out by the LUKE's Tree Breeding Group, giving a direct access to selected pedigree materials. Zygotic embryos originating in the controlled crossings among the top trees of the breeding program, (i.e., the parent trees having a breeding value based on the comparative genetic testing) have been used as initial explants in SE. In addition, a small portion of the explants has been collected from special forms of Norway spruce in order to propagate ornamental trees.

During the SE pipeline development, by the end of 2022, 12,954 explants were used, originating in the 132 full-sib families, representing 75 mother genotypes and 91 father genotypes (Varis et al. 2023). The current number of established and cryopreserved SE lines that is available for further studies or applications is over 5,600. Most of these materials represents genotypes suitable for forest regeneration; only 117 are ornamental ones.

As described in several publications about the development of various steps of SE protocols (see Methods), as broad material as possible have been used for experiments. Most often, a new method was tested first with 5-20 genotypes, and then promising results confirmed with a broader selection of genotypes before taking them as a part of routine propagation procedure.

Methods

In the present paper, the emphasis is not on detailed presentation of the SE methodology but on overall consideration of all the factors affecting building a SE-pipeline. Thus, the propagation methodology will be only shortly referred followed by description of other important factors, such as the development of automation, coping with regulations, collaboration with stakeholders, and finally, communication issues together with public acceptance.

Development of SE and cryopreservation methodology

In the research work done to improve the various steps of SE propagation, a few principles have been followed: (1) robustness of the developed methodology, meaning that it needs to be suitable for most of genotypes; and (2) keeping in mind the final goal i.e., cost-efficient commercial propagation. In practice, this has firstly meant testing the methods with big number of SE lines. Secondly, addition of any extra or more expensive steps/work/ingredients is acceptable only if it improves the outcome (amount and/or quality of the somatic plants ready for planting), also when counting in the costs. The detailed description of the SE methodology tested in Norway spruce in Finland can be found in the following publications, listed according to the propagation steps:

- Initiation and proliferation: embryogenic lines were initiated and proliferated by applying the methods (developed by Litvay et al. 1985, Klimaszewska et al. 2001 and Lelu-Walter et al. 2008) described in Varis et al. (2017). Proliferation in temporary immersion system was studied in Välimäki et al. (2020); suspension cultures was tested in Välimäki et al. (2021) and filters in semi-solid media in Välimäki et al. (2022).
- Cryopreservation: the development of the selection of lines for cryopreservation and methods used over the years are described in Varis et al. (2023). Developed methods using semi-solid media are described in Varis et al. (2017) and Varis et al. (2022).
- Maturation: methods used in 2011-2014 experiments are described in Varis et al. (2017), and the method used after that is introduced in Tikkinen et al. (2018a). The pre-maturation on hormone-free semi-solid media was tested in Välimäki et al. (2022).
- Embryo storage, germination, acclimatisation, and nursery phase: the effects of in vitro embryo storage and germination treatments and *ex vitro* growing techniques on the survival and growth of emblings was studied in Tikkinen et al. (2018b). The adopted methods are shortly described in Edesi et al. (2021). The embryo storage compound profile was analysed after post-maturation cold storage treatments in Välimäki et al. (2022).

- Added value-traits in SE material (pest and pathogen resistance): Edesi et al. (2021) showed that the root rot resistance locus PaLAR3B is successfully delivered from elite Norway spruce parent trees to their SE progeny through the SE propagation method. Berggren et al. (manuscript submitted) studied the effect of the propagation method on healing of bark damages which can be caused, e.g., by bark-feeding pine weevils.

Development of automation

The need for automation has been recognised in a vast number of publications. In Finland the development of automation was launched after the cost structure of different phases in SE was estimated. Most of the production cost in the laboratory was created during germination (Tikkinen 2018), highlighting the need to focus the development of automation to this particular phase. The development of the germination robotics was launched in 2018, with the initiative to automate the embryo selection and disposition to germination plates (Bernhard et al. 2020). In 2022, the first germination cell, including three robot units, was established at LUKE, being integral to the current efforts to scale up the SE plant production to a commercially relevant scale. The development of robots is still ongoing to improve their accuracy and speed, e.g., by integration of AI (Havia et al. 2022). In 2024, the application of the developed robots to radiata pine was initiated together with Scion (now the New Zealand Institute of Bioeconomy Science) in New Zealand. Currently, LUKE and XAMK are developing more automation to related phases of SE.

The goal for development of automation, i.e., robot's tasks were determined to include picking up individual embryos, evaluating them and either placing them on germination media or discarding them. Handling living material involves certain special requirements as the robot needs to handle the embryos without damaging or predisposing them to contamination. Therefore, all components that are in contact with the embryos need to be autoclavable. It was also important for the robot to be easy to use, so that staff could be quickly trained to operate it.

Acknowledging the regulatory framework

Finland is a member of the European Union (EU). Within the EU, forestry is under national legislation, but EU regulates marketing of forest reproductive material (FRM) based on Council Directive 1999/105/EC (Council of the European Union 1999). Organisation for Economic Co-operation and Development (OECD), working world-wide with more than 100 countries, has similar regulations for international trade. The EU regulations are currently under revision, but the main principles will remain the same. In Finland, the Directive 1999/105/EC has been implemented by the Act on Trade in Forest Reproductive Material (241/2002) and the Decree on the Trade in Forest Reproductive Material (1055/2002). These EU and national regulations set the requirements and limits for all the FRM to be marketed in Finland, including SE materials.

Registration of the basic material in reforestation is required in EU markets. In addition, Finnish national regulations rule the minimum number of genotypes to be propagated, and the maximum number of plants produced from a single genotype, depending on the basic material type and category.

In practice, SE materials can be registered with their basic material being either "Parents of family/ies", "Clone" or "Clone mixture". With the "Parents of family" option, the FRM is evaluated on the basis of the characteristics of their parent trees, while in "Clone" options the propagated clonal genotypes themselves are evaluated. Further, the different types of basic materials can be either in the category "Qualified" or "Tested". "Qualified" is phenotypically selected at individual level, and "Tested" has superiority shown by proper genetic evaluation, meaning generally long-lasting field experiments with reference plants.

Getting to know the regulatory framework, and requirements set for FRM was considered important from the very beginning of the SE development. A representative of Finnish national authority controlling FRM marketing, The Finnish Food Authority, was invited into steering committee for the

RDI projects in SE development and consulted when selecting materials for SE initiations to ensure that material fulfils the requirements. Later, LUKE has performed the registration process with the Norway spruce SE materials twice, in 2017 and 2024, according to national authority's process. As a prerequisite for this, LUKE has created a specialised database containing information on every SE line, i.e., genotype (e.g., pedigree, test results, cryostored samples, etc.). In practice, every SE line carries a unique identification code through the whole propagation, including cryostorage, and propagation history and ramet numbers are recorded.

Collaboration with stakeholders

Stakeholders recognised for building the SE pipeline include forest companies, forest plant producers, professionals in forest administration and advisory sector, and forest owners. In addition, also stakeholders representing e.g., Christmas tree producers and users of ornamental conifers, such as representatives of cities, have been involved. It should be noted that in Finland most of the forestry land, around 52%, is owned by private individuals, the state owns approximately 35% and the companies only about 8% (Vaahtera et al. 2023).

Stakeholders were originally involved by inviting them into steering committees of the RDI projects for SE development that were financed by public funding, e.g., by European Regional Development Fund (ERDF) and LUKE. Already during this time, an opportunity was provided to stakeholders to get SE germinants from LUKE to test their growing in private nurseries, with several of them taking this opportunity. Following the successful realisation of the first RDI projects, the stakeholders were also asked to give financial support for SE development, and the later RDI projects have been realised using LUKE's Public-Private-Partnership (PPP) project model, in which costs are jointly covered by private partners and Luke. The personnel of the PPP partners have been able to familiarise themselves with SE propagation in practice in Luke premises, guided by LUKE personnel. In addition of the PPP projects, customer funded SE pilot propagations have been performed, i.e., LUKE has produced SE germinants to private parties according to their orders.

Survey about stakeholders' preferences and acceptance

Further, in order to understand and acknowledge stakeholder views more broadly, a survey on interest in vegetatively propagated Norway spruce materials was conducted among forest owners and forest professionals (Tikkinen et al. 2021). In this survey, perceptions towards tree breeding and vegetative propagation were investigated, including acceptance and concerns. Additionally, the respondents were asked which traits they considered important to be improved by breeding, and their willingness to pay for these improved traits, if available e.g., by usage of SE material.

Preparation of commercialisation

As a first step for commercialisation, the cost structure of SE propagation has been estimated, as described by Tikkinen (2018). Further, more information on realised costs have been obtained from LUKE's pilot propagations, while gains achieved can be evaluated based on the field experiments.

High quality plant material is vital for successful outcome. The genotypes need to be screened for both laboratory and field performance. Additionally, the propagation methods need to be robust enough to minimise compromises in line selection due to the performance of the lines in laboratory. It is important to preserve the juvenility of the cell lines in the production to avoid losing their embryo production capacity due to physical age by cryopreserving them systematically as soon as possible after initiation and thawing. The refined propagation and testing protocols, together with registered forest regeneration materials and developed robotics form the foundation for the commercialisation of the SE pipeline.

In the Laukansaari Giants project funded by Business Finland and launched in 2024, LUKE and XAMK are together preparing SE pipeline commercialisation. The project also aims to produce SE plant lots approaching scale relevant to forestry as a proof of concept. Simultaneously, further applied research is conducted to refine the protocols and develop the automation. New improved plant materials from crossings of the national tree breeding programme are constantly initiated and evaluated.

Communication efforts
Communication with stakeholders has taken place via discussions at the project steering committees, and by working with PPP-partners, customer-funded pilot propagations, and numerous visits by the interested parties to LUKE SE technology platform at Savonlinna (Finland) and field trials. Also, presentations and discussions at workshops and meetings organised for professionals, such as Annual Forest Plant Nursery Days, and articles in professional magazines are part of the stakeholder communication.

Beyond the stakeholder communication, public discussion on SE in Finland has so far been very limited, although forests and forestry in general have been a widely discussed topics in the recent years. For the SE pipeline development, following the public discussion both on national and EU level is important to identify the prevailing and raising views and perceptions, and to be able to address SE-related questions with science-based information. Communication strategy and plans are being prepared for application of SE in Finnish forestry.

LUKE has also made communication efforts to increase the public awareness on forest tree breeding more generally, and on vegetative propagation options including SE, as a part of this. In March 2023, LUKE launched an open website and educational materials for tree breeding, with information on vegetative propagation (Natural Resources Institute Finland 2023). This information is in Finnish, being currently translated into English. Further, close to LUKE SE technology platform, at LUKE Punkaharju Research Park, there is a Norway spruce SE demonstration area, as well as several SE field experiments. The Punkaharju Research Park is open to public and easily accessible, having several marked routes with interactive signs containing also QR codes to e.g., video presentations including more information. The Punkaharju Research Park exists also as a Virtual Research Park allowing remote access (Natural Resources Institute Finland 2020).

Results and Discussion

Biological challenges mostly solved
In Norway spruce, recalcitrance in SE is not an issue. Based on LUKE's experience, with the optimised protocol, SE initiation rates are high, over 80% genotypes (Varis et al. 2023). Even though there is some variation among families, capture of different genetic backgrounds succeeds well, and the risk of unintentional selection due to big proportion of recalcitrant genotypes is low. Storage of established SE lines by the developed cryopreservation technique is reliable (Varis et al. 2022), 75% of genotypes recovering after thawing. There are several options for realisation of proliferation and maturation phases, of which maturation on semi-solid medium, potentially preceded by short scale-up in suspension culture appears the best now (Välimäki et al.2021). The mature somatic embryos can then be either directly germinated or safely cold-stored for months without any adverse effects on their later performance (Konrádová et al. 2003, Tikkinen et al. 2018b, Välimäki et. al 2022). On the contrary, a couple of months in cold storage can improve the greenhouse outcome. For plant conversion, the short *in vitro* germination method is efficient, the resulting germinants being suitable for either direct transplanting into final nursery containers or to be grown in mini plugs (Tikkinen et al. 2018b). For all the SE propagation phases, the developed methods are robust, i.e., work well with most of the genotypes, based not only on broad testing but also on the pilot propagations realised.

Economics of propagation
The higher price of SE plants compared with the seedlings is one of the major challenges for application of SE in forestry. The higher costs could be balanced e.g., by increased productivity or reduced rotation length, or improved traits such as resistance to pests or pathogens. For the forest owner, however, the planting stock cost is immediate, but the profits are realised much later, at the end of rotation, which in the case of Norway spruce in Finland having a rotation time of 50-80 years is quite often later than the forest owners' life expectancy. In a recent survey (Tikkinen et al. 2021), forest owners expressed willingness to pay potentially up to 24% more on the improved material, setting a goal for SE

propagation costs, too. To reach acceptable price level, cost efficiency of SE propagation needs to be improved by increasing success rate of all, but especially the later SE steps and by developing technologies to replace manual work.

According to Tikkinen (2018), the SE cost structure highlights the labour intensity of the process, especially in maturation and germination stages (Table 1). The maturation and germination outcomes were improved without increasing the production costs by reducing the ABA concentration in maturation media and shortening the germination period (Tikkinen et al. 2018a, b). However, automation for the germination and transplantation stages are still vital to reach commercial production. In the later stages, embryos are more and more handled individually, which increases the costs when done manually. Additionally, the loss of a SE plant later in the process is more costly, as more effort has been invested in its propagation.

Table 1. Cost structure in Norway spruce embling production for different phases of SE propagation. Estimation of labour and material costs in different phases together with the proportion of the overall costs in each phase. Originally published by Tikkinen (2018).

Production phase	Labour cost (%)	Material cost (%)	Overall cost (%)
Planning	100		6
Thawing	92	8	4
Proliferation	88	12	10
Maturation	83	17	20
Germination	98	2	60

Liquid cultures, application of bioreactors and automation, as well as encapsulating somatic embryos into artificial seed have all been suggested as tools to provide better scalability and cost-efficiency (Egerstdotter et al. 2019). In conifers, production of somatic embryos in bioreactors has succeeded in some species including Norway spruce. However, experiences on cost efficiency vary, as spruce somatic embryos matured in bioreactor require a separate desiccation step to be able to germinate properly. Also, the handling of the bioreactors can be surprisingly laborious (Välimäki et al. 2020). Automated technology has been developed for harvesting mature somatic embryos. In Canada, robotics has already been implemented for several years into practical propagation of SE planting stock of spruces by JD Irving Ltd. (Andrew McCartney, personal communication). Another example is the SE Fluidics System handling conifer somatic embryos in liquid and selecting good-quality embryos for germination using image analyses by comparing individual embryos with pre-set criteria. The system has been tested in Sweden for Norway spruce since 2014 (Egerstsdotter et al. 2019).

To enable mass production of SE plants, specialised robotics for machine vision-based selection and picking up, orienting and placing of conifer somatic embryos for germination have recently been developed in Finland (Bernhardt et al. 2020, Havia et al. 2022). The robot is being tested for scaling-up of Norway spruce SE propagation. The robot comprises two units: one for picking up, evaluating and placing embryos, and the other for feeding empty and removing full germination Petri plates. Compared to existing SE automation solutions, the developed robot is compact, can be set up into a regular laminar flow hood and is easily movable if needed. Its modular design allows for modifications based on user needs. The robot's dry operation reduces contamination risk, as it does not require the material to be sorted in any liquid. This also makes changing the cell line without the fear of cross-contamination easy and fast. Usability has been a key focus during development, and the robot can be operated with minimal training, especially for those already familiar with tissue culture and aseptic work. Currently, the image analysis is carried out using traditional machine vision methods based on criteria by Tikkinen et al. (2018b), but a neural network-based solution is being implemented to the robots to improve the selection results.

Many of the parts of the robot are 3D printed, which has been a significant advantage during the development. Through rapid prototyping using 3D printed components, it has been possible to quickly test solutions for encountered issues (Havia et al. 2022). During the testing, constant feedback was provided to the programmer to report bugs and improve the user interface. The robot's modularity enhances its potential to be used in different laboratories using various types of plant material or labware. Currently, the robot is being tested for radiata pine by Scion (now the New Zealand Institute of Bioeconomy Science) in New Zealand.

The development of automation is necessary for the cost-efficiency of the SE propagation, but not sufficient by itself. For ensuring the economical outcomes, it is important to be able to propagate the desired genotypes in large numbers. The performance of SE lines varies a lot, both considering their field characteristics and their somatic embryo production capacity. From a big stock of SE materials, it is easier to select the ones having both good embryo production capacity and desired field performance, as these are not connected to each other. Of course, it requires the testing of numerous genotypes both in laboratory and in the field, and especially field experiments are long-lasting and expensive.

At LUKE, the Norway spruce SE materials are firstly tested at lab for their embryo production capacity following cryo-storage. Then mature embryos are germinated, acclimatised and the embryogenic lines producing enough plants tested in the field following layout and procedures for clonal tests established for breeding purposes with rooted cuttings. In Finland, the field testing of embryogenic lines is carried out using the same procedures as the Finnish tree breeding programme. Field testing consists of a series of trials replicated at four to six locations within and outside the region of provenance. The test materials are thus subjected to wider climatic variation than the actual reforestation material. Most trials utilise a randomised complete block design with 8 to 10 ramets per clone planted in single-tree plots. The trees are assessed for height and diameter growth, stem defects, disease symptoms and wood density at several ages up to 10-20 years. The genotypes to be utilised in commercial clone mixtures are selected for both their field performance and laboratory production characteristics. The resulting clone mixtures will, in turn, be tested in realised gain trials and demonstration plots to evaluate the performance of the commercial reforestation material.

Further, in the Business Finland funded Laukansaari Giants project, the avenues for commercialising SE are currently being explored. This project has already performed a robot germination pilot with over 70 000 embryos sorted as good in a single lot and a more extensive pilot is being prepared. Also, extension of robotics to other phases of SE propagation is ongoing, simultaneously with preparation of commercialisation.

Acknowledgement of regulatory framework

The regulations are often seen as a challenge for application of SE in forestry. The regulations have been developed to reduce potential risks of using vegetatively propagated FRM and set guidelines for sustainable production. If the regulations are known and considered from the beginning, they don't form an obstacle for SE propagation, based on LUKE's experience. The best way of fulfilling requirements is close cooperation with breeding programme able to provide proper genetic data on materials to be propagated. For LUKE, being also responsible for tree breeding in Finland, this has been easy.

LUKE has registered two SE basic materials as FRM, the basic material in both cases being of "Parents of families". The first registration took place at the end of 2017, as the first one in Europe, and the second one in the beginning of 2024. Both registered basic materials consist of 12 full-sib families and have their own deployments areas (Figure 1). The 2017 registered material "Laukansaari" is of category "Qualified" in which the Finnish national regulations allow production of 4 million plants per a family, i.e., 48 million plants altogether. The 2024 registered material "Sammonvarvas" is of category "Tested" with no restrictions on the propagation numbers. Further, for both categories, the national regulations require every family to be represented in each propagation lot with reasonable number of genotypes, as evenly as possible, in the same proportion as given in the master certificate. The registration enables marketing of SE materials as FRM, i.e., commercial propagation. In practice, The Finnish Food

Authority as a national authority should issue a master certificate for all the material derived from these approved basic materials.

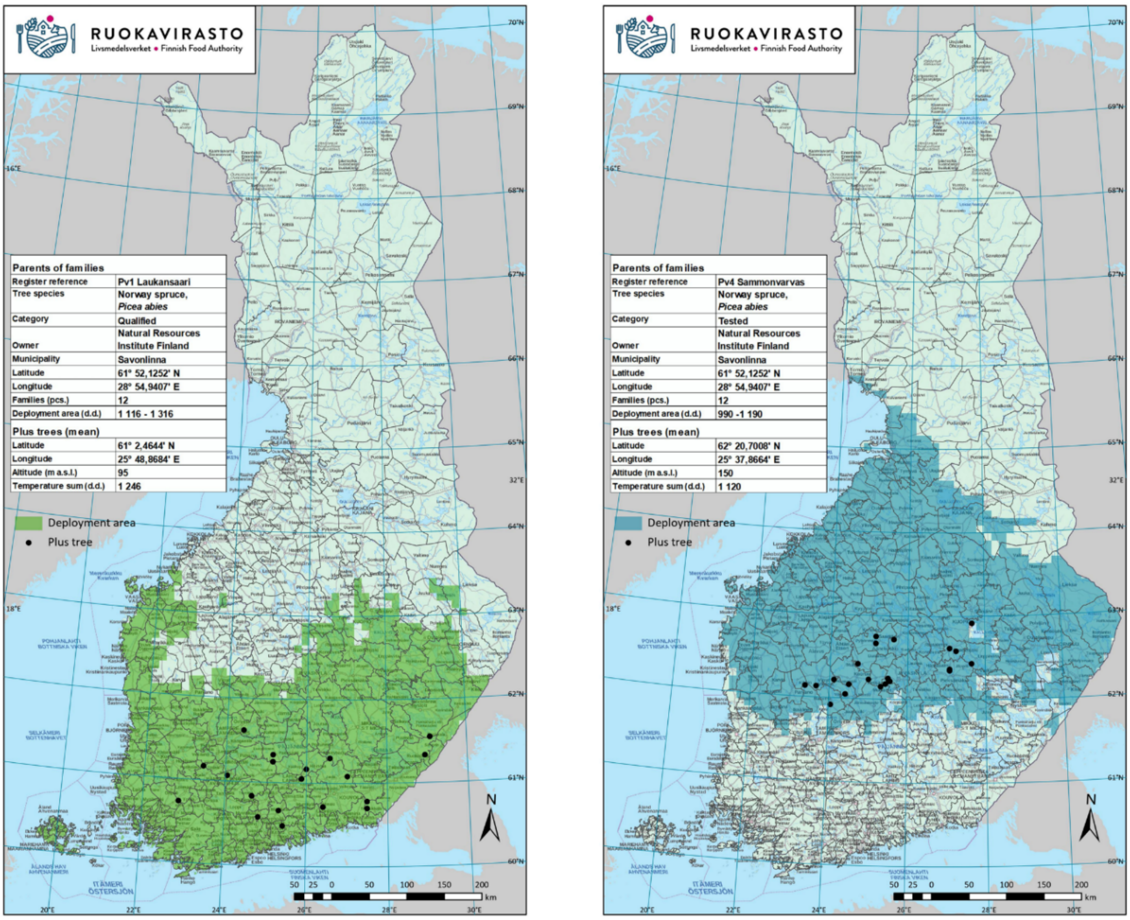

Figure 1. Somatic embryogenesis derived basic materials registered as Forest Reproductive Material of Norway spruce in Finland: Pv1 Laukansaari (left) and Pv4 Sammonvarvas (right). The maps show the original location of the parent trees as black dots, as well as recommended deployment zones for the material in colour.

Addressing stakeholder preferences

In the survey performed among the forest owners and professionals (Tikkinen et al. 2021), there were over 3000 respondents who represented 65 000 invited by email. As the forest ownership in Finland is very widely distributed, only forest owners having an average sized estate or more forest (>30.5 ha) were asked to participate. The respondents considered the most desired traits in the improved material to be better resilience in the climate change, improved pest and pathogen resistance, and securing species' gene pool. In addition, they wanted increased growth and improved wood quality, as well as replacing import of seedlings with domestic material. If having this kind of improved material, the respondents said they would pay 7–15% more and even possibly up to 24% more. When asked to take genetic diversity issues into account and evaluate how much of Finnish forest plant production could be vegetatively propagated, the respondents accepted, on an average, 36%, median being 32%. Responses varied a lot, from 0 (2% of respondents) to 100 (1% of respondents). Acceptance level was also observed to rise with the amount of experience on vegetative propagation. When asking about potential risks related to vegetative propagation, the biggest concern was depletion of genetic diversity, and unknown risks. Lack of information and prejudices together with economic concerns were also recognised (Tikkinen et al. 2021). The results of the survey have also been confirmed in discussion with the stakeholders involved in the RDI projects.

How to address stakeholder wishes?
Of the desired traits, both improved resilience and growth are best addressed in SE propagation by using the best trees from tree breeding programme as a source of explants. At LUKE approach, both the parent trees and SE lines produced from them are field-tested on multiple locations, within and outside the deployment area to ensure resilience, and only health and well-growing genotypes are selected for propagation, resulting in improved growth and performance. In Norway spruce, genetic gain in volume growth when using seed-orchard seed is appr. from 21 to 37% (Haapanen 2020). Based on LUKE's first SE field trials, increase in volume growth could be much more using SE material, depending on selection intensity. Further, the recent studies show that Norway spruces propagated via SE have been found to be less damaged by harmful forest pests i.e., bark-feeding pine weevils than regular nursery seedlings. This effect is synergistic to application of plant defence hormone methyl jasmonate (MeJa) and last for several years. Together SE and MeJa treatment showed 86-87% less damage at field conditions than untreated seedlings (Berggren et al. 2023; Puentes et al.2018, Berggren et al. Ms submitted). SE is also well compatible with both marker-assisted and genomic selection, and in Norway spruce, SE pipeline was shown to be suitable for multiplication of material having a specific gene allele connected with improved root rot resistance (Edesi et al. 2021), more root rot resistance related genes being actively searched. To replace seedling import, SE itself can provide an answer.

Stakeholders were mostly concerned about reduced genetic diversity. In public discussion, this is often worried to lead to lack of resistance among the propagated trees e.g., to novel pests or diseases that may appear in the changing climate. These risks can be minimised with proper field testing of the materials, as discussed above. Another concern is that every genotype uses resources in its own unique way, and within a monoclonal plantation increased competition could thus lead to yield losses. It is also feared that reduced genetic diversity of ecosystem key species, trees, might be less supportive for other species, or reduce recreational values in multiuse forests that are common in Finland, too. These concerns are, however, not only dependent on the genetic diversity of the planting stock but also on forest management practices.

Although in SE propagation the number of different genotypes is always smaller than in seed propagation, genetic diversity can still be high on allelic level, if propagating high enough number of genotypes from unrelated families. Suggested numbers are overall around 5–30, or for Northern conditions with long rotation times 15–25 genotypes (Roswall et al. 2019). In Finland, diversity of the SE stock has been emphasised. The registered Norway spruce SE basic materials contain 12 full-sib families, every family being represented in propagation lots by many genotypes, e.g., 5–10. Propagation of several genotypes per family ensures that the material represents the characteristics of the parents, which cannot be guaranteed with a single untested offspring genotype. To avoid even smaller monoclonal blocks, the plants from different SE lines – although kept separate during propagation – are mixed when transplanted into nursery containers.

Diversity of forest stands can also be increased by mixing SE plants and seedlings or by planting SE spruces only on part of the prepared planting sites and letting naturally born seedlings to occupy the remaining ones. This could also reduce establishment costs and favour mixed stands. As spruces propagated via SE represent the best growing improved material, they are better to cope with competing broadleaves than unimproved spruces. The SE materials also change over time due to progressing breeding and have their own deployment areas, i.e., with time passing SE lots will be replaced with new and even more improved ones and the same genotypes are not grown everywhere. Again, having broad SE materials helps to keep as wide genetic diversity as possible and still select for desired traits.

Public acceptance
The views of the general public on the application of SE in Finland are yet not known, as there has not been much discussion on this topic. At the time of making the stakeholder survey, there was one writing on "From the readers" page in the leading newspaper in Finland, Helsingin Sanomat, expressing concerns on diversity issues. LUKE answered that, and there was no further discussion. Also, the

visitors of the LUKE SE Technology Platform and field demonstration site often raise the same question on diversity. In our experience, the discussions among the visitors and the experts have been constructive and the precautions taken by LUKE to ensure a safe level of genetic diversity understood. However, it should be noted that so far only a small number of people have been involved in the discussions, with only a few representatives of Non-Governmental Organisations among them. Even though the annual number of people visiting LUKE's Research and Virtual Park containing SE demonstration site and information, is rather high, around 20000 and 1000, respectively, it is not known if they have familiarised themselves with the SE topic. At the LUKE tree breeding web site, around 500 visitors were recorded in four months from its publication in 2023, vegetative propagation being among the web site's most popular subtopics. More discussion will probably appear, if not earlier, at least when SE materials will be more actively marketed.

More generally, during the recent years, forestry has been among the hot topics of political and public discussion both in Finland and in the EU. The role of forests in carbon sequestration and storage, loss of biodiversity and actions to be taken to prevent it, forest-management methods and the amounts of cuttings required have all been debated. There has also been a change in emphasis of public discussions during the period of the SE pipeline development with diversity issues and climate change mitigation being constantly important.

The EU has launched initiatives, strategies and guidelines that may have either direct or indirect effect on the SE application potentials too, including e.g., EU Forest Strategy for 2030 ((European Commission, Directorate-General for Agriculture and Rural Development 2021), and EU Biodiversity Strategy for 2030 (European Commission, Directorate-General for Environment 2021). The EU Forest Strategy aims to achieve both the EU's biodiversity objectives as well as greenhouse gas emission reduction target of at least 55% by 2030, and climate neutrality by 2050. The goals of the EU Biodiversity Strategy contain increasing the protected areas, including all the remaining primary and old-growth forests, as well as restoration of forest habitats. The EU is also currently revising its regulations for FRM (European Commission, Directorate-General for Health and Food Safety 2023), and the LUKE SE researchers have participated in the process as invited experts.

SE propagation can, on the one hand, contribute to the EU targets, but on the other hand, the EU guidelines may also work against application of SE. Usage of more productive reforestation material in production forests could decrease pressure to use the remaining old forests, and at the same time increase carbon sequestration. Efficient SE propagation may also help in reaching the EU's pledge for planting 3 billion additional trees by 2030 as a part of the EU Forest Strategy, or e.g., in planting more urban trees as recommended by EU Guidelines on biodiversity-friendly afforestation, reforestation and tree planting (European Commission, Directorate-General for Environment 2023). The same guidelines, however, recommend natural regeneration, or, if using improved material, avoidance of decrease in genetic diversity – what always happens to some extent with vegetatively propagated material. Recognising these contradictions and following public debate, as well as being prepared to provide science -based information may be crucial for the acceptance of SE propagation in forestry.

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