forests

MDPI

Review

Somatic Embryogenesis of Norway Spruce and Scots Pine: Possibility of Application in Modern Forestry

Teresa Hazubska-Przybył [1], Mikołaj Krzysztof Wawrzyniak [1,*], Joanna Kijowska-Oberc [1], Aleksandra Maria Staszak [2] and Ewelina Ratajczak [1]

[1] Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kornik, Poland; hazubska@man.poznan.pl (T.H.-P.); joberc@man.poznan.pl (J.K.-O.); eratajcz@man.poznan.pl (E.R.)
[2] Laboratory of Plant Physiology, Department of Plant Biology and Ecology, Faculty of Biology, University of Bialystok, Ciolkowskiego 1J, 15-245 Bialystok, Poland; a.staszak@uwb.edu.pl
* Correspondence: mikwaw@man.poznan.pl

Abstract: Somatic embryogenesis (SE) is an important method for the vegetative propagation of trees. SE is the developmental in vitro process in which embryos are produced from somatic cells. This method can be integrated with other biotechnological techniques, genomic breeding and cryopreservation, which enables commercial-scale sapling production of selected high-yielding genotypes in wood production combined with fast breeding cycles. The SE is potential tool to improve plant stock in comparison with seed orchards. It can be useful for ecologically and economically important species, such as Norway spruce (*Picea abies* L. Karst.) and Scots pine (*Pinus sylvestris* L.), ensuring stable production in the era of climate change and biodiversity crisis. In this review, we summarize the current state of research on problems associated with somatic embryogenesis in *P. abies* and *P. sylvestris*.

Keywords: Norway spruce; Scots pine; somatic embryogenesis

Citation: Hazubska-Przybył, T.; Wawrzyniak, M.K.; Kijowska-Oberc, J.; Staszak, A.M.; Ratajczak, E. Somatic Embryogenesis of Norway Spruce and Scots Pine: Possibility of Application in Modern Forestry. *Forests* **2022**, *13*, 155. https://doi.org/10.3390/f13020155

Academic Editors: Ricardo Javier Ordás and José Manuel Álvarez Díaz

Received: 15 December 2021
Accepted: 17 January 2022
Published: 20 January 2022

1. Introduction

The demand for wood products is predicted to increase in response to a growing population and its needs [1]. This demand results from wood being a versatile, ecological and renewable resource used as an energy source, building compound or raw material in many industries [2,3]. Unfortunately, forest areas are still shrinking not only as a consequence of wood harvesting but also as a consequence of large-scale land operations, such as rapid urbanization and agriculture [4,5]. Deforestation leads to fragmentation of forest ecosystems, and the result is population division and modification of interspecies interactions [6]. This phenomenon generates both microclimate changes [7,8] and global-scale modifications of climate conditions [9–11], and consequently, the risks to biodiversity decrease [12,13]. Therefore, to obtain economically important timber without damaging natural forests, high-yield plantations have been established [2,14]. However, successful and efficient plantations need to be based on high-yielding genotypes of native timber species. This approach can be supported by genomic selection and somatic embryogenesis (SE), together with cryopreservation. Using vegetative propagation (e.g., SE) can possibly accelerate the breeding process around 20–30 years in comparison with seed orchards [15]. Integrating these methods can help in the near future with the climate crisis, providing clonal forests with new varieties of trees adapted to changed environmental conditions [16].

Two of the most important timber species in Europe are Norway spruce (*Picea abies* (L.) H. Karst) and Scots pine (*Pinus sylvestris* L.). Both belong to the Pinaceae family and are the primary species in forest management in many European countries (Sweden, Finland, France, Poland and the Baltic States). *P. abies* is distributed in the boreal forest zone covering Scandinavia to the Ural Mountains and in the mountainous areas of the temperate zone [17]. This species is also cultivated outside its natural range in warmer

and drier regions of Europe for commercial purposes due to its valuable wood and is harvested mainly for production of cellulose and use by the construction industry. *P. abies* is wind-pollinated, *monoecious* and matures between 25–65 years of age. The species can be self-pollinated, although flower positioning on the trees and shift in flowering time minimize the process. In plantations, a strong relationship between the number single tree progenies and the probability of allele losses has been observed [18]. *P. sylvestris* is a very valuable forest tree species with a broad natural range that covers a large area of Eurasia [19]. Similar to spruce wood, pine wood is an important building material. It is also a source of cellulose and is used as fuel. *P. sylvestris* grows very well in poor habitats; thus, it is important as a foundational coniferous species commonly used in the reforestation of degraded lands and former arable lands [20,21]. Similarly to *P. abies*, *P. sylvestris* is wind-pollinated with male and female flowers present on the same tree. It mature between 25–30 years. Populations are genetically variable, due to effective geneflow and high mobility of pollen [22]. Unfortunately, over the past few decades, climate change has had a significant impact on the condition of Europe's boreal forests and forestry [23–25]. *P. abies* and *P. sylvestris* are among the species most threatened by the effects of climate change. Their distribution is modelled to decline over the next few years in Europe [26].

To ensure the desired breeding characteristics are retained in the offspring, propagation based on the use of vegetative methods is needed. A root cutting method is routinely used for the vegetative propagation of *P. abies*, e.g., in Finland and Sweden. However, due to high production costs, this method has not been introduced into mass plant production [27]. Such possibilities are potentially offered by the SE. SE is potentially the most efficient method of vegetative propagation of trees that could be used for breeding and selection of forest trees on a commercial scale, however its use is still usually limited to juvenile explants [28–30]. This technology is particularly useful both for tree species that are difficult to propagate using conventional methods and for vegetative propagation of valuable varieties [31]. It enables the production of many somatic seedlings in a relatively short time from the induction of embryogenic cultures [28]. Moreover, embryogenic tissues can be stored in liquid nitrogen (LN; −196 °C) in the juvenile stage for potentially hundreds of years, while the somatic seedlings obtained from them may be planted whenever necessary.

The SE has been intensively developed for spruce and pine species. Although the costs of somatic seedling production are still high, it is expected that the future automation of the process will allow a significant reduction in the costs generated and the implementation of SE in large-scale forest tree production. *P. sylvestris* is recalcitrant to vegetative propagation, and attempts to propagate it based on rooted cuttings have thus far not yielded satisfactory results [19,32]. SE may be the only way to obtain vegetative progeny for this coniferous species; hence, the development of propagation protocols based on this method seems justified. Unfortunately, although the SE system is advanced for many conifers (including spruces and pines), its extensive use for practical propagation is strictly limited due to its application to only a few selected genotypes and to high somatic seedling production handling costs. Therefore, it is still necessary to resolve several challenges so that SE could be used universally [33–35]. In this review, we summarize the current state of research on the problem of SE in *P. abies* and *P. sylvestris*. The efficiency of the SE process varies greatly from one stage to the next, so developing universal protocols for different tree species is a great challenge. Studies undertaken in recent years from both biochemical and molecular aspects to better understand the mechanisms controlling the SE process in conifers, as well as efforts aimed at improving the conditions for somatic embryo development, germination and acclimatization, give hope for the development of increasingly efficient protocols for these economically important forest tree species. We expect that it could result in the implementation of SE in European breeding programmes in the near future and will help the forest sector overcome the problems caused by climate change and market demands for wood products.

2. Explants and Initiation of SE

P. abies was the first conifer in which SE was successfully performed using mature zygotic embryos as explants [36]. However, for some coniferous species, including pines and common juniper (*Juniperus communis* L.), it is possible to induce SE only when zygotic embryos at the polyembryonic cleavage stage are used [37–39]. This is very inconvenient from a practical point of view because explants at this stage must be taken within a strictly defined, narrow time period. In contrast, mature explants may be used even from seeds stored in seed banks for many years, although the risk of lowering their SE induction potential increases over time [40].

In *Picea* spp., embryogenic cultures are usually induced from immature or mature zygotic embryos, but in most cases, the SE induction frequency is higher for the immature embryos [34]. The induction and proliferation media contain 2,4-dichlorophenoxyacetic acid (2,4-D) or rarely 1-naphthaleneacetic acid (NAA) or 4-amino-3,5,6-trichloropicolinic acid (Picloram) at 9.0–10.0 µM and benzyladenine (BA) at 4.5–5.0 µM [41–44]. In contrast, other explants derived from old trees are recalcitrant to SE induction. The oldest vegetative explants from which induction of embryogenic tissue has been achieved thus far were primordial shoots derived from 2- to 10-year-old white spruce (*Picea glauca* (Moench) Voss) [45] and 4- to 6-year-old trees obtained via SE [46]. Of the 17 clonal trees of *P. glauca*, five clones induced SE, while for, 5% of genotypes from 39 clonal trees tested had a positive response to this explant type [45,46]. For comparison, explants from trees of zygotic origin are much less susceptible to SE induction. Earlier, Ruaud et al. [47] demonstrated that in *P. abies*, explants of zygotic origin showed a significantly lower ability to induce SE (10%) than explants from SE-derived plantlets (80%) at the same age. The cause of these differences is not well understood. Klimaszewska et al. [45] hypothesized that during the first stages of SE, the capacity for this process undergoes permanent fixation, which is primarily associated with changes at the epigenetic level.

In many studies, it has been highlighted that the physiological state of the explant may be a major determinant of its ability to induce SE. Rutledge et al. [48] suggested that the underlying cause of this explant resistance is not a lack of activity of specific genes or other SE determinants but a potential activation of biotic defences that can work against this process. Based on the gene expression profile of shoot primordial explants originating from adult trees of *P. glauca*, for responsive and nonresponsive genotypes, high activity levels of the four candidate genes in the nonresponsive genotype were demonstrated. All these genes encoded proteins similar to angiosperm proteins, whose high activity over a prolonged period is associated with activation of the biotic defence response. At the same time, a more moderate response was obtained for the responsive genotype, indicating adaptation to stress conditions. This means a potential relationship between biotic defence and SE induction recalcitrance. Recently, Aronen et al. [49] drew attention to the possible connection between telomere length and SE induction, embryogenic tissue proliferation period and somatic embryo regeneration of *P. abies*. Telomeres are fragments of DNA with a repeating sequence that are found at the end of chromosomes. They have a specific structure; in plants, they are usually repeated DNA sequences of (TTTAGGG)n, and their role is to protect and stabilize DNA during cell division. However, telomeres may be shortened under the influence of various factors, such as exposure to stressors, chemicals or pollutants, pathogen attack, dietary patterns and weather harshness [50]. Under in vitro culture conditions, oxidative stress may be a significant contributor to telomere damage. The consequences of telomere shortening are changes in gene expression and cell cycle function, which ultimately lead to ageing and programmed cell death [49,50]. Analyses conducted for *P. abies* showed a clear correlation between maximum telomere length in explants and SE induction frequency [49]. It was found that the longer the telomeres are, the higher the ability of explants to regenerate embryogenic tissue. Changes in telomere length were probably the result of multiple stress factors that acted at the SE induction stage on the explants (wounding, harsh conditions and chemical treatments), inducing oxidative stress, which was a direct cause of telomere shortening. Interestingly, telomere

shortening was not observed in either induced embryogenic tissues or somatic embryos that regenerated from these tissues [49]. However, prolonged in vitro multiplication of up to one year significantly affected telomere shortening, which as a consequence, could result in a decrease in the level of tissue productivity.

On the other hand, *P. sylvestris* is more recalcitrant to SE processes than the other pine species [19]. The first successful SE for *P. sylvestris* was reported in 1996 by Keinonen-Mettälä et al. [51]. In the beginning, it was assumed that the SE methods developed for spruce species would also apply to other coniferous species. However, it turned out that pines require significant modification. The most important differences result from the difficulty in obtaining SE initiation from mature zygotic embryos and from the quality of somatic embryos as determined by higher ABA concentration and higher osmolarity in the maturation medium [52–54]. Generally, in *Pinus* spp., SE induction is limited to the first weeks of zygotic embryo development, when the embryo is in a cleavage stage [39,51] or before it reaches the cotyledonary stage [55]; however, induction was also obtained from mature embryos [56]. According to Aronen and co-workers [57], the best explant type for this pine species is the intact gametophyte with immature zygotic embryo. The induction frequency was relatively low, reaching a maximum of 42%, and dependent on the mother tree [55,58]. Recently, Trontin et al. [59] induced embryogenic-looking tissues using slices from developing shoot buds. Only a low number of induced cell lines are able to proliferate and regenerate high-quality mature embryos. According to Abrahamsson et al. [60], this proliferation may be associated with the cleavage stage and the degeneration pattern of early and late embryos, which occur in a different way in normal and abnormal cell lines. In embryogenic cultures of *P. sylvestris*, a significant proportion of embryos at these stages degenerate. Studies have shown that in normal-cotyledon embryos regenerating cell lines, degenerating embryos are eliminated, as are subordinate embryos during zygotic embryogenesis. In contrast, in abnormal embryos regenerating cell lines, there is continuous degeneration and differentiation of new embryos [60]. According to the authors, this phenomenon already occurs during the initiation of the SE process and leads to an increased risk of producing abnormal embryos by lines induced from early zygotic embryos (at the cleavage polyembryony stage). To eliminate this risk, the authors suggest further exploration of other sources of explants for SE induction in pines. A recent analysis on the molecular regulation of somatic embryo development from these two types of *P. sylvestris* lines showed that there are differences in the expression patterns of selected transcripts. These were associated with phenomena such as the transition from morphogenesis to maturation, embryo degeneration or apical-basal polarization. Several genes probably related to the cleavage process were also differentially expressed during the development of somatic *P. sylvestris* and *P. abies* embryos. For example, upregulation of the SERK1 gene stimulated lobing of the embryonal mass, the first step of the cleavage process in *P. sylvestris*, which does not occur in *P. abies* [61].

Generally, embryogenic cultures both *P. abies* and *P. sylvestris* are induced and proliferated on the solid or semisolid media [51,52,62], although embryogenic tissues of *P. abies* had the ability to multiplication in liquid cultures [34,63]. For *P. sylvestris*, such attempts, to our knowledge, have not yet been undertaken. However, studies for black pine (*Pinus nigra* J.F. Arnold) conducted by Salaj et al. [64] showed such possibility in *Pinus* spp. Embryogenic cultures of *P. abies* are usually induced and maintained at 20–24 °C [62,63,65] and at 24–25 °C for *P. sylvestris* cultures [52,57]. For spruce SE, the most commonly used media are recommended by von Arnold and Eriksson [66] (LP) and by Litvay et al. [67] (LM); for pine SE, the most useful media are described by Gupta and Durzan [68] (DCR), Becwar et al. [69], Litvay et al. [67] (modified to contain half-strength macroelements; $\frac{1}{2}$ LM), Smith [70] and Teasdale et al. [71] (modified). According to Aronen [19], in the case of *P. sylvestris*, the most useful are modified Litvay's [67] and Gupta and Durzan's [68] media. In contrast to spruce species, induction of SE in pines usually requires a higher or lower concentration of auxin and cytokinin or a lack of these PGRs, plus supplementation of the induction medium with ABA and amino acids [42,72–74]. For example, Lelu-Walter et al. [52] applied 9.0 or 2.2 µM

of 2,4-D and 4.4 or 2.3 BA to the induction medium with lower concentrations of PGRs in the proliferation medium. Induced ETs began to lose their potential for SE very quickly, even at 6 months of subculture [54]. For *P. nigra*, the potential for induction lasted slightly longer—2 or maximally 3 years [68]. To improve the multiplication rate of ETs filter paper discs were used [19,52].

Research conducted recently by García-Mendiguren et al. [74] for Monterey pine (*Pinus radiata* D. Don) showed that a lower initiation temperature (18 °C) promoted the SE process compared to that for the cultures incubated at 28 °C, indicating that the initiation stage has a long-term effect on embryogenesis in this species. The initiation rate was the highest at 18 °C (17%) and did not differ from the control variant 23 °C (13%), but was significantly lower at 28 °C (4%). The proliferation of embryonal masses initiated in 18 °C and 23 °C (54%) was higher as compared to 28 °C (15%). Moreover, the highest percentage of embryogenic cell lines was obtained at these lower temperatures as compared to 28 °C. Earlier, Montálban et al. [75] demonstrated that cold storage of cones for over 1 month increases SE initiation frequency. On the other hand, Gao et al. [76] proved that the success of the induction step in some pine species is also dependent on the family origin and collection date of explants. Open-pollinated cones of three families with explants were collected from June to July 2015. The age of each family was 28 years. The dates of seed collection were 23 June, 30 June, 6 July and 13 July (representing explants in four phases).

In chir pine (*Pinus roxburghii* Sargent), the role of salicylic acid (SA) in SE induction has been considered. SA could promote the inhibition of ethylene biosynthesis, which acts in plant differentiation, or by inhibiting enzyme detoxification of H_2O_2 [77]. ROS concentration and subcellular distribution in plants are carefully regulated, as imbalances cause redox state disturbances that have crucial effects on cell fate [78]. The steady state of ROS in cells is maintained through ROS-scavenging enzymes, including superoxide dismutases (SODs), ascorbate peroxidases (APXs), catalases (CATs), glutathione peroxidase (GPX), glutathione transferase (GST) and antioxidant molecules, such as glutathione and ascorbic acid [78].

An attempt to perform SE with explants derived from old trees for *P. sylvestris* was made by Trontin et al. [59]. Slices from developing shoot buds were used as explants, from which embryogenic tissues containing primordia were induced. However, only a few cell lines were able to proliferate and regenerate somatic embryos, but these were of poor quality and unable to germinate.

3. Maturation of Somatic Embryos

The lack of sufficient synchronization of the development of early somatic embryos (proembryos, PEMs) is still a serious factor reducing the efficiency of regeneration of somatic embryos in *Picea* spp. from embryogenic tissues. Only properly formed early somatic embryos develop into fully mature embryos, while others are eliminated. Synchronized embryo development is dependent on both the culture conditions and the inherent ability of the embryogenic cell lines to generate specific types of early somatic embryos [79]. During the maturation step, the multiplication process of early somatic embryos is stopped, and the accumulation of storage reserves (starch, proteins and lipids) in developing embryos starts. As a result, somatic embryos grow and go through successive, specific stages of development (globular, heart, torpedo, early cotyledonary and cotyledonary stages). The maturation of spruce somatic embryos is conditioned by the presence of abscisic acid (ABA) and their exposure to the osmotic stress provided by saccharides [80,81]. According to Varis et al. [62] the best ABA concentration for *P. abies* somatic embryos maturation was 30 µM. It was also proven that high-molecular-weight compounds such as polyethylene glycol (PEG) 4000 had a positive effect on somatic embryo quality and increased the number of mature spruce embryos [80,82,83]. However, their impact on post-maturation development is still unclear, and further investigations are needed [80,84]. An alternative to osmotic stress treatment during the maturation stage may be reduced glutathione (GSH) application to the medium [85] or low-temperature treatment [86,87]. Earlier, it

was shown that *P. glauca* somatic embryos after treatment with GHS during maturation were characterized by improved shoot:root conversion and a higher frequency of embryos generating functional roots and shoots [85]. Additionally, they were able to develop into properly growing plants to a greater extent than the germinated control embryos. However, low-temperature treatment of mature somatic embryos has proven to be an effective approach in *P. abies*. Research results published by Varies et al. [88] indicated that treatment of mature somatic embryos of *P. abies* with a temperature of +4 °C did not interfere with their proper germination. Recent studies have shown that cold storage of mature embryos before their germination positively influences their further development during germination and conversion into plants, which could then be planted after a nursery period one year earlier than that of the control variant [87].

Despite the many protocols available, somatic embryo maturation in pines is not always successful [89]. The main reasons for this include asynchronous embryo production, abnormal morphology or poor root development. As in most pine species, the development of mature somatic embryos in *P. sylvestris* is dependent on the presence of high concentrations of ABA (80–90 µM) and osmoticum (9–10 g/L gellan gum) in the medium [52,57]. Recently, Salo et al. [90] demonstrated on the basis of changes in polyamine metabolism in this pine that ABA + PEG treatment may act in different ways on the cells in embryo-producing lines and in lines unable to produce somatic embryos. In the former, it is recognized as a signal to trigger the embryogenic pathway; in the latter, it is perceived as osmotic stress, which leads to the activation of stress defence mechanisms in the cells. Therefore, manipulation of the stress response pathways seems to be a promising solution for improving the somatic embryo production of recalcitrant *P. sylvestris* lines.

Some studies have revealed that when using improved protocols, up to 95% of established embryogenic cell lines of *P. sylvestris* are able to produce properly developed somatic embryos capable of germination [91]. Aronen [57] reported a germination rate greater than 90% if well-developed somatic embryos were used. The protocol developed at the Finnish Forest Research Institute allowed the production of high-quality *P. sylvestris* saplings, which were tested in the field [19]. Despite this, many genotypes remain resistant to propagation procedures through SE, and further research is needed in this area.

As previous studies have shown, SE is a good micropropagation system for *P. sylvestris* [52,57,91]. However, the need to develop efficient protocols and the lower demand for improved forest regeneration material in this species slow down the application of SE in breeding practice and for forestry purposes.

4. Growth Conditions

4.1. Nutrients

A prerequisite for the correct development of somatic embryos of *P. abies* (and other coniferous species) under in vitro culture is that they are provided with an adequate dose of both carbon (C) and nitrogen (N) in the medium. Under natural conditions, during the development of the zygotic embryo in the seed, these components are supplied by the surrounding megagametophyte [92], which do not occur during the SE process. Some studies have revealed that N and the form in which it is applied to the media have a significant influence on the maturation and germination of coniferous somatic embryos and further on the survival rate of somatic seedlings [92,93]. N is added to the nutrient solution either in inorganic (NH_4^+ and NO_3^-) or organic forms (Gln or casein hydrolysates) or as mixtures [92]. In *Picea* spp., enrichment of the medium with organic nitrogen additionally improved the frequency of initiation, multiplication of embryogenic tissue and the quality of mature somatic embryos [65,94,95]. Carlsson et al. [96] demonstrated the importance of Gln in *P. abies* PEM multiplication, suggesting that it is a significant source of N for germinating somatic embryos. This hypothesis was confirmed in recent studies, which showed that 50% of assimilated N was supplied by Gln [92]. Therefore, it must be assumed that manipulation with this medium component may be crucial for improving SE efficiency in some coniferous species. SE is a complex process affected by many factors, and over

time, research has shown that stress factors play an important role during SE [97]. Several stress treatments, such as low or high temperature, osmotic stress, and heavy metals, may be key factors for inducing the SE process, even without the presence of PGRs in the medium [98]. In turn, studies on Japanese larch (*Larix kaempferi* (Lamb.) Carr.) SE have revealed that addition of ABA to the medium led to induction of this process not only in the presence of high H_2O_2 levels but also with increasing levels of CAT, SOD, and APX gene expression [99]. This indicates that oxidative stress conditions with high levels of ROS are needed for SE induction rather than low concentrations of ROS, as was previously considered in the context of the messenger role of ROS under physiological conditions, e.g., in seeds.

4.2. Light

Light is one of the main determinants of somatic embryo morphogenesis in conifers. Its presence or absence, intensity, spectrum and photoperiod are essential for the efficiency of embryogenic cultures. While SE induction, ET proliferation and/or somatic embryo maturation in coniferous species require darkness [41,100], the presence of light promotes germination and root growth [62,101]. Detailed research concerning light intensity and its spectra, using various light sources, on the particular stages of SE in *P. abies* was published recently by the research group from the LUKE Institute in Finland [62]. Low-intensity LED light was found to have little positive effect on the basic parameters of ET growth and somatic embryo production and survival. The proliferation of ET exposed to green light resulted in an increase in embryo productivity, but the quality of the germinating embryos was lower than that of embryos obtained from ET proliferated under far red light or in darkness. However, maturation under green light had a positive effect on root and shoot growth. The effect of blue light on the proliferation of ET was negative, similar to previous findings reported by Latkowska [102]. During maturation no or low intensity light was applied [41,63,65]. However, for the further growth of *P. abies* somatic seedlings the light intensity was increased for example from 5 µmol m^{-2} s^{-2} to 100–150 µmol m^{-2} s^{-2} at 20 °C during germination and plant regeneration [65]. However, different laboratories used various germinating conditions in this spruce somatic seedling development. In the case of *P. sylvestris* Lelu-Walter and coworkers [52] reported good results after treatment of mature cotyledonary embryos with darkness for 10–14 days at day/night temperatures of 24/21 °C and then with 10 µmol m^{-2} s^{-2} at the same temperatures scheme.

Additionally, germination performed under blue light resulted in shorter shoot and root development in this study. Generally, the spectral type and light intensity applied during germination had a significant influence on both shoot and root growth and somatic seedling survival. A beneficial effect on shoot growth was observed in the presence of red light. In previous investigations, it was reported that this light spectrum promoted somatic embryo maturation and germination in *P. abies* and in some *Pinus* spp. [101,103]. In turn, research results revealed that low-intensity red light favoured N storage during *P. abies* somatic embryo germination [92]. Based on the results presented by Varis et al. [62], it can be expected that maintaining an adequate balance between the different light spectra and the optimal light intensity will enable the effective control of the final stage of conifer somatic seedling production.

5. Germination and Acclimatization

At the germination and conversion stages PGRs are usually omitted in the media, both in *Picea* and *Pinus* spp. [41,52,65,87]. However, germination and acclimatization of saplings still remain major constraints for somatic embryos of spruces and pines due to low hypocotyl growth and subsequent abnormalities in growth [104]. These can lead to significant losses, as germination and acclimatization are the last stages of high-cost tissue culture. However, the successful production of high-quality saplings is highly dependent on previous steps of SE. Genotype, maturation method, duration of maturation and embryo quality have all been proven to influence the performance of somatic saplings [57]. Unbal-

anced root: shoot growth during germination may be a response to redox stress suffered by explants during the previous stages of SE [105]. The role of the redox state in the SE process was studied in white spruce and loblolly pine [106,107]. The addition of a low level of GSH during *P. glauca* somatic embryo formation led to higher production of embryos and root growth during the postembryonic phase. At higher concentrations, GSH inhibited these processes. When glutathione disulfide (GSSG) was added at a high concentration (1 mM), better-quality embryos were produced according to the control treatment. Supplementation of medium with GSSG led to a 20% increase in conversion frequency during postembryonic development [106]. A similar result was obtained for loblolly pine, where the addition of GSSG to germination medium led to an increase in SE germination. These results suggest that the level of oxidative stress during SE is high and that the process of oxidation of glutathione is very important [107].

In many spruce and pine species, desiccation of mature somatic embryos has been proven to considerably increase germination [108]. It is especially important in embryos matured in medium with the addition of PEG. Although PEG is beneficial to embryo maturation, it can inhibit later germination. For example, 2–3 weeks of desiccation was reported to be beneficial for egg-cone pine (*Pinus oocarpa* Schiede ex Schltdl.), increasing germination. Without this treatment, germinated embryos produce abnormal saplings incapable of further growth [109]. Apart from desiccation, additional cold stratification was proven to increase germination in slash pine (*Pinus elliottii* Engelm.) [110], Taiwan spruce (*Picea morrisonicola* Hayata) [111] and Fraser fir (*Abies fraseri* (Pursh.) Poir.) [112].

Saplings grown in vitro generally show altered morphology, physiology and anatomy, which result in their high mortality in field conditions. In vitro saplings have large stomata with changed shapes and structures, which result in increased transpiration. Their guard cells have thinner cell walls and contain more starch and chloroplasts [113]. In contrast, successfully acclimatized saplings generally have increased leaf thickness and decreased stomatal density. Subsequent development of cuticles, epicuticular waxes and effective stomatal regulation of transpiration leads to stabilization of transpiration in saplings [114]. Therefore, to ensure successful acclimatization, saplings should be exposed to ex vitro conditions slowly at high light intensity and low humidity. Additionally, carbohydrate accessibility plays a significant role in the acclimatization process as saplings change conditions from heterotrophic to autotrophic [115]. Biotization of micropropagated plants with either endophytic bacteria or mycorrhizal fungi promotes plant growth, survival and general persistence. Mycorrhizal inoculation has been proven to increase the survival rate and mean number of branches and roots, as well as the root length of stone pine (*Pinus pinea* L.) [116].

6. SE—The Future of Forestry?

6.1. Applied Biotechnology and SE

Elite trees with characteristics desired for forest management can be grown on plantations as part of breeding programs with strictly defined assumptions. For example, in New Zealand, the tree improvement program is based on the levels of genetic advantage gained by using previous generations of tree breeding [117,118]. Improved clones that allow for the commercial deployment of clones to plantations are an excellent opportunity for realizing higher genetic gains for breeding by applying genomic selection (GS). Benefits from the genomic methods implementing GS and its integration with SE are significant. They include reductions in identification errors, accurate estimates of relatedness and improved accuracy in genotyping for better control of breeding values. GS allows shortened breeding cycles and therefore results in greater flexibility than conventional conifer improvement, which is characterized by long breeding cycles necessary to make selections and reach the production stage [119]. It is likely that a drastic reduction in breeding cycle time while maximizing genetic gain is possible with the integration of GS selection and SE. This integration will lead to high synergies for implementation in the near future of multi-varietal forestry—the tested tree varieties in plantation forestry—and will be an effective way of maintaining

the productivity and adaptation capacity of conifer plantations [119–121]. This solution to tree improvement will allow the deployment of genetically improved reforestation stock for long-living conifers, such as spruces. It is very important, especially in the face of climate change and the changing needs of wood product markets, to improve planting material for the various needs in a short time. In forest practice, tree breeding programs combine clonal testing with progeny testing to achieve the best selection accuracy [122]. In the case of plant material obtained via SE, such testing would be greatly facilitated by significant shortening of the breeding cycle and the possibility of long-term cryostorage of embryogenic cultures. The clonal populations will show the greatest gains for traits having low heritability, and the progeny will allow testing of large numbers of individuals for traits with higher heritability. Relatedly, a new strategy of breeding applied by New Zealand and New South Wales includes an elite population of *P. radiata* tested both as progeny and as clones [118].

The long-term breeding program of Sweden aims to adapt trees to different conditions (current and future), including changing climatic conditions, to conserve genetic diversity and to improve tree features important to timber production [123,124]. The main advantage of using vegetative reproduction in Sweden compared to seed orchards is the elimination of the 20–30 year time delay from breeding progress to the distribution of nursery material, which will increase breeding profits but also increase the stability of crops grown under different environmental conditions because of replication of the breeding program field test system [15]. In Finland, where the increased planting of *P. abies* and fluctuating seed yields caused shortages of improved regeneration material [125], SE may be a promising method for clonal propagation of conifers on a large scale to offset this lack of improved seeds [34].

Apart of GS, genetic engineering is also expected to be included on a larger scale in breeding programs in forestry management in the future [126]. It is considered that the combination of SE and genetic transformation will allow for the rapid and precise introduction of desired features into breeding material and, consequently, to improve production efficiency of species used in clonal forestry. However, the introduction of foreign DNA into the tree genome and subsequent regeneration of transformed plants is very difficult. A crucial factor determining successful genetic manipulation is the choice of explants competent for transformation and regeneration [127,128], such as embryogenic tissues, embryogenic suspensions and mature somatic [129–131]. The most convenient plant material is embryogenic tissue, which can multiply in vitro over a longer period of time and provide an excellent basis for experiments. In the case of somatic embryos, their availability may be limited [132].

Currently, several reports concerning the genetic transformation of *P. abies* embryogenic cultures are available [133–136]. Most studies were carried out on embryogenic tissues using *Agrobacterium tumefaciens*-mediated transformation. For example, Wenck et al. [133] obtained over 100 transformed embryogenic cell lines after their co-cultivation with *Agrobacterium* strains containing additional copies of virulence genes (either a constitutively active *virG* or extra copies of *virG* and *virB*, both from pTiBo542). Additionally, Briža et al. [136] reported 70 transgenic lines, which were transformed with *Agrobacterium tumefaciens*. In this experiment the modified versions of *Cry3A* gene of *Bacillus thuringiensis*, with the increased toxicity against spruce bark beetle, were introduced into embryogenic tissues. For comparison, when using the biolistic method, the authors obtained only 18 transformed cell lines. Earlier Walter et al. [134] obtained stable integration of *uidA* and *nptII* into the *P. abies* genome using also the biolistic transformation technology. Transgenic plants of *P. abies* were obtained after transformation of embryogenic cultures with an antisense construct of the *P. abies* gene encoding cinnamoyl CoA reductase (*CCR*), one of the genes that regulates lignin biosynthesis. The underexpression of the sense *CCR* gene in transgenic plants resulted in as much as an 8% decrease in lignin content, and stable expression was maintained in the plants for over five years [137]. Literature concerning the *P. abies* somatic embryos genetic transformation is very scarce due to difficulty in obtaining transgenic

callus. In a study conducted by Pavingerová et al. [135], only one of the nine tested lines of cotyledonary somatic embryos were able to produce transgenic callus.

Until now, no studies were performed on the genetic transformation of *P. sylvestris* embryogenic cultures, although such attempts were carried out for its transformed pollen [138,139] and for other pine species [64,133,140,141]. Grace et al. [140] reported the regeneration of transgenic *P. radiata* plants transformed with the *cry1Ac* gene, which improved the level of resistance to larvae of the painted apple moth. For comparison, Salaj and coworkers [64] demonstrated the expression of the *uidA* reporter gene in five transgenic embryogenic tissue lines of *P. nigra* after one year of culture; however, poor or no transgenic plantlets were obtained.

Molecular studies have identified some genes regulating the process of SE. These include *SERK*, *LEA*, *LEC*, *YUC*, *AUX*/*IAA*, *BBM* or *WOX* genes. The activity of these genes was also detected during SE of trees. Introducing of these genes into explants (for example zygotic embryos, primordial shoots) or embryogenic tissues with low embryogenic potential would presumably improve induction and further embryo development of coniferous species somatic embryos. Thus, this research area is worth further exploration.

6.2. Possibility of Automation

The utilization of SE tissue culture protocols to produce millions of somatic seedlings for forestry with the aim of forest regeneration requires a long time because it is a multistage process. In most laboratories, conifer somatic embryo production is carried out on solid media. However, this system does not allow for the generation of large numbers of high-quality embryos and is expensive due to the considerable labour involved [33]. The solution to this problem may be to scale up by culturing in liquid media in suspension cultures or in bioreactors [142]. To achieve cost-efficient mass propagation, automation of SE is necessary, which will be possible via temporary immersion system bioreactor-based culture solutions. The choice of scale-up in bioreactors is possible thanks to the application of a multiplication step in liquid medium, which is feasible for most species [143]. However, although the use of suspension cultures improved the level of synchronization of the development of differentiating somatic embryos, the development of embryos at later stages in coniferous species remains problematic [33,64], and many more studies are needed in this area. To reduce the cost of somatic seedling production, investigations on bioreactors based on SE for some tree conifer species have been undertaken [144–147]. In the 1990s, perfusion bioreactors and regular immersion bioreactors were effective in the maturation of somatic embryos of several *Picea* spp. and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) [148]. However, in the last decade, TIS bioreactors have been successfully applied for Caucasian fir (*Abies nordmanniania* Spach.) [145] and *P. abies* [144,149]. The promising results of somatic embryo production after testing a TIS bioreactor platform for *P. abies* were demonstrated by Välimäki et al. [147]. It was found that both embryo production and survival were dependent on the frequency of bioreactor aeration and the support pad material used as a base. Moreover, the post-maturation desiccation of the mature embryos enabled the improvement of final somatic seedling survival rates. Embryo maturation in TIS was the most efficient on filter papers on plastic netting with 20 min/4 h aeration and when the mature embryos were desiccated at +2 °C for 5 wk on nested plates. However, the authors emphasize that to enable mass use of bioreactors for *P. abies* sapling production, it is still necessary to optimize the SE process in this spruce species.

The fluidics-based technology recently proposed by Swedish forestry companies is another promising solution for massive SE plant production [143]. In this approach, a special temporary immersion bioreactor model was designed for *P. abies* embryogenic cultures, allowing improvement of the quality and synchronization of the development of produced embryos. After reaching maturation, somatic embryos are harvested with the SE fluidics system; each embryo is documented individually and sorted by an image analysis system according to its morphological measurements [34,150]. It is important that the automation of maturation, germination and planting steps in this system enables the

significant reduction of production time and costs of the SE process. Temporary immersion bioreactors may have advantages over other types of bioreactors based on total immersion and mixing of the culture. They provide support for the growth of culture by promoting the directional growth of PEM and the formation of polarized embryos at an early stage. In the case of SE coniferous cultures, reproduction and maturation can occur sequentially in the same bioreactor without disturbing the culture [143]. It is expected that the development of appropriate criteria for the selection of somatic embryos will allow for the rapid production of valuable material on a large scale. Therefore, it seems that *P. abies* SE process automation will be realized in the near future. If it can be carried out for one species, it will only be a matter of time before it can for others.

7. Concluding Remarks

In the last decade, significantly more research has been conducted to understand the mechanisms controlling tree embryogenesis and in vitro cultivation. SE still remains as a method with an unfulfilled potential. Despite being used commercially in some instances, application of SE in forestry is not well developed. Still, the main limiting factors appear to be the costs and difficulties in establishing cultures from some genotypes. However, in order to rapidly accelerate many tree improvement programs, an effective and practically reproducible technique must be developed to produce somatic plants from adult vegetative material (Figure 1). Especially in the era of climate change as well as biodiversity collapse possibility of combining SE with other biotechnological methods, cryopreservation, genome sequencing or genetic modification is an asset, which cannot be overestimated. It is especially helpful in trees that are generally characterized as having a long life span and therefore, slow reaction for changes.

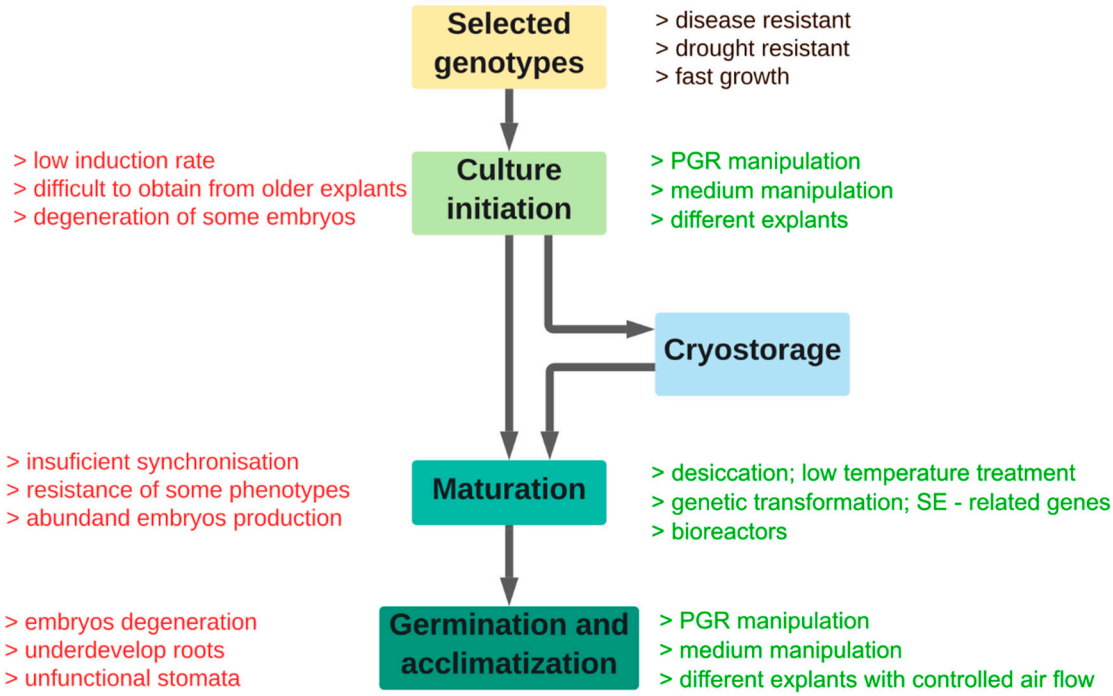

Figure 1. Steps of SE process of *Picea abies* and *Pinus sylvestris*. Major challenges are presented in red and potential solutions in green.

Amongst the challenges is to improve the general quality of mature somatic embryos and increase the conversion rate of somatic embryos and the survival rate of acclimatized saplings. This last part is especially significant, as losses in the number of received saplings increase the overall cost of their production. Improvements at each step of the SE process will contribute to the final success rate.

Author Contributions: Conceptualization, T.H.-P. and E.R. writing—original draft preparation, T.H.-P., M.K.W., J.K.-O., A.M.S. and E.R.; writing—review and editing, T.H.-P., M.K.W.; visualization, A.M.S.; supervision: T.H.-P., M.K.W.; All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by statutory research of the Institute of Dendrology, Polish Academy of Sciences.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bruinsma, J. *World Agriculture: Towards 2015/2030: An FAO Study*; Earthscan Publications Ltd.: London, UK, 2017; p. 431.
2. Sutton, W.R.J. The Need for Planted Forests and the Example of *Radiata* Pine. *New For.* **1999**, *17*, 95–110. [CrossRef]
3. Pintilii, R.-D.; Andronache, I.; Diaconu, D.C.; Dobrea, R.C.; Zeleňáková, M.; Fensholt, R.; Peptenatu, D.; Drăghici, C.-C.; Ciobotaru, A.-M. Using Fractal Analysis in Modeling the Dynamics of Forest Areas and Economic Impact Assessment: Maramureș County, Romania, as a Case Study. *Forests* **2017**, *8*, 25. [CrossRef]
4. Keenan, R.J.; Reams, G.A.; Achard, F.; de Freitas, J.V.; Grainger, A.; Lindquist, E. Dynamics of Global Forest Area: Results from the FAO Global Forest Resources Assessment 2015. *For. Ecol. Manag.* **2015**, *352*, 9–20. [CrossRef]
5. Singha, K.; Sahariah, D.; Saikia, A. Shrinking Forest and Contested Frontiers: A Case of Changing Human-Forest Interface along the Protected Areas of Nagaon District, Assam, India. *Eur. J. Geogr.* **2019**, *10*, 120–136.
6. Andronache, I.; Marin, M.; Fischer, R.; Ahammer, H.; Radulovic, M.; Ciobotaru, A.-M.; Jelinek, H.F.; Di Ieva, A.; Pintilii, R.-D.; Drăghici, C.-C.; et al. Dynamics of Forest Fragmentation and Connectivity Using Particle and Fractal Analysis. *Sci. Rep.* **2019**, *9*, 12228. [CrossRef] [PubMed]
7. Kupfer, J.A.; Malanson, G.P.; Franklin, S.B. Not Seeing the Ocean for the Islands: The Mediating Influence of Matrix-Based Processes on Forest Fragmentation Effects. *Glob. Ecol. Biogeogr.* **2006**, *15*, 8–20. [CrossRef]
8. Cao, Q.; Liu, Y.; Georgescu, M.; Wu, J. Impacts of Landscape Changes on Local and Regional Climate: A Systematic Review. *Landsc. Ecol.* **2020**, *35*, 1269–1290. [CrossRef]
9. Davin, E.L.; de Noblet-Ducoudre, N. Climatic Impact of Global-Scale Deforestation: Radiative versus Nonradiative Processes. *J. Clim.* **2010**, *23*, 97–112. [CrossRef]
10. Strandberg, G.; Kjellström, E. Climate Impacts from Afforestation and Deforestation in Europe. *Earth Interact.* **2019**, *23*, 1–27. [CrossRef]
11. Mendes, C.B.; Prevedello, J.A. Does Habitat Fragmentation Affect Landscape-Level Temperatures? A Global Analysis. *Landsc. Ecol.* **2020**, *35*, 1743–1756. [CrossRef]
12. Wu, J. Key Concepts and Research Topics in Landscape Ecology Revisited: 30 Years after the Allerton Park workshop. *Landsc. Ecol.* **2013**, *28*, 1–11. [CrossRef]
13. Acharya, K.P.; Paudel, P.K.; Jnawali, S.R.; Neupane, P.R.; Köhl, M. Can Forest Fragmentation and Configuration Work as Indicators of Human–Wildlife Conflict? Evidences from Human Death and Injury by Wildlife Attacks in Nepal. *Ecol. Indic.* **2017**, *80*, 74–83. [CrossRef]
14. Hazubska-Przybyl, T.; Bojarczuk, K. Tree Somatic Embryogenesis in Science and Forestry. *Dendrobiology* **2016**, *76*, 105–116. [CrossRef]
15. Rosvall, O. Using Norway Spruce Clones in Swedish Forestry: General Overview and Concepts. *Scand. J. For. Res.* **2019**, *34*, 336–341. [CrossRef]
16. Park, Y.-S.; Beaulieu, J.; Bousquet, J. Multi-Varietal Forestry Integrating Genomic Selection and Somatic Embryogenesis. In *Vegetative Propagation of Forest Trees*; Park, Y.S., Bonga, J.M., Moon, H.-K., Eds.; National Institute of Forest Science (Nifos): Seoul, Korea, 2016; pp. 302–318.
17. Montwé, D.; Spiecker, H.; Hamann, A. An Experimentally Controlled Extreme Drought in a Norway Spruce Forest Reveals Fast Hydraulic Response and Subsequent Recovery of Growth Rates. *Trees Struct. Funct.* **2014**, *28*, 891–900. [CrossRef]
18. Skrøppa, T. *EUFORGEN Technical Guidelines for Genetic Conservation and Use for Norway Spruce (Picea abies)*; International Plant Genetic Resources Institute: Rome, Italy, 2003.
19. Aronen, T. From Lab to Field-Current State of Somatic Embryogenesis in Scots Pine. In *Vegetative Propagation of Forest Trees*; Park, Y.S., Bonga, J.M., Moon, H.-K., Eds.; National Institute of Forest Science (Nifos): Seoul, Korea, 2016; pp. 515–527.
20. Hytönen, J.; Jylhä, P. Long-Term Response of Weed Control Intensity on Scots Pine Survival, Growth and Nutrition on Former Arable Land. *Eur. J. For. Res.* **2011**, *130*, 91–98. [CrossRef]
21. O'Reilly-Wapstra, J.M.; Moore, B.D.; Brewer, M.; Beaton, J.; Sim, D.; Wiggins, N.L.; Iason, G.R. *Pinus sylvestris* Sapling Growth and Recovery from Mammalian Browsing. *For. Ecol. Manag.* **2014**, *325*, 18–25. [CrossRef]

22. Mátyás, C.; Ackzell, L.; Samuel, C.J.A. *EUFORGEN Technical Guidelines for Genetic Conservation and Use for Scots Pine (Pinus sylvestris)*; International Plant Genetic Resources Institute: Rome, Italy, 2004.
23. Kellomäki, S.; Peltola, H.; Nuutinen, T.; Korhonen, K.T.; Strandman, H. Sensitivity of Managed Boreal Forests in Finland to Climate Change, with Implications for Adaptive Management. *Philos. Trans. R. Soc. B Biol. Sci.* **2008**, *363*, 2341–2351. [CrossRef] [PubMed]
24. Subramanian, N.; Bergh, J.; Johansson, U.; Nilsson, U.; Sallnäs, O. Adaptation of Forest Management Regimes in Southern Sweden to Increased Risks Associated with Climate Change. *Forests* **2016**, *7*, 8. [CrossRef]
25. Sierota, Z.; Grodzki, W.; Szczepkowski, A. Abiotic and Biotic Disturbances Affecting Forest Health in Poland over the Past 30 Years: Impacts of Climate and Forest Management. *Forests* **2019**, *10*, 75. [CrossRef]
26. Dyderski, M.K.; Paź, S.; Frelich, L.E.; Jagodziński, A.M. How Much Does Climate Change Threaten European Forest Tree Species Distributions? *Glob. Chang. Biol.* **2018**, *24*, 1150–1163. [CrossRef] [PubMed]
27. Högberg, K.; Varis, S. Vegetative Propagation of Norway Spruce: Experiences and Present Situation in Sweden and Finland. In *Vegetative Propagation of Forest Trees*; Park, Y.S., Bonga, J.M., Moon, H.-K., Eds.; National Institute of Forest Science (Nifos): Seoul, Korea, 2016; pp. 528–550.
28. Cyr, D.R.; Attree, S.M.; El-Kassaby, Y.A.; Ellis, D.D.; Polonenko, D.R.; Sutton, B.C.S. Application of Somatic Embryogenesis to Tree Improvement in Conifers. *Prog. Biotechnol.* **2001**, *18*, 305–312. [CrossRef]
29. Lelu-Walter, M.-A.; Thompson, D.; Harvengt, L.; Sanchez, L.; Toribio, M.; Pâques, L.E. Somatic Embryogenesis in Forestry with a Focus on Europe: State-of-the-Art, Benefits, Challenges and Future Direction. *Tree Genet. Genomes* **2013**, *9*, 883–899. [CrossRef]
30. Egertsdotter, U. Plant Physiological and Genetical Aspects of the Somatic Embryogenesis Process in Conifers. *Scand. J. For. Res.* **2019**, *34*, 360–369. [CrossRef]
31. Isah, T. Induction of Somatic Embryogenesis in Woody Plants. *Acta Physiol. Plant.* **2016**, *38*, 118. [CrossRef]
32. Högberg, K.-A.; Hajek, J.; Gailis, A.; Stenvall, N.; Zarina, I.; Teivonen, S.; Aronen, T. *Practical Testing of Scots Pine Cutting Propagation—A Joint Metla-Skogforsk-Silava Project*; Finnish Forest Research Institute: Vantaa, Finland, 2011; p. 20.
33. Thompson, D. Challenges for the Large-Scale Propagation of Forest Trees by Somatic Embryogenesis–a Review. In Proceedings of the Third International Conference of the IUFRO Unit 2.09.02 on Woody Plant Production Integrating Genetic and Vegetative Propagation Technologies, Vitoria-Gasteiz, Spain, 8–12 September 2014; pp. 81–91.
34. Bonga, J. Conifer Clonal Propagation in Tree Improvement Programs. In *Vegetative Propagation of Forest Trees*; Park, Y.S., Bonga, J.M., Moon, H.-K., Eds.; National Institute of Forest Science (NIFoS): Seoul, Korea, 2016; pp. 3–31.
35. Lelu-Walter, M.A.; Klimaszewska, K.; Miguel, C.; Aronen, T.; Hargreaves, C.; Teyssier, C.; Trontin, J.F. Somatic Embryogenesis for More Effective Breeding and Deployment of Improved Varieties in *Pinus* spp.: Bottlenecks and Recent Advances. *Somat. Embryog. Fundam. AsP. Appl.* **2016**, 319–365. [CrossRef]
36. Toon, S.M. Somatic Embryogenesis of Lodgepole Pine (*Pinus contorta*) and Subalpine Larch (*Larix Lyallii*) for Conservation Purposes. Bachelor's Thesis, University of British Columbia, Vancouver, BC, Canada, 12 April 2010.
37. Park, Y.S.; Lelu-Walter, M.A.; Harvenght, L.; Trontin, J.F.; MacEacheron, I.; Klimaszewska, K.; Bonga, J.M. Initiation of Somatic Embryogenesis in *Pinus* Banksiana, *P. strobus*, *P. pinaster*, and *P. sylvestris* at Three Laboratories in Canada and France. *Plant Cell Tissue Organ Cult.* **2006**, *86*, 87–101. [CrossRef]
38. Helmersson, A.; von Arnold, S. Embryogenic Cell Lines of *Juniperus Communis*; Easy Establishment and Embryo Maturation, Limited Germination. *Plant Cell Tissue Organ Cult. PCTOC* **2009**, *96*, 211–217. [CrossRef]
39. Abrahamsson, M.; Valladares, S.; Larsson, E.; Clapham, D.; von Arnold, S. Patterning during Somatic Embryogenesis in Scots Pine in Relation to Polar Auxin Transport and Programmed Cell Death. *Plant Cell Tissue Organ Cult.* **2012**, *109*, 391–400. [CrossRef]
40. Park, Y.S.; Barrett, J.D.; Bonga, J.M. Application of Somatic Embryogenesis in High-Value Clonal Forestry: Deployment, Genetic Control, and Stability of Cryopreserved Clones. *Vitr. Cell. Dev. Biol. Plant* **1998**, *34*, 231–239. [CrossRef]
41. Hazubska-Przybył, T.; Ratajczak, E.; Obarska, A.; Pers-Kamczyc, E. Different Roles of Auxins in Somatic Embryogenesis Efficiency in Two *Picea* Species. *Int. J. Mol. Sci.* **2020**, *21*, 3394. [CrossRef]
42. Klimaszewska, K.; Cyr, D.R. Conifer Somatic Embryogenesis: I. Development. *Dendrobiology* **2002**, *48*, 31–39.
43. Lu, C.-Y.; Thorpe, T.A. Somatic Embryogenesis and Plantlet Regeneration in Cultured Immature Embryos of *Picea glauca*. *J. Plant Physiol.* **1987**, *128*, 297–302. [CrossRef]
44. Ramarosandratana, A.V.; Van Staden, J. Tissue Position, Explant Orientation and Naphthaleneacetic Acid (NAA) Affect Initiation of Somatic Embryos and Callus Proliferation in Norway Spruce (*Picea abies*). *Plant Cell Tissue Organ Cult.* **2003**, *74*, 249–255. [CrossRef]
45. Klimaszewska, K.; Overton, C.; Stewart, D.; Rutledge, R.G. Initiation of Somatic Embryos and Regeneration of Plants from Primordial Shoots of 10-Year-Old Somatic White Spruce and Expression Profiles of 11 Genes Followed during the Tissue Culture Process. *Planta* **2011**, *233*, 635–647. [CrossRef]
46. Varis, S.; Klimaszewska, K.; Aronen, T. Somatic Embryogenesis and Plant Regeneration from Primordial Shoot Explants of *Picea abies* (L.) H. Karst. Somatic Trees. *Front. Plant Sci.* **2018**, *9*, 1551. [CrossRef]
47. Ruaud, J.-N.; Bercetche, J.; Pâques, M. First Evidence of Somatic Embryogenesis from Needles of 1-Year-Old *Picea Abies* Plants. *Plant Cell ReP.* **1992**, *11*, 563–566. [CrossRef]

48. Rutledge, R.G.; Stewart, D.; Caron, S.; Overton, C.; Boyle, B.; MacKay, J.; Klimaszewska, K. 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 Biol.* **2013**, *13*, 116. [CrossRef] [PubMed]
49. Aronen, T.; Virta, S.; Varis, S. Telomere Length in Norway Spruce during Somatic Embryogenesis and Cryopreservation. *Plants* **2021**, *10*, 416. [CrossRef] [PubMed]
50. Chatelain, M.; Drobniak, S.M.; Szulkin, M. The Association between Stressors and Telomeres in Non-Human Vertebrates: A Meta-Analysis. *Ecol. Lett.* **2020**, *23*, 381–398. [CrossRef] [PubMed]
51. Keinonen–Mettälä, K.; Jalonen, P.; Eurola, P.; von Arnold, S.; von Weissenberg, K. Somatic Embryogenesis of *Pinus sylvestris*. *Scand. J. For. Res.* **1996**, *11*, 242–250. [CrossRef]
52. Lelu-Walter, M.-A.; Bernier-Cardou, M.; Klimaszewska, K. Clonal Plant Production from Self- and Cross-Pollinated Seed Families of *Pinus sylvestris* (L.) through Somatic Embryogenesis. *Plant Cell Tissue Organ Cult.* **2008**, *92*, 31–45. [CrossRef]
53. Yang, F.; Xia, X.-R.; Ke, X.; Ye, J.; Zhu, L.-H. Somatic Embryogenesis in Slash Pine (*Pinus elliottii* Engelm): Improving Initiation of Embryogenic Tissues and Maturation of Somatic Embryos. *Plant Cell Tissue Organ Cult.* **2020**, *143*, 159–171. [CrossRef]
54. Peng, C.; Gao, F.; Wang, H.; Shen, H.; Yang, L. Optimization of Maturation Process for Somatic Embryo Production and Cryopreservation of Embryogenic Tissue in *Pinus koraiensis*. *Plant Cell Tissue Organ Cult.* **2021**, *144*, 185–194. [CrossRef]
55. Lelu, M.-A.; Bastien, C.; Drugeault, A.; Gouez, M.-L.; Klimaszewska, K. Somatic Embryogenesis and Plantlet Development in *Pinus sylvestris* and *Pinus pinaster* on Medium with and without Growth Regulators. *Physiol. Plant.* **1999**, *105*, 719–728. [CrossRef]
56. Hohtola, A. Somatic Embryogenesis in Scots Pine (*Pinus sylvestris* L.). In *Somatic Embryogenesis in Woody Plants. Forestry Sciences*; Jain, S.M., Gupta, P.K., Newton, R.J., Eds.; Springer: Berlin/Heidelberg, Germany, 1995; Volume 44–46, pp. 269–285.
57. Aronen, T.; Pehkonen, T.; Ryynänen, L. Enhancement of Somatic Embryogenesis from Immature Zygotic Embryos of *Pinus sylvestris*. *Scand. J. For. Res.* **2009**, *24*, 372–383. [CrossRef]
58. Niskanen, A.-M.; Lu, J.; Seitz, S.; Keinonen, K.; Von Weissenberg, K.; Pappinen, A. Effect of Parent Genotype on Somatic Embryogenesis in Scots Pine (*Pinus sylvestris*). *Tree Physiol.* **2004**, *24*, 1259–1265. [CrossRef]
59. Trontin, J.F.; Aronen, T.; Hargreaves, C.; Montalbán, I.A.; Reeves, C.; Quoniou, S.; Lelu-Walter, M.A.; Klimaszewska, K. International Effort to Induce Somatic Embryogenesis in Adult Pine Trees. In *Vegetative Propagation of Forest Trees*; Park, Y.S., Bonga, J.M., Moon, H.-K., Eds.; National Institute of Forest Science (Nifos): Seoul, Korea, 2016.
60. Abrahamsson, M.; Valladares, S.; Merino, I.; Larsson, E.; von Arnold, S. Degeneration Pattern in Somatic Embryos of *Pinus sylvestris* L. *Vitr. Cell. Dev. Biol. Plant* **2017**, *53*, 86–96. [CrossRef] [PubMed]
61. Merino, I.; Abrahamsson, M.; Larsson, E.; von Arnold, S. Identification of Molecular Processes That Differ among Scots Pine Somatic Embryogenic Cell Lines Leading to the Development of Normal or Abnormal Cotyledonary Embryos. *Tree Genet. Genomes* **2018**, *14*, 1–17. [CrossRef]
62. Varis, S.; Tikkinen, M.; Välimäki, S.; Aronen, T. Light Spectra during Somatic Embryogenesis of Norway Spruce—Impact on Growth, Embryo Productivity, and Embling Survival. *Forests* **2021**, *12*, 301. [CrossRef]
63. Filonova, L.H.; Bozhkov, P.V.; Brukhin, V.B.; Daniel, G.; Zhivotovsky, B.; von Arnold, S. Two waves of programmed cell death occur during formation and development of somatic embryos in the gymnosperm, Norway spruce. *J. Cell Sci.* **2000**, *113*, 4399–4411. [CrossRef] [PubMed]
64. Salaj, T.; Blehová, A.; Salaj, J. Embryogenic Suspension Cultures of *Pinus* Nigra Arn.: Growth Parameters and Maturation Ability. *Acta Physiol. Plant.* **2007**, *29*, 225–231. [CrossRef]
65. Dahrendorf, J.; Clapham, D.; Egertsdotter, U. Analysis of Nitrogen Utilization Capability during the Proliferation and Maturation Phases of Norway Spruce (*Picea abies* (L.) H. Karst.) Somatic Embryogenesis. *Forests* **2018**, *9*, 288. [CrossRef]
66. Arnold, S.V.; Eriksson, T. In vitro Studies of Adventitious Shoot Formation in *Pinus contorta*. *Can. J. Bot.* **1981**, *59*, 870–874. [CrossRef]
67. Litvay, J.D.; Verma, D.C.; Johnson, M.A. Influence of a Loblolly Pine (*Pinus taeda* L.). Culture Medium and Its Components on Growth and Somatic Embryogenesis of the Wild Carrot (*Daucus carota* L.). *Plant Cell Rep.* **1985**, *4*, 325–328. [CrossRef] [PubMed]
68. Gupta, P.K.; Durzan, D.J. Shoot Multiplication from Mature Trees of Douglas-Fir (*Pseudotsuga menziesii*) and Sugar Pine (*Pinus lambertiana*). *Plant Cell ReP.* **1985**, *4*, 177–179. [CrossRef]
69. Becwar, M.R.; Nagmani, R.; Wann, S.R. Initiation of Embryogenic Cultures and Somatic Embryo Development in Loblolly Pine (*Pinus taeda*). *Can. J. For. Res.* **1990**, *20*, 810–817. [CrossRef]
70. Smith. Growth Medium. U.S. Patent 5,565,355, 15 October 1996.
71. Teasdale, R.; Buxton, P.A. Culture of *Pinus radiata* embryos with reference to artificial seed production. *NZJ For. Sci.* **1986**, *16*, 387–391.
72. Montalbán, I.A.; Setién-Olarra, A.; Hargreaves, C.L.; Moncaleán, P. Somatic Embryogenesis in *Pinus halepensis* Mill.: An Important Ecological Species from the Mediterranean Forest. *Trees* **2013**, *27*, 1339–1351. [CrossRef]
73. Salaj, T.; Klubicová, K.; Matusova, R.; Salaj, J. Somatic Embryogenesis in Selected Conifer Trees *Pinus nigra* Arn. And *Abies* Hybrids. *Front. Plant Sci.* **2019**, *10*, 13. [CrossRef]
74. García-Mendiguren, O.; Montalbán, I.A.; Goicoa, T.; Ugarte, M.D.; Moncaleán, P. Environmental Conditions at the Initial Stages of *Pinus radiata* Somatic Embryogenesis Affect the Production of Somatic Embryos. *Trees Struct. Funct.* **2016**, *30*, 949–958. [CrossRef]
75. Montalbán, I.A.; García-Mendiguren, O.; Goicoa, T.; Ugarte, M.D.; Moncaleán, P. Cold Storage of Initial Plant Material Affects Positively Somatic Embryogenesis in *Pinus radiata*. *New For.* **2015**, *46*, 309–317. [CrossRef]

76. Gao, F.; Peng, C.; Wang, H.; Tretyakova, I.N.; Nosov, A.M.; Shen, H.; Yang, L. Key Techniques for Somatic Embryogenesis and Plant Regeneration of *Pinus koraiensis*. *Forests* **2020**, *11*, 912. [CrossRef]
77. Malabadi, R.B.; Teixeira da Silva, J.A.; Nataraja, K. Salic Acid Induces Somatic Embryogenesis from Mature Trees of *Pinus roxburghii* (Chir Pine) Using TCL Technology. In *Tree Forest Science and Biotechnology*; Global Science Books Ltd: East Sussex, UK; Ikanobe: Takamatsu, Japan, 2008; pp. 34–39.
78. Kocsy, G.; Tari, I.; Vanková, R.; Zechmann, B.; Gulyás, Z.; Poór, P.; Galiba, G. Redox Control of Plant Growth and Development. *Plant Sci.* **2013**, *211*, 77–91. [CrossRef]
79. Filonova, L.H.; Bozhkov, P.V.; Von Arnold, S. Developmental Pathway of Somatic Embryogenesis in *Picea Abies* as Revealed by Time-Lapse Tracking. *J. Exp. Bot.* **2000**, *51*, 249–264. [CrossRef] [PubMed]
80. Hudec, L.; Konrádová, H.; Hašková, A.; Lipavská, H. Norway Spruce Embryogenesis: Changes in Carbohydrate Profile, Structural Development and Response to Polyethylene Glycol. *Tree Physiol.* **2016**, *36*, 548–561. [CrossRef] [PubMed]
81. Tremblay, L.; Tremblay, F.M. Carbohydrate Requirements for the Development of Black Spruce (*Picea* Mariana (Mill.) B.S.P.) and Red Spruce (*P. rubens* Sarg.) Somatic Embryos. *Plant Cell Tissue Organ Cult.* **1991**, *27*, 95–103. [CrossRef]
82. Chen, S.; Chen, S.; Chen, F.; Wu, T.; Wang, Y.; Yi, S. Somatic Embryogenesis in Mature Zygotic Embryos of *Picea likiangensis*. *Biologia* **2010**, *65*, 853–858. [CrossRef]
83. Stasolla, C.; Yeung, E.C. Recent Advances in Conifer Somatic Embryogenesis: Improving Somatic Embryo Quality. *Plant Cell Tissue Organ Cult.* **2003**, *74*, 15–35. [CrossRef]
84. Hazubska-Przybył, T.; Wawrzyniak, M. Stimulation of Somatic Embryo Growth and Development in *Picea* spp. by Polyethylene Glycol. *Dendrobiology* **2017**, *78*, 168–178. [CrossRef]
85. Stasolla, C. The Effect of Reduced Glutathione on Morphology and Gene Expression of White Spruce (*Picea glauca*) Somatic Embryos. *J. ExP. Bot.* **2004**, *55*, 695–709. [CrossRef]
86. Pond, S.E. The Effect of Temperature on Conversion of White Spruce Somatic Embryos. *Propag Ornam Plants* **2005**, *5*, 35–44.
87. Tikkinen, M.; Varis, S.; Peltola, H.; Aronen, T. Improved Germination Conditions for Norway Spruce Somatic Cotyledonary Embryos Increased Survival and Height Growth of Emblings. *Trees* **2018**, *32*, 1489–1504. [CrossRef]
88. Varis, S.; Ahola, S.; Jaakola, L.; Aronen, T. Reliable and Practical Methods for Cryopreservation of Embryogenic Cultures and Cold Storage of Somatic Embryos of Norway Spruce. *Cryobiology* **2017**, *76*, 8–17. [CrossRef]
89. Montalbán, I.A.; De Diego, N.; Moncaleán, P. Bottlenecks in *Pinus radiata* Somatic Embryogenesis: Improving Maturation and Germination. *Trees Struct. Funct.* **2010**, *24*, 1061–1071. [CrossRef]
90. Salo, H.M.; Sarjala, T.; Jokela, A.; Häggman, H.; Vuosku, J. Moderate Stress Responses and Specific Changes in Polyamine Metabolism Characterize Scots Pine Somatic Embryogenesis. *Tree Physiol.* **2016**, *36*, 392–402. [CrossRef] [PubMed]
91. Krakau, U.-K.; Liesebach, M.; Aronen, T.; Lelu-Walter, M.-A.; Schneck, V. Scots Pine (*Pinus Sylvestris* L.). In *Forest Tree Breeding in Europe: Current State-of-the-Art and Perspectives*; Pâques, L.E., Ed.; Managing Forest Ecosystems; Springer: Dordrecht, The Netherlands, 2013; pp. 267–323, ISBN 978-94-007-6146-9.
92. Carlsson, J.; Egertsdotter, U.; Ganeteg, U.; Svennerstam, H. Nitrogen Utilization during Germination of Somatic Embryos of Norway Spruce: Revealing the Importance of Supplied Glutamine for Nitrogen Metabolism. *Trees Struct. Funct.* **2019**, *33*, 383–394. [CrossRef]
93. Llebrés, M.T.; Avila, C.; Cánovas, F.M.; Klimaszewska, K. Root Growth of Somatic Plants of Hybrid *Pinus strobus* (L.) and *P. wallichiana* (A. B. Jacks.) Is Affected by the Nitrogen Composition of the Somatic Embryo Germination Medium. *Trees Struct. Funct.* **2018**, *32*, 371–381. [CrossRef]
94. Bozhkov, P.V.; Mikhlina, S.B.; Shiryaeva, G.A.; Lebedenko, L.A. Influence of Nitrogen Balance of Culture Medium on Norway Spruce [*Picea abies* (L.) Karst] Somatic Polyembryogenesis: High Frequency Establishment of Embryonal-Suspensor Mass Lines from Mature Zygotic Embryos. *J. Plant Physiol.* **1993**, *142*, 735–741. [CrossRef]
95. Barrett, J.D.; Park, Y.S.; Bonga, J.M. The Effectiveness of Various Nitrogen Sources in White Spruce [*Picea glauca* (Moench) Voss] Somatic Embryogenesis. *Plant Cell ReP.* **1997**, *16*, 411–415. [CrossRef] [PubMed]
96. Carlsson, J.; Svennerstam, H.; Moritz, T.; Egertsdotter, U.; Ganeteg, U. Nitrogen Uptake and Assimilation in Proliferating Embryogenic Cultures of Norway Spruce—Investigating the Specific Role of Glutamine. *PLoS ONE* **2017**, *12*, e0181785. [CrossRef] [PubMed]
97. Jin, F.; Hu, L.; Yuan, D.; Xu, J.; Gao, W.; He, L.; Yang, X.; Zhang, X. Comparative Transcriptome Analysis between Somatic Embryos (SEs) and Zygotic Embryos in Cotton: Evidence for Stress Response Functions in SE Development. *Plant Biotechnol. J.* **2014**, *12*, 161–173. [CrossRef]
98. Nic-Can, G.I.; Avilez-Montalvo, J.R.; Aviles-Montalvo, R.N.; Márquez-López, R.E.; Mellado-Mojica, E.; Galaz-Ávalos, R.M.; Loyola-Vargas, V.M. The Relationship between Stress and Somatic Embryogenesis. In *Somatic Embryogenesis: Fundamental Aspects and Applications*; Springer International Publishing: Cham, Switzerland, 2016; pp. 121–170.
99. Zhang, S.-G.; Han, S.-Y.; Yang, W.-H.; Wei, H.-L.; Zhang, M.; Qi, L.-W. Changes in H_2O_2 Content and Antioxidant Enzyme Gene Expression during the Somatic Embryogenesis of *Larix leptolepis*. *Plant Cell Tissue Organ Cult.* **2010**, *100*, 21–29. [CrossRef]
100. Salajova, T.; Salaj, J.; Kormutak, A. Initiation of Embryogenic Tissues and Plantlet Regeneration from Somatic Embryos of *Pinus nigra* Arn. *Plant Sci.* **1999**, *145*, 33–40. [CrossRef]
101. Kvaalen, H.; Appelgren, M. Light Quality Influences Germination, Root Growth and Hypocotyl Elongation in Somatic Embryos but Not in Seedlings of Norway Spruce. *Vitr. Cell. Dev. Biol. Plant* **1999**, *35*, 437–441. [CrossRef]

102. Latkowska, M.J.; Kvaalen, H.; Appelgren, M. Genotype Dependent Blue and Red Light Inhibition of the Proliferation of the Embryogenic Tissue of Norway Spruce. *Vitr. Cell. Dev. Biol. Plant* **2000**, *36*, 57–60. [CrossRef]
103. Merkle, S.A.; Montello, P.M.; Xia, X.; Upchurch, B.L.; Smith, D.R. Light Quality Treatments Enhance Somatic Seedling Production in Three Southern Pine Species. *Tree Physiol.* **2006**, *26*, 187–194. [CrossRef] [PubMed]
104. Montalbán, I.A.; Moncaleán, P. Rooting of *Pinus radiata* Somatic Embryos: Factors Involved in the Success of the Process. *J. For. Res.* **2019**, *1*, 65–71. [CrossRef]
105. Bonga, J.M.; Klimaszewska, K.K.; von Aderkas, P. Recalcitrance in Clonal Propagation, in Particular of Conifers. *Plant Cell Tissue Organ Cult. PCTOC* **2010**, *100*, 241–254. [CrossRef]
106. Belmonte, M.F.; Yeung, E.C. The Effects of Reduced and Oxidized Glutathione on White Spruce Somatic Embryogenesis. *Vitr. Cell. Dev. Biol. Plant* **2004**, *40*, 61–66. [CrossRef]
107. Pullman, G.S.; Zeng, X.; Copeland-Kamp, B.; Crockett, J.; Lucrezi, J.; May, S.W.; Bucalo, K. Conifer Somatic Embryogenesis: Improvements by Supplementation of Medium with Oxidation-Reduction Agents. *Tree Physiol.* **2015**, *35*, 209–224. [CrossRef] [PubMed]
108. Maruyama, T.E.; Hosoi, Y. Progress in Somatic Embryogenesis of Japanese Pines. *Front. Plant Sci.* **2019**, *10*, 31. [CrossRef]
109. Lara-Chavez, A.; Flinn, B.S.; Egertsdotter, U. Initiation of Somatic Embryogenesis from Immature Zygotic Embryos of Oocarpa Pine (*Pinus oocarpa* Schiede Ex Schlectendal). *Tree Physiol.* **2011**, *31*, 539–554. [CrossRef] [PubMed]
110. Liao, Y.K.; Amerson, H.V. Slash Pine (*Pinus elliottii* Engelm.) Somatic Embryogenesis I. Initiation of Embryogenic Cultures from Immature Zygotic Embryos. *New For.* **1995**, *10*, 145–163. [CrossRef]
111. Liao, Y.K.; Juan, I.-P. Improving the Germination of Somatic Embryos of *Picea morrisonicola* Hayata: Effects of Cold Storage and Partial Drying. *J. For. Res.* **2015**, *20*, 114–124. [CrossRef]
112. Pullman, G.S.; Olson, K.; Fischer, T.; Egertsdotter, U.; Frampton, J.; Bucalo, K. Fraser Fir Somatic Embryogenesis: High Frequency Initiation, Maintenance, Embryo Development, Germination and Cryopreservation. *New For.* **2016**, *47*, 453–480. [CrossRef]
113. Marin, J.A.; Gella, R.; Herrero, M. Stomatal Structure and Functioning as a Response to Environmental Changes in Acclimatized Micropropagated *Prunus cerasus* L. *Ann. Bot.* **1988**, *62*, 663–670.
114. Pospóšilová, J.; Tichá, I.; Kadleček, P.; Haisel, D.; Plzáková, Š. Acclimatization of Micropropagated Plants to Ex Vitro Conditions. *Biol. Plant.* **1999**, *42*, 481–497. [CrossRef]
115. Chandra, S.; Bandopadhyay, R.; Kumar, V.; Chandra, R. Acclimatization of Tissue Cultured Plantlets: From Laboratory to Land. *Biotechnol. Lett.* **2010**, *32*, 1199–1205. [CrossRef] [PubMed]
116. Ragonezi, C.; Caldeira, A.T.; Martins, M.R.; Dias, L.S.; Santos-Silva, C.; Ganhão, E.; Miralto, O.; Pereira, I.; Louro, R.; Klimaszewska, K.; et al. *Pisolithus arhizus* (Scop.) Rauschert Improves Growth of Adventitious Roots and Acclimatization of in vitro Regenerated Plantlets of *Pinus pinea* L. *Propag. Ornam. Plants* **2012**, *12*, 139–147.
117. Jayawickrama, K.J.S.; Carson, M.J. A Breeding Strategy for the New Zealand *Radiata* Pine Breeding Cooperative. *Silvae Genet. Ger.* **2000**, *49*, 82–89.
118. Dungey, H.S.; Brawner, J.T.; Burger, F.; Carson, M.; Henson, M.; Jefferson, P.; Matheson, A.C. A New Breeding Strategy for *Pinus Radiata* in New Zealand and New South Wales. *Silvae Genet.* **2009**, *58*, 28–38. [CrossRef]
119. Weng, Y.H.; Park, Y.S.; Krasowski, M.J.; Mullin, T.J. Allocation of Varietal Testing Efforst for Implementing Conifer Multi-Varietal Forestry Using White Spruce as a Model Species. *Ann. For. Sci.* **2011**, *68*, 129–138. [CrossRef]
120. Park, Y.-S. Implementation of Conifer Somatic Embryogenesis in Clonal Forestry: Technical Requirements and Deployment Considerations. *Ann. For. Sci.* **2002**, *59*, 651–656. [CrossRef]
121. Plomion, C.; Bastien, C.; Bogeat-Triboulot, M.-B.; Bouffier, L.; Déjardin, A.; Duplessis, S.; Fady, B.; Heuertz, M.; Le Gac, A.-L.; Le Provost, G.; et al. Forest Tree Genomics: 10 Achievements from the Past 10 Years and Future Prospects. *Ann. For. Sci.* **2016**, *73*, 77–103. [CrossRef]
122. Baltunis, B.S.; Wu, H.X.; Dungey, H.S.; Mullin, T.J.; Brawner, J.T. Comparisons of Genetic Parameters and Clonal Value Predictions from Clonal Trials and Seedling Base Population Trials of *Radiata* Pine. *Tree Genet. Genomes* **2009**, *5*, 269–278. [CrossRef]
123. Danell, Ö. Breeding Programmes in Sweden. General Approach. In Proceedings of the Progeny Testing and Breeding Strategies. Proceedings from a Meeting with the Nordic Group for Tree Breeding, Edinburg, UK, 6–10 October 1993.
124. Rosvall, O. Review of the Swedish Tree Breeding Program. *Skogforsk Upps. Swed.* **2011**, 4–37.
125. Jansson, G.; Hansen, J.K.; Haapanen, M.; Kvaalen, H.; Steffenrem, A. The Genetic and Economic Gains from Forest Tree Breeding Programmes in Scandinavia and Finland. *Scand. J. For. Res.* **2017**, *32*, 273–286. [CrossRef]
126. Find, J.I.; Charity, J.A.; Grace, L.J.; Kristensen, M.M.M.H.; Krogstrup, P.; Walter, C. Stable genetic transformation of embryogenic cultures of *Abies nordmanniana* (Nordmann fir) and regeneration of transgenic plants. *In vitro Cell. Dev. Biol. Plant* **2005**, *41*, 725–730. [CrossRef]
127. Giri, C.C.; Shyamkumar, B.; Anjaneyulu, C. Progress in tissue culture, genetic transformation and applications of biotechnology to trees: An overview. *Trees Struct. Funct.* **2004**, *18*, 115–135. [CrossRef]
128. Sun, Z.-L.; Li, X.; Zhou, W.; Yan, J.-D.; Gao, Y.-R.; Li, X.-W.; Sun, J.-C.; Fang, K.-F.; Zhang, Q.; Xing, Y.; et al. *Agrobacterium*-mediated genetic transformation of Chinese chestnut (*Castanea mollissima* Blume). *Plant Cell Tissue Organ Cult.* **2020**, *140*, 95–103. [CrossRef]
129. Tereso, S.; Miguel, C.; Zoglauer, K.; Valle-Piquera, C.; Oliveira, M.M. Stable *Agrobacterium*-mediated transformation of embryogenic tissues from *Pinus pinaster* Portuguese genotypes. *Plant Growth Regul.* **2006**, *50*, 57–68. [CrossRef]

130. Teixeira da Silva, J.A.; Kher, M.M.; Soner, D.; Page, T.; Zhang, X.; Nataraj, M.; Ma, G. Sandalwood: Basic biology, tissue culture, and genetic transformation. *Planta* **2016**, *243*, 847–887. [CrossRef] [PubMed]
131. McGuigan, L.; Fernandes, P.; Oakes, A.; Stewart, K.; Powell, W. Transformation of American chestnut (*Castanea dentata* (marsh.) borkh) using Rita® temporary immersion bioreactors and in vitro containers. *Forests* **2020**, *11*, 1196. [CrossRef]
132. Klimaszewska, K.; Lachance, D.; Pelletier, G.; Lelu, M.-A.; Séguin, A. Regeneration of transgenic *Picea glauca*, *P. mariana*, and *P. abies* after cocultivation of embryogenic tissue with *Agrobacterium tumefaciens*. *In Vitro Cell.-Dev. Biol.-Plant* **2001**, *37*, 748–755. [CrossRef]
133. Wenck, R.A.; Quinn, M.; Whetten, R.W.; Pullman, G.; Sederoff, R. High-efficiency *Agrobacterium*-mediated transformation of Norway spruce (*Picea abies*) and loblolly pine (*Pinus taeda*). *Plant Mol. Biol.* **1999**, *39*, 407–416. [CrossRef]
134. Walter, C.; Grace, L.J.; Donaldson, S.S.; Moody, J.; Gemmell, J.E.; van der Maas, S.; Kvaalen, H.; Lönneborg, A. An efficient Biolistic® transformation protocol for *Picea abies* embryogenic tissue and regeneration of transgenic plants. *Can. J. For. Res.* **1999**, *29*, 1539–1546. [CrossRef]
135. Pavingerová, D.; Bříza, J.; Niedermeierová, H.; Vlasák, J. Stable *Agrobacterium*-mediated transformation of Norway spruce embryogenic tissues using somatic embryo explants. *J. For. Sci.* **2011**, *57*, 277–280. [CrossRef]
136. Bříza, J.; Pavingerová, D.; Vlasák, J.; Niedermeierová, H. Norway spruce (*Picea abies*) genetic transformation with modified Cry3A gene of *Bacillus thuringiensis*. *Acta Biochim. Pol.* **2013**, *60*, 395–400. [CrossRef]
137. Wadenbäck, J.; Von Arnold, S.; Egertsdotter, U.; Walter, M.H.; Grima-Pettenati, J.; Goffner, D.; Gellerstedt, G.; Gullion, T.; Clapham, D. Lignin biosynthesis in transgenic Norway spruce plants harboring an antisense construct for cinnamoyl CoA reductase (CCR). *Transgenic Res.* **2008**, *17*, 379–392. [CrossRef]
138. Häggman, H.M.; Aronen, T.S.; Nikkanen, T.O. Gene transfer by particle bombardment to Norway spruce and Scots pine pollen. *Can. J. For. Res.* **1997**, *27*, 928–935. [CrossRef]
139. Aronen, T.S.; Nikkanen, T.O.; Häggman, H.M. The Production of Transgenic Scots Pine (*Pinus sylvestris* L.) via the Application of Transformed Pollen in Controlled Crossings. *Transgenic Res.* **2003**, *12*, 375–378. [CrossRef] [PubMed]
140. Grace, L.J.; Charity, J.A.; Gresham, B.; Kay, N.; Walter, C. Insect-resistant transgenic *Pinus radiata*. *Plant Cell Rep.* **2005**, *24*, 103–111. [CrossRef]
141. Alvarez, J.M.; Ordás, R.J. Stable *Agrobacterium* -mediated transformation of maritime pine based on kanamycin selection. *Sci. World J.* **2013**, *2013*, 681792. [CrossRef] [PubMed]
142. Mamun, N.H.A.; Egertsdotter, U.; Aidun, C.K. Bioreactor Technology for Clonal Propagation of Plants and Metabolite Production. *Front. Biol.* **2015**, *10*, 177–193. [CrossRef]
143. Egertsdotter, U.; Ahmad, I.; Clapham, D. Automation and Scale Up of Somatic Embryogenesis for Commercial Plant Production, With Emphasis on Conifers. *Front. Plant Sci.* **2019**, *10*, 109. [CrossRef] [PubMed]
144. Aidun, C.K.; Egertsdotter, U. SE Fluidics System. In *Step Wise Protocols for Somatic Embryogenesis of Important Woody Plants.*; Jain, S.M., Gupta, P., Eds.; Springer International Publishing: Berlin/Heidelberg, Germany, 2018; Volume I, pp. 211–227.
145. Businge, E.; Trifonova, A.; Schneider, C.; Rödel, P.; Egertsdotter, U. Evaluation of a New Temporary Immersion Bioreactor System for Micropropagation of Cultivars of Eucalyptus, Birch and Fir. *Forests* **2017**, *8*, 196. [CrossRef]
146. Salonen, F.; Varis, S.; Aronen, T.S. From Petri Dishes to Bioreactors—First Experiences on Optimization of Norway Spruce SE-Process for Bioreactors. In Proceedings of the 4th International Conference of the IUFRO Unit 2.09.02. on Development and Application of Vegetative Propagation Technologies in Plantation Forestry to Cope with a Changing Climate and Environment, La Plata, Argentina, 19–23 September 2016; pp. 293–297.
147. Välimäki, S.; Paavilainen, L.; Tikkinen, M.; Salonen, F.; Varis, S.; Aronen, T. Production of Norway Spruce Embryos in a Temporary Immersion System (TIS). *Vitr. Cell. Dev. Biol. Plant* **2020**, *56*, 430–439. [CrossRef]
148. Yoeup, P.K.; Chakrabarty, D. Micropropagation of Woody Plants Using Bioreactor. In *Micropropagation of Woody Trees and Fruits*; Jain, S.M., Ishii, K., Eds.; Forestry Sciences; Springer Netherlands: Dordrecht, The Netherlands, 2003; pp. 735–755, ISBN 978-94-010-0125-0.
149. Mamun, N.H.A.; Aidun, C.K.; Egertsdotter, U. Improved and Synchronized Maturation of Norway Spruce (*Picea abies* (L.) H. Karst.) Somatic Embryos in Temporary Immersion Bioreactors. *Vitr. Cell. Dev. Biol. Plant* **2018**, *54*, 612–620. [CrossRef]
150. Le, K.-C.; Weerasekara, A.B.; Ranade, S.S.; Egertsdotter, E.-M.U. Evaluation of Parameters to Characterize Germination-Competent Mature Somatic Embryos of Norway Spruce (*Picea abies*). *Biosyst. Eng.* **2021**, *203*, 55–59. [CrossRef]