DENDROBIOLOGY

2016, vol. 76, 105–116

http://dx.doi.org/10.12657/denbio.076.010

Teresa Hazubska-Przybył, Krystyna Bojarczuk

Tree somatic embryogenesis in science and forestry

Received: 9 February 2016; Accepted: 18 July 2016

Abstract: Somatic embryogenesis is the latest, and potentially the most efficient, method for the vegetative micropropagation of plants. Over the past three decades, numerous laboratory studies have investigated somatic embryogenesis of forest trees, yielding positive results for a number of economically important tree species. The first test trials were run and plantations were planted with interior spruce in the 90s by CellFor Inc. (Canada). However, at the beginning of the XXI century, the program to produce spruce and Douglas fir somatic seedlings was stopped for economic reasons. Thus, currently no operational program is ongoing except on a small scale in New Brunswick. In order to integrate somatic embryogenesis technology into operational reforestation programs, the production costs of forest tree somatic seedlings needs to be reduced, and the awareness of foresters and forest landowners that the material obtained through somatic embryogenesis is valuable needs to be increased. This awareness would enable implementation of this technology on a large scale for production and forest management throughout Europe including Poland. In this review, the importance of somatic embryogenesis in scientific research and in global and European forestry is presented. Our main aims are to provide basic information on the challenges in researching somatic embryogenesis of forest trees and to raise interest in this tree propagation technique in both scientists and foresters.

Key words: *in vitro* culture, somatic seedlings, forestry management

Address: T. Hazubska-Przybył, K. Bojarczuk, Institute of Dendrology, Parkowa 5, 62-035 Kórnik, Poland, e-mail: hazubska@o2.pl

Introduction

Vegetative propagation of forest trees as a method for the production of new trees via regeneration from the tissues of the donor tree has increasingly been used in breeding programmes (Szczygieł & Hazubska-Przybył, 2010). This type of propagation retains desirable breeding traits in the progeny to a higher extent than sexual propagation. The progeny obtained vegetatively has the same genotype as the donor tree, and thus, is its clone. Vegetative progeny is obtained using various methods of tree propagation, such as rooting of shoot or root cuttings, layering or grafting. The effectiveness of these methods is often dependent on the age and physiological state of the donor plant. Micropropagation (i.e., propagation in vitro) by axillary shoot multiplication, organogenesis or somatic embryogenesis is in many cases a more useful method of vegetative propagation of trees as compared to the traditional ones. As a result of organogenesis, microshoots prime for rooting are obtained after the treatment of plant fragments with

plant growth regulators added to the medium. In somatic embryogenesis, vegetative embryos (somatic embryos) are produced from a single cells of explants as a bipolar structures with a root and shoot axis, capable of germination and development into somatic seedlings (emblings).

Currently, forest areas are quickly shrinking while the demand for wood continues to increase. To obtain an additional source of raw timber outside the forest ecosystem's, large-scale plantations of various forest tree species were created (Sutton, 1999; Lane, 2004). On these plantations, Douglas fir (*Pseudotsuga menziesii*), loblolly pine (*Pinus taeda*), radiata pine (*Pinus radiata*), poplars (*Populus* spp.), and gum trees (*Eucalyptus* spp.) predominate, which are characterized by rapid growth and high resistance to diseases and pests and are sources of high-quality wood (Lane, 2004). Although forestry plantations account for only a small percentage of the forested areas worldwide, one third of industrial round wood production comes from plantations; this trend is growing (Bonga, 2016). To achieve high productivity of plantation crops, high quality of the planting stock must be guaranteed. The genetically improved planting stock is obtained from high quality seeds from seeds orchards, both by open and controlled pollination (Bonga, 2016). However, seed orchards have some limitations (e.g., not all trees produce seeds, some seeds yield poor progeny because of self-pollination, etc.) (Lelu-Walter et al., 2013). Therefore, in many cases, vegetative propagation is a better solution. For instance, good results were obtained after application of the cuttings of the rootings in species such as *Eucalyptus grandis*, *Pinus radiata* or *Picea abies* (Titon et al., 2006; Szczygieł & Hazubska-Przybył, 2010; Högberg & Varis, 2016; Bonga, 2016). However, this technique is often limited to certain clones because of poor rooting of cuttings (Titon et al., 2006). Therefore, currently, many forest companies are interested in applying micropropagation techniques for the production of high quality planting stock (Adams et al., 2016; Goh & Monteuuis, 2016; Pinto et al., 2016). In vitro microcuttings were successfully applied, e.g., in mass propagation of *Tectona grandis* (Goh & Monteuuis, 2016) and *Eucalyptus ssp.* (Pinto et al., 2016). Somatic seedlings have been used in commercial propagation of *Picea glauca* and *Picea glauca* (Moench) Voss × *P. engelmannii* Parry ex. Engelm. Silvagen Inc. produced over 300 000 seedlings in 1997–98 (Grossnickle & Sutton, 1999). This program increased to produce one million seedlings as Silvagen Inc. transitioned into being part of CellFor in 2001. No production level information has been published recently.

Within the last thirty years of research on somatic embryogenesis in many forest tree species worldwide, including Europe, much attention has been paid to its efficiency in the multiplication of this group of plants (Bajaj, 1995; Klimaszewska et al., 2007; Hazubska-Przybył & Bojarczuk, 2008; Lelu-Walter et al., 2010; Nawrot-Chorabik, 2012). Although many protocols have been developed, the practical application of this micropropagation method is still limited to a small amount of forest tree species. Currently, numerous field tests of somatic seedlings of some forest tree species in different countries have been performed in order to introduce somatic embryogenesis on a wider scale in forestry and in commerce (Högberg & Varies, 2016; Find, 2016; Lelu-Walter et al., 2016). According to Sutton et al. (2004), nursery and field trials were ongoing with somatic seedlings of some spruce species (e.g., *Picea mariana, P. abies, P. glauca, P. glauca x engelmanii* complex), pine species (*Pinus taeda*, *P. radiata*) or *Pseudotsuga menziesii*. Since 2008, comprehensive research on the optimization of somatic seedling production of *Picea* spp. mainly, by J.D. Irving, Limited (JDI), has been undertaken (Adams et al., 2016). The current use of SE seedlings in forest programs is being implemented primarily by Arborgen under the term multi-varietal forestry (http://www.arborgen.com/pine-varietal-technology/); Arborgen purchased this micropropagation system from CellFor Inc. when CellFor Inc. closed in 2012. Previously, both companies produced conifer seedlings for the commercial market in North America (Grossnickle & Pait, 2008).

Somatic embryogenesis could be applied to the propagation of selected high-value genotypes of various trees species. Further development of research on somatic embryogenesis would allow the application of this system in forestry on a global scale. Implementation of somatic embryogenesis for the production of forest trees in the framework of clonal forestry in the future will enable a greater flexibility and higher efficiency and production rate of genetically improved material, as compared to the rooting of cuttings, thereby increasing the probability of covering the ever-growing demand for raw timber (Lane, 2004; Lelu-Walter et al., 2010; 2013).

In this review, the basic information on forest tree somatic embryogenesis is presented in the light of scientific research and its practical applications in forestry. Our objective is to explain the challenges related to research on somatic embryogenesis of forest trees both on the global scale and in European countries and to arouse interest in this reproduction technique in both the scientific and forestry communities.

Somatic embryogenesis as a research tool

Micropropagation by somatic embryogenesis

Somatic embryogenesis is an excellent tool in tree biology research, enabling the study of tree development, physiology, disease resistance, genomics, metabolomics, proteomics, epigenetics, and other areas of research (Park & Bonga, 2010). Currently, a significant part of tree somatic embryogenesis research is focused on tree development, starting from the induction of somatic embryos and finishing with the acclimation of the developed somatic seedlings to natural conditions. In trees, somatic embryogenesis is a highly complex process (Roberts et al., 1991; Bozhkov et al., 2002; Hazubska-Przybył & Bojarczuk, 2008) and is often difficult to trigger (Hazubska-Przybył et al., 2015). Somatic embryogenesis is divided into several steps: culture initiation, proliferation, maturation and in vitro germination. It is induced from various explant types, such as immature or mature embryos obtained from seeds, buds, needles, leaves (Toribio et al., 2004; Nawrot-Chorabik, 2012) or shoot apex explants (San-José et al., 2010). Some explants regenerate a specific tissue type (embryogenic tissue) in the presence of plant growth regulators (usually cytokinins and/or auxins). Embryogenic tissues contain early-stage somatic embryo structures, which are mostly analogous to particular stages of zygotic embryos present in normal seeds. These tissues may be maintained for a long time under in vitro conditions or in liquid nitrogen. Under specific physicochemical conditions (ABA, BA, light, increased osmoticum), parts of the tissues are capable of producing somatic embryos (Klimaszewska et al., 2000; Bozhkov et al., 2002; Hazubska-Przybył & Bojarczuk, 2008). The most productive embryogenic tissue lines (clones) are a source of mature somatic embryos (Fig. 1). Mature embryos (at the cotyledonary stage), after drying in high relative humidity (Roberts et al., 1991) or pre-germination cold treatment (Liao & Juan, 2015) and finally after transfer onto the germination medium, may convert into somatic seedlings (Fig. 2). Somatic seedlings are subjected to acclimation to ex vitro conditions, and finally somatic plants capable of growth and development in nurseries and plantations are obtained (Grossnickle & Folk, 2007; South, 2009). However, the last step is very difficult because the transition from a petri plate into a nursery environment requires a sophisticated transition handling process and environmental control. For example, to obtain good quality somatic seedlings, the somatic embryo in vitro germination must be done within a computerized incubation room with precisely controlled temperature, humidity and light. Next, the germinants must be hand-transported into miniplags and finally delivered to the forestry nursery production within a specific time period. This period of time is 2–3 months of the calendar year when seedlings can be transplanted into the soil (Sutton et al., 2004).

Currently, the somatic embryogenesis technique is highly effective for a small number of tree species including several larches, spruces, and pines (Bonga, 2016). Until now, mature and immature zygotic embryos as for example for *Q. robur* (Chmielarz, 1999) were the most useful explants for the induction of somatic embryogenesis in many tree species. However, during the last decade, important advances have been made in this area, and other explant types (e.g., leaves, apices, primordial shoots) derived from older trees were used to induce this process. Toribio et al. (2004) achieved somatic embryo induction from leaves derived from mature hundred-year-old trees of *Quercus robur*. Although the frequency was low (0.3–3.6 depending on genotype and collection date),

Fig. 1 Somatic embryos obtained from embryogenic tissue, originating from a mature zygotic embryo of *Picea abies*

Fig. 2 Somatic seedlings of *Picea omorika* under in vitro conditions

a large number of somatic embryos were obtained by secondary embryogenesis. In *Quercus suber,* somatic embryogenesis was obtained both from leaves derived from 2- to 4-month-old seedlings and from hundred-year-old trees (Toribio et al., 2005). Some leaves began to regenerate early-stage somatic embryos after approximately 60 days in culture. Corredoira et al. (2012) induced somatic embryogenesis from shoot apices and leaf explants of shoot cultures derived from 6- to 7-year-old *Quercus alba* trees. Somatic embryogenesis induction was obtained for two out of the three tested genotypes, and its frequencies ranged up to 3.4% and 50.7% for individual genotypes. Recently, Klimaszewska et al. (2011) reported somatic embryogenesis from primordial shoot explants for *Picea glauca* excised from the shoot buds of 10-year-old somatic embryo-derived trees. The somatic embryogenesis induction frequency ranged from 7 to 82% for five tested clones using this type of explant.

One of the current challenges in research on the somatic embryogenesis of forest trees is to improve the quality of somatic embryos and to reduce the costs of somatic plant production to the level that allows the implementation of this technique to forestry production (Lelu-Walter et al., 2013; Thompson, 2014). Thus, the research on the optimization of the efficiency of forest tree somatic embryogenesis is still carried out (Krajňáková et al., 2013; Pérez et al., 2013; Pullman & Bucalo, 2014). For example, some researches replace the costly solid media, which is routinely used in the production of somatic embryos, with liquid media (suspension cultures) or temporary immersion systems (TIS) (Mallón et al., 2012; Lelu-Walter et al., 2013; Pérez et al., 2013). Application of both the suspension cultures and TIS may increase the number of embryos that are produced and considerably improve their quality (Lelu-Walter et al., 2013; Ballester et al., 2016). According to Dai et al. (2004), by using a system based on liquid media, it is possible to produce 24 600 somatic embryos per 0.5 g of embryogenic tissue of tulip tree hybrids (*Liriodendron tulipifera* × *L. chinense*) that are capable of germination. However, in gymnosperms, the development and maturation of somatic embryos in liquid cultures is much more problematic despite the high productivity level of immature embryos (Gupta & Timmis, 2005).

Application of TIS improved the productivity of embryogenic cultures in some tree species. For example, in the case of the English oak (*Quercus robur*), it was estimated that after using the TIS system the production of somatic embryos was 85 000/m^2, whereas for semisolid cultures, it was approximately 12 000/m^2 (Mallón et al., 2012). It was found that TIS had a significant effect on somatic embryo synchronization and on the number of obtained well-developed cotyledonary embryos (Mallón et al., 2012). This is very important because the quality of embryos dictates their conversion rate in the nursery. Recently, Pérez et al. (2013) improved the growth intensity and embryogenic tissue productivity of cork oak (*Q. suber*) using one of the TIS - RITA® (Recipient for Automated Temporary Immersion). It was demonstrated that the fresh weight increase of embryogenic tissues was much higher in temporary immersion cultures than in semisolid ones. The number of somatic embryos that could be obtained was also much higher after cultivation in a RITA® system.

However, the authors note the need for further studies to determine the influence of the immersion frequency on the quality and further growth of somatic embryos (maturation, germination). Currently, an important issue to consider is the impact of the TIS on the final conversion rate in the nursery. Good results obtained for TIS has already allowed the use of this micropropagation technique for commercial production of somatic embryos of some woody crops such as *Coffea canephora* (Ducos et al., 2011).

Cryopreservation of embryogenic cultures

A great advantage of somatic embryogenesis is that this method does not require the use of nurseries from which the cuttings for rooting are collected. Instead it allows the long-term storage of embryogenic tissue or somatic embryos of the selected lines of specific genotypes in liquid nitrogen (–196ºC; cryopreservation), which has been confirmed by numerous studies (Park et al., 1998; Cyr, 1999, Hägmann et al., 2000) for various tree species, for example: *Quercus robur* (Chmielarz et al., 2005), *Pinus nigra* (Salaj et al., 2007) or *Picea abies* (Hazubska-Przybył et al., 2013).

Good results were obtained, for example, for somatic embryos of *Quercus robur* (Chmielarz et al., 2005), *Picea sitchensis* (Gale et al., 2008), and *Alnus glutinosa* (San-José et al., 2015) and also for embryogenic tissues of *Picea omorika* and *Persea americana* (Hazubska-Przybył et al., 2010; Guzman-Garcia et al., 2013), after application of new cryopreservation techniques, based on vitrification. Using a pregrowth dehydration method, almost a 100% recovery rate was obtained for *Picea omorika* embryogenic tissue (Hazubska-Przybył et al., 2010). For *Persea americana,* recovery rates from 77.78 to 100% were obtained (Guzman-Garcia et al., 2013) when applying the droplet-vitrification method. These results suggests that new methods may equal or in many cases exceed the results obtained for the traditional controlled-rate cooling method. Moreover, Kong and Aderkas (2011) recently proposed a revolutionary

method of cryopreservation without using cryoprotectants. In this method, the authors induced cryotolerance in the somatic embryos of interior spruce and Douglas fir when embryos were matured or pretreated at 5˚C for 4–8 weeks. In this way, the embryos were prepared to successfully survive a rapid cooling process and freezing in liquid nitrogen.

The possibility of the storage of plant material obtained via somatic embryogenesis in liquid nitrogen can prevent the loss of embryogenic potential by individual lines and lower the risk of genetic changes (Lopes et al., 2006; Park & Bonga, 2010; Hazubska-Przybył et al., 2013). However, some reports revealed that cryopreservation may induce genetic changes in this type of cultures (DeVerno et al., 1999; Harding, 2004; Krajňáková et al., 2011). For example, De Verno et al. (1999) found some genetic changes in embryogenic cultures of *Picea glauca* after 2 and 12 months after recovery from liquid nitrogen. These changes were not observed in plantlets regenerated from recovered cultures. The authors hypothesized that genetic instability might result from repeated subculturing rather than from cryopreservation procedures. Similarly, Krajňáková et al. (2011) demonstrated some changes in the RAPD profiles in embryogenic cultures of *Abies cephalonica* that had been cryostored for 6 years. However, the authors suggest that these genetic aberrations might be the consequence of long-term cryopreservation or somaclonal variation during the proliferation stage. The monitoring of genetic stability in plants obtained both directly after somatic embryogenesis procedures and from cryostored embryogenic cultures remains very important, especially when dealing with forest trees. The appearance of new research methods that can estimate changes not only at genetic but also at epigenetic levels will soon allow better understanding of this problem (Michalak et al., 2014).

Currently, most embryogenic cultures are established primarily from young explants as immature or mature zygotic embryos; cryopreservation enables the ability to retain the juvenility and regenerability of these cultures. This technique plays an important role in the progeny testing of individual genotypes obtained via somatic embryogenesis in the field to confirm their superiority (Adams et al., 2016). Cryostorage also enables the characteristics of the obtained embryogenic lines (e.g., their embryogenic potential). Moreover, it may also be applied for long-term gene conservation of valuable cultures established from selected elite trees in breeding programs (Häggman et al., 2000).

Use of embryogenic cultures for genetic transformation

Somatic embryogenesis is more frequently applied as an excellent tool for the production of genetically modified plants (Wadenbäck, 2008; Vidal et al., 2010), mainly due to the competence to express introduced DNA at a high level and to differentiate plants from single cells (Ellis, 1995). In forestry tree species, positive results of genetic transformation for both embryogenic tissues (Salaj et al., 2005; Tereso et al., 2006; Shekhawat et al., 2008) and somatic embryos (Polin et al., 2006; Corredoira et al., 2007; Vidal et al., 2010) were obtained. The genetic transformation using embryogenic cultures was successful for such species as *Picea glauca, P. mariana, P. abies* (Klimaszewska et al., 2001), *Pinus radiata* (Walter et al., 1994), *P. patula* (Nigro et al., 2004), *P. nigra* (Salaj et al., 2005), *P. pinaster* (Tereso et al., 2006), *Pinus wallichiana* (Malabadi & Nataraja, 2007), *Abies nordmanniana* (Find et al., 2005), *Santalum album* (Shekhawat et al., 2008), *Castanea sativa* (Corredoira et al., 2007), *Quercus robur* (Vidal et al., 2010) etc. The success of a specific method provides a stable transformation of a particular gene and the ability to regenerate transgenic plants. In trees the biolistic method resulted in stable transformation for *Pinus radiata* embryogenic tissues (Walter et al., 1994). The authors also achieved the regeneration of transgenic plants, in contrast to Salaj et al. (2005), who did not succeed in regenerating of somatic embryos, despite the positive results of transformation for embryogenic *Pinus nigra* by this method.

Some reports showed that the efficiency of genetic transformation is related to genotype (Corredoira et al., 2007; Lin & Zhang, 2005). Polin et al. (2006) reported that five of six tested lines were transformed by *Agrobacterium tumefaciens,* but only four of them were capable of proliferation. Corredoira et al. (2007) demonstrated that both the genotype and initial explant type (if somatic embryos were used) played a crucial role in the successful transformation of *Castanea sativa*. The highest transformation rates were obtained for embryos at the globular stage compared to the cotyledonary stage.

Although positive results for the transformation of many forest tree species have been obtained, the implementation of this technology to an operational program is still in conflict with public opinion, which is opposed to cultivation of the transgenic trees. Consequently, all field trials require special permission from the government in all countries. Experimentation on transgenic trees is carried out under strict control for research purposes only (Malabadi & Nataraja, 2007). Today, despite a large number of limited field trials that have been globally established (Ahuja, 2009), almost no commercial plantations of

transgenic forest trees, with the exception of China where insect-resistant transgenic poplars are cultivated commercially, exist (Ewald et al., 2006).

Somatic embryogenesis in global forestry

As a result of the progress in research on tree somatic embryogenesis and the development of the technology in some countries (Canada, the USA, Australia, and New Zealand), clonal plantations of selected coniferous trees, e.g., of the genera *Pinus* and *Picea*, have begun to be established (Lane, 2004). More than 20 years ago, in the province of New Brunswick in Canada were created clonal plantations of *Picea glauca*, *P. mariana*, *Pinus strobus*, and *P. banksiana* (Park, 2002).

In the last decade, some organizations have been working on commercialization programs for *Picea* ssp., *Pinus taeda*, *P. radiata* and *Pseudotsuga menziesii*, for example. In North America, CellFor Inc. delivered varieties of *Pinus taeda* seedlings to the market (Grossnickle & Pait, 2008) before it was acquired by Arborgen. Currently, a forest products company, JDI in northeastern North America, is also interested in the vegetative propagation of some conifers species through somatic embryogenesis (Adams et al., 2016).

In New Zealand and Ireland, somatic seedlings are used as juvenile stock plants for large – scale production of cuttings for rooting by some organizations. This method has been very effective for *Pinus radiata* and *Picea sitchensis,* for example. It enables decreasing the production costs of a large number of cuttings that can be harvested several times from the stock plants during their life (Lelu-Walter et al., 2013).

Currently, the production costs of the tree seedlings obtained via somatic embryogenesis still remain high; this is the biggest impediment to integrating this technology into forest programs. The price for the final product is higher compared with the material obtained using classical vegetative propagation methods (South, 2009), mainly because of the critical lab to nursery transition stage (i.e., converting a somatic embryo germinant into a seedling). However, until this issue is solved, somatic technology will be too costly for a seedling production system for standard forest regeneration programs. According to South (2009), the unit price of somatic seedlings in North America, depending on the order size and type of planting material, is approximately 0.28 EUR when ordering 30 000 bare-root seedlings. However, for 2 000 000 seedlings, the cost would decrease to approximately 0.195 EUR. Thus, few customers are interested in purchasing somatic seedlings. High costs of somatic seedling production primarily come from the need to individually handle embryos/seedlings during maturation, desiccation, and germination/conversion of the somatic embryogenesis process. These costs may be decreased by using a computer to select quality somatic seedlings and using robotic handling for the seedlings (Lelu-Walter et al., 2013). Lelu-Walter et al. (2006) showed that costs for *Pinus pinaster* may be reduced by eliminating the tissue and embryo subculture during the initiation and maturation stages by cryopreservation without using a programmable freezer and by eliminating the need for acclimatization and further growth of seedlings in greenhouses. Moreover, the reduction of costs may also be achieved by applying the "SE Fluidics System" (Aiduin & Egertsdotter, 2012) or "artificial seed" (Lulsdorf et al., 1993). In Europe, the production cost of somatic spruce and pine seedlings is between eight and ten times the price of traditionally produced seedlings (Lelu-Walter et al., 2013). In the US in 2008, the cost for *Pinus taeda* somatic seedlings was between five and six times higher compared with traditionally produced seedlings. However, the scientific community is convinced that the production costs of somatic seedlings can be reduced by improving their quality, automation of production processes, and increasing the number of valuable genotypes available on the market due to gene banks (Park, 2002; Thompson, 2014).

To increase interest in products of somatic embryogenesis, it is important to convince forest owners and foresters that the material produced using this technique is valuable and worthy of attention. Furthermore, the somatic seedlings may be a very good planting materials that meet the defined standards, which has been corroborated by research evidence (Grossnickle, 2011). For example, over 20 years ago, the Forest Biotechnology Centre in Vancouver implemented a programme of somatic embryogenesis technology into the vegetative propagation of trees on a large scale (Grossnickle, 2011). Within this programme, the quality of somatic seedlings of white spruce and the Engelmann spruce hybrid (*Picea glauca* (Moench) Voss × *P. engelmannii* Parry ex. Engelm.) were evaluated. It was found that both the zygotic and somatic seedlings exhibited comparable growth potential when they were planted in restrictive cold and drought conditions (Grossnickle & Folk, 2005; 2007). Additional field trials showed that somatic seedlings of this spruce hybrid demonstrated similar physiological efficiency in relation to the environmental conditions occurring in the afforested areas (both in the summer and winter), such that they would be a suitable materials for the afforestation of boreal areas (Grossnickle & Major, 1994).

Somatic embryogenesis in European forestry?

Many multidimensional studies using somatic embryogenesis technique were performed in most European countries (Lelu-Walter et al., 2013), especially for species of the genera *Picea* (Bozhkov et al., 2002; Wadenbäck et al., 2008; Hazubska-Przybył et al., 2013), *Pinus* (Salaj et al., 2007), *Abies* (Krajňáková et al., 2011; Find, 2005), and *Quercus* (Chmielarz, 1999; Mallón et al., 2012). However, the major objective of these studies was basic or basic/applied research. Considerably less attention has been paid to research concerning implementation of somatic embryogenesis to breeding programes. Consequently, this technique has not yet been implemented both in industry and in forestry on the old continent (Lelu-Walter et al., 2013). According to Lelu-Walter et al. (2013) in Sweden in 2004, several forestry companies showed interest in somatic embryogenesis, which facilitated the improvement of the genetic quality of Norway spruce trees (*Picea abies*). However, the somatic embryogenesis technique is still too expensive to be used both commercially and as a tool in breeding. A similar situation is present in Finland (Högberg & Varis, 2016). One of the main solutions is development of automated somatic seedling production that will enable the reduction of plant production costs. Therefore, scientists have begun conducting research in this area in both countries (Högberg & Varis, 2016). In France, somatic embryogenesis has been used for the production of somatic maritime pine trees (*Pinus pinaster*) mainly for field trials. Lelu-Walter et al. (2013) assumed that commercial production of somatic seedlings in this country will begin probably in 10 years. Thus, somatic seedlings of *Pinus sylvestris* were used to establish a field experiment in 2009 in Finland by the Finish Research Institute to verify the effect of somatic embryo quality on the further growth of plants (Aronen, 2016). In Denmark, Caucasian fir somatic trees (*Abies nordmanniana)* are produced as Christmas trees for commercial purposes. Since 2007, clonal trials have been established with somatic seedlings from this conifer species to investigate the growth of plants in the field. In 2014, approximately 4 000 2-year-old plants were planted in two locations in Denmark to test the effect of environmental conditions on their growth and development. In 2015, approximately 5 000 plants was used for clonal trials. It was found that the micropropagation of A. *nordmanniana* via the somatic method is effective in laboratory conditions and that the growth of the plants is comparable to traditional seedlings (Find, 2016). Regarding deciduous trees, in Spain, somatic seedlings of cork oak (*Quercus suber*) were used to establish the field trials of propagated clones of this tree species (Hernández et al., 2011).

In Europe, somatic embryogenesis is still perceived mostly as a tool for research rather than as a technique that can be implemented in gardening and forest management. The main reasons remain the high cost of somatic seedling production, a lack of appreciation by foresters of the value of the plant material obtained with this technique, and the weak interest of potential purchasers of this plant material. The most important reason is the lack of a well-developed market for the improved plant material obtained by somatic embryogenesis (Lelu-Walter et al., 2013). However, it seems that the present state of knowledge, skills, and improved material in Europe will enable the implementation of somatic embryogenesis technology to European forest management in the near future.

Somatic embryogenesis research in Poland

In Poland, studies of somatic embryogenesis of forest tree species were initiated approximately a dozen years ago (Szczygieł & Sułkowska, 1996; Latkowska et al., 2000; Olszewska, 2000). Using this modern biotechnological method, somatic seedlings of *Picea abies*, *P. omorika*, *Larix decidua*, *Abies alba* (Szczygieł et al., 2007; Hazubska-Przybył & Bojarczuk, 2008) and *Quercus robur* (Chmielarz, 1999) were obtained. A high adaptation level to ex vitro conditions for *Larix decidua* was achieved, whereas for the other species, this process has been much more difficult (Szczygieł et al., 2007). In contrast, attempts to achieve somatic embryogenesis of broad-leaved tree species, e.g., oak and beech (Olszewska, 2000; Hazubska-Przybył et al., 2015), have not yielded positive results, although there was recently new information concerning the initiation of somatic embryogenesis from leaves derived from in vitro cultures established from the shoots of thirty-year-old *Quercus robur* trees (Kotlarski et al., 2015). In some countries, selected species of oak trees have been successfully propagated using this method (Wilhelm, 2000; Vieitez et al., 2012); while in the case of beech, research on somatic embryogenesis has been rarely undertaken (Vieitez et al., 1992; Naujoks, 2003). The reason is most likely the general difficulty to achieve vegetative reproduction of this tree species (Hazubska-Przybył et al., 2015).

Recently, Hazubska-Przybył et al. (2013) have obtained successful cryopreservation of *Picea abies* embryogenic tissues using pregrowth-dehydration method. Preliminary DNA analysis of *Picea abies* embryogenic tissues stored using this method has shown no changes within 5 tested microsatellite loci. This finding is very important because this cryopreservation method allows for the effective stor-

age both *Picea abies* and *P. omorika* embryogenic tissues (Hazubska-Przybył et al., 2010; 2013) without the use of dimethylsulfoxide (DMSO), a substance that is present in a majority of cryopreservation procedures and may contribute to genetic, epigenetic and other changes in cells (Finkle et al., 1985). Currently, further research on the potential application of this technique to a greater range of conifer species is in progress. Additionally, the simplified cryopreservation technique could provide an alternative method to store embryogenic cultures of conifers in gene banks, thereby avoiding the negative impact of DMSO on this type of plant material.

Conclusions

Somatic embryogenesis technique carries a very high scientific, practical, and economical potential. Despite numerous research many issues related to the phenomenon and technique of somatic embryogenesis have not yet been finally clarified and resolved. Thus, further research both at the basic and application level is very important to practical use of tree somatic embryogenesis in gardening and forestry.

The initial issue brought up by foresters was whether somatic embryogenesis seedlings were comparable to zygotic seedlings. After this issue was resolved, the major impediment to integrating somatic embryogenesis technology into operational reforestation programs is cost. Once the production system is created where somatic seedlings costs are comparable to zygotic seedlings, this technology will be readily accepted by the forest industry.

Acknowledgments

This article was written thanks to the funding of the project NN 309 705 240, approved by the National Research Centre (NCN) in Krakow, Poland.

References

Adams GW, Kunze HA, McCartney A, Millican S & Park YS (2016) An industrial perspective on the use of advanced reforestation stock technologies: Vegetative Propagation of Forest Trees (ed. by YS Park, JM Bonga & HK Moon) National Institute of Forest Science, pp. 323–334.

Ahuja MR (2009) Transgene stability and dispersal in forest trees. Trees 23: 1125–1135.

Aidun CK & Egertsdotter EMU (2012) Fluidics-based automation of clonal propagation via somatic embryogenesis: SE-Fluidics System: Proceedings of the IUFRO Working Party 2.09.02: "Somatic Embryogenesis of Trees" conference on "Integrating vegetative propagation, biotechnologies and genetic improvement for tree production and sustainable forest management" June, 25–28 2012, Brno, Czech Republic, S3–3.

Aronen T (2016) From lab to field – current state of somatic embryogenesis in Scots pine: Vegetative Propagation of Forest Trees (ed. by YS Park, JM Bonga & HK Moon) National Institute of Forest Science, pp. 515–527.

Bajaj YPS (1995) Somatic embryogenesis and its applications for crop improvement: Biotechnology in Agriculture and Forestry. vol. 30, Somatic embryogenesis and synthetic seeds I (ed. by YPS Bajaj) Springer-Verlag, Berlin Heidelberg, pp. 221–233.

Ballester A, Corredoira E & Vieitez AM (2016) Limitations of somatic embryogenesis in hardwood trees: Vegetative propagation of forest trees (ed. by YS Park, JM Bonga & HK Moon) National Institute of Forest Science, Seoul, Korea, pp. 56–74.

Bonga JM (2016) Conifer clonal propagation in tree improvement programs: Vegetative propagation of forest trees (ed. by YS Park, JM Bonga & HK Moon) National Institute of Forest Science, Seoul, Korea, pp. 3–31.

Bozhkov PV, Filonova LH & von Arnold S (2002) A key developmental switch during Norway spruce somatic embryogenesis is induced by a withdrawal of growth regulators and is associated with cell death and extracellular acidification. Biotechnology and Bioengineering 77: 658–667.

Chmielarz P (1999) Somatic embryogenesis of *Quercus robur* L. and cryopreservation of somatic embryos in liquid nitrogen: Mitteilungen aus der Biologischen Bundesanstalt für Land-Forstwirtschaft. Berlin-Dahlem, 365. Fortschritte bei de Lagerungstechnologie von Eichensaatgut. Braunschweig. Parey Buchverlag Berlin, pp. 49–59.

Chmielarz P, Grenier-de March G & de Boucaud MT (2005) Cryopreservation of *Quercus robur* L. embryogenic calli. CryoLetters 26: 349–355.

Corredoira E, San-José MC, Vieitez AM & Ballester A (2007) Improving genetic transformation of European chestnut and cryopreservation of transgenic lines. Plant Cell, Tissue and Organ Culture 91: 281–288.

Corredoira E, San-José MC & Vieitez AM (2012) Induction of somatic embryogenesis from different explants of shoot cultures derived from young *Quercus alba* trees. Trees 26: 881–891.

Cyr DR (1999) Cryopreservation of embryogenic cultures of conifers and its application to clonal forestry: Somatic embryogenesis in woody plants (ed. by SM Jain, PK Gupta & RJ Newton) Kluwer Academic Publishers, Netherlands, pp. 239–261.

Dai J, Vendrame WA & Merkle SA (2004) Enhancing the productivity of hybrid yellow-poplar and

hybrid sweetgum embryogenic cultures. In Vitro Cellular & Developmental Biology - Plant 40: 376–383.

DeVerno LL, Park YS, Bonga JM, Barrett JD & Simpson C (1999) Somaclonal variation in cryopreserved embryogenic clones of white spruce (*Picea glauca* (Moench) Voss.). Plant Cell Reports 18: 948–953.

Ducos JP, Gibault E, Broun P & Lambot C (2011) Coffee propagation by somatic embryogenesis at Nestlé R&D Center-Tours. Proceedings of the IUFRO Working Party 2.09.02 conference, Suwon, Republic of Korea, pp. 72–77.

Ellis D (1995) Genetic transformation of somatic embryos: Biotechnology in agriculture and forestry, vol. 30. Somatic embryogenesis and synthetic seeds I (ed. by YPS Bajaj), Springer-Verlag, Berlin Heidelberg, pp. 207–220.

Ewald D, Hu J & Yang M (2006) Transgenic forest trees in China: Tree transgenesis: recent developments (ed. by M Fladung & D Ewald) Springer, Berlin, pp. 25–45.

Find JI, Charity JA, Grace LJ, Kristensen MMMH, Krogstrup P & Walter C (2005) Stable genetic transformation of embryogenic cultures of *Abies nordmanniana* (Nordman fir) and regeneration of transgenic plants. In Vitro Cellular & Developmental Biology–Plant 41: 725–730.

Find JI (2016) Towards industrial production of tree varieties through somatic embryogenesis and other vegetative propagation technologies: Nordmanns fir (*Abies nordmanniana* (Steven) Spach) – From research laboratory to production: Vegetative Propagation of Forest Trees (ed. by YS Park, JM Bonga & HK Moon) National Institute of Forest Science, pp. 528–537.

Finkle BJ, Zavala ME & Ulrich JM (1985) Cryopreservation of Plant Cells and Organs. Cryoprotective compounds in the viable freezing of plant tissues: Cryopreservation of plant cells and organs (ed. by KK Kartha) CRC Press, Boca Raton, FL, pp. 75–113.

Gale S, John A, Harding K & Benson EE (2008) Developing cryopreservation for *Picea sitchensis* (Sitka spruce) somatic embryos: a comparison of vitrification protocols. CryoLetters 29: 135–144.

Goh D & Monteuuis O (2016) Teak: Vegetative Propagation of Forest Trees (ed. by YS Park, JM Bonga & HK Moon) National Institute of Forest Science, pp. 425–440.

Grossnickle SC (2011) Tissue culture of conifer seedlings – 20 years on: Viewed through the lens of seedling quality. USDA Forest Service Proceedings RMRS-P-65: 139–146.

Grossnickle SC & Major JE (1994) Interior spruce seedlings compared with emblings produced from somatic embryogenesis. III. Physiological response and morphological development on a reforestation site. Canadian Journal of Forest Research 24: 1397–1407.

Grossnickle SC & Sutton BCS (1999) Applications of biotechnology for forest regeneration. New Forests 17: 213–226.

Grossnickle SC & Folk RS (2005) Stock quality assessment of a somatic interior spruce seedlot. Northern Journal of Applied Forestry 22: 197–202.

Grossnickle SC & Folk R (2007) Field performance potential of a somatic interior spruce seedlot. New Forests 34: 51–72.

Grossnickle SC & Pait J (2008) Somatic embryogenesis tissue culture for applying varietal forestry to conifer species. USDA Forest Service Proceedings: RMRS-P-57: 135–139.

Gupta PK & Timmis R (2005) Mass propagation of conifer species in liquid cultures-progress towards commercialization. Plant Cell, Tissue and Organ Culture 81: 339–346.

Guzman-Garcia E, Bradaï F & Sánchez-Romero C (2013) Cryopreservation of avocado embryogenic cultures using the droplet-vitrification method. Acta Physiologiae Plantarum 35: 183–193.

Häggman H, Aronen T & Ryynänen LA (2000) Cryopreservation of embryogenic cultures of conifers: Somatic embryogenesis in woody plants, vol. 6 (ed. by SM Jain, PK Gupta & RJ Newton) Kluwer Academic Publishers, pp. 707–728.

Harding K (2004) Genetic integrity of cryopreserved plant cells: a review. CryoLetters 25: 3–22.

Hazubska-Przybył T & Bojarczuk K (2008) Somatic embryogenesis of selected spruce species (*Picea abies*, *P. omorika*, *P. pungens* 'Glauca' and *P. breweriana*). Acta Societatis Botanicorum Poloniae 77: 189–199.

Hazubska-Przybył T, Chmielarz P, Michalak M & Bojarczuk K (2010) Cryopreservation of embryogenic tissues of *Picea omorika* (Serbian spruce). Plant Cell, Tissue and Organ Culture 102: 35–44.

Hazubska-Przybył T, Chmielarz P, Michalak M, Dering M & Bojarczuk K (2013) Survival and genetic stability of *Picea abies* embryogenic cultures after cryopreservation using a pregrowth-dehydration method. Plant Cell, Tissue and Organ Culture 113: 303–313.

Hazubska-Przybył T, Chmielarz P & Bojarczuk K (2015) In vitro responses of various explants of *Fagus sylvatica*. Dendrobiology 73: 135–144.

Hernández I, Cuenca B, Carneros E, Alonso-Blázquez N, Ruiz M, Celestino C, Ocaña L, Alegre J & Toribio M (2011) Application of plant regeneration of selected cork oak trees by somatic embryogenesis to implement multivarietal forestry for cork production. Tree and Forestry Science and Biotechnology 5: 19–26.

Högberg KA & Varis S (2016) Vegetative propagation of Norway spruce: Experiments and present situation in Sweden and Finland: Vegetative Propagation of Forest Trees (ed. by YS Park, JM Bonga & HK Moon) National Institute of Forest Science, pp. 538–550.

Klimaszewska K, Cardou MB, Cyr DR & Sutton BCS (2000) Influence of gelling agents on culture medium gel strength, water availability, tissue water potential, and maturation response in embryogenic cultures of *Pinus strobus* L. In Vitro Cellular & Developmental Biology – Plant 36: 279–286.

Klimaszewska K, Lachance D, Pelletier G, Lelu MA & Sèguin A (2001) Regeneration of transgenic *Picea glauca*, *P. Mariana* and *P. abies* after cocultivation of embryogenic tissue with *Agrobacterium tumefaciens*. In Vitro Cellular & Developmental Biology–Plant 37: 748–755.Klimaszewska K, Trontin JF, Becwar M, Devillard C, Park YS & Lelu-Walter MA (2007) Recent progress in somatic embryogenesis of four *Pinus* spp. Tree and Forestry Science and Biotechnology 1: 11–25.

Klimaszewska K, Trontin JF, Becwar MR, Devillard C, Park YS & Lelu-Walter MA (2007) Recent progress in somatic embryogenesis of four *Pinus* spp. Tree and Forestry Science and Biotechnology 1: 11–25.

Klimaszewska K, Overton C, Stewart D & Rutledge RG (2011) 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 233: 635–647.

Kong L & von Aderkas P (2011) A novel method of cryopreservation without a cryoprotectant for immature somatic embryos of conifer. Plant Cell, Tissue and Organ Culture 106: 115–125.

Kotlarski S, Plitta-Michalak B, Barciszewska MZ, Barciszewski J, Tylkowski T, Sobczak M, Pilarz P, Jaworska K, Litkowiec M, Lewandowski A, Michalak M & Chmielarz P (2015) The possibilities of preserving the genetic resources of the oldest pedunculate oaks (*Quercus robur* L.) in Poland. Proceedings of the national scientific conference Biology and technology in the trees and shrubs seed multiplication, Puszczykowo, Poland, pp. 72–73.

Krajňáková J, Sutela S, Aronen T, Gömöry D, Vianello A & Häggman H (2011) Long-term cryopreservation of Greek fir embryogenic cell lines: Recovery, maturation and genetic fidelity. Cryobiology 63: 17–25.

Krajňáková J, Bertolini A, Gömöry D, Vianello A & Häggman H (2013) Initiation, long-term cryopreservation, and recovery of *Abies alba* Mill. embryogenic cell lines. In Vitro Cellular & Developmental Biology – Plant 49: 560–571.

Lane A (2004) Attack of the clones: somatic embryogenesis in forestry. BioTech Journal 2: 13–17.

Latkowska MJ, Kvaalen H & Appelgren M (2000) Genotype dependent blue and red light inhibition of the proliferation of the embryogenic tissue of Norway spruce. In Vitro Cellular & Developmental Biology – Plant 36: 57–60.

Lelu-Walter MA, Bernier-Cardou M & Klimaszewska K (2006) Simplified and improved somatic embryogenesis for clonal propagation of *Pinus pinaster* (Ait.). Plant Cell Reports 25: 767–776.

Lelu-Walter MA, Pâques L, Harvenght L & Thompson D (2010) Somatic Embryogenesis of Forest Trees in Europe: What's Going on? An Overview. Proceedings of the IUFRO Working Party 2.09.02 conference, Suwon, Republic of Korea, pp. 97–99.

Lelu-Walter MA, Thompson D, Harvengt L, Sanchez L, Toribio M & Pâques LE (2013) Somatic embryogenesis in forestry with a focus on Europe: state-of-the-art, benefits, challenges and future direction. Tree Genetics & Genomes 9.

Lelu-Walter MA, Teyssier C, Guérin V, Pâques LE (2016) Vegetative propagation of larch species: somatic embryogenesis improvement towards its integration in breeding programs: Vegetative Propagation of Forest Trees (ed. by YS Park, JM Bonga & HK Moon) National Institute of Forest Science, pp. 551–571.

Liao YK & Juan IP (2015) Improving the germination of somatic embryos of *Picea morrisonicola* Hayata: effects of cold storage and partial drying. Journal of Forest Research 20: 114–124.

Lin YJ & Zhang QF (2005) Optimising the tissue culture conditions for high efficiency transformation of indica rice. Plant Cell Reports 23: 540–547.

Lopes T, Pinto G, Loureiro J, Costa A & Santos C (2006) Determination of genetic stability in long-term somatic embryogenic cultures and derived plantlets of cork oak using microsatellite markers. Tree Physiology 26: 1145–1152.

Lulsdorf MM, Tautorus TE, Kikcio SI, Bethune TD & Dunstan DI (1993) Germination of encapsulated embryos of interior spruce (*Picea glauca engelmanni* complex) and black spruce (*Picea mariana* Mill.). Plant Cell Reports 12: 385–389.

Malabadi RB & Nataraja K (2007) Production of transgenic plants via *Agrobacterium*-mediated genetic transformation in *Pinus wallichiana* (Himalayan blue pine). Transgenic Plant Journal 1: 376–383.

Mallón R, Covelo P & Vieitez AM (2012) Improving secondary embryogenesis in *Quercus robur*: Application of temporary immersion for mass propagation. Trees – Structure and Function 26: 731–741.

Michalak M, Plitta BP, Kotlarski S & Chmielarz P (2014) Cryopreservation – is it safe? Possible changes in cryostored plant genetic resources.

Sylwan 158: 795–800.Naujoks G (2003) Somatic embryogenesis in beech (*Fagus sylvatica*). Biologia 58: 83–87.

Nawrot-Chorabik K (2012) Somatic embryogenesis in forest plants. (ed. by K Sato) Embryogenesis. InTech 20: 423–446.

Nigro SA, Makunga NP, Jones NB & van Staden J (2004) A biolistic approach towards producing transgenic *Pinus patula* embryonal suspensor masses. Plant Growth Regulation 44: 187–197.

Olszewska A (2000) Micropropagation and somatic embryogenesis of oak *Quercus* sp. Mikrorozmnażanie i embriogeneza somatyczna dębu – *Quercus* sp. University of Life Science Poznań. PhD thesis.

Park YS (2002) Implementation of conifer somatic embryogenesis in clonal forestry: technical requirements and deployment considerations. Annals of Forest Science 59: 651–656.

Park YS & Bonga JM (2010) Application of somatic embryogenesis in forest management and research. Proceedings of the IUFRO Working Party 2.09.02 conference, Suwon, Republic of Korea, pp. 3–8.

Park YS, Barret JD & Bonga JM (1998) Application of somatic embryogenesis in high-value clonal forestry: deployment, genetic control and stability of cryopreserved clones. In Vitro Cell Developmental Biology – Plant 34: 231–239.

Pérez M, Bueno MA, Escalona M, Toorop P, Rodríguez R & Cañal MJ (2013) Temporary immersion systems (RITA®) for the improvement of cork oak somatic embryogenic culture proliferation and somatic embryo production. Trees 27: 1277–1284.

Pinto G, Correia S, Corredoira E, Ballester A, Correia B, Neves L & Canhoto J (2016) In vitro culture of *Eucalyptus*: where we stand? Vegetative propagation of forest trees (ed. by YS Park, JM Bonga & HK Moon) National Institute of Forest Science, Seoul, Korea, pp. 441–462.

Polin LD, Liang H, Rothrock RE, Nishii M, Diehl DL, Newhouse AE, Nairn CJ, Powell WA & Maynard CA (2006) *Agrobacterium*-mediated transformation of American chestnut (*Castanea dentata* (Marsh.) Borkh.) somatic embryos. Plant Cell, Tissue and Organ Culture 84: 69–79.

Pullman GS & Bucalo K (2014) Pine somatic embryogenesis: analyses of seed tissue and medium to improve protocol development. New Forests 25: 353–377.

Roberts DR, Lazaroff WR & Webster FB (1991) Interaction between maturation and high relative humidity treatments and their effects on germination of Sitka spruce somatic embryos. Journal of Plant Physiology 138: 1–6.

Salaj T, Moravčíková J, Grec-Niqet L & Salaj J (2005) Stable transformation of embryogenic tissues of *Pinus nigra* Arn. using a biolistic method. Biotechnology Letters 27: 899–903.

Salaj T, Panis B, Swennen R & Salaj J (2007) Cryopreservation of embryogenic tissues of *Pinus nigra* Arn. by a slow freezing method. CryoLetters 28: 69–76.

San-José MC, Corredoira E, Martínez MT, Vidal N, Valladares S, Mallón R & Vieitez AM (2010) Shoot apex explants for induction of somatic embryogenesis in mature *Quercus robur* L. trees. Plant Cell Reports 29: 661–671.

San-José MC, Corredoira E, Oliveira H & Santos C (2015) Cryopreservation of somatic embryos of *Alnus glutinosa* (L.) Gaertn. and confirmation of ploidy stability by flow cytometry. Plant Cell, Tissue and Organ Culture 123: 489–499.

Shekhawat UKS, Ganapathi TR, Srinivas L, Bapat VA & Rathore TS (2008) *Agrobacterium*-mediated genetic transformation of embryogenic cell suspension cultures of *Santalum album* L. Plant Cell, Tissue and Organ Culture 92: 261–271.

South DB (2009) Economics of clonal forestry: Vegetative propagation and deployment of varieties – the scope for Europe. Treebreedex, Liverpool, England.

Sutton WRJ (1999) The need for planted forests and the example of Radiata pine. New Forests 17: 95–109.

Sutton BCS, Attree SA, El-Kassaby YA, Grossnickle SC & Polonenko DR (2004) 17 Commercialization of somatic embryogenesis for plantation forestry: Plantation Forest Biotechnology for the 21st Century (ed. by C Walter & M Carson) Research Signpost, pp. 275–301.

Szczygieł K & Sułkowska M (1996) Using isoenzyme analysis for estimation somaclonal variation during somatic embryogenesis of some Polish provenances of Norway spruce (*Picea abies* (L.) Karst.). Proceedings of the 10th FESPP conference. From molecular to the plant: an integrated approach, Florencja, Italy. Plant Physiology and Biochemistry (Special issue): 41.

Szczygieł K, Hazubska-Przybył T & Bojarczuk K (2007) Somatic embryogenesis of selected coniferous tree species of the genera *Picea, Abies* and *Larix*. Acta Societatis Botanicorum Poloniae 76: 7–15.

Szczygieł K & Hazubska-Przybył T (2010) Światowe tendencje wykorzystania metod wegetatywnego rozmnażania drzew. Leśne Prace Badawcze 71: 208–211.

Tereso S, Miguel C, Zoglauer K, Valle-Piquera C & Oliveira MM (2006) Stable *Agrobacterium*-mediated transformation of embryogenic tissues from *Pinus pinaster* Portuguese genotypes. Plant Growth Regulation 50: 57–68.

Thompson D (2014) Challenges for the large-scale propagation of forest trees by somatic embryogenesis – a review. 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, pp. 81–91.

Titon M, Xavier A & Otoni WC (2006) Clonal propagation of *Eucalyptus grandis* using the mini-cutting and micro-cutting techniques. Scienta Forestalis 71: 109–117.

Toribio M, Celestino C & Molinas M (2005) Cork oak, *Quercus suber* L.: Protocol for somatic embryogenesis in woody plants. (ed. by SM Jain & PK Gupta), Springer-Dordrecht, Netherlands, pp. 445–457.

Toribio M, Fernández C, Celestino C, Martínez MT, San-José MC & Vieitez AM (2004) Somatic embryogenesis in mature *Quercus robur* trees. Plant Cell, Tissue and Organ Culture 76: 283–287.

Vieitez JF, Ballester A & Vieitez AM (1992) Somatic embryogenesis and plantlet regeneration from cell suspension cultures of *Fagus sylvatica* L. Plant Cell Reports 11: 609–613.

Vieitez AM, Corredoira E, Martínez MT, San-José MC, Sánchez C, Valladares S, Vidal N & Ballester A (2012) Application of biotechnological tools to *Quercus* improvement. European Journal of Forest Research 131: 519–539.

Vidal N, Mallon R, Valladares S, Meijomın AM & Vieitez AM (2010) Regeneration of transgenic plants by *Agrobacterium*-mediated transformation of somatic embryos of juvenile and mature *Quercus robur*. Plant Cell Reports 29: 1411–1422.

Wadenbäck J, Arnold von S, Egertsdotter U, Walter MH, Grima-Pettenati J, Goffner D, Gellerstedt G, Gullion T & Clapham D (2008) Lignin biosynthesis in transgenic Norway spruce plants harboring an antisense construct for cinnamoyl CoA reductase (CCR). Transgenic Research 17: 379–392.

Walter C, Grace LJ, Wagner A, White DRW, Walden AR, Donaldson SS, Hinton H, Gardner RC & Smith DR (1994) Stable transformation and regeneration of transgenic plants of *Pinus radiata*. Plant Cell Reports 17: 460–468.

Wilhelm E (2000) Somatic embryogenesis in oak (*Quercus* ssp.). In Vitro Cellular & Developmental Biology – Plant 36: 349–357.