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Review article

Application of ectomycorrhizal fungi in vegetative propagation of conifers

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Abstract

In forestry, vegetative propagation is important for the production of selected genotypes and shortening the selection cycles in genetic improvement programs. *In vivo* cutting production, *in vitro* organogenesis and somatic embryogenesis are applicable with conifers. However, with most coniferous species these methods are not yet suitable for commercial application. Large-scale production of clonal material using cuttings or organogenesis is hindered by rooting problems and difficulties in the maturation and conversion limit the use of somatic embryogenesis. Economically important conifers form symbiotic relationship mostly with ectomycorrhizal (ECM) fungi, which increase the fitness of the host tree. Several studies have shown the potential of using ECM fungi in conifer vegetative propagation. Inoculation with specific fungi can enhance root formation and/or subsequent root branching of *in vivo* cuttings and *in vitro* adventitious shoots. Germination of somatic embryos and subsequent root growth can also be improved by the use of ECM fungi. In addition, inoculation can increase the tree's ability to overcome the stress related to *ex vitro* transfer. A specific interaction between a fungal strain and tree clone occurs during root induction and germination of somatic embryos. Multiple rooting factors exist in this interaction that complicate the predictability of the response to inoculation. Fungal-specific factors that influence rooting responses to inoculation may include plant growth regulator production, modification of the rooting environment, and interactions with beneficial microbes. A combination of these factors may act synergistically to result in positive responses in tree genotypes that are compatible with the fungus.

Abbreviations: ECM – ectomycorrhiza (l); IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; MHB – mycorrhization helper bacteria; PA – polyamine; PGR – plant growth regulator

Vegetative propagation of conifers

Vegetative propagation provides the possibility to multiply the favorable genetic combination of a selected superior tree and to produce genetically homogenous plant material that will grow predictably and uniformly. Current techniques for *in vivo* production of rooted cuttings and *in vitro* organogenesis allow, in most species, multiplication using seedling explants and in some cases also explants from mature trees.

Somatic embryogenesis, that is, production of embryos from vegetative cells, mostly derived from immature or mature zygotic embryos, is applicable for several coniferous species and has the most potential for mass propagation of specific clones. For proper selection of clones, the propagated material derived from juvenile material has to be cryopreserved (reviewed by Häggman et al., 2000), and just after field testing the cryopreserved clones may be retrieved for tree breeding purposes, mass propagation, and cultivation.

In vivo *rooting of cuttings*

With conifers, commercial scale cutting propagation has been reported only for radiata pine (*Pinus radiata* Don.), Norway spruce (*Picea abies* [L.] Karst.), Sitka spruce (*Picea sitchensis* [Bong.] Carr.), black spruce (*Picea mariana* [Mill.] B.S.P.), and sugi (*Cryptomeria japonica* D. Don) (Ritchie, 1991; Ahuja and Libby, 1993; Menzies et al., 2001). For most coniferous species, large-scale commercial use of cutting propagation methods is hindered by rooting problems. Rooting frequency varies considerably between genotypes, and decreases with increasing age of the donor tree. Rooted cuttings may also show plagiotrophic growth (Ahuja and Libby, 1993; Browne et al., 1997; Niemi et al., 2000). To investigate problems associated with rooting of conifer cuttings *in vivo* and factors affecting root formation, hypocotyl cuttings induced to form roots *in vitro* have been used in several studies (e.g. Grönroos and von Arnold, 1988; Greenwood and Weir, 1995; Niemi et al., 2002a, b).

In vitro *rooting of adventitious shoots*

Organogenesis techniques have been tested with several coniferous species as a method of *in vitro* propagation. As with *in vivo* cutting production, radiata pine is an example of commercial propagation success using organogenesis in conifers (Menzies et al., 2001). With most other coniferous species, the method has only been applicable on a limited research scale, which is primarily because of the rooting problems and plagiotrophic growth, as with *in vivo* cuttings (e.g. Supriyanto and Rohr, 1994; Häggman et al., 1996; Drake et al., 1997; Gonzales et al., 1998; Tang and Guo, 2001).

Adventitious root induction using exogenous plant growth regulators

Application of exogenous auxin, usually in the form of indole-3-butyric acid (IBA) or 1-napthaleneacetic acid (NAA) is a general practice to induce *in vitro* and *in vivo* adventitious rooting in conifers (e.g. Grönroos and von Arnold, 1988; Greenwood and Weir, 1995; Browne et al., 1997, 2000; Niemi et al., 2002b). The studies with different hardwood species have indicated that the polyamine (PA; referred to here as a plant growth regulator) putrescine interacts with auxins in root formation because its concentration increases soon after the transfer of the *in vitro* shoots to rooting medium containing auxins. Furthermore, application of putrescine can promote root formation even in the absence of exogenous auxins (Hausman et al., 1994; Kevers et al., 1997; Tonon et al., 2001). However, with Scots pine hypocotyl cuttings the effects of exogenous putrescine and cadaverine on root formation have been variable (Niemi et al., 2002a). Ethylene synthesis is closely associated with PA metabolism because spermidine and spermine are derived from the same precursor, S-adenosylmethionine (SAM), as ethylene. In lodgepole pine (*Pinus contorta* L.) hypocotyl cuttings two genes encoding SAM synthase expressed differentially during the formation of adventitious roots (Lindroth et al., 2001).

In vitro *somatic embryogenesis*

Since the first reports on somatic embryogenesis of Norway spruce (Chalupa, 1985; Hakman et al., 1985), research has extended to a wide range of coniferous species, and somatic embryogenesis has been reported to succeed more or less completely in several species (e.g. Jain et al., 1995; Timmis, 1998). Somatic embryo development relies upon changes in culture conditions during initiation, proliferation, maturation, and conversion (i.e. germination and subsequent acclimatization). Recent advances in somatic embryogenesis for conifers have been reviewed by Stasollo and Yeung (2003) and are therefore not described here. For germination, plant growth regulators (PGRs) are usually omitted and concentration of sugar and other nutrients are lowered, which results in rapid utilization of storage compounds. However, exogenous abscisic acid included in the maturation phase may still influence the conversion phase (von Aderkas et al., 2002). Successful germination and subsequent growth and acclimatization are known to be dependent on seed family and genotype, as well as quality of somatic embryos (Timmis, 1998; Högberg et al., 2001), and it is still a challenge to find optimal conditions for germination over a wide range of genotypes.

Mycorrhizal symbiosis of conifers

Symbiotic partners in ectomycorrhizas

Mycorrhiza refers to a symbiosis between plants and fungi that colonize their roots. In this interaction, nutrients taken up by the fungus are exchanged for carbohydrates derived from the host plant. Economically important coniferous species, including *Abies*, *Larix, Picea, Pinus* and *Pseudotsuga* predominantly form ectomycorrhizas (ECM). The majority of fungal partners in ECM symbiosis are basidiomycetes (e.g. fungi in the Boletales, Russulales, Telephorales), but there are also several ascomycetes and some zygomycetes that form ECMs (reviewed by Smith and Read, 1997).

Most conifers form ECM symbiosis with a broad range of fungi, and several fungal species may exist concomitantly in the same root system (Smith and Read, 1997). The interactions for initiation and maintenance of ECM symbiosis appear to be highly specific and dependent on both the fungus and tree genotype (Dixon et al., 1987; Debaud et al., 1995). Host trees may release specific metabolites into the rhizosphere attracting suitable fungi to grow towards developing roots. Subsequent release of signal molecules, including adhesins, hydrolases, PGRs, and molecules related to plant pathogen defense, directs interaction and results in either initiation of mycorrhiza formation between the root and a compatible fungal strains or inhibition of incompatible strains (reviewed by Martin et al., 2001). Recently, symbiosis of plants with bacteria and endomycorrhizal fungi have been found to rely on partially overlapping genetic programs with the molecular basis being the orthologous SYMRK (symbiosis receptor-like kinase) genes being required for both fungal and bacterial recognition (Stracke et al., 2002).

Ectomycorrhizal structures and water and nutrient uptake

The importance of ECM fungi to the growth and survival of individual trees and forests is well established. Formation of ECM symbiosis occurs continuously and concomitantly with root growth and it directly influences most aspects of root biology including the uptake of nutrients and water and tolerance to pathogens and toxic heavy metals (reviewed by Smith and Read, 1997).

ECM conifers have heterorhizic root systems consisting of long and short roots. Ectomycorrhizal fungi primarily colonize short roots with a rounded apex and restricted growth (Piché et al., 1983; Brundrett et al., 1990). During the formation of ECM, root hair elongation in the short roots is inhibited and fungal hyphae replace root hairs for absorbing water and nutrients (Béguiristain and Lapeyrie, 1997; Ditengou et al., 2000). The extraradical hyphae extend through the soil and can take up nutrients from a much larger soil volume than root hairs (reviewed by Agerer, 2001). Nutrients taken up by the extraradical hyphae are transported to a hyphal mantle, which encloses the short root and serves as a site of nutrient and carbohydrate storage (Agerer, 2001) (Figure 1). Transfer of nutrients from the fungus to the root cells and of carbohydrates in the opposite direction occurs via a highly branched hyphal structure called Hartig net and epidermal and cortical cells (Figure 1) (Smith and Read, 1997).

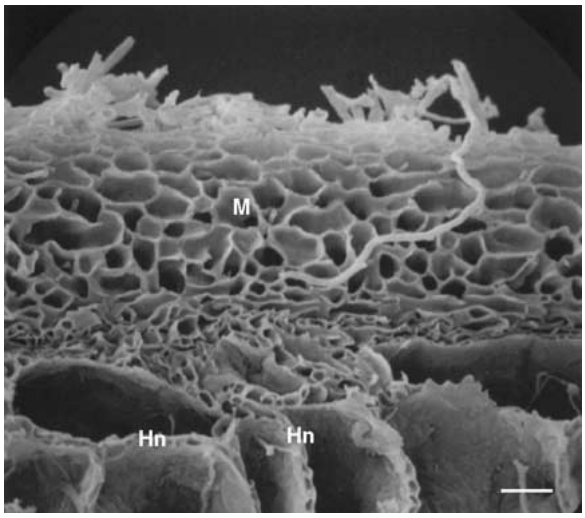

Figure 1. General structure of an ectomycorrhiza of Norway spruce (*P. abies* (L.) Karst.) in the natural forest. Fungal hyphae cover the short root as a mantle consisting of several cell layers. The hyphae also penetrate into the intercellular space of the root and form a Hartig net. M: mantle; Hn: Hartig net. Scale bar 10 μm.

Production of plant growth regulators by the fungus and root development

Ectomycorrhizal fungi produce different PGRs including auxins, cytokinins, ethylene and PAs (e.g. Ho, 1987; Kraigher et al., 1991; Strzelczyk et al., 1992, 1994; Zarb and Walters, 1994; Scagel and Linderman, 1998; Niemi et al., 2000, 2003). Slankis (1973) was the first to postulate the involvement of production of indole-3-acetic acid (IAA) by the ECM fungus

to root morphology and mycorrhiza formation. The use of an IAA overproducing mutant of *Hebeloma cylindrosporum* Romagnesi, which lacks normal feedback inhibition for tryptophan biosynthesis, has shown the importance of fungal auxin in the establishment of ECM symbiosis *in vitro* (Gay et al., 1994; Tranvan et al., 2000). On the other hand, Kaska et al. (1999) found that in pine roots ethylene acts as a main trigger of dichotomous branching of short roots, a phenomenon characteristic of pine ECMs, and IAA may act by indirectly inducing ethylene production. In addition to auxin and ethylene, certain PAs formed by ECM fungi may also be involved in root branching and mycorrhiza formation *in vitro* (Niemi et al., 2002a).

Ectomycorrhizal fungi during vegetative propagation

In vitro *root induction using ectomycorrhizal fungi*

Because of the importance of ECM fungi for plant growth it has been attempted to use them as promoting agents for adventitious rooting. With conifers, fungal effects on adventitious root formation have mainly been studied using hypocotyl cuttings *in vitro* (Figure 2, Table 1). However, adventitious shoots of Scots pine and maritime pine (*Pinus pinaster* (Ait.) Sol.) raised by organogenesis have also been rooted in the presence of ECM fungi *in vitro* (Table 1). In *in vitro* studies, inoculation has been performed using mycelium agar plugs cut from the edge of 3–4-week-old mycelial cultures. The agar plugs were placed close to the base of the cutting or adventitious shoot at the time of transfer or, alternatively, the fungal mycelium was pre-cultivated on the medium before transfer of the explant.

In vitro studies have shown a positive effect of ECM inoculation on adventitious root formation. However, the mechanisms through which fungi exert an effect appear to depend on the fungus strain and culture conditions. Gay (1990) and Karabaghli et al. (1998) increased rooting on hypocotyl cuttings of Aleppo pine (*Pinus halepensis* Mill.) and Norway spruce, respectively, with ECM fungi, but only in the presence of tryptophan, the precursor of IAA. In addition, over-production of IAA by a *Hebeloma cylindrosporum* mutant favored rooting of adventitious shoots of a Scots pine clone (Normand et al., 1996).

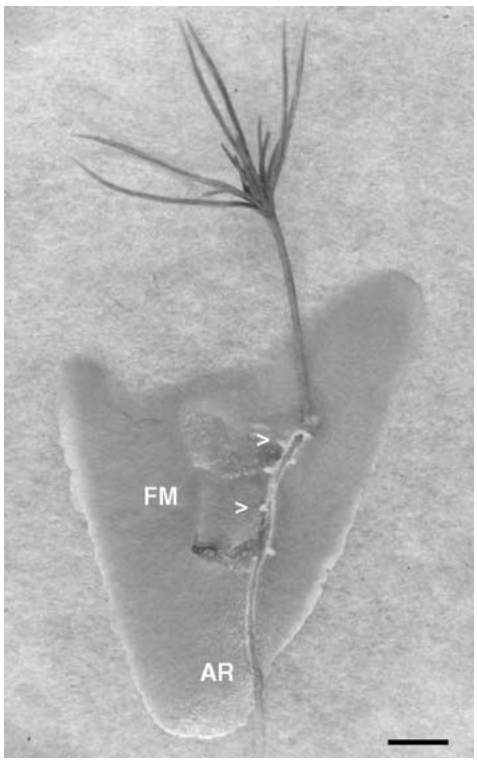

Figure 2. In vitro hypocotyl cutting of Scots pine has rooted in the presence of an ectomycorrhizal fungus *P. tinctorius*. The fungal mycelium covers lateral roots as a mantle. AR: adventitious root; FM: fungal mycelium; >: lateral root. Scale bar 0.5 cm.

The results of these studies suggest that ECM production of IAA plays an important role in fungal-induced adventitious rooting. However, using two maritime pine clones, Normand et al. (1996) showed no increase in root formation in the presence of excess fungal IAA. The authors suggest that the low production of IAA by the wild-type mycelium was sufficient to enhance root formation on the maritime pine clones that were highly responsive to exogenous auxin.

PGRs other than auxin produced by the fungi may be equally important in root induction. For instance, a *Paxillus involutus* strain with very low *in vitro* IAA production (Niemi et al., 2000, 2002b) that induced rooting of Scots pine hypocotyl cuttings also produced a high amount of putrescine *in vitro*. The combination of this fungus and exogenous putrescine also had a synergistic effect on root formation (Niemi et al., 2002a). Stein and Fortin (1990) studied the role of fungal ethylene in root induction of *Larix laricina* (Du Roi) K. Koch, and found that *Laccaria bicolor* Maire (Orton) induced an upward distribution of roots along hypocotyl cuttings, as did an ethylene-releasing compound ethephon.

Table 1. Use of ectomycorrhizal fungi in adventitious root formation of conifers *in vitro* and *in vivo*

Tree species	Explant	Fungus[b,c,d]	Research object	References
L. decidua	*In vivo* cuttings	*L. bicolor*[b], *S. cavipes*[b]	Rooting, root growth	Stein et al. (1990)
L. laricina	*In vitro* hypocotyls	*L. bicolor*[a]	Rooting	Stein and Fortin (1990)
P. abies	*In vitro* hypocotyls	*L. bicolor*[a]	Rooting, root growth	Karabaghli et al. (1998)
P. mariana	*In vivo* cuttings	*L. bicolor*[b], *S. cavipes*[b]	Rooting, root growth	Stein et al. (1990)
P. halepensis	*In vitro* hypocotyls	*Hebeloma hiemale*[a]	Rooting	Gay (1990)
P. pinaster	*In vitro* hypocotyls	*Hebeloma cylindrosporum*[a]	Rooting	Normand et al. (1996)
	In vitro adventitious shoots	*H. cylindrosporum*[a]	Rooting, acclimatization	Normand et al. (1996)
P. sylvestris	*In vitro* hypocotyls	*P. involutus*[a], *P. tinctorius*[a]	Rooting, root growth	Niemi et al. (2002a, b)
	In vitro adventitious shoots	*H. cylindrosporum*[a]	Rooting	Normand et al. (1996)
		H. cylindrosporum[a]	Root growth, acclimatization	David et al. (1983), Normand et al. (1996), Supriyanto and Rohr (1994)
		P. tinctorius[a]		David et al. (1983)
	In vivo cuttings	*P. involutus*[d], *P. tinctorius*[d]	Rooting, root growth	Niemi et al. (2000)
Pseudotsuga menziesii	*In vivo* cuttings	*L. bicolor*[b], *Melanogaster ambiguus*[c], *Rhizopogon subareolatus*[c]	Rooting, root growth	Parladé et al. (1999)

[a] Inoculations of hypocotyls and adventitious shoots *in vitro* were performed using mycelial agar plugs.
[b] Under *in vivo* conditions, cuttings were rooted in the soil inoculated with mycelium.
[c] Under *in vivo* conditions, cuttings were rooted in the soil inoculated with spores.
[d] Under *in vivo* conditions, cuttings were dipped into mycelium slurry before rooting.

Specific ECM fungi not only induce formation of adventitious roots *in vitro*, but also support subsequent root elongation and lateral root formation (Supriyanto and Rohr, 1994; Karabaghli et al., 1998; Niemi et al., 2002a, b). In the study of Karabaghli et al. (1998), both an ECM fungus *L. bicolor* and a bacterium *Pseudomonas fluorescens* produced IAA, but only the fungus induced root elongation and lateral root formation on the hypocotyl cuttings of Norway spruce *in vitro*. The authors suggested that IAA was not the only factor regulating root growth. This is supported by the observation that inoculation with *Pisolithus tinctorius*, which is able to produce cadaverine, enhanced root growth on Scots pine cuttings and that the fungus and exogenous cadaverine had a synergistic effect on root growth (Niemi et al., 2002a). In addition, the fungi that induce rooting and root growth can also promote shoot growth by increasing secondary needle production, and epicotyl (Karabaghli et al., 1998) and primary needle elongation (Niemi et al., 2002b) of the rooted hypocotyl cuttings.

In vivo *root induction using ectomycorrhizal fungi*

Reactions of conifer cuttings to ECM inoculation *in vivo* are complex and difficult to relate to PGR production by the fungi (Table 1). Stein et al. (1990) inoculated the rooting substrate of black spruce cuttings with *L. bicolor* and *Suillus cavipes* (Opat.) Smith

Table 2. Use of ectomycorrhizal fungi in somatic embryogenesis of conifers *in vitro*

Tree species	Plant material	Fungus[a]	Research object	References
Larix × *eurolepis*	Somatic embryo plants	Four different fungus species	Root growth	Piola et al. (1995)
P. sitchensis	Somatic embryo plants	Six different fungus species	Root growth, acclimatization	Sasa and Krogstrup (1991)
P. sylvestris	Embryogenic cell mass	Six different fungus species	Proliferation	Niemi et al. (1998)
	Mature somatic embryos	*P. tinctorius*	Germination, root growth, acclimatization	Niemi and Häggman (2002)

[a] Inoculations were performed using mycelial agar plugs.

and Thiers and found that both fungi stimulated root formation and subsequent root growth of the cuttings. However, inoculation did not result in as good a rooting response as treating the cutting bases with IBA. On European larch (*Larix decidua* Mill.) cuttings, which rooted easily, the fungi had no effect, and IBA increased the number of adventitious roots per cutting, but not rooting frequency or subsequent root growth (Stein et al., 1990). The ability of the fungi used in the inoculations to produce different PGRs was not determined and, therefore, it was impossible to know the role of fungal PGRs in root induction. When Scots pine fascicular shoots, pre-treated with IBA, were dipped into a mycelium slurry of ECM fungi with different abilities to produce IAA *in vitro*, a highly variable rooting response occurred depending on the plant/fungus genotypic interaction (Niemi et al., 2000). Similar genotype-dependent rooting reactions *in vivo* to *P. tinctorius* have been found with cuttings of hybrid poplars (Navratil and Rochon, 1981). The use of a mixed inoculum consisting of different species and strains of fungi might result in positive rooting responses across a wider range of genotypes than the use of a single strain. However, to ensure that one fungus strain does not reduce the activity of another, their interactions should be carefully studied before performing large-scale inoculations.

For pre-inoculation of the rooting substrate (Navratil and Rochon, 1981; Stein et al., 1990; Parladé et al., 1999), as well as for treatment of the cutting bases with homogenized mycelium slurry (Niemi et al., 2000), large amounts of fungal material are needed. For mass production of vegetative mycelium, both solid-substrate and submerged aerated cultures are used. The development of the latter technique is hindered by the fact that certain fungal species do not grow in submerged cultures (reviewed by Kuek, 1994).

Effects of ectomycorrhizal fungi on somatic embryogenesis

Less attention has been paid to the possibility to enhance somatic embryogenesis, especially germination and root growth, by means of ECM fungi than to improve rooting of cuttings (Table 2). Niemi et al. (1998) cultivated specific ECM fungi and embryogenic cell masses of Scots pine on proliferation medium rich in nutrients and sugar. The responses of the cell masses to co-culture, in which a fungus and the cell mass grew far enough from each other to avoid physical contact, varied from a significant increase to strong decrease in proliferation growth. When the fungus and the cell mass were let to reach each other some cell lines continued normal proliferation, whereas other cell lines stopped growing and the cell mass became brown and necrotic and the fungus grew aggressively over it. The reactions on the proliferation medium were highly dependent on the plant/fungus genotypic interaction. Release of specific PGRs by the fungi to the medium was not determined, and therefore, their role in proliferation growth of the cell masses is not known.

An imbalance between symbiotic partners *in vitro*, due to aggressive growth of the fungus, was evident when the mycelium was in physical contact with germinating Scots pine somatic embryos (Niemi and Häggman, unpublished data) and with Sitka spruce somatic embryo plants with short radicles (Sasa and Krogstrup, 1991). The germination of Scots pine somatic embryos was successful only when the distance between the fungus and the somatic embryo

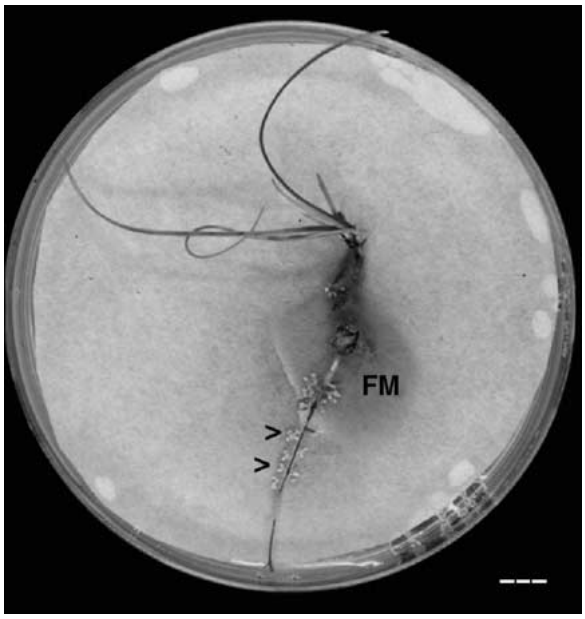

Figure 3. In vitro somatic embryo plant of Scots pine forms mycorrhizas with an ectomycorrhizal fungus *P. tinctorius*. FM: fungal mycelium; >: dichotomously branched lateral root covered by hyphae. Scale bar 0.8 cm (Niemi and Häggman, 2002).

was long enough to avoid physical contact (Niemi and Häggman, 2002). Four out of five cell lines of Scots pine germinated better in the presence of *P. tinctorius* than in control culture, the degree of enhanced germination depending on the cell line. Subsequent inoculation of the germinated somatic embryo plants on a modified Melin-Norkrans medium (Marx, 1969) with reduced nutrient and sugar concentrations resulted in dichotomous branching of lateral roots and mycorrhiza formation (Figure 3). Similar results were obtained by Piola et al. (1995) with germinated somatic embryos of hybrid larch (*Larix* × *eurolepis* Henry) inoculated with selected ECM fungi *in vitro*. These studies indicate the importance of both the developmental stage of the somatic embryos and the nutrient composition of the medium in obtaining a balanced interaction with the fungus.

Ectomycorrhizal fungi and ex vitro *acclimatization*

Transfer of rooted adventitious shoots or somatic embryo plants from *in vitro* to *ex vitro* conditions in the greenhouse is one of the most critical steps in the propagation process. Poorly developed root systems may cause water stress and insufficient nutrient supply resulting in growth reduction and increased susceptibility to root pathogens. To overcome acclimatization problems, adventitious shoots have been inoculated with ECM fungi before (Supriyanto and Rohr, 1994; Normand et al., 1996) the *ex vitro* transfer, whereas germinated somatic embryos have been inoculated at the time of transfer (Sasa and Krogstrup, 1991; Niemi and Häggman, 2002). Survival of the rooted adventitious shoots of Scots pine increased from 20 to 70 percent as a result of mycorrhizal symbiosis (Supriyanto and Rohr, 1994). However, with somatic embryo plants of Sitka spruce *ex vitro*, the plants with the highest mycorrhiza colonization frequency had only slightly higher shoot and root dry weights than the control plants (Sasa and Krogstrup, 1991).

Improved adaptation after inoculation with ECM fungi has also been observed in the absence of mycorrhizal structures. When somatic embryo plants of Scots pine were inoculated with *P. tinctorius*, plant survival increased even though there was no Hartig net development in the roots. Inoculation resulted in either higher survival percentage or better shoot and root growth depending on the Scots pine cell line (Niemi and Häggman, 2002). The absence of the mycorrhizal structures in the developing root system suggests that the fungus modified the rhizosphere in a way that facilitated plant growth. ECM fungi may attract beneficial bacteria to grow in the rhizosphere. These, so-called mycorrhization helper bacteria (MHB) have been shown to stimulate mycorrhiza formation (Garbaye, 1994), but ECM fungi and MHB together may also reduce the growth of certain pathogenic fungi threatening the root system (Schelkle and Peterson, 1997).

Conclusion

Inoculation with specific ECM fungi has potential as a tool to improve adventitious root formation *in vitro* and *in vivo*, as well as acclimatization of the rooted *in vitro* shoots to *ex vitro* conditions. Studies on adventitious rooting have shown that PGRs produced by the fungi, such as IAA, ethylene and PAs, play a regulatory role in the interaction between specific ECM fungi and cuttings. However, the results have been highly variable depending on plant and fungus species and genotypes, as well as on culture conditions. ECM fungi may have different strain-specific factors associated with rooting, which alone or synergistically result in a positive response in compatible plant genotypes. In addition to PGRs produced by the fungi, strain-

specific effects on rooting substrate and soil microbes may be involved in the root–fungus interaction.

High concentrations of sugar and other nutrients in the media disturb the use of ECM fungi in somatic embryogenesis. The growth of the fungus may be aggressive, resulting in imbalance in the plant–fungus interaction. For somatic embryos the concentrations of nutrients are lowered in the germination medium, and germination can occur when a fungus and an embryo are not in physical contact. After the root of the germinated embryo has reached about 1 cm in length, physical contact is no longer detrimental and improves growth. Therefore, the phases of somatic embryogenesis with the highest potential for application of ECM fungi are the germination of mature somatic embryos *in vitro* and acclimatization of somatic embryo plants to *ex vitro*.

Propagation using cuttings is useful only when the growth of the rooted cuttings in the field is comparable to that of normal seedlings. Subsequent mycorrhiza formation does not appear to be necessary for root induction by the fungus on *in vitro* and *in vivo* cuttings. However, the further survival and subsequent growth may be improved by true ECM structures and, therefore, it is important to study whether the fungi that increased rooting also favor acclimatization in the greenhouse and field.

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