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REVIEW

Adventitious rooting of conifers: influence of physical and chemical factors

Carla Ragonezi · Krystyna Klimaszewska · Mário Rui Castro · Mónica Lima · Paulo de Oliveira · Maria Amely Zavattieri

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Abstract In conifers, vegetative propagation of superior genotypes is the most direct means for making large genetic gains, because it allows a large proportion of genetic diversity to be captured in a single cycle of selection. There are two aims of vegetative propagation, namely large-scale multiplication of select genotypes and production of large numbers of plants from scarce and costly seed that originates from controlled seed orchard pollinations. This can be achieved, in some species, either through rooted cuttings or rooted microshoots, the latter regenerated through tissue culture in vitro. Thus far, both strategies have been used but often achieved limited success mainly because of difficult and inefficient rooting process. In this overview of technology, we focus on the progress in defining the physical and chemical factors that help the conifer cuttings and microshoots to develop adventitious roots. These factors include plant growth regulators, carbohydrates, light quality, temperature and rooting substrates/media as major variables for development of reliable adventitious rooting protocols for different conifer species.

Keywords Cuttings · Gymnosperms · In vitro culture · Micropropagation · Microshoots

Abbreviations

ACC	1-Aminocyclopropane-1-carboxylic acid
AOA	Aminooxyacetic acid
ARF	Adventitious root formation
AVG	Aminoethoxyvinylglycine
BA	6-Benzyladenine
cGMP	Cyclic guanosine monophosphate
CW	Cool white light
DCR	Gupta and Durzan (1985)
GA_3	Gibberellic acid
GD medium	Gresshoff and Doy (1972)
GL	Growth-lux
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
L9 medium	Ewald (2007b)
LP medium	Quorin and Lepoivre (1977)
MAPK	Mitogen-activated protein kinase
MS medium	Murashige and Skoog (1962)
NAA	Naphthalene acetic acid
PGR	Plant growth regulator
PPFD	Photosynthetic photon flux densities
PS medium	*Pinus strobus* medium-Tang and Newton (2005a)
PBZ	Paclobutrazol
RD	Red-rich daylight
RIM medium	Abo El-Nil (1982)
RW medium	Risser and White (1964)
SH medium	Schenk and Hildebrandt (1972)

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C. Ragonezi · M. R. Castro · M. Lima · M. A. Zavattieri (✉)
Laboratory of Breeding and Plant Biotechnology,
Institute of Mediterranean Agricultural Sciences,
University of Évora, 7002-554 Évora, Portugal
e-mail: zavattieri@uevora.pt

K. Klimaszewska
Natural Resources Canada, Canadian Forest Service,
Laurentian Forestry Centre, 1055 du PEPS, P.O. Box 10380, Stn.
Sainte-Foy, Québec, QC G1V 4C7, Canada

P. de Oliveira
Laboratory of Soil Microbiology,
Institute of Mediterranean Agricultural Sciences,
University of Évora, 7002-554 Évora, Portugal

STS	Silverthiosulfate
TE medium	Tang et al. (1998)
TDZ	Thidiazuron
WPM medium	Lloyd and McCown (1981)
WW	Warm white

Introduction

Vegetative propagation of trees has been a useful tool in traditional tree improvement and holds important prospects for reforestation (Libby 1986). It provides the possibility for multiplication of select superior trees with favorable genetic combination and to produce genetically homogenous plant material that will grow predictably and uniformly. In addition, improved efficiency in management and finished product utilization may also be achieved (Sutton 2002).

Conifers (cone-bearing trees) are the best known and most important economically among gymnosperms, covering approximately 60% of the forested areas of the world, and are mostly used for the production of softwood lumber, pulp and paper (Wenger 1984). Conifers comprise eight families, 68 genera and 629 species (Farjon 1998) including pines (*Pinus* spp.), spruces (*Picea* spp.), cowtail pine (*Cephalotaxus* spp.), cypress pine (*Callitris* spp.), firs (*Abies* spp.), larches (*Larix* spp.), bald cypresses (*Taxodium* spp.), yellowwood (*Podocarpus* spp.), yews (*Taxus* spp.), arbor vitae (*Thuja* spp.) and junipers (*Juniperus* spp.) (Farjon 1998).

In spite of the major role conifers are bound to play in reforestation strategies, current research on their vegetative propagation is not sufficiently developed (Sutton 2002). In part, this is due to the slow progress in propagation methods, mainly because of rooting problems associated with the tree maturation phase, an age-related developmental process that affects reproductive competence, morphology, and growth rate (Greenwood and Hutchison 1993).This notwithstanding, commercial scale propagation through rooted cuttings of young trees has been reported for radiata pine (*Pinus radiata* D. 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) (Menzies et al. 2001). For a few economically important forest conifer species, an alternative vegetative, large-scale in vitro propagation technology has been developed, called somatic embryogenesis that utilizes mature or immature seed embryos as starting explants (reviewed by Klimaszewska et al. 2007). The advantages of somatic embryogenesis over rooted cuttings are: unlimited number of clonal somatic plants that can be produced from a single seed embryo (without a need for a separate step involving adventitious rooting) and the possibility of long-term storage of a given genotype in liquid nitrogen (cryopreservation). For example, in Norway spruce, both means of vegetative propagation, namely somatic embryogenesis and rooted cuttings of donor somatic trees, are being combined for clonal selection and commercial production of genetically superior seed families (Lamhamedi and Tousignant 2008).

Adventitious roots are post-embryonic roots that arise from the stem and leaves and from non-pericycle tissues in old roots. These roots may form naturally from stem tissue or may be induced by stressful environmental conditions, by mechanical damage or following tissue culture regeneration of shoots (Li et al. 2009). Adventitious root formation (ARF) is a critical step in vegetative propagation. An efficient rooting treatment can lead to a high percentage of rooting and a higher quality of the root system (De Klerk et al. 1997). Quality involves root number and length, and the absence of callus at the base of a shoot, all of which influence the performance of the plants after transfer to soil (Mohammed and Vidaver 1990). Many factors, during the rooting phases, can cause poor quality of the shoots at the time of planting, thus affecting growth (De Klerk et al. 1999; Hartmann et al. 2002; Mohammed and Vidaver 1990).

Adventitious rooting is a complex developmental process that consists of three successive but interdependent physiological phases: induction, initiation and expression, and each of these phases have different requirements. The induction phase comprises molecular and biochemical events without visible changes. The initiation phase is characterized by cell divisions and root primordia organization. The expression phase is characterized by intra-stem growth of root primordia and root emergence (Li et al. 2009). The chemical and physical factors that affect rooting include plant growth regulators (PGRs) (Wiesman et al. 1989; Davis and Haissig 1990), nutrients (the carbohydrate source foremost) (Wiesman and Lavee 1995), temperature and light (Haissig 1990; Corrêa and Fett-Neto 2004). Increasing body of knowledge on ARF pathway activation is generated from research on angiosperms. Recently, it has been discovered that nitrate, both a nitrogen source and a signal molecule, is transported by the NRT1.1 nitrate transporter and the transduction of nitrate signal is associated with a modification of auxin transport (Krouk et al. 2010). Thus, the NRT1.1 represses lateral root growth in *Arabidopsis* at low nitrate concentration by promoting auxin transport out of these roots. In mung bean, the adventitious root induction phase was regulated by a complex set of cellular messengers, among which some were activated by hydrogen peroxide, nitric oxide and

calcium (Li and Xue 2010). However, the signaling network responsible for root development has not been discovered yet.

In tissue culture, plant regeneration can be achieved either through the development of axillary shoot buds or through adventitious shoot formation, most frequently from callus. In both cases, the microshoots have to be rooted through adventitious rooting. The process of in vitro organ regeneration from the explants occurs through an apparent reversal of cell differentiation and acquisition of meristematic characteristics (Gahan 2007). Because many environmental and endogenous factors regulate rooting, some aspects of this regulation can be exploited to control rooting in vitro, through the application of chemicals, light and/or temperature control, or biotization. Different types of chemicals may be applied: PGRs to promote cell dedifferentiation, trigger the initial meristematic activity (Wiesman et al. 1989; Davis and Haissig 1990) and to promote the elongation and development of formed roots (Hartmann et al. 2002; Wiesman et al. 1989); nutrients to promote growth of the new roots (Wiesman and Lavee 1995); and protecting agents, such as biocides, to help protect against pathogens during the entire rooting period if carried out in a non-sterile substrate (Henrique et al. 2006).

Adventitious rooting in conifers has long been discussed, but the available information is fragmentary and circumstantial. Since the extensive articles by Gaspar and Coumans (1987) and Mohammed and Vidaver (1988) appeared over 20 years ago, no other review on root production and plantlet development in conifers has been published. Some aspects, widely discussed in those reviews, such as biological factors, root morphogenesis, genetic stability and acclimatization, are not covered in the present review. Instead, we focused on the progress in defining the chemical and physical factors that help the conifer cuttings and in vitro regenerated microshoots to develop adventitious roots. We compiled (mostly in the tabular form) the most successful, for a given conifer species, protocol/s of chemical treatments and physical factors that promoted adventitious rooting in both propagation systems.

Rooting of conifer cuttings

Four discrete stages of adventitious root formation in cuttings can be distinguished (Hamann 1998): (1) proliferation of cells at the base of the cutting, (2) differentiation of wound vascular tissue and periderm, (3) dedifferentiation of a zone near the wound cambium and wound phloem to form a root initial, and (4) formation of a root meristem. To obtain high-quality young plants in the shortest possible time, cuttings must root quickly and abundantly. Cuttings must also be able to produce lateral branching and grow fast after rooting (Moe and Andersen 1988). Propagation by cuttings has long been established in many conifer species. Ritchie (1991) calculated that more than 65 million rooted conifer cuttings were already produced around the world, and that half of this production was for sugi (*C. japonica*) in Japan, at least 10 million for radiata pine (*P. radiata*) in Australia and New Zealand, and about 21 million for Norway spruce (*P. abies*), Sitka spruce (*P. sitchensis*) and black spruce (*P. mariana*) in Canada, Scandinavia and the British Isles together.

In conifers, in addition to chemical and physical rooting treatments, the success of propagation by cuttings depends on a variety of other factors that include cutting collection time and season, cutting size, whether the needles are kept or not, condition and age of the source plant, plant nutritional condition, pruning treatments, and type and health of the cuttings at collection (Silva 1985). The role of donor plant growing conditions has long been recognized as important in influencing the rooting capacity of cuttings (Hartmann and Kester 1983; Moe and Andersen 1988).

Plant growth regulators

Auxins

For many decades, IBA has been applied to different plant species to induce adventitious roots, and conifers follow the rule. Nordström et al. (1991) attributed this preference, relative to IAA, to the higher stability of IBA. On the other hand, in many conifers the cuttings respond well to a pulse treatment with NAA. The combinations of various types of PGRs, the concentrations and application are extensive and are summarized in Table 1. Although IBA promoted rooting of cuttings in most of the conifers, in *Pinus* spp. NAA was also used at concentrations that varied between 1.6 and 2.7 mM. IBA was most frequently used at 24.6 or 49 μM mixed with talc or in water solution, and usually involved a quick dip or pulse treatment of the cut surfaces, with or without additional wounding, and was followed by transfer of the cuttings to substrates or to water nutrient solutions for rooting. In most cases, mixtures of sand, perlite and/or vermiculite were used in the substrates without any particular preference. The highest mean rooting percentage obtained in various experiments was 86% (Table 1).

Polyamines

Polyamines are generally considered to be growth regulators that are implicated in a range of developmental processes (Martin-Tanguy 2001; Kaur-Sawhney et al. 2003; Couée et al. 2004). It has been reported that the inhibition

Table 1 Treatments and growth conditions applied in two phases of rooting of conifer cuttings

Conifer species	Plant material	Root induction	Root growth	Environmental conditions	Rooting	References
Abies fraseri	Cuttings	IBA at 4 mM, 3 s dip	Horticultural perlite:peat 3:2 (v/v)	26.1 ± 2/20.5 ± 2°C day/night	31%	Rosier et al. (2004a, b)
Cedrus deodara	Cuttings	IBA at 5,000 ppm in talc or NAA at 10,000 ppm with activated charcoal, both with 1% captan and 1% sucrose	Unknown	Unknown	69%	Shamet and Bhardwaj (1995)
Cedrus deodara 'Shalimar'	Cuttings collected in late fall to early winter	IBA at 5,000 ppm, quick dip	Sand:perlite	Bottom heat maintained at 24°C. Greenhouse, 120 days under intermittent mist	67%	Nicholson (1984)
Chamaecyparis lawsoniana Parl.	Apical cuttings 15 cm long	IBA at 10,000 ppm for 5 s	Vermiculite	Not mentioned	99%	Stumpf et al. (1999)
Cupressus dupreziana Camus	Cuttings collected in early winter	IBA at 500–1,000 ppm, 24 h soaking in a solution	Coarse sand:perlite (1:1, v/v)	Bottom heat intermittent mist with ambient temperature of 15.5–23.8°C	90%	Nicholson et al. (1999)
Cupressocyparis leylandii Dallim. and A. B. Jackson	Callused cuttings, with a callus size of c. 1 cm diameter	IBA at 10,000 ppm and double wounding	Sterilized pumice (particle size 1–15 mm) and one part Southland peat (v/v).	Tunnel house, less than 25°C. Sun, tunnel shading of c. 40–50%.	43%	De Silva et al. (2005)
Juniperus scopulorum "Skyrocket"	Cuttings	K-IBA in 0.9% talc powder	Peat:perlite (2:1) medium under low polytunnels, no mist greenhouse	Unknown	96%	Bielenin (2003)
Larix x eurolepis (European × Japanese larch)	Cuttings	IBA at 0.5%in talc powder including a fungicide, dipped in the solution	Peat:compost:pouzzolane (2:1:3) under greenhouse conditions	Unknown	87%	Pâques and Cornu (1991)
Picea sitchensis (Bong.) Carr.	Cuttings	IBA at 10^{-6} to 10^{-5} M, water solution	PGRs were prepared as aqueous solutions and no nutrients were used	20°C, 16 h L, 70–90 LI, CW fluorescent lamps	70%, 20 days	Selby et al. (1992)
Pinus banksiana	Cuttings	NAA at 5.4 mM, pulse treatment for 10 s and then 1:1 (v/v) forestry mix/ vermiculite	Conventional polyethylene-covered greenhouse (poly-house)	10–30°C, 16 h L, 1,500 (sunny) 350 LI (cloudy)	87%	Browne et al. (2000)
Pinus banksiana	Cuttings	IBA at 25 mM in 100% ethanol, brief wetting of the cut basal surface of the cuttings	Intermittent water–mist in a sand–perlite substrate	750–900 LI (sunny) to 200 LI (cloudy)	94%, 25 days	Haissig (1990)
Pinus banksiana	Cuttings (central axis, 3 months)	NAA at 5.4 mM, pulse treatment for 10 s and then 1:1 (v/v) Forestry mix/ vermiculite	Phosphoglucoisomerase multipots (PGI-45, 110 ml, Plastiques Gagnon, Que.) filled with medium (1:1 v/v forestry mix/ vermiculite)	20–28/14 to18°C day/night, 16 h L and 350–700 (sunny) to 150–270 LI (cloudy), 500-W high-pressure sodium lamps	95%	Browne et al. (1996)
Pinus caribaea var. *hondurensis* Morelet	Cuttings	IBA at 19.7 mM, immersion in a gel solution for 2 s	50% carbonized rice hulls and 50% vermiculite	Intermittent mist system in an enclosed polyethylene propagation house	95%	Henrique et al. (2006)
Pinus contorta	Cuttings (hypocotyl)	IBA at 1.23 mM pulse treatment for 6 h and then in Hoagland nutrient solution	Brief rinse in water and culture in Hoagland solution	22°C and 200 LI, fluorescent tubes (Philips TLD 58 W/84) and incandescent light	100%	Lindroth et al. (2001)

Table 1 continued

Conifer species	Plant material	Root induction	Root growth	Environmental conditions	Rooting	References
Pinus elliottii var. *elliottii* × *P. caribaea* var. *hondurensis*	Cuttings (lateral tip)	Pasteurized coarse perlite:pine bark peat 1:1:1 (v/v/v), with additions of 0.5 kg Micromax m^{-3} (Granular by Scotts Australia) and 2.5 kg Osmocote m^{-3} (Low Start 5–6 month by Scotts Australia)	Controlled environment glasshouse with the appropriate temperature treatment	25°C, 11.5–13 h L, 70% of natural daylight irradiance (about 2,300–2500 micro-Einstein's in SE QLD)	83%, 12 weeks	Rasmussen et al. (2009)
Pinus radiata	Derooted seedling cuttings	IBA at 44.3 μM in 1/2 MS, 0.8% agar, 2% sucrose, for 10 d	Plant growth room	22°C with continuous lighting at 80 LI	95%	Li and Leung (2000)
Pinus sylvestris	Cuttings	IBA at 4,000 ppm dipped for 10 s in a solution of 95% ethanol and the PGR	Inserted in 90 ml containers filled with 60% peat and 40% perlite	25°C and then 20°C	54.4%	Högberg (2005)
Pinus strobus L.	Cuttings	NAA at 1.6 mM, 5 min pulse treatment	Moist silica:sand	24°C, 16 h L, 40 LI CW fluorescent tubes	97%, 4 weeks	Goldfarb et al. (1998)
Pinus taeda L.	Cuttings (seedlings and hedged donor plants)	Unknown	Perlite:vermiculite 1:1 (v/v)	20–27°C, 14 h L, CW fluorescent tubes	80%	Hamann (1998)
Pinus taeda L.	Cuttings (hypocotyl)	IBA at 10 μM, pulse treatment then transferred to distilled water for rooting	Unknown	27/20°C day/night, 16 h L, 100 LI, CW fluorescent tubes	82%, 15–30 days	Diaz-Sala et al. (1996)
Pinus taeda	Cuttings	NAA at 2.7 M, 5 min pulse as described by Diaz-Sala et al. (1996)	Unknown	16 h L, 90 LI, CW fluorescent tubes	Greater than 80%, 30 days	Greenwood et al. (2001)
Pinus taeda	Cuttings (hypocotyl)	IBA 10 μM in distilled water was stuck through holes in with styrofoamrafts (Hansen and Ernstsen 1982) and floated in trays made of PVC	The IBA solution was replaced with distilled water after 11 days	27/20°C day/night, 100 LI, fluorescent and incandescent lamps	94%, <25 days	Greenwood and Weir (1994)
Pinus virginiana	Cuttings (open-pollinated progeny)	IBA or NAA at 6 mM, applied for 3 s	Horticultural perlite:peat 3:2 (v/v)	25.5 ± 2/20.0 ± 2°C day/night temperatures	47% (semi-hard woody)	Rosier et al. (2004a, b)
Pseudotsuga menziesii (Mirb.) Franco	Cuttings, 15 cm long	IBA at 12.3–123 mM or NAA at 2.5–7.5 mM dipped for 10 s	Peat moss:fine sand 2:1 (v/v)	Rooting medium heated to 21°C and intermittent mist. Natural light in a conventional glasshouse	68%	Copes and Mandel (2000)
Thuja occidentalis 'Smaragd'	Cuttings	K-IBA 0.6% in talc powder, dipped	Peat:perlite 2:1 (v/v) under low polytunnels greenhouse without misting	Unknown	100%	Bielenin (2003)

LI light intensity in μmol m^{-2} s^{-1}

L photoperiod

of polyamine synthesis blocks the mitotic cell cycle by blocking the transition between G1 and S phase where increased levels of spermidine and spermine have been found (Couée et al. 2004). Both Martin-Tanguy and Carré (1993) and Tarenghi et al. (1995) hypothesized that the endogenous concentrations of polyamines might be growth limiting based on observations of the developmental stimulation of higher plants. A direct relationship between high polyamine content, such as putrescine and spermine, and the onset of ARF has been demonstrated, which accentuates the possible participation of these substances in the general cellular processes of division and differentiation in the rooting process (Couée et al. 2004; Martinez-Pastur et al. 2007). Polyamine metabolism has also been pointed as responsive to environmental circumstances, therefore playing an important role in the relations between plant and external conditions (Couée et al. 2004; Tang and Newton 2005b).

Tang and Newton (2005b) tested the influence of polyamines on the overall rooting frequency of *Pinus virginiana*. In their trials, the administration of 0.001 mM putrecine or spermidine in the NAA supplemented medium resulted in a 25% increase of rooting frequency, whereas 0.001 mM spermine caused a 6.7% decrease of rooting frequency.

Ethylene

The information concerning ARF in cuttings of conifers and ethylene is limited to a few articles. In general, Ethrel (a commercial formulation of the slow-release ethylene compound ethephon) promoted rooting and root growth in Engelmann spruce 2 weeks after planting (Scagel and Linderman 2000). Ethrel induced changes in root initiation of cuttings of Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] 2 weeks after application and these changes were positively correlated with subsequent increases in shoot growth. In these studies, the results indicated that exogenously applied Ethrel influenced root initiation indirectly by increasing levels of free IAA at the rooting site (Scagel et al. 2000). Ethrel was also reported to increase IAA conjugates in roots of Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), lodgepole pine (*Pinus contorta* Dougl.) and Douglas fir. On the other hand, Bollmark and Eliasson (1990) concluded that the enhanced rooting of Norway spruce (*P. abies*) hypocotyl cuttings, promoted by the treatment with ACC or Ethrel, was attributed to the ethylene-mediated acceleration of the breakdown of cytokinins.

When cuttings of Japanese black pine (*Pinus thunbergii* Parl.) were soaked for 10 min in Ethrel solution (69.2 μM ethephon) 24 h prior to soaking in Oxyberon (19.7 mM IBA solution), a significantly higher rooting ability was observed compared with the controls without the pretreatment with Ethrel (Mori, Miyahara, Tsutisumi, Kondo, unpublished). Similarly in *P. abies* L. (Karst), the hypocotyl cuttings produced 64 adventitious roots after 28-days treatment with 0.1 μM Ethephon compared with 22 roots in untreated controls and with two roots after treatment with the ethylene inhibitor $CoCl_2$ at 10 μM (Wang and Pan 2006).

Ethylene inhibitors

Several ethylene inhibitors, compiled by Kumar et al. (1998), inhibit both ethylene biosynthesis or ethylene actions in a plant. Among the most commonly used are aminoethoxyvinylglycine (AVG) and aminooxyacetic acid (AOA), both inhibiting 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and cobalt ions that inhibit the conversion of ACC to ethylene (Biddington 1992). Another inhibitor widely used is the silver ion, either as nitrate or as more mobile thiosulphate (STS), which excels in ethylene action inhibition (Beyer 1976). Inhibition of ethylene may lead to a lower number of adventitious roots, decrease of response to endogenous and exogenous auxins and reduced root hair formation (Clark et al. 1999). Ethylene itself has been reported to have no effect or even inhibit rooting depending on its concentration and genotypes, and therefore its role is still disputed (Mudge 1988). Inhibitors, when at high concentrations can promote stress, resulting in the synthesis of ethylene and root formation, thus defeating the purpose of their use. De Klerk et al. (1999) reported that STS may induce ethylene formation because silver is a heavy metal and damages the tissue. When STS was added along with auxins, the appearance of the rooted microcuttings at the time of transplanting was strongly improved (De Klerk et al. 1999).

As described by Kumar et al. (1998), ethylene inhibitors work within specific concentrations. The use of these substances under or above the recommended levels, might not have an inhibiting effect as desirable, or might promote the ethylene synthesis due to tissue damage (De Klerk et al. 1999). Also, there is not much work relating conifers with ethylene inhibiting substances, since the actual major scientific goal is to promote and improve conifer rooting and not the opposite. Nonetheless, such a study is fundamental to fully comprehend the role of ethylene in conifer ARF.

Plant growth retardants

Plant growth retardants are organic compounds that retard cell division and cell elongation (Arteca 1995). A number of growth retardants and inhibitors have been tested for their ability to influence rooting of cuttings. They are responsible for antagonizing the activity or inhibiting the

synthesis of gibberellins, which normally inhibit rooting (Hartmann and Kester 1983; Davis et al. 1988).

Henrique et al. (2006) investigated the effect of different levels of auxins (NAA, IBA) and gibberellin synthesis inhibitor (PBZ) on the rooting of 4–6 cm long shoots obtained from cuttings of *Pinus caribaea* var. *hondurensis* Morelet. Sixty days after planting, the IBA-treated cuttings rooted at a higher frequency than those treated with NAA, but IBA applied together with PBZ was the most effective treatment. However, daminozide (currently used as a growth retardant for many plants) inhibited rooting of *Chamaecyparis obutusa* seedling cuttings after spray application of 2,000 ppm wettable solution (Shigehiro 2006).

Carbohydrates

Non-structural carbohydrates usually accumulate in needle fascicles during propagation, sometimes after an initial decrease. However, concentrations of specific carbohydrates such as sucrose or glucose in needle fascicles may not be uniform among tissues or with time during propagation (Veierskov 1988). Perhaps, the changing concentrations of specific carbohydrates in cuttings during propagation are linked to the direct control of ARF, for instance, because auxin treatments often concomitantly promote adventitious rooting and modify concentrations of individual carbohydrates within specific regions of cuttings during propagation (Haissig 1990).

Nevertheless, in a study with Sitka spruce cuttings, little correlation between rooting and concentration of sugars in stems and foliage was found (Van den Driessche 1983). It is possible that interaction between carbohydrates and hormones, nitrogen and carbon ratios, light and carbohydrate and also temperature pre-treatments, as well as the carbohydrates status of the mother plants, make the comparison of results from different studies difficult.

Light

Roots of *P. radiata* cuttings from seedlings had the highest dry weight under high PPFD, which had a red to far-red ratio similar to daylight (Wenger 1984). In other experiments with *P. radiata*, the best rooting conditions for short shoots were: treatment with 50 ppm IBA for 24 h, then planting at 20–25°C under a 12-h photoperiod (as compared to 18-h photoperiod or continuous illumination). The needle fascicles rooted better if they were collected during winter or early spring, suggesting a direct influence of short days (Kummerow 1966).

Both McClelland et al. (1990) and Corrêa et al. (2005) have stressed the need for increased efficiency of the process and that rooting should be carried out in the dark for the first few days. However, rooting can be influenced by light intensities, as reported by Kunneman and Ruesink (1997) who showed better responses to 66–83 μmol s^{-1} m^{-2} in a few *Juniperus* cultivars, while 27–37 μmol s^{-1} m^{-2} were more suitable for the *Chamaecyparis* and *Cupressocyparis* cultivars.

Temperature

A temperature range between 18 and 27°C is commonly used during rooting of the cuttings. The effect of artificial light, CO_2 and temperature on rooting in ornamental cultivars of *Chamaecyparis*, *Cupressocyparis* and *Juniperus* was studied by Kunneman and Ruesink (1997). These authors demonstrated that rooting was best at a constant temperature of 23°C, compared with 17 and 29°C. On the other hand, for *Pinus taeda* L., the best rooting of cuttings (seedlings and hedged donor plants) (80%) was obtained by maintaining the air temperature at approximately 27°C during the day (14-h photoperiod) and 20°C at night (Hamann 1998), and the temperature of the rooting medium was kept at 25°C using a root zone heating system.

Cedrus spp., in general, are difficult to root, for example *Cedrus libani* A. Rich. is considered almost impossible to propagate by cuttings; however, *Cedrus deodara* (Roxb.) G.Don 'Shalimar' can be rooted to 67% if cuttings are collected in late fall to early winter and, after a quick dip in IBA solution, placed in a sand–perlite medium maintained at 24°C with bottom heat (Nicholson 1984 cited by Pijut 2000). In Table 1, other examples of temperature treatments for adventitious root formation in conifer cuttings are listed.

Substrates for rooting

The blends of propagation substrates should create suitable air and drainage characteristics and remain moist, but not waterlogged during the period of time that roots are initiated. Different substrates can be used to promote rooting in conifer cuttings, the most common being vermiculite, perlite or a combination of both. However, the requirements of various species can be very different. Davidescu et al. (2003) found that propagation by cuttings of *Thuja occidentalis* L. 'Columna' and *T. occidentalis* 'Danica' was best in peat substrate in August. Six substrates were used in *Picea* cuttings by Mazăre et al. (2007): sand, perlite, peat, sand with perlite, sand with peat and perlite with peat, all at 1:1. The rooted cuttings were at a higher proportion in sand with peat, but increased rooting index (13% as visually established based on the number of primary and secondary roots) was obtained in perlite with peat.

Rooting of loblolly pine (*P. taeda*) cuttings has been extensively studied, but the description of substrates used

Table 2 Treatments and growth conditions applied in two phases of rooting of conifer microshoots in vitro

Conifer species	Plant material	Root induction	Root growth	Environmental conditions	Rooting (%)	References
Juniperus oxycedrus	Shoots (terminal shoots from lateral branches—5 cm length)	NAA, IAA or IBA separately, or in combination in solidified medium B (SH macronutrients supplemented with 3% sucrose, 0.7% agar) for 30 days	The same medium without auxins	26 ± 2°C, 16 h L, 80 LI, Gro-lux (F36W/GRO)	None of the auxins or auxin combinations tested promoted satisfactory rooting. The frequency of rooted shoots ranged from 7 to 10%	Gómez and Segura (1994)
Juniperus phoenicea	Shoots 2–3 cm long (from axillary buds)	IBA at 2.4 μM, 5 min dipping and cultured on medium without PGRs	Plantlets with 2 cm long roots were transferred to pots with sterilized mixture of peat:perlite 3:2 (v/v)	22 ± 1°C, 16 h L, 400 LI, *OSRAM* (Munich, Germany) L36W/21lamps	40%	Loureiro et al. (2007)
Larix sp.	Shoots (newly formed shoot tips, approximately 3–4 cm long and without any visible bud primordia)	NAA at 10.7 μM in L9 medium for 2 weeks	After 2 weeks, the induced shoots were transferred directly into Jiffy-7 peat pellets (ø 42 mm) saturated with water	17°C and 16 h L, white light (OSRAM L58W/31–830)	100%	Ewald (2007a)
Picea sitchensis	Adventitious shoots (from cotyledons)	PGR-free ½MS, 0.7% agar	Transfer to a mixture of equal volumes of Levington M3 compost (Fisons, Ipswich, UK), perlite and vermiculite (William Sinclair Horticulture Ltd., Lincoln, UK) in 9 cm diameter plastic pots	20 ± 1°C, 16 h L, 1.5 LI, CW fluorescent lamps	84%, 22 weeks	Drake et al. (1997)
Picea chihuahuana	Adventitious shoots (from embryos)	IBA at 14.8 or 24.6 μM in ½ SH liquid medium, placed vertically on filter paper for 48 h	Fresh liquid medium (1/2 SH) without PGRs	26 ± 2°C, 16 h L, 46–48 LI	±8.5%	López-Escamilla et al. (2000)
Pinus armandii var. *amamiana*	Adventitious shoots (from embryos)	IBA at 4.9–14.8 μM in RIM medium	Transfer to pots with florialite containing 0.1% hyponex for 2 weeks under 100% humidity	25°C, 16 h L, 70 LI, fluorescent light	Unknown	Ishii et al. (2007)
Pinus ayacahuite	Adventitious shoots (from zygotic embryos)	NAA at 100 μM solution for 8 h	Transfer to ½ GD medium PGR-free with 0.05% activated charcoal, 30 mM sucrose and 1% agar	25 ± 1°C, 16 h L, 60–80 LI, Sylvania Gro-Lux F40T12 Gro-WS lights	40%	Saborio et al. (1997)
Pinus contorta	Adventitious shoots (from embryos)	IBA at 1.23 mM for 6 h and then liquid medium	12 weeks later potted in mineral wool	Unknown	70%	Högberg et al. (2005)
Pinus contorta	Adventitious shoots (from embryos)	IBA at 1.23 mM, pulse treated for 6 h	Cultured in liquid mineral nutrient solution, composed according to the nutrient requirements of *P. sylvestris* given by Ingestad (1979) with N concentration at 4.5 mM	20°C, 110 LI, fluorescent light supplemented with incandescent light	77%	Flygh et al. (1998)
Pinus heldreichii	Adventitious shoots (from embryos)	IBA at 1 mM, pulse treatment for 2 or 5 h, afterward transferred to ½GD with 2% sucrose	Transfer to a greenhouse	25 ± 2°C, 8 h L, 47 LI, white fluorescent tubes	14%, for up to 18 weeks	Stojicic et al. (1999)

Table 2 continued

Conifer species	Plant material	Root induction	Root growth	Environmental conditions	Rooting (%)	References
Pinus brutia × *Pinus halepensis*	Shoots (from fascicle buds—clone A and B)	IBA at 2.46 μM plus NAA at 2.7 μM plus 0.65% agar (Sigma) plus 1.5% sucrose	Transfer after 7 days to a mixture of peat:perlite 1:1 (v/v)	14–19°C, 18 h L, 65–70 LI, high-pressure lamps (HPI/T, SON, 400 W)	84% clone A, 32% clone B, 10–16 weeks	Scaltsoyiannes et al. (1994)
Pinus canariensis	Adventitious shoots (from adventitious buds induced from cotyledon explants)	IBA at 1 mM, 4 h liquid pulse treatment	Transfer to peat:vermiculite, 1:1 (v/v)	Unknown	83%	Martínez Pulido et al. (1990)
Pinus eldarica	Adventitious shoots (from adventitious buds induced from cotyledon explants)	BA at 0.22 μM, IBA at 10 μM and NAA at 5 μM in ½ SH medium	½ SH medium with 1% activated charcoal. Vermiculite:perlite:peat 2:2:1 (v/v) in plastic bags to maintain high humidity for 4 weeks	16 h L, 26/18°C day/night, 250 LI	78%	Sen et al. (1994)
Pinus massoniana L.	Adventitious shoots (from adventitious buds induced from mature embryos)	IBA at 9.8 μM, BA at 2.2 μM and 2% sucrose in ½ GD medium for 1 week	Subculture onto ½ GD medium with IBA at 0.98 μM and BA at 2.2 μM for 4 weeks. Then, transfer to pots filled with a mixture of vermiculite and perlite 3:1 (v/v)	25 ± 2°C, 14 h L, 80 LI, CW fluorescent tubes	70%	Zhang et al. (2006)
Pinus elliotti	Adventitious shoots (induced from callus)	IAA at 1 μM and IBA at 1 μM	Perlite:peat moss:vermiculite 1:1:1 (v/v/v)	23°C, 16 h L, 100 LI, CW fluorescent tubes	26–35%	Tang and Newton (2007)
Pinus kesiya	Adventitious shoots (from 2 to 3 week old seedling explants)	NAA at 16.1 μM in GD medium for 24 or 120 h	Subculture to the same medium without PGR	25 ± 1°C, 16 h L, 50–70 LI, CW fluorescent and incandescent lamps	67%	Nandwani et al. (2001)
Pinus pinaster	Adventitious shoots (from adventitious buds induced from embryos)	NAA at 5.4 μM in induction medium (RW macroelements, ½ MS microelements, 50 mg l^{-1} myo-inositol, 2 mg l^{-1} glycine, 1 mg l^{-1} thiamine, 1 mg l^{-1} pyridoxine, 1 mg l^{-1} nicotinic acid, 1% sucrose)	Same medium without PGRs with 3% sucrose. Then, transfer to a sterile peat:vermiculite, 1:1 (v/v)	25 ± 1°C, 16 h L, 80 ± 5 LI	86%, 3 weeks	Álvarez et al. (2009)
Pinus pinaster	Shoots (from axillary buds)	NAA at 10^{-6} M for the first 16 days in basal nutrient medium with nitrate at 3.3 mM and glutamine at 2 mM	Unknown	Unknown	92%, 30 days	Faye et al. (1989)
Pinus pinea L.	Adventitious shoots (from cotyledons)	IBA at 10 μM in DCR (½ macroelements) with 3% sucrose for 10 days	Peat:sand:perlite (2:1:1 v/v) mixture	23 ± 1°C, 16 h L, 80–100 LI	34%	Capuana and Giannini (1995)

Table 2 continued

Conifer species	Plant material	Root induction	Root growth	Environmental conditions	Rooting (%)	References
Pinus pinea L.	Adventitious shoots (from embryos)	NAA at 10 μM in ½ LPC (LP medium with 0.5% (w/v) of activated charcoal), 20% glucose and 0.8% Roko-Agar	The same medium without PGRs and then transferred to sterile peat:perlite 1:4 (v/v)	1 week at 19°C dark, 2 weeks at 19°C, 16 L, 100 LI and then 21°C 16 h L, 100 LI, white fluorescent tubes	68%, 3–6 weeks	Alonso et al. (2006); Ordás et al. (1999)
Pinus pinea L.	Adventitious shoots (from cotyledons)	NAA at 0.05 μM in ½ MS medium	Unknown	23°C, 16 h L, 60–70 LI, CW fluorescent tubes	15–20%	Sul and Korban (2004)
Pinus pinea L.	Adventitious shoots (from cotyledons)	NAA at 10.7 μM in ½ WPM macroelements with 0.117 M glucose and 0.8% agar	The same without PGR and with 58.4 mM glucose	1 week at 19°C dark, 2 weeks at 19°C, 16 h L, 90 LI and then 21°C, 16 h L and 90 LI, CW fluorescent tubes	70%, 4 weeks	Ragonezi et al. (2010)
Pinus pinea L.	Adventitious microshoots (from cotyledons)	NAA at 10.7 μM with 0.117 of sucrose or 0.117 M of glucose in WPM gelled with 0.65% Difco Bacto-agar	The same without PGR and with 58.4 mM sucrose	Induction for 2 weeks and then for expression 25/19°C day/night 16 h L	Average 53% when 0.117 M of glucose was used in the medium	Zavattieri et al. (2009)
Pinus radiata	Shoots (from isolated meristems)	IBA at 8.2 mM and NAA at 5.4 mM in 5% National Midesa agar for 5 d	Transfer to ½ LP medium supplemented with 10% sucrose	24 ± 2°C, 16 h L, 20–30 LI, CW fluorescent tubes	28%	Prehn et al. (2003)
Pinus radiata	Shoots (seedlings from a mixed population of open-pollinated seed)	NAA, IBA and BAP at 2.7, 5.0 and 0.11 μM, respectively, for 10 days in SH macro- and micro-salts, 3% sucrose	Transfer to ½ SH medium with 1% sucrose and without PGR	23 ± 2°C, 16 h L 80 LI CW—fluorescence tubes	43%	Schestibratov et al. (2003)
Pinus roxburghii Sarg	Shoots (from axillary buds)	BA at 10 μM in ½ MS medium with 2% sucrose and 0.6–0.8% agar	Transfer to semisolid (0.6% agar) and liquid ½ MS with filter paper bridges for elongation of roots. The rooted plantlets were washed thoroughly and transferred to liquid ¼ MS medium containing 1% sucrose and absorbent cotton	25 ± 2°C, 16 h L, 30 LI, CW fluorescent tubes	97%	Kalia et al. (2007)
Pinus sylvestris	Shoots (from axillary buds)	NAA at 53.8 μM in 0.6% water agar. Shoots were placed for 24 h	Transfer to l/2-strength basal medium (1/8-strength MS medium as modified by Cheng (1975), supplemented with 3% sucrose, 1% Difco Bacto-agar) with 1% sucrose and 1% agar	26 ± 2°C, 16 h L, 107–240 LI, Sylvania Gro-Lux and fluorescent LV 20	64%	Zel et al. (1988)

Table 2 continued

Conifer species	Plant material	Root induction	Root growth	Environmental conditions	Rooting (%)	References
Pinus sylvestris	Adventitious shoots (from zygotic embryos)	NAA at 0.67 mM in 1/6 MS medium for 24 h	The same medium without PGR	Unknown	33%	Sonia Tsai and Huang (1985)
Pinus sylvestris	Adventitious shoots (from cotyledons)	NAA at 2.7 μ*M* ½ GD medium ½ micronutrients, ½ macronutrients, and ½ organics of those in GD medium 1% agar	The same medium without PGR for 4 week	Unknown	6%	Häggman et al. (1996)
Pinus strobus L.	Adventitious shoots (from zygotic embryos)	IAA at 0.01 mM and IBA at 0.01 mM in PS medium	Perlite:peat moss:vermiculite (1:1:1 v/v) in a greenhouse	24°C, 16 h L, 50 LI, CW fluorescent tubes	36%, 6 weeks	Tang and Newton (2005a, b)
Pinus taeda L.	Adventitious shoots (from zygotic embryos)	IBA at 2.46 μM, GA_3 at 1.44 μM and BA at 4.43 μM in TE medium	Vermiculite:commercial compost 3:1 (v/v)	25°C, 16 h L, 100 LI, CW fluorescent tubes	Unknown	Tang and Guo (2001)
Pinus virginiana Mill.	Adventitious shoots (from zygotic embryos)	NAA at 0.05 μM in TE medium for 6 weeks	Established in soil in a greenhouse	24°C, 16 h L, 50 LI, CW fluorescent tubes	18%	Tang et al. (2004)
Pseudotsuga menziesii (Mirb.) Franco	Adventitious shoots (from cotyledons)	NAA at 10.7 μM in ½DCR with 1% sucrose for 6 days (2 days in darkness and 4 days in light)	The same without PGR (only in light)	Unknown	40%, 4 weeks	Hutzell and Durzan (1993)
Sequoia sempervirens (Lamb.) Endl	Shoots (from axillary buds)	IBA at 12.3 μM in ½ MS medium with 2% sucrose, 0.75% Difco agar, for 3 months	The same without PGR	22 ± 3°C, 9 h L, 60 LI, CW fluorescent tubes	61%	Blazkova et al. (1997)
Taxus meirei	Shoots (from steckling)	IBA at 12.5 μM ½ MS medium supplemented with 20 g l^{-1} sucrose for 3 months	The same without PGR	24 ± 1°C, 16 h L, 45 LI, CW fluorescent tubes	55%	Chang et al. (2001)
Taxus brevifolia	Adventitious shoots (from zygotic embryos)	Treated with ABT rooting powder (ABT Research Center, Beijing, China)	Transfer rooted shoots to 2-inch pots of plant growth medium (vermiculite: perlite 1:1 (v/v), J. Mollenma Co., Grand Rapids, MI, USA)	26°C, 16 h L, 80 LI	58%	Chee (1995)
Taxus baccata L.	Shoots (from closed buds or shoot tips)	IBA at 9.8 μM, spermidine at 6.88 mM and TDZ at 4.5 mM in 1/3 L9 medium with 0.5% sucrose	JIFFY 7 peat pellets saturated with water	15–17°C, 16 h L, 30 LI, white light radiation	Unknown	Ewald (2007b)
Thuja occidentalis L.	Adventitious shoots (from zygotic embryos)	IBA at 25 mM in 1/3 MS, 3% sucrose and 0.7% agar	Transfer to autoclaved Redi-Earth (W.R. Grace & Co., Ontario)	20°C, 16 h L, 30–40 LI	60%, 3–4 weeks	Harry et al. (1987)

LI light intensity in μmol m^{-2} s^{-1}

L photoperiod

was not always included. Hamann (1998), working with this species, used a substrate consisting of equal parts of perlite and coarse vermiculite, to a depth of 1.5 ± 2 cm and obtained 80% rooting. In Brazil, with the same species, the cuttings were placed in plastic tubes containing Mecplant (substrate composed of biostabilized pine bark) overlaid with vermiculite (Alcantara et al. 2007). Table 1 shows a compilation of different substrates applied to conifer cuttings.

In vitro rooting of conifer microshoots

Many basic studies on rooting are now carried out in vitro. Using seedling explants and in some cases also explants from mature trees, it has become current, in some species, to produce rooted micropropagated shoots (microcuttings) by in vitro organogenesis (Niemi et al. 2004). Tissue culture method facilitates administration of PGRs and other compounds and avoids microbial degradation of applied compounds (De Klerk et al. 1999). According to many workers, further research is required on the influence of factors such as donor plant age, genotype and type of explant, microcutting quality, auxin treatment, root system and environmental conditions on rooting and acclimatization (Fett-Neto et al. 2001; Greenwood et al. 2001; Bielenin 2003; Henrique et al. 2006). Adventitious rooting of microshoots is characterized by the same four phases as rooting of cuttings (see above).

Rooting medium

The success of plant tissue culture as a means of plant propagation is greatly influenced by the composition of the culture medium. In vitro rooting of conifer microshoots usually occurs in gelled nutrient media (mostly agar- and gellan-gum based) as substrate. This ensures the consistent distribution of PGRs, macro- and micronutrients, and also provides a better contact between shoots and substrate, resulting in more synchronous rooting (Mohammed and Vidaver 1990). However, the quality of produced roots is not always satisfactory. Gelled media probably obstruct gas exchange and inhibit the development of the vascular system in roots, as well as the production of root hairs (Skolmen and Mapes 1978). Culture media and physical supports currently used for rooting of conifer shoots are listed in Table 2. Nutrients are usually reduced to half the strength of that used for shoot production (Blazkova et al. 1997). In general, it has been reported that lower concentration of salts in the culture medium, particularly nitrogen, seems to favor the adventitious rooting of cuttings (Ordás et al. 1985). In our experience, reducing the WPM macronutrients to half strength increases significantly the percentage of rooted microshoots of *Pinus pinea* L. (Ragonezi et al. 2010).

Plant growth regulators

In vitro organogenesis is a complex series of events that a cell or groups of cells undergo in response to external/internal stimuli such as phytohormones. According to Thorpe (1980), organogenesis is a developmental process that comprises (a) attainment of competence or pre-induction phase, (b) induction or determination phase, and (c) expression phase or post-initiation phase. Cell/tissue responses to form adventitious roots may be different according to species, physiological status of the explants, the phase of the rooting process, and the interaction of the chemical and physical factors of the culture. Table 2 summarizes the available information in the scientific literature, including species, rooting induction treatments, culture media, physical conditions, light regimes and rooting percentages.

Most frequently, the treatments involved IBA (13 references) or NAA (15 references). For five species, a mixture of IBA either with NAA or IAA was used. On the other hand, one research report cited 97% rooted shoots of *Pinus roxburghii* when 10 μM BA was applied before transfer to a liquid medium for root expression. In one experiment, *Taxus brevifolia* treated with ABT rooting powder (developed by ABT Research and Development of Chinese Academy of Forestry) produced roots in 58% of the microshoots derived from cotyledon explants. In one case, it was possible to root 84% of the microshoots of *P. sitchensis* derived from cotyledons without any application of PGRs and by rooting directly in a substrate mix. IBA was applied at concentrations that ranged from 1 to 25 μM, most often between 2.5 and 14.8 μM, and only in one study IBA was applied at 25 μM. NAA concentrations varied between as low as 0.05 and 100 μM, with 10.7 μM being the most commonly used. However, the best results were obtained with NAA at concentrations higher than 50 μM, while low concentrations gave poor rooting percentages. The physical support for the shoots during the root expression phase was either a substrate (16 references) or a culture medium without PGRs (18 references).

Carbohydrates

Sucrose is commonly used in tissue culture media because it is the main sugar translocated in the phloem of many plants. However, other carbohydrates such as glucose and fructose have been also used to improve organogenesis (Faye et al. 1989; Ordás et al. 1999; Zavattieri et al. 2009). The exogenous sucrose (in the presence or absence of auxin) is beneficial for the rooting of most herbaceous and

woody plants (Haissig 1982). Generally, enrichment with sucrose improves rooting, but this has its limits, as sucrose at high concentrations tends to have negative effects, especially during the root expression phase. A negative interaction between carbohydrates and light could emerge at such high concentrations, either through transformation of added sugars into soluble and storage forms, or through altered nitrogen/sucrose or auxin/sucrose ratios (Moncousin 1991).

In conifers, the data on the influence of carbohydrates in adventitious rooting are limited. Zavattieri et al. (2009) made a direct comparison between different carbon sources (sucrose or glucose at different concentrations) for the induction and expression phases of the adventitious roots in microshoots of *Pinus pinaea*. An increased number of roots per shoot and an accelerated root formation were consistently obtained using glucose. However, there were no differences in the overall frequency of rooting. Light (16-h photoperiod, 25/19°C day/night) and less sugar were beneficial for the ensuing root expression phase. Large differences in the ability to form roots were observed among clones with the rooting percentages ranging between 0 and over 75%. Other examples can be found in Table 2.

Light

Plants grown in vitro have been in most cases subjected to fluorescent lamps. These fluorescent lamps have a broad emission peak in the yellow–red region of the spectrum with different spectral emissions and wavelengths from 350 to 750 nm. However, little attention has been given to the wavelength specificity and its effect on organogenesis, especially in ARF.

Broad-spectrum CW light is often used in rooting studies conducted in vitro (Flygh et al. 1998; Stojičić et al. 1999; Zhang et al. 2006; Ishii et al. 2007; Tang and Newton 2007). Different types of light sources such as Growth-lux (GL) (Gómez and Segura 1994), high-pressure lamps (Scaltsoyiannes et al. 1994) and their combinations such as fluorescent light supplemented with incandescent light (Flygh et al. 1998) are also applied. Different light quality influenced the rooting frequencies according to the PPFD used. Under the intensity of 90 µmol m^{-2} s^{-1} from CW lamps, 70% microshoots of *P. pinea* rooted; however, under GL lamps, with the same intensity, rooting was <50% (Ragonezi et al. 2010).

In the case of shoots of Sitka spruce (*P. sitchensis*), the rooting frequency was high (84%) when cultivated under low illumination (1.5 µmol m^{-2} s^{-1}) (Drake et al. 1997), but in *Juniperus phoenicea* 40% of rooting was achieved under light intensity of 400 µmol m^{-2} s^{-1}. The majority of studies relating to ARF applied light intensities that varied from 60 to 80 µmol m^{-2} s^{-1} (Gómez and Segura 1994; Scaltsoyiannes et al. 1994; Nandwani et al. 2001; Zhang et al. 2006; Ishii et al. 2007). The influence of light (quality) on ARF in shoots of other species is listed in Table 2.

Photoperiod

A photoperiod of 16 h is generally used for in vitro rooting of most conifers (Anderson and Ievinsh 2002; Parasharami et al. 2003). However, different photoperiodism requirements to induce ARF have been published. As an example Burkhart and Meyer (1991), while testing the effect of GA inhibitors to promote in vitro rooting of axillary shoots of white pine (*Pinus strobus* L.), obtained 43% rooted shoots with a pulse treatment of NAA under a long (18 h) photoperiod with CW lamps at 50 µmol m^{-2} s^{-1}. In *Picea glauca* (Moench) Voss, a higher percentage of shoots rooted under continuous light (Campbell and Durzan 1975).

Temperature

Most rooting protocols reported temperatures in the range of 23–27°C (Table 2). Apart from the example of *T. occidentalis* shoots that rooted at 60 and 10% at 25 and 20°C, respectively (Harry et al. 1987), the lower temperature ranges tended to be beneficial in the induction of roots from shoots of many other gymnosperms. *Picea glauca* rooting was greater at 20/18°C day/night temperature regimes, compared with 24/18, 20/15 or 25/25°C (Rumary and Thorpe 1984); in Douglas fir, 19°C promoted rooting and normal plants, while at 24°C few roots formed along with callus at the stem/root junction, causing discontinuity in the vascular system (Cheng 1977). In white pine (*P. strobus*), the highest rooting frequency was obtained after elongated shoots were treated at 4°C for 4 weeks (Tang and Newton 2005a). The positive effect of combining low temperature and darkness in the induction phase (for the first 2 weeks) was observed in *P. pinea* L., which rooted at a higher percentage at 19°C compared with 25°C (Ragonezi et al. 2010). This is in agreement with the results reported for *P. menziesii* (Cheng and Voqui 1977) and *P. radiata* (Smith 1986). A possible explanation for the effect of low temperature and darkness in promoting ARF was given by Hartmann et al. (2002). They noted that under these physical environmental conditions, fewer cell wall deposits, less vascular tissue and thinner walls might have facilitated the movements of exogenous PGRs to regeneration sites. In loblolly pine (*P. taeda*), Hutchison et al. (1999) observed that during the first 2 days of the rooting process the cambium layer of the hypocotyls dedifferentiated into parenchyma cells in both hypocotyls and epicotyls. Since dedifferentiation

is a part of the regeneration process (Christianson and Warnick 1983), a higher proportion of already undifferentiated cells may improve shoot or root organogenesis. A possible effect of low temperature and darkness could be explained by their influence on auxin metabolism in relation to rooting through modification of peroxidase activities and formation of endogenous phenolic compounds (Druart et al. 1982).

Conclusions

Although difficult to unify, the research results covered in this review highlight some tentative suggestions to explore the physicochemical variables in experimental rooting of conifers, as a guideline for development of more effective conditions for each species. This review also shows the difficulty in establishing correlations between species, PGR concentrations and treatments or any other of the variables cited.

However, an ongoing research on elucidating important aspects of ARF signaling network in angiosperms should eventually provide a better understanding of the process and aid in developing efficient rooting protocols. Whether the same or similar molecular events will be identified in conifers, the evolutionary and physiologically different organisms, remains unknown. A study undertaken by Brinker et al. (2004) in *P. contorta* showed that the transcription level of 200 genes changed from root induction to development suggesting a complex network of interactions in this conifer species.

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