TRACHEARY ELEMENT DIFFERENTIATION AND SECONDARY CELL-WALL FORMATION IN CELL CULTURES OF CONIFEROUS GYMNOSPERMS*

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ABSTRACT

Tracheary element differentiation and secondary cell-wall formation have been studied in cell cultures of coniferous gymnosperms. Factors that influence tracheary element differentiation are (i) the sucrose concentration in the media, (ii) the concentration of nutrients in the media, (iii) temperature and light, and (iv) the types and concentrations of phytohormones in the media. There are advantages and disadvantages in using cell cultures for studying tracheary element differentiation and secondary cell-wall formation, but in combination with *in planta* studies the cell culture approach is very useful for advancing our understanding of these processes.

Keywords: tracheary elements; tracheids; secondary cell wall; cell differentiation; plant cell culture; functional gene testing; model systems; xylogenesis.

INTRODUCTION

Tracheary element (TE) differentiation *in vitro* has been studied for more than 50 years using many different plant species (reviewed by Torrey *et al.* 1971; Roberts 1976; Fukuda & Komamine 1985; Roberts *et al.* 1988; Fukuda 1992; Bolwell & Robertson 2000; Leitch & Savidge 2000). Tracheary elements can easily be recognised under the microscope and their differentiation can be induced under controlled culture conditions. Tracheary element differentiation *in vitro* is therefore an excellent model for studying plant cell differentiation.

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The *Zinnia elegans* L. mesophyll cell culture system has emerged as the preferred experimental model system for analysing molecular and biochemical aspects of tracheary element differentiation in angiosperms (reviewed by Fukuda 1992; McCann 1997; McCann, Domingo, Stacey, Milioni, & Roberts 2000). However, tracheary element differentiation and secondary cell-wall formation have also been studied in cell cultures of coniferous gymnosperms (Durzan *et al.* 1973; Washer *et al.* 1977; Ramsden & Northcote 1987; Havel *et al.* 1997; Möller *et al.* 2003, 2005; Möller, Ball, Henderson, Modzel, & Find 2006; Möller, Koch, Nanayakkara, & Schmitt 2006). These studies are important from both a scientific and an economic point of view. Coniferous gymnosperms evolved much earlier than angiosperms and have a different vascular system. Also, secondary cell-wall structure and composition of tracheary elements from coniferous gymnosperms is different to that of tracheary elements from angiosperms. From an economic point of view the coniferous gymnosperms are of major importance as a source for biomaterials. Tracheary element differentiation *in vitro* is important for cell-wall and biomaterials research because of the biosynthesis of secondary cell walls during tracheary element development. The wood of coniferous gymnosperms consists mainly of tracheary elements, and the composition and structure of their secondary walls influence the properties of biomaterials produced from this raw material. Tracheary element differentiation *in vitro* can serve as a model to advance our understanding of secondary cell-wall formation and to discover biotechnological ways to design secondary cell walls with desired properties.

This paper discusses the advantages and disadvantages of using cell cultures for studying tracheary element differentiation and secondary cell-wall formation and reviews the use of cell cultures of coniferous gymnosperms in this interesting field of research.

TRACHEARY ELEMENTS

Tracheary elements are nonliving, water-conducting cells with lignified walls or wall thickenings that are present in the xylem tissue of plants (Torrey *et al.* 1971; McCann 1997). According to Esau (1965a), the term "tracheary element" is derived from "trachea", a name originally applied to certain primary xylem elements resembling insect tracheae. Tracheary element now applies to tracheids and vessel members or elements. Although both tracheids and vessel members occur in angiosperms, only tracheids occur in the xylem of coniferous gymnosperms (Core *et al.* 1979). In intact plants, tracheary elements normally differentiate from meristematic cells of the procambium and/or cambium (Esau 1965b; Larson 1994). The tracheary elements derived from the procambium become part of the primary xylem, whereas those derived from the vascular cambium become part of the secondary xylem (wood). Tracheary elements can also *trans*-differentiate from

parenchyma cells after wounding, during lateral root formation, host-graft interactions, or host-parasite interactions, to re-establish a continuous water conduction system for the plant (Jacobs 1952; McCann 1997). Furthermore, tracheary elements can differentiate from cells in callus and suspension cultures (Fig.1and 2) (Torrey 1975; Roberts 1976; Bengochea *et al.* 1983; Fukuda & Komamine 1985; Roberts *et al.* 1988; Fukuda 1992; Dodds & Roberts 1995; Blee *et al.* 2001; Möller *et al.* 2003; Oda *et al.* 2005).

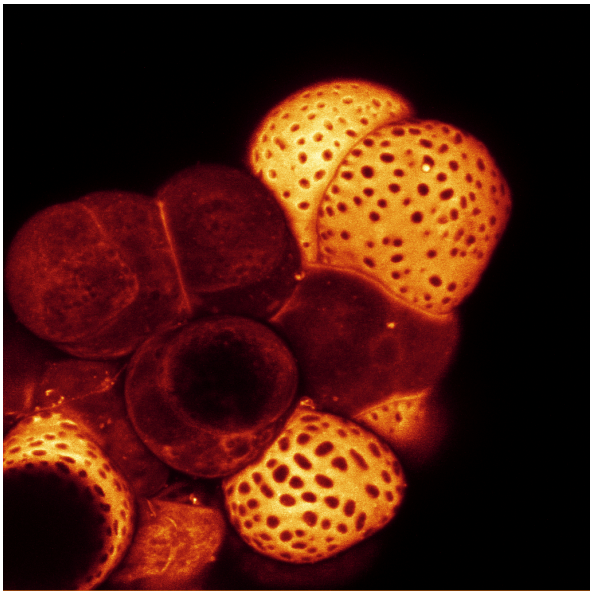

FIG. 1–Confocal laser scanning micrograph of tracheary elements produced in callus cultures of *Pinus radiata*. The tracheary elements have a spherical cell shape and pitted or reticulate cell-wall patterns. The cells without cell-wall pattern are parenchymatous callus cells.

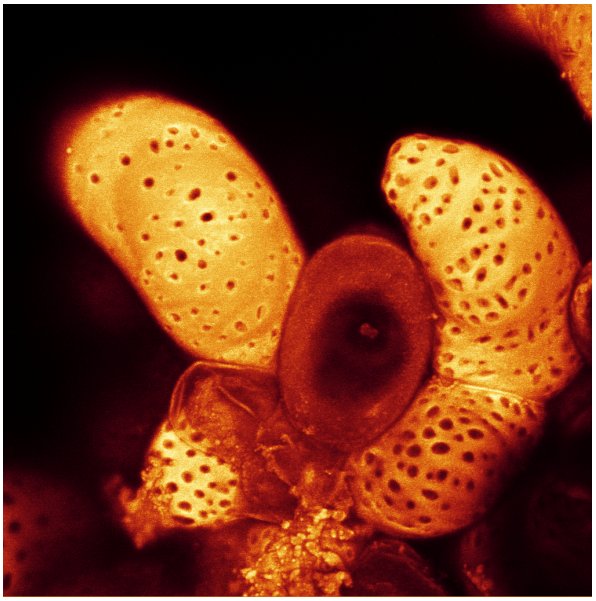

FIG. 2–Confocal laser scanning micrograph of tracheary elements produced in callus cultures of *Pinus radiata*. Two elongated tracheary elements with pitted cell-wall patterns, and spherical tracheary elements with pitted or reticulate cell-wall patterns.

Tracheary elements differentiate by a distinctive process, which is characterised by a series of cytological changes (Barnett 1981; Roberts & McCann 2000; Samuels *et al.* 2002). The initial cells, which have only primary cell walls, often first elongate and expand. At the same time, new cell-wall components, such as cellulose, non-cellulosic polysaccharides, proteins, and hydroxycinnamic acids are synthesised and incorporated into the expanding primary cell wall. Once cell expansion has ceased, a secondary cell wall consisting of cellulose, different non-cellulosic polysaccharides, proteins, and lignin is synthesised (Fukuda 1992). As long as the secondary wall is not fully formed, the tracheary elements have living protoplasts containing nuclei and various cytoplasmic structures. Finally, the cells undergo programmed cell death (Groover *et al.* 1997; McCann, Stacey, & Roberts 2000; Dahiya 2003).

The secondary cell walls of tracheary elements are deposited in distinctive patterns (Bierhorst 1960; Esau 1965a). These secondary cell wall patterns often occur in the following ontogenetic series: *annular* thickenings, which are rings of secondary wall thickenings not connected with each other; *helical* (*spiral*) thickenings, which are helically wound around a cell; *scalariform* (ladder-like) thickenings, which are helices that are interconnected in certain areas; *reticulate* thickenings, which are net-like patterns of thickenings; and *pitted* elements, where the secondary wall thickenings cover the entire primary wall except for pits (Esau 1960). Similar patterns of secondary wall thickenings have also been found in tracheary elements formed *in vitro* (Roberts 1976; Falconer & Seagull 1985; Roberts *et al.* 1988; Fukuda 1992).

CELL CULTURES AS MODEL SYSTEMS FOR TRACHEARY ELEMENT DIFFERENTIATION

One of the major difficulties in studying tracheary element differentiation in plants is that tracheary elements differentiate within complex tissues in the plant body. For example, during secondary-xylem formation the developing tracheary elements are protected by the bark and are not easily accessible. Tracheary elements in different stages of development are attached to each other, and cell populations that are in specific developmental stages have been isolated only by using a method that was developed at the Swedish University of Agricultural Sciences in Umeå (Uggla *et al.* 1996; Hertzberg *et al.* 2001; Schrader *et al.* 2004). This method involves rapid freezing of the cambial zone and tangential cryo-sectioning of the samples.

Cell cultures that produce tracheary elements in a synchronous fashion offer easier access to tracheary elements that are in specific stages of development (Table 1). Cell cultures that differentiate a high percentage of tracheary elements can be collected at different time points after induction of tracheary element differentiation, and the changes in cell-wall biochemistry, gene expression, and the proteome or

TABLE 1–Advantages and disadvantages of using cell culture systems for studying tracheary element (TE) differentiation and secondary cell-wall formation.

	Source
Advantages	
Cultures are often homogeneous in cell composition and grow fast	Fukuda (1992)
Cell cultures can be manipulated by controlling concentrations of nutrients and phytohormones in defined culture media and by controlling growth conditions, such as temperature or light intensity	Leitch & Savidge (2000)
TE are readily identified by their patterned secondary cell walls using phase contrast or polarised light microscopy	Fukuda & Komamine (1985)
Cell cultures can be genetically transformed and used for functional analysis of genes involved in cell-wall formation	Möller *et al.* (2005)
Cell cultures are independent of the growing season and studies can be carried out throughout the year	
Cell cultures can be used as model systems for some aspects of xylogenesis *in planta*	Haigler (1994) McCann, Domingo, Stacey, Milioni, & Roberts (2000)
Large amounts of homogeneous cell walls can be isolated for chemical analysis	Edashige & Ishii (1996)
Cell cultures can provide a large source of protein, DNA, RNA or metabolites	
Aspects of cell signalling can be studied by isolation and characterisation of conditioned medium	Roberts *et al*. (1997) Motose *et al.* (2001) Motose *et al.* (2004) Fukuda (2004)
TE are easily accessible and can be collected at specific stages of differentiation	
Differentiating TE can be observed live under the microscope	Groover *et al.* (1997)
Transgene effects that are lethal *in planta* can be studied in cell cultures	
Disadvantages	
Differentiation frequency and synchronicity can be low	Fukuda & Komamine (1985)
Habituation to plant hormones can occur in long term cultures. This can lead to a change in induction conditions, or to a loss of the potential to differentiate.	Webb (1981) Fukuda & Komamine (1985) Leitch & Savidge (2000)
Patterns of TE differentiation can not be studied because TEs often occur as single cells or in random groups	Fukuda (1992)
TE differentiation *in vitro* can probably not model the complete process of xylogenesis *in planta*, e.g., structure-function relationships between different cell types in xylem, earlywood-latewood formation, or heartwood formation.	Leitch & Savidge (2000)
Somaclonal variation, mutations, and DNA methylation can occur in long-term cultures	Leitch & Savidge (2000)

metabolome can be studied (Milioni *et al.* 2001, 2002; Demura *et al.* 2002; Kubo *et al.* 2005; Möller, Ball, Henderson, Modzel, & Find 2006; Möller, Koch, Nanayakkara, & Schmitt 2006). Furthermore, tracheary elements can be separated from the cell cultures and their cell walls analysed chemically (Möller *et al.* 2005). Cell separation has been achieved for *Pinus radiata* D. Don cultures by breaking up of cell aggregates followed by slow circular movement of the resulting cell suspension in Petri dishes. The tracheary elements are heavier than the parenchymatous cells, migrate to the centre of the Petri dish, and can be collected with a glass pipette. Cell separation is an important aspect with regard to functional testing of genes involved in secondary cell-wall formation, because changes in cell-wall composition of the secondary wall can be detected more easily when the interfering primary cell walls of the other cell types in the cultures are removed.

Cell cultures can be genetically modified and used for the functional testing of genes related to cell differentiation and cell-wall formation (Möller *et al.* 2005). This approach is especially interesting for evaluating gene function in conifers, because the production of genetically modified trees is labour-intensive and time-consuming. It takes approximately 2 years until a genetically modified pine tree can be analysed for changes in cell-wall biochemistry. In contrast, genetically modified cell cultures that can be induced to produce tracheary elements with lignified secondary cell walls, can be analysed after 5 months (Möller *et al*. 2005). Furthermore, tracheary element differentiation in cell cultures is independent of the growing season, allowing studies to be carried out throughout the year.

It is also possible to study the development of tracheary elements in real time under the microscope (Groover *et al.* 1997). This will potentially allow the examination of protein localisation during cell differentiation in cell cultures expressing green fluorescent protein(GFP)-tagged proteins, and will offer another interesting insight into the processes of tracheary element differentiation.

A major limitation of using cell culture systems is that patterns of tracheary element differentiation cannot be studied. Tracheary elements often differentiate as single cells or in random groups and do not form vascular tissue, such as wood. Also, seasonal processes, such as earlywood, latewood formation, and heartwood formation probably cannot be studied in cell cultures.

DIFFERENTIATION OF TRACHEARY ELEMENTS IN CELL CULTURES OF CONIFEROUS GYMNOSPERMS

Tracheary elements have been reported to occur in cell cultures of various species of gymnosperms (Table 2). However, most of these studies were carried out not to study tracheary element differentiation, but to establish *in vitro* cultures of gymnosperms. They were designed to study their cytology, or to produce cells that

TABLE 2–Plant species and *in vitro* techniques used for studying tracheary element differentiation in coniferous gymnosperms.

Plant species	*In vitro* technique	Source
Cryptomeria japonica D. Don	Callus culture	Mehra & Anand (1979); Makino & Kuroda (1985); Edashige & Ishii (1996)
Cupressus sempervirens L.	Callus culture	Havel *et al.* (1997)
Ginkgo biloba L.	Callus culture	Makino & Kuroda (1985)
Larix laricina K. Koch	Explant from cambial zone of mature tree	Savidge (1993) Leitch & Savidge (1995)
Picea glauca (Moench) Voss	Suspension culture	Durzan *et al.* (1973)
P. glauca	Callus culture	White & Gilbey (1966) White (1967)
Pinus banksiana Lamb.	Callus culture	Geissbühler & Skoog (1957)
Pinus cembra L. var *sibirica*	Callus culture	Salmia (1975)
Pinus contorta Dougl.	Callus culture	Savidge (1983)
P. contorta	Suspension culture	Webb (1981)
Pinus gerardiana Wall.	Callus culture	Konar (1963, 1974)
Pinus radiata D. Don	Callus culture	Washer *et al.* (1977); Möller *et al.* (2003, 2005); Möller, Koch, Nanyakkara, & Schmitt (2006); Möller, Ball, Henderson, Modzel, & Find (2006)
Pinus roxburghii Sargent	Callus culture	Mehra & Anand (1983)
Pinus sylvestris L.	Suspension culture	Ramsden & Northcote (1987)
Pinus thunbergii Parl.	Callus culture	Makino & Kuroda (1985)
Sequoia sempervirens Endl.	Callus culture	Ball (1950)

had only primary cell walls for chemical analysis of these cell walls (Geissbuehler & Skoog 1957; Durzan *et al*. 1973; Salmia 1975; Mehra & Anand 1979, 1983; Makino & Kuroda 1985; Edashige & Ishii 1996). Although the factors influencing tracheary element differentiation were mentioned in some of these studies, detailed analyses of *how* they influence differentiation are often lacking.

Factors that influence tracheary element differentiation and have been investigated in some detail are (i) sucrose concentration in the media, (ii) concentration of nutrients in the media, (iii) temperature and light, and (iv) types and concentrations of phytohormones in the media.

Sucrose

The influence of sucrose concentration in the medium on tracheary element differentiation was described by Konar (1974). He found that "tracheid-like cells" differentiated in callus cultures derived from hypocotyl explants of 1- to 4-month-

old seedlings of *Pinus gerardiana* Wall. With increasing concentrations of sucrose (1–5%) in the media, tracheary elements were found in the calli. The optimal sucrose concentration was not reported. Washer *et al.* (1977) reported that nodules containing "parenchymatous cells with simple pitting" and with secondary cell walls differentiated in *P. radiata* callus cultures (derived from hypocotyl-explants) when the concentration of sucrose in the medium was reduced from 3% to 1.5%.

Nutrients

Washer *et al.* (1977) also observed in *P. radiata* callus cultures that the differentiation of nodules containing tracheary elements and cambial and phloem cells was influenced by the nutrient concentration in the media. The nodules developed at the surface of the calli, when the concentration of the major nutrients was reduced by half and the sucrose concentration was reduced to 1.5%. All media were supplemented with the auxin indole-3-butyric acid (IBA) at 5 mg/litre. Therefore, the influence of different auxin concentrations was not tested and the authors concluded that tracheary element differentiation *in vitro* was influenced by a number of unspecified factors, including the concentration of nutrients.

Webb (1981) studied tracheary element differentiation in suspension cultures of *P. contorta* Loudon derived from hypocotyl explants. Up to 40% of the cells differentiated into tracheary elements with thick, sculptured, secondary cell walls. Tracheary element differentiation was highest (20–40% of the cells) after the initiation of the suspension cultures, and declined with the duration of culture to 5–10% of the cells. Both the age of the culture and the concentrations of nitrogen and phytohormones in the media influenced tracheary element differentiation. However, a detailed analysis was reported only for the effect of different concentrations of boric acid. Media containing only traces of boric acid resulted in a lower percentage of tracheary elements than media containing 124 mg boric acid/litre. Mehra & Anand (1979) also found that the percentage of cells in a callus culture of *Cryptomeria japonica* (L.f.) D.Don differentiating into tracheary elements decreased with increasing age of the cultures. The calli were grown on MS-medium (Murashige & Skoog 1962) supplemented with sucrose (2% w/v) and IAA (22.8 μM). In 2 -month-old calli up to 34% of the cells were tracheary elements, whereas in $1^1/_2$-year-old calli only 2–3% of the cells were tracheary elements. The media were further supplemented with different concentrations of IAA, NAA, 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin, and coconut milk, but the effect of these supplements on tracheary element differentiation was not specified.

The source of nitrogen in combination with a light treatment has been found to influence tracheary element differentiation in callus cultures of *Picea glauca* (Moench) Voss (White & Gilbey 1966; White 1967). When cultures were grown on a medium containing ammonium chloride (NH_4Cl) or ammonium nitrate

(NH_4NO_3) instead of glutamine as a nitrogen source, tracheary elements with reticulate secondary cell-wall structures developed.

Temperature and Light

Temperature and light have been found to influence tracheary element differentiation in suspension cultures of 11-day-old *P. glauca* derived from hypocotyl explants (Durzan *et al.* 1973). When the cultures were grown under continuous light at 22.5°C, clumps of cells developed that contained tracheary elements in their centres. This kind of differentiation was not observed when the cultures were maintained under alternating light/dark conditions. As indicated above, White & Gilbey (1966) and White (1967) observed induction of tracheary element differentiation in *P. glauca* callus cultures when these were grown in light with a 16-h photoperiod; however, the tracheary element differentiation rate was not reported. Möller, Ball, Henderson, Modzel, & Find (2006) found that exposure of *P. radiata* callus cultures to light increased tracheary element differentiation from 20% to 45%. They also found that tracheary elements had differentiated after only 2 days as compared to 5 days in callus cultures grown in the dark. Exposure to light therefore seems to be very effective in inducing tracheary element differentiation in cell cultures of coniferous gymnosperms.

Phytohormones

The influence of different phytohormone concentrations on tracheary element differentiation has been studied by Savidge (1983), Ramsden & Northcote (1987), and Möller *et al.* (2003). Ramsden & Northcote (1987) studied a suspension culture obtained from hypocotyl explants of *Pinus sylvestris* L. seedlings. Tracheary element differentiation was induced by subculturing from a maintenance medium containing 2,4-D to an induction medium containing NAA and kinetin (Ramsden & Northcote 1987). The highest percentage of tracheary elements (16% of the cells) formed in a medium containing 10 mg NAA/litre, 2 mg kinetin/litre, and 6% sucrose. In contrast, Savidge (1983) found that the application of various phytohormones did not induce tracheary element differentiation in callus cultures from 6- to 10-year-old cambium of *Pinus contorta*. However, Savidge (1983) regarded as tracheary elements only those cells that had circular bordered pits and did not mention whether the differentiation of cells that he referred to as sclereids was influenced by the application of phytohormones. He found that about 3% of the callus cells differentiated into these so-called sclereids. Möller *et al.* (2003) evaluated the effect of various substances on tracheary element and sclereid differentiation in callus cultures of *P. radiata*. These substances included the auxins 2,4-D and NAA, the cytokinin BAP, the inhibitor of auxin action *p*-chlorophenoxyisobutyric acid (PCIB), and the auxin transport inhibitor

2,3,5-triiodobenzoic acid (TIBA). None of these substances significantly increased tracheary element differentiation in these cultures.

Based on reports that activated charcoal stimulated embryogenesis, and shoot and root initiation, in calli of a number of angiosperm species (George 1993; Pan & van Staden 1998; Von Aderkas *et al.* 2002), Möller *et al.* (2003) also used activated charcoal as a medium supplement and found that a concentration of 2 g activated charcoal/litre induced tracheary element differentiation of up to 25%. Subsequently, Möller, Ball, Henderson, Modzel, & Find (2006) found that the differentiation rate was dependent on the concentration of activated charcoal in the medium, the optimal concentration being 5 g/litre. The mechanism by which activated charcoal induces tracheary element differentiation has not yet been identified. Activated charcoal has been shown to adsorb phytohormones and phenolic substances (Toering & Pullman 2005) and it might be that the auxin concentration in *P. radiata* calli is too high for differentiation to occur. Tracheary element differentiation was inhibited when induction medium was supplemented with 200 mg 2,4-D/litre (Ralf Möller unpubl. data). The inhibitory effect of 2,4-D has also been described by Oda *et al.* (2005) in suspension cultures of Arabidopsis.

To my knowledge, no studies other than those described above investigated in detail factors influencing tracheary element differentiation in cell cultures of coniferous gymnosperms. In other studies the factors influencing tracheary element differentiation were not specified. Ball (1950) induced callus from stem segments of *Sequoia sempervirens* Endl. and found groups of differentiated tracheary elements, some of which had surrounding cambium and phloem cells. Geissbuehler & Skoog (1957) reported the occurrence of "tracheid-like cells" in callus cultures of *Pinus banksiana* Lamb., and Konar (1963) reported the development of tracheary elements in callus cultures of *P. gerardiana*. Salmia (1975) induced callus cultures from hypocotyl explants of 7-day-old *P. cembra* L. var *sibirica* and observed tracheary elements with spiral, annular, or reticulate secondary cell wall thickenings. Mehra & Anand (1983) also described the development of tracheary elements with reticulate secondary thickenings and interspersed bordered pits in callus cultures of *P. roxburghii* Sargent induced from hypocotyls, cotyledons, shoot-tips, or roots of 3- to 5-week-old seedlings. Makino & Kuroda (1985) studied the formation and morphology of calli induced from young shoots of *C. japonica*, *P. thunbergii* Parl., *S. sempervirens,* and petioles of the gymnosperm *Ginkgo biloba* L. After 2 months they observed the formation of vascular nodules containing phloem, cambium, and xylem within the calli. The tracheary elements had secondary cell walls with interspersed bordered pits. Tracheary element differentiation also occurred in calli derived from megagametophytes of *Cupressus sempervirens* L. cultured on a B5-medium (Gamborg *et al.* 1968) containing 1 mg 2,4-D/litre and 0.1 mg 6-benzylaminopurine (BAP)/litre (M.T.Scarano pers. comm.). Tracheary

elements in the *C. sempervirens* calli differentiated in 2.5% of all cell clusters, which comprised 70% of the total cell population on the slides examined (Havel *et al.* 1997). The number of tracheary elements per cell cluster ranged from a maximum of 101 to a minimum of two, but no isolated tracheary elements were found. Finally, Edashige & Ishii (1996a) mentioned the presence of cells with secondary cell walls in suspension cultures of *C. japonica*.

Another three papers have described cell-wall thickening and lignification in suspension cultures of *Pinus taeda* L. (Eberhardt *et al.* 1993; Nose *et al.* 1995; Stasolla *et al.* 2003). Although the authors did not specify the cell types, they concluded that secondary cell wall formation occurred. Eberhardt *et al.* (1993) and Stasolla *et al.* (2003) maintained *P. taeda* suspension cultures in a medium containing 2,4-D. Changing the auxin to 11 μM NAA induced thickening and lignification of the cell walls. After studying the cell wall ultrastructure Eberhardt *et al.* (1993) concluded that an S1-secondary wall layer had been deposited. However, the walls were not birefringent when examined by polarised light microscopy. It was concluded that the cellulose microfibrils were not oriented as a result of either the constant motion of the culture flasks and/or the lack of a longitudinal growth axis along which the microfibrils can align. Nose *et al.* (1995) transferred the cells of their *P. taeda* suspension cultures to a sterile sucrose solution (8%) and observed irregular cell wall thickening, lignification, and extracellular lignin precipitation. However, they did not carry out further analysis of the developing cell walls.

In summary, factors such as media composition, temperature/light intensity, and the type/concentration of phytohormones have been found to influence tracheary element differentiation in cell cultures of coniferous gymnosperms. However, the results obtained were not consistent and were even sometimes contradictory, which is not surprising given the variety of plant species and techniques used.

CONCLUSION AND PERSPECTIVES

Although cell cultures offer new ways to study tracheary element differentiation and secondary cell-wall formation, they can only be useful tools but cannot replace *in planta* studies. A challenge that lies ahead is to relate results obtained from cell culture studies to xylogenesis in whole plants. In particular, the processes of tracheary element determination and initiation of differentiation in cell cultures by altering medium conditions and culture conditions are artificial and difficult to relate to these processes in plants. However, once tracheary element differentiation is initiated, the developmental process seems to be very similar in cell cultures and in plants. Cell cultures will be very useful to study this process by analysing gene expression and changes in the proteome and metabolome. The *P. radiata* culture system (Möller *et al.* 2003) will also be very useful for fast analyses of the function

of genes related to secondary cell-wall formation, because it can be genetically engineered allowing the suppression and overexpression of candidate genes. If the insights gained from cell culture approaches are combined with tracheary element differentiation studies in model plants such as *Arabidopsis* (Nieminen *et al.* 2004) or *Populus* (Hertzberg *et al.* 2001; van Raemdonck *et al.* 2005), we will quickly advance our understanding of the fascinating process of tracheary element differentiation and secondary cell-wall formation.

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