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REVIEW

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Progress in tissue culture, genetic transformation and applications of biotechnology to trees: an overview

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Abstract Trees are an integral part of human life, and a vital component of biodiversity. Forest trees in particular are renewable sources of food, fodder, fuel wood, timber and other valuable non-timber products. Due to the rapid growth of population and the human desire to progress, there has been a tremendous reduction in forest cover from the earth's surface. To maintain and sustain forest vegetation, conventional approaches have been exploited in the past for propagation and improvement. However, such efforts are confronted with several inherent bottlenecks. Biotechnological interventions for in vitro regeneration, mass micropropagation and gene transfer methods in forest tree species have been practised with success, especially in the last decade. Against the background of the limitations of long juvenile phases and life span, development of plant regeneration protocols and genetic engineering of tree species are gaining importance. Genetic engineering assumes additional significance, because of the possibility of introducing a desired gene in a single step for precision breeding of forest trees. There are no comprehensive and detailed reviews available combining research developments with major emphases on tissue culture and basic genetic transformation in tree species. The present communication attempts to overview the progress in tissue culture, genetic transformation and biotechnological applications in the last decade and future implications.

Keywords Tissue culture · Somatic embryogenesis · Genetic transformation · Trees · Biotechnology

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Introduction

Since ancient times trees have been an integral part of human life and a vital component of biodiversity. Forest trees are renewable sources of food, fodder, fuel wood, fiber, timber and other valuable non-timber products. Due to rapid deforestation, depletion of genetic resources coupled with escalating human needs the forest cover is being reduced tremendously from the earth's surface. There are alarming threats to forests in particular and biodiversity in general. Amongst others, agricultural plants and trees in general, and forest species in particular, which account for two-thirds of global photosynthesis may be soft targets. There is a great need to conserve tree ecosystems for both their environmental and aesthetic values. To maintain and sustain forest vegetation, conventional approaches have been exploited for propagation and improvement, but tree breeding efforts are restricted to the most valuable and fast growing species. However, such methods are limited with several inherent bottlenecks because trees are generally slow growing, long-lived, sexually self-incompatible and highly heterozygous plants. Due to the prevalence of high heterozygocity in these species, a number of recessive deleterious alleles are retained within populations, resulting in high genetic load and inbreeding depression. This limits the use of traditional breeding methods such as selfing and backcrossing, and makes it difficult to fix desirable alleles in a particular genetic background (Williams and Savolainen 1996). Thus conventional breeding is rather slow and less productive and cannot be used efficiently for the genetic improvement of trees. To circumvent these impediments clonal or vegetative propagation has been deployed for recovering dominant, additive and epistatic genetic effects to select superior genotypes. Plant tissue culture and genetic transformation methods offer an important option for effective multiplication and improvement of trees within a limited time frame. Interventions of biotechnological approaches for in vitro regeneration, mass micropropagation techniques and gene transfer studies in tree species have been encourag-

ing, particularly in the last decade. Somaclonal variation was first reported for woody plants in *Citrus grandis* (Chaturvedi and Mitra 1975). Therefore, it should be common practice to assure the trueness of tissue culture plants after regeneration. However, somaclonal variation is of immense importance for the isolation of improved clones of forest trees (Ahuja 1993). Recently with the development of molecular techniques it has been revealed that chromosomal variation in tissue culture is probably the rule rather than an exception (Rani and Raina 2000; Endemann et al. 2001; Rahmann and Rajora 2001). Superior trees can be cloned or selected from such in vitro raised plants with judicious assistance of molecular techniques. The availability of protoplast to plant technology for various tree species is of practical importance. Direct application to tree improvement may include parasexual hybridization through protoplast fusion and in vitro selection.

Clonal fidelity is a major consideration in commercial micropropagation using in vitro tissue culture methods. Micropropagation of tree species offers a rapid means of producing clonal planting stock for forestation programmes, woody bio-mass production and conservation of elite and rare germplasm. Plantation or clonal forestry is widely discussed to cope with the expected increasing demand for wood during the next few decades (Fenning and Gersheazon 2002). In general woody trees are difficult to regenerate under in vitro conditions. The sticking constraint in the propagation of trees in vitro is the comparatively poor success with mature explants from adult trees. Most of the trees can be propagated by vegetative means during the juvenile phase. As trees grow and attain maturity the ability of vegetative propagules to root declines. In the past four developmental phases for maturation (ontogenetic aging), each characterized by a unique array of competence for morphogenesis, have been envisaged (Greenwood 1987). These are the embryogenetic phase, the seedling phase (equivalent to juvenile phase), the transition phase (i.e. acquisition of reproductive competence) and the aged or mature phase (i.e. highest reproductive competence and lowest growth competence). It is well established that juvenile tissues facilitate propagation of mature trees. It has also been emphasized that the juvenile characters may be preserved at the base of the tree (ontogenetically young tissue) whereas maturation occurs at the periphery of the plant in tissue that is ontogenetically older but young chronologically. When such material (explants) for the initiation of in vitro culture is not available, some manipulations for reversal of aging or partial rejuvenation are helpful. In such cases conventional methods such as hedging, severe pruning, use of root suckers, spraying with plant growth regulators and stimulating stem segments for epicormic bud flushing have been adopted (Evers et al. 1996). Alternatively, in vitro methods include culture of selected explants such as epicormic buds, repeated sub-culturing, micro-grafting into juvenile root stalks, adventitious bud formation and somatic embryogenesis. During the last few years micropropagation techniques have been used for the rapid and large-scale propagation of forest trees. Successful micropropagation, especially for difficult and recalcitrant species, is primarily dependent on the quality of explants and plant growth regulators used in culture media. Micropropagation is the only aspect of plant tissue culture that has been convincingly documented with regard to its feasibility for mass scale propagation commercially. Success of in vitro regeneration depends on the control of morphogenesis, which is influenced by several factors namely genetic background, kinds of tissue or explants, nutritional components, growth regulators and culture environment. Two of the basic strategies used for micropropagation of forest tree species are direct regeneration and indirect regeneration via an intermediate callus phase. Indirect regeneration often results in somaclonal variation making the strategy less desirable for large scale clonal multiplication. Therefore, direct regeneration without a callus phase is a reliable method for clonal propagation.

Somatic embryogenesis on the other hand offers immense potential to speed up the propagation of forest trees (Attree and Fowke 1993; Gupta et al. 1993). However, very few protocols for commercial application have been developed which ensure clonal fidelity involving forest tree species. Somatic embryogenesis has been used for mass scale propagation and genetic transformation. The paucity of knowledge controlling somatic embryogenesis, the synchrony of somatic embryo development and low frequency true to type embryonic efficiency are responsible for its reduced commercial application in forest trees.

Somatic embryogenesis has been reported in relatively few woody species (Bonneau et al. 1994). Somatic embryogenesis is of importance in forestry biotechnology for two reasons. First, this system offers the capability to produce unlimited numbers of somatic embryo derived propagules (Attree et al. 1994) and artificial seeds (Lulsdorf 1993). Secondly, the embryogenic culture system could be used efficiently for genetic transformation studies. To obtain a complete conversion into plantlets it is necessary to provide optimum nutritive and environmental conditions. Practical applications of somatic embryogenesis in woody conifers have been demonstrated. The production of artificial seeds using somatic embryos is an obvious choice for efficient transport and storage. Compared to other plant species active research on somatic embryogenesis involving forest trees has been slow. Development of in vitro plant regeneration protocols is a pre-requisite for genetic transformation studies. Transfer of chimeric genes of academic and agronomic importance to the genome of the recipient species through genetic transformation of forest trees has been demonstrated; there have been several recent reviews (Pena and Seguin 2001; van Raemdonck et al. 2001; Fenning and Gershenzon 2002; Herschbach and Kopriva 2002; Campbell et al. 2003). However, there are no comprehensive and detailed reviews available depicting biotechnological research developments with emphasis on tissue culture (micropropagation, somatic

Table 1 Micropropagation of some important tree species in vitro. [*A* Apical, *AA* Ascorbic acid, *Ab* Axillary bud, *Ac* Activated charcoal, *AC* Aspen culture medium, *AM* Axillary meristem, *AR* Anderson's Rhododendron medium, *AS* Adenine sulphate, *B5* Gamborg's medium (Gamborg et al. 1968), *CN* Cotyledonary node, *CW* Coconut water, *DI* Dark incubation, *DKW* Driver and Kuniyuki (1984) medium, *GE* Germinated embryo, *Glu* Glutamine, *Gtn* Glutathione, *H* Hypocotyl, *IN* Internode, *L* Leaf, *Lp* Leaf petiole, *LP* Quoirin and Lepoivre medium, *LS* Linsmaier and Skoog (1965) medium, *MS* Murashige and Skoog (1962) medium, *MSI* Multiple shoot induction, *MT* Mature tree, *N* Nodal, *ORG* Organogenesis, *P* Plumule, *PG* Phloroglucinol, *PGR* Plant growth regulator, *R* Root, *RWM* Risser and White's medium(1964), *S* Seedling, *Sb* Shoot bud, *Sc* Stemcutting, *SH* Schenk and Hilderbrandt (1972) medium, *SN* Shoot node, *STS* , Silver thiosulphate, *Su* Sucrose, *WPM* Woody plant medium, *WS* Wolter and Skoog (1966) medium]

Plant species	Explant source	Media compositions		Culture response	Rooting response		References
		Media + Additives	PGR + Additives		Media	PGR	
Acacia auriculiformis	N	MS	BA 1.1 mg/l NAA 0.2 mg/l	MSI	MS	IBA 0.5 mg/l	Reddy et al. 1995
A. catechu	N	MS	BA 4.0 mg/l NAA 0.5 mg/l AS 25.0 mg/l AA 20.0 mg/l	MSI	-	-	Kaur et al. 1998
A. mearnsii	IS	MS	BA 2.0 mg/l IAA 0.01 mg/l	MSI	1/2MS	NAA0.6 mg/l	Huang et al. 1994
Acer pseudoplantanus	P, H, R	MS	BA 0.25 mg/l TDZ 0.004 mg/l to 0.02 mg/l	MSI	MS	IBA 25.0 mg/l	Wilhelm 1999
Aegle marmelos	SN	MS	BA 2.5 mg/l	MSI	-	-	Ajithkumar and Seeni 1998
Albizzia julibrissin	R	B5	BA 2.8 mg/l Zeatin 2.1 mg/l TDZ 0.01 mg/l	MSI	B5	IBA 0.1 mg/l	Sankhla et al. 1996
Ancistrocladus abbreviatus	N	1/5th LS	BA 10.0 mg/l NAA 0.01 mg/l 10.05 mg/l TDZ 0.004 mg/l	MSI	AR	IBA 0.8 mg/l	Bringmann et al. 1999
Anacardium occidentale	CN	MS	BA 1.0 mg/l KN 0.5 mg/l Zeatin 2.0 mg/l	MSI	MS	IBA+AC 0.5% NAA+AC IBA+NAA	Das et al. 1996
A. occidentale	SN	1/2 MS+AC	KN 4.0 mg/l Zeatin 4.0 mg/l	MSI	MS	IBA 20.0 mg/l	Boggetti et al. 1999
Annona squamosa	H, N	WPM	BA 7.5 mg/l STS 0.5 mg/l	MSI	WPM	AC 10.0 g/l NAA 8.0 mg/l IBA 8.0 mg/l	Lemos and Blake 1996
Aralia sieboldianus	SN	WPM	BA 2.1 mg/l TDZ 0.4 mg/l CPPU4.95 mg/l	MSI	-	-	Yang and Read 1997
Azadirachta indica	L	MS	BA 2.0 mg/l IAA 0.1 mg/l	MSI	MS	IBA 1.0 mg/l	Salvi et al. 2001
Catalpa ovata	N	SH	BA 0.5 mg/l IAA 0.1 mg/l	ORG	-	-	Lisowska and Wysokinska 2000
Cercis canadensis	ST	AR, WPM	BA 15–5.0 mg/l	MSI	1/2 WPM	NAA 1.3 mg/l	MacKay et al. 1995
C. canadensis	CN	DKW	BA 2.5–3.5 mg/l TDZ 0.1 mg/l or 0.2 mg/l	MSI	1/2 WPM	IBA2–40.0 mg/l	Distabanjong and Geneve 1997
Cleistanthus collinus	N	WPM	BA 0.5 mg/l	MSI	1/2 MS	IAA 4.0 mg/l DI	Quraishi et al. 1996
Croton sublyratus	SB	MS	BA 0.6 mg/l	MSI	MS	-	Shibata et al. 1996
Cycas revoluta	C	SH	BA 0.9 mg/l NAA 1.0 mg/l	ORG	-	-	Rinaldi and Leva 1995
Dalbergia sissoo	CN	MS	BA 2.0 mg/l 2IP 2.0 mg/l BA 1.0 mg/l	MSI	1/2 MS	IAA1.0 mg/l IBA1.0 mg/l IPA 1.5 mg/l	Pradhan et al. 1998
Dendrocalamus strictus	S, Ab	MS+CW	BA 0.25 to 2.0 mg/l KN 0.5 to 1.0 mg/l	MSI	MS	IBA 0.25 mg/l	Ravikumar et al. 1998
Elaeagnus angustifolia	L	WPM	BA 0.25 mg/l, 2.5 mg/l GA_3 0.4 mg/l	MSI	-	-	Economou and Maloupa 1995

Table 1 (continued)

Plant species	Explant source	Media compositions		Culture response	Rooting response		References
		Media + Additives	PGR + Additives		Media	PGR	
Fagus sylvatica	IN	WPM	BA 0.5 mg/l Zeatin 2.0 mg/l IAA 0.1 mg/l	MSI	-	-	Cuenca et al. 2000
Fragus grandifolia	ST, Sb, MT	ACM, WS	BA 0.2 mg/l NAA 0.05 mg/l	MSI	1/2 ACM	IBA 1.0 mg/l	Barker et al. 1997
Gentiana sp.	N	MS,WPM	BA 2.0 mg/l IAA 0.2 mg/l	MSI	MS	NAA 1.0 mg/l	Momcilovic et al. 1997
Gmelina arborea	N	MS	BA 0.25 mg/l	MSI	MS	IBA 45.0 mg/l	Kannan and Jasrai 1996
Hopea odorata	CN S	MS WPM	BA 0.5 mg/l 2.0 mg/l	MSI	-	-	Scott et al. 1995
Iora coccinea	SC	WPM	BA 0.6 mg/l Kn 2.0 mg/l 2IP 4.0 mg/l	MSI	WPM	NAA 0.2 mg/l	Lakshmanan et al. 1997
Litchi chinensis	S	MS	BA 20.0 mg/l	MSI	1/2MS	IBA 2.0 mg/l	Das et al. 1999
Liquidambar styraciflua	H	RWM	TDZ 1.0 mg/l 2,4 D 0.01 mg/l	MSI	RWM	IBA 25.0 mg/l	Kim et al. 1997
Madhuca longifolia	Ab, Am	MS	BA 1.0 mg/l NAA 1.0 mg/l IBA 1.0 mg/l	MSI	MS	IBA 1.0 mg/l	Rout and Das 1993
Melia azedarach	N, S	MS	BA 4.0 mg/l	MSI	1/2 MS	IBA 1.0 mg/l	Thakur et al. 1998
Mimosa tenuiflora	Ab, In	MS	IAA 0.1 mg/l KN 0.3 mg/l IBA 1.0 mg/l	MSI	MS	KN 0.1 mg/l,	Villarreal and Rojas 1996
Morus sp.	Ab, Sb, MT	MS	BA 0.5–1.0 mg/l GA_3 0.4 mg/l	MSI	MS	IAA, IBA, IPA 1.0 mg/l	Pattnaik and Chand 1997
Nothofagus alpina	L, N	WPM	TDZ 0.1 mg/l IBA 0.01 mg/l	MSI	WPM	BA 0.01 mg/l IBA 0.5 mg/l	Jordan et al. 1996
Paulownia sp.	L	MS	IAA 1.5 mg/l BA 11.3 mg/l	MSI	MS	-	Rao et al. 1996
Photinia fraseri	Ab	MS PG	BA 1.0 mg/l BA 10.0 mg/l	MSI	MS	IBA 45.0 mg/l	Malagon et al. 1997
Picea omorika	Sb, S	1/2 MS	BA 2.5 mg/l KN 2.2 mg/l	MSI	1/3 MS	-	Kolevska-Pletikapic and Buturmic-Deric 1995
P.sitchensis	C, S	MS	BA 100.0 mg/l	MSI	1/2 MS	-	Drake et al. 1997b
Pinus wallichiana	GE	LP	BA 1.0 mg/l NAA 0.5 mg/l	MSI	-	-	Bastola et al. 2000
Pistacia vara	N	MS	BA 2.0 mg/l	MSI	MS	IBA 4.0 mg/l	Onay 2000
Prunus mume	Ab	WPM, 3% sorbitol	BA 0.25–1.0 mg/l	MSI	WPM	IBA or NAA 1.0–2.0 mg/l	Harada and Murai 1996
P. avium	Sb, MT	MS	BA 1.0 mg/l GA3 0.1 mg/l	MSI	MS	IBA 2.75 mg/l	Hammatt and Grant 1997
Psidium guajava	C, Sn	MS	BA 2.0 mg/l BA 1.0 mg/l	MSI	MS+AC	IBA 2.0 mg/l	Yasseen et al. 1995
Pterocarpus marsupium	ST	B5	BA 0.1 mg/l	MSI	1/2MS	IAA 4.0 mg/l	Anuradha and Pullaiah 1999
P.santalinus	C	MS	BA 1.0 mg/l NAA 0.1 mg/l KN 1.0 mg/l	MSI	1/4MS	IAA 1.0 mg/l	Arockiasamy et al. 2000
Pyrus communis	L	LP	BA 2.0 mg/l	MSI	-	-	Caboni et al. 1999
Sapium sebiferum	N, MT	MS	BA 0.2–2.0 mg/l NAA 0.1 mg/l BA 0.5 mg/l, NAA 0.05 mg/l	MSI	1/2 MS	IBA 2.0 mg/l	Siril and Dhar 1997
Sapindus mukorossi	S	MS	BA 0.1 mg/l GA3 0.15 mg/l	MSI	MS	IBA 0.75 mg/l	Philomina and Rao 2000
Sesbania grandiflora	C, S	MS	NAA 1.0 mg/l BA 1.0 mg/l	MSI	MS	NAA 1.0 mg/l IBA 1.0 mg/l	Detrez et al. 1994

Table 1 (continued)

Plant species	Explant source	Media compositions		Culture response	Rooting response		References
		Media + Additives	PGR + Additives		Media	PGR	
Simarouba glauca	C	MS	BA 2.5 mg/l NAA 0.25 mg/l	MSI	1/2 MS	NAA 0.1–0.5 mg/l	Rout and Das 1994a
Spathoglottis plicata	N, L	MS	BA 2.0 mg/l NAA 0.5 mg/l	MSI	MS	NAA 2.0 mg/l BA 2.0 mg/l	Teng et al. 1997
Stryphnodendrun polyphythum	C	MS	BA 4.0 mg/l	MSI	1/2 MS	NAA 1.0 mg/l	Franca et al. 1995
Swartzia madagascariensis	N, S	WPM, B_5	BA 0.5 mg/l	MSI	1/2 MS	NAA 5.0 mg/l	Berger and Schaffner 1995
Swertia chirata	R	MS	BA 0.6 mg/l	MSI	MS 1/2 MS	IBA 2.0 mg/l NAA 2.0 mg/l IAA 2.0 mg/l	Wawrosch et al. 1999
Tamarix gallica	N	LS	BA 0.8 mg/l Gtn 200 mg/l	MSI	LS	IBA 0.1 mg/l	Lucchesini et al. 1993
Tectona grandis	Sb	MS	BA 1.0 mg/l KN1.0 mg/l	MSI	RWM	IBA 2.0 mg/l	Devi et al. 1994
Thevetia peruviana	E	MS	BA 1.0 mg/l KN 1.0 mg/l	MSI	1/2MS	NAA 1.0 mg/l	Kumar and Kumar 1995
Ulmus pumila	N, MT	MS	BA 0.5 mg/l	MSI	1/2 MS	NAA 0.1 mg/l	Corrchete et al. 1993
U. pumila	ST	MS	BA 0.25 mg/l	MSI	-	-	Cheng and Shi 1995
Vaccinium macrocarpona	L, MT	AR	TDZ 2.2 mg/l NAA 0.1 mg/l	MSI	-	-	Marcotrigiano et al. 1996

embryogenesis etc.) and basic gene transfer studies in forest tree species. The present review is an attempt towards making a comprehensive study of the development of in vitro regeneration protocols, genetic transformation studies and some of the recent novel approaches for genetic improvement of trees.

Micropropagation of tree without intervening callus phase

Research work on plant tissue culture has been ongoing for decades. The first complete plants from tissue culture of tree species were regenerated by Winton (1968) from leaf explants of black cotton wood (*Populus trichocarpa*). In addition there are general tissue culture-related challenges such as the production of chimeras, somaclonal variation, and endogenous bacterial contamination; the regeneration of woody plant species is still considered recalcitrant because of the effects related to ontogenetic aging. Some forest tree explants do show positive responses while others are still very recalcitrant in vitro. The term response implies that the tissues will be able to grow, differentiate and that plantlets can be regenerated in vitro. Tree propagation in vitro has been a difficult proposition compared to other plants. Only a few tree species with explants from mature trees have been propagated by tissue culture methods. In general juvenile tissues from forest trees are more responsive to in vitro manipulation than that of mature tissues. The longer life span of trees may add to the problem of contamination in vitro by the symbiotic association of microorganisms. Besides the age of the tree, the response of explants is primarily determined by genotype, physiological state of the tissue, and time of the year when the explants are collected and cultured. The composition of the media used for establishment of aseptic cultures is important. Several limitations such as low shoot proliferation in forest trees, excessive phenolic exudation (Linington 1991), basal callusing (Marks and Simpson 1994), vitrification (Monsalede et al. 1995), and shoot tip necrosis (Bargchi and Alderson 1996) are pronounced in tree tissue culture. Further, difficulty in rooting (Noiton et al. 1992; Harada and Murai 1996) has also had a negative effect on micropropagation of woody forest tree species in vitro. In this section we have attempted to describe the trend of research activities during the last decade. However, a reproducible in vitro micropropagation protocol been demonstrated in only a few tree species.

Micropropagation without an intervening callus phase is advantageous over conventional vegetation propagation in terms of quantity, quality and economics (Altmann and Loberant 1998). In general three modes of in vitro plant regeneration have been in practice, organogenesis, embryogenesis and axillary proliferation. The difference mainly matters when it relates to the genetic stability of the resulting micropropagated plants; the obvious option then would be axillary and adventitious shoot proliferation. In vitro micropropagation has proved in the recent past a means for supply of planting material for forestry (Ahuja 1993; Laximisita and Raghavaswamy 1998). The use of a protocol to promote axillary and apical shoot bud proliferation in vitro has been used for the propagation of forest tree species. Different basal media, plant growth

regulators, media additives and carbohydrate sources are being used to manipulate culture conditions in vitro for propagation of forest trees (Table 1). The plant growth regulators such as auxins (NAA: α-napthalene acetic acid; NOA: β-napthoxy acetic acid; IAA: indole-3-acetic acid; IBA: indole-3-butyric acid; and GA3:gibberellic acid) and cytokinins (BA: 6-benzyladenine; KN: kinetin; CPPU: *N* -(2-chloro-4-pyridyl)- *N* -phenylurea; 2ip: 2-isopentenyl adenine; and TDZ: thiadiazuron and Zeatin) were used for multiple shoot induction in trees. It has become a necessity to standardize media formulations when dealing with woody forest tree species.

It is evident that there are few micropropagation protocols from mature tree explants. However, a recent report of successful micropropagation of 1,000-year-old field grown mature *Taxus mairei* tree is innovative and encouraging (Chang et al. 2001). Micropropagation of forest trees in vitro is not only a means for mass scale propagation of superior clones of tree species but it can be used for developing transgenic plants and conservation of germplasm through cryopreservation. Cryopreservation of white poplar (*Populus alba* L.) by one-step vitrification of shoot tips from in vitro-grown cold hardened stock plants has been achieved (Lambardi et al. 2000). Recently, an in vitro protocol based on axillary bud proliferation has been developed for mature female Mediterranean trees (*Ceratonia siliqua*) and a field trial has been established to study their agronomic behaviour (Romano et al. 2002). A protocol has been developed to induce adventitious shoot organogenesis from semi-mature and mature cotyledons lacking the embryonic axis of *Dalbergia sissoo* (Singh et al. 2002). Multiple shoots of Himalayan oaks were induced from intact embryos and cotyledonary nodes (Purohit et al. 2002). Recently, a method for adventitious shoot regeneration from leaf explants of micropropagated peach shoots has been developed (Gentile et al. 2002).

Somatic embryogenesis / organogenesis from in vitro cultures of tree species

Different media such as MS, B5, WPM, mWPM, AE (Arnold and Erikksson 1981), LM (Litvay et al. 1985), and MCM (Bornman and Jansson 1981) have been used for the induction of somatic embryogenesis. Different plant growth regulators, such as 2,4-D (2, 4-dichloro phenoxyacetic acid) NAA, IAA, IBA, IPA (indole propanoic acid) BA, KN, TDZ, 2iP, Zeatin, CPPU, TIBA (tri-iodobenzoic acid) GA3, and ABA (abscisic acid) were used for callus induction, somatic embryo formation, shoot organogenesis and for the conversion of somatic embryos into plantlets (Table 2). In some cases somatic embryo formation occurred directly on the callus induction medium whereas in some cases different media and different hormones were needed for callus induction, somatic embryo formation, shoot organogenesis and plant conversion.

Different media additives such as PVP, coconut water (CW), CH (casein hydrosylate) and glutamine were used for shoot organogenesis and for somatic embryogenesis. CW and ammonium chloride proved to be effective for embryo germination and plant formation in *Sapindus mukorossi* (Sinha et al. 2000). Addition of $AgNO_3$ and fructose enhanced somatic embryogenesis in *Coffea canephora* (Fuentes et al.2000). Immature zygotic embryos were cultured on MS medium supplemented with B5 vitamins and 2, 4-D produced embryogenic cultures. Somatic embryos were also produced on MS medium containing NAA, KN, and glutamine in *Litchi* (Yu et al. 2000). Somatic embryogenesis and plant regeneration from leaf tissues of Jojoba have been obtained on medium supplemented with 2,4-D, BA and or synthetic cytokinins such as *N* -(2-chloro-4 pyridyl)- *N* -phenyl urea or [E]-6-[3-(trifluromethyl)-but-2-cnylamino] purine (Hamama et al. 2001).

Recently, propagation of loblolly pine through direct somatic embryogenesis from mature cotyledons has also been reported as a two step procedure for high frequency multiple shoot production (Tang and Guo 2001). Direct somatic embryogenesis from zygotic embryos of a timber yielding leguminous tree *Hardwickia binata* has been reported for high frequency somatic embryogenesis and subsequent conversion (Chand and Singh 2001). Using cotyledon explants with high concentrations of picloram or IBA somatic embryos have been produced from in vitro cultures of *Eucalyptus globulus* (Nugent et al. 2001). In a recent finding minute concentrations of salicylic acid promoted cellular growth and enhanced somatic embryogenesis in *Coffea arabica* (Quiroz-Figueroa et al. 2001). Automation may enhance the use of somatic embryos for commercialization of this pathway of micropropagation. Recently techniques related to automation of different processes such as evaluation of embryogenic cultures, embryo development, harvesting and conversion have been studied (Ibaraki and Kurata 2001). Automation can enhance commercial prospects of somatic embryogenesis, however, certain limitations may hinder the process. It has recently been found that the morphological variability within a population of *Coffea* somatic embryos produced in a bioreactor affects the regeneration and the performance of plants in the nursery (Barry-Etienne et al. 2002). Therefore, more parameters and factors need to be addressed to develop an efficient protocol for regeneration of tree species.

Plant embryogenesis is a very complicated and simultaneously an organized process. Recently, the molecular basis of somatic embryogenesis and isolation and cloning of embryogenesis associated cDNAs from somatic embryos of *Picea glauca* have been compared with zygotic embryogenesis (Dong and Dunstan 1999). There is a need to understand the genetic basis of somatic embryogenesis. Recently, random amplified polymorphic DNA analysis has indicated a polymorphism between the genome of individual species that were capable of embryogenesis and those were not. Two specific polymorphic bands have been found that can be correlated to a gene for embryogenesis by cloning and sequencing of marker bands (Imani et al. 2001).

Table 2 Somatic embryogenesis/organogenesis and plant regeneration from in vitro cultures of important tree species. (*A* Anther, *AA* Ascorbic acid, *AC* Activated charcoal, *Adb* Adventitious bud, *Ade* Adenine, *AdE* Adventitious embryos, *Apib* Apical bud, *Axib* Axillary bud, *BN* Bourgin and Nitsch (1967) medium, *C* Callus, *CA* Caffeic acid, *Cag* Cell aggregates, *CE/MH1* Carron and Enjalric medium, *CH* Casein hydrosylate, *Cot* Cotyledon, *CPPU N*-(2-Chloro-4-pyridyl)-*N*-phenylurea, *CSE* Cotyledonary somatic embryo, *CW* Coconut water, *DCR* Gupta and Durzan medium, *DE* Developing embryo, *E* Embryo, *EA* Embryonic axis, *EC* Embryogenic callus, *EP* Embryogenic performance, *ES* Embryogenic suspension, *ESM* Embryogenic suspension masses, *ESPt* Embryogenic suspension protoplast, *Ger* Germination, *Glu* Glutamine, *GSE* Globular somatic embryo, *GS* Globular structures, *H* Hypocotyl, *ImCot* Immature cotyledon, *ImS* Immature seeds, *Intg* Integument, *KM* Kao and Michayluk (1975) medium, *L* Leaf, *LH* Lactalbumin hydrosylate, *LM* Litvay's et al. (1985) medium, *LP/QP* Quoirin and Lepoivre (1977) medium, *M4E/PCM* modified B5, *mBL* Witham medium, *MeSo* Merkle and Sommer medium, *MiSp* Microspores, *mMCM* Bornman and Jansson (1981) medium, *MSE* Mature somatic embryo, *MT* Murashige and Tucker (1969) medium, *N* Nitsch (1969) medium, *NS* Nodal segment, *Nuc* Nucellus, *O* Ovule, *Pro* Proline, *ProE* Proembryos, *ProEM* Proembryogenic masses, *Pt* Protoplast, *PtC* Protoplast derived callus, *PtGC* Protoplast derived globular callus, *PVP* Polyvinyl pyrrolidine, *R* Root, *S* Stem, *SE* Somatic embryo, *Sho* shoot, *SL* Seedling, *Som* Sommer et al. (1975) medium, *SSE* Secondary somatic embryo, *StIn* Staminate inflorescence, *Su* Sucrose, *Sus* Suspension, *SusC* Suspension derived callus, *ZE* Zygotic embryo)

Plant species	Explant	Media	PGR	Media additives	Culture response	Plant conversion	References
Abis alba	ZE	mMCM	BA 0.5 mg/l, Kn 0.5 mg/l	-	ESM	-	Hristoforroglu et al. 1995
	ESM	mMCM	0.4 mg/l 2,4-D	-	ESMCL	-	
	ESMCL	mMCM	0.4 mg/l 2,4-D	500–1,000.0 mg/l CH, 500.0 mg/l Glu, 40.0 g/l Su, 10.0 g/l AC	GSE	-	
	GSE	mMCM			MSE	-	
	MSE	mMCM	5.2 mg/l ABA	18.0 g/l maltose	-	Yes	
Acacia catechu	ImCot	WPM	3.0 mg/l KN 0.5 mg/l NAA	0.4 g/l Pro	SE	-	Rout et al. 1995
	SE	1/2 MS	-	2% Su	-	Yes	
Aegle marmelos	ZE	MS	0.22 mg/l 2,4-D 0.22 mg/l BA	20% CM	SE	-	Islam et al. 1996
	SE	1/2MS	0.22 mg/l BA			Yes	
A. marmelos	EA	MS	KN 1–2.0 mg/l IAA 0.1 mg/l	-	Sho	-	Islam et al. 1995
	Sho	MS	IBA 1.0 mg/l	-	R	Yes	
Albizia julibrissin	O	MS	10.0 mg/l 2,4-D	-	PE	-	Burns and Wetzstein 1998
	PEM	FN	10.0 mg/l 2,4-D	1.4 g/l Glu	EP	-	
	EP	MS	-	-	CSE	-	
	CSE	MS	-	-	-	Yes	
Azadirachta indica	A	NB	1.75 mg/l IAA, 0.25 mg/l BA	-	C	-	Gautam et al. 1993
	C	MS	1.0 mg/l BA, 0.1 mg/l NAA	PVP 0.18 g/l	Sho		
	Sho	MS	0.1 mg/l IBA	PVP 0.18 g/l	R	Yes	
	A	MS	IAA 1.75 mg/l, BA 0.22 mg/l	-	C	Yes	
	C	MS	NAA 0.09 mg/l, BA 1.0 mg/l	-	Sho		
Azadirachta indica A	Cot, H	MS	0.5 mg/l NAA, 1.0 mg/l BA	1 g/l CH 50.0 g/l Su	EC	-	Su et al. 1997
	EC	MS	0.5 mg/l IAA	50.0 g/l Su	GS	-	
	GS	MS	0.2 mg/l zeatin	50.0 g/l Su	SE	-	
	SE	1/2 MS	-	10.0 g/l Su	-	Yes	
Betula platyphylla	Pt	1/2MS	0.18 mg/l PU, 0.18 mg/l NAA	0.1 g/l mannitol 0.3 g/l Su	PtGC	-	Watika et al. 1996
	PtGC	1/2 MS	0.18 mg/l PU	-		-	
			2.2 mg/l zeatin	-	Sho		
	Sho	MS	0.5 mg/l IBA 1.0 mg/l NAA	-	-	Yes	
Coffea arabica	Intg	MS	1.0 mg/l TIBA	50.0 mg/l cysteine 100.0 mg/l PVP	C	-	Sreenath et al. 1995
	C	mMS	0.2 mg/l 2,4-D, 2.5 mg/l 2ip		SE	-	
	SE	MS	1.0 mg/l ABA	-	MSE		
	MSE	1/2MS	0.1 mg/l KN	-	-	Yes	
C. canephora	L	MS	0.5 mg/l 2,4-D 0.5 mg/l IBA 2.0 mg/l 2ip	-	C	-	Berthouly and Michau-Ferriere 1996
	C	MS	1.0 mg/l 2,4-D 4.0 mg/l BA	-	SE	-	

Table 2 (continued)

Plant species	Explant	Media	PGR	Media additives	Culture response	Plant conversion	References
Castanea dentata	O	WPM	4.0 mg/l 2,4-D, 0.25 mg/l BA	1.0 g/l CH N vitamins	ProEM	-	Xing et al. 1999
	ProEM	B5	0.1 mg/l BA, 0.1 mg/l NAA	20.0 g/l Su	CSE	-	-
Ceratozamia hildae	Pinnae	mB5	0.25 mg/l KN 1.0 mg/l 2,4-D	60.0 g/l Su 0.4 g/l Glu 0.1 g/l arginine 0.1 g/l aspargine	SE	-	Litz et al. 1995
Cupressus sempervirens	ZE	DCR	1.0 mg/l NAA 1.12 mg/l BA	-	EC	-	Lambardi et al. 1995
	ZE	1/2 QP	2.25 mg/l BA	-	Adb	-	
	Adb	1/2 QP	-	-	Sho	Yes	
Dalbergia latifolia	L	MS	1.0 mg/l 2,4-D 5.0 mg/l NAA 1.0mg/l BA	CW 10%	C	-	Laximisita and Raghavaswamy 1993
	C	MS, WPM	5.0 mg/l BA 0.5 mg/l NAA	-	Sho	-	
	Sho	mWPM	2.0 mg/l IBA	-	R	Yes	
D. latifolia	H	MS	2.0 mg/l NAA, 0.5 mg/l BA	10% CW	C	-	Pradhan et al. 1998
	C	MS	2.0 mg/l NAA, 0.5 mg/l BA	10% CW	Sus	-	
	SusC	MS	0.5 mg/l BA 3.0 mg/l BA	-	Sho	-	
	Sho	1/2 MS	1.0 mg/l IAA, 1.0 mg/l IBA, 1.0 mg/l IPA	-	R	Yes	
D. sissoo	ZE I	MS	0.1–0.25 mg/l KN 1.5–2.0 mg/l 2,4-D	-	SE	-	Das et al. 1997
	SE	1/2 MS	0.5 mg/l ABA	2% Su	Ger	-	
Encephalartos natalensis	ZE	mB5	1.0 mg/l 2,4-D 1.0 mg/l KN	-	C	-	0
	C	mB5	0.5 mg/l 2,4-D 1.0 mg/l KN	-	SE	-	
E. dyerianus	ZE	mB5	1.0 mg/l 2,4-D 1.0 mg/l KN	- -	C	- -	
		C	0.5 mg/l 2,4-D 1.0 mg/l KN	-	SE	-	Jager and Staden (1996)
Eucalyptus dunnii	SL	B5	3.6 mg/l NAA 1.0 mg/l 2,4-D	1.0 g/l CH	EC	-	Termignoni et al. 1996
	SL	MS	3.0 mg/l NAA 1.0 mg/l 2,4-D		EC		
	EC	B5	-	1.0 g/l CH, 10%CW	SE	-	
	EC	MS	-	-	SE	-	
Feronia limonia	Cot	MS	1.0 mg/l BA 1.0 mg/l KN	-	Sho	-	Hossain et al. 1994
	Sho	1/2 MS	0.1 mg/l IAA, 0.1 mg/l IBA	-	R	Yes	
Gingko biloba	Misp	BN	2.0 mg/l IAA, 0.2 mg/l KN	- -	EC	-	Laurain et al. 1993
G. biloba	ZE	MT	2.25 mg/l BA	-	SE	-	Laurain et al. 1996
Gnetum ula	ZE	MS	5.0 mg/l BA	-	EC	-	Augustine and D'Souza 1997
	EC	1/2 MS	-	2.5 g/l CH 0.5 g/l Glu 20.0 g/lSu	SE	-	
	SE	1/2 MS	10 mg/l ABA	60.0 g/l Su	MSE	-	
Hardwickia binata	Cot	mMS	1.0 mg/l KN 1.0 mg/l NAA	-	EC	-	Das et al. 1995
	EC	MS	0.02 mg/l GA3	300.0 mg/l Glu	SE	-	
	SE	1/2 MS	0.25 mg/l IBA	2% Su	MSE	-	
H. binata	ZE	MS	0.5 mg/l	2,4-D	-	SE	Chand and Singh 2001
	SE	MS	0.03 mg/l NAA 0.5 mg/l BA 0.5 mg/l ABA	-	SE	-	

Table 2 (continued)

Plant species	Explant	Media	PGR	Media additives	Culture response	Plant conversion	References
Hevea brasiliensis	IS	MH1	2.0 mg/l 3,4-D 2.0 mg/l BA	-	EC	-	Veisseire et al. 1994
	EC	MH1	0.26 mg/l ABA	-	DE	-	
H. brasiliensis	A	mMS	2 mg/l 2,4-D 0.5 mg/l KN	-	C	-	Jayasree et al. 1999
	C	MS	0.7 mg/l KN 0.2 mg/l NAA	-	SE	-	
	SE	MS	-	-	-	Yes	
Hevea brasiliensis	ImS	CE	-	84.0 g/l maltose	SE	-	Blanc et al. 1999
Juglans nigra	Cot	WPM	1.0 mg/l TDZ 1.0 mg/l 2,4-D	1.0 g/l CH 30.0 g/lSu	SE	-	Neuman et al. 1993
Liriodendron chinense × *L. tulipifera*	ZE, End ProEM/SE	MrSo	2.0 mg/l 2,4-D 0.25 mg/l BA 2.0 mg/l 2,4-D, 0.25 mg/l BA	1.0 g/l CH 40.0 g/lsu -	ProEM/SE Ger	- Yes	Merkle et al. 1993
Larix leptolepis	ZE	LP	0.25 mg/l BA 1.0 mg/l BA	1.85 g/l Glu 20.0 g/l Su	EC	-	Kim et al. 1999
	EC	1/2 LM	1.0 mg/l BA	1.22 g/l Glu 20.0 g/l Su	EC	-	
	EC	1/2 LM	1.0 mg/l BA	20.0 g/l Su	SE	-	
	SE	1/2 LM	-	20.0 g/l Su	-	Yes	
Litchi chinensis	Pt	MS	1.0 mg/l KN 0.1 mg/l NAA	500.0 mg/l Glu 80.0 g/l Su	SE	-	Yu et al. 2000
	SE	mMS		500.0 mg/l Glu, 5% CW	MSE	-	
	MSE	mMS	1.0 mg/l KN 5.0 mg/l GA3	5% Su,5%CW	Ger	Yes	
Liquidambar styraciflua	StIn	mBL	1.0 mg/l NAA	1.0 g/l CH	SE	-	Merkle and Battle 2000
Malus × *domestica*	Pt	MS	0.5 mg/l BA 0.5 mg/l NAA 0.5 mg/l 2,4-D	-	PtC	-	Perales and Schieder 1993
	PtC	MS	2.5 mg/l TDZ 0.75 mg/l NAA	50.0 mg/l CH	Sho	-	
	Sho	MS	2.5 mg/l IBA	-	R	Yes	
Malus × *domestica*	L, Cot	MS	2.0 mg/l 2,4-D 1.0 mg/l BA	-	C	-	Ai-Ping et al. 1995
	C	MS	2.0 mg/l 2,4-D, 1.0 mg/l BA	2.0 mg/l AA 100.0 mg/l LH	Sus	-	
	Pt	MS	2.0 mg/l NAA 0.1 mg/l BA	- -	PtC -	- -	
	PtC	MS	0.1 mg/l IAA 1 mg/l BA or 1–2.0 mg/l TDZ	20.0 mg/l Ade 500.0 mg/l LH	Sho	-	
	Sho	MS	0.8–1.0 mg/l IBA	-	R	Yes	
Malus × *domestica*	Pt	KM8P	0.1 mg/l IAA, 1.0 mg/l ABA 2.0 mg/l TDZ	-	Sho	Yes	Saito and Suzuki 1999
Mangifera indica	Nu	mB5	1.0 mg/l 2,4-D	-	EC	-	Litz et al. 1998
M. indica	Nu	1/2 MS	0.88 mg/l 2,4-D 4.5 mg/l BA	6% Su	C	-	Laxmi et al. 1999
	C	1/2 MS	4.5 mg/l BA	-	SE		
	SE	B5	0.5 mg/l BA 0.5 mg/l GA3	-	Ger	Yes	
M. indica	Nu	mB5	1.0 mg/l 2,4-D	-	C	-	Ara et al. 2000b
	C	mB5	-	-	SE		
	SE	1/2 B5	1.0 mg/l GA3	-	-	Yes	
Myrtus communis	ZE	MS	0.5 mg/l 2,4-D	-	SE	-	Canhoto et al. 1999
	SE	MS	0.1–0.5 mg/l GA_3 0.2 mg/l BA	-	Ger	Yes	
Octotea catherinensis	ZE	mMS	80 mg/l 2,4-D	0.3% AC 2% Su	EC	-	Moura-Costa et al. 1993
	EC	WPM	40.0 mg/l 2,4-D	0.3% AC, 2% Su	SE	-	
	SE	1/2 WPM	20.0 mg/l 2,4-D	0.3% AC, 2% Su	SEP	-	
	SE	1/2 WPM	0.2 mg/l 2,4-D 0.5 mg/l GA_3	2% Su	-	Yes	

Table 2 (continued)

Plant species	Explant	Media	PGR	Media additives	Culture response	Plant conversion	References
Picea mariana	SE	1/2 LM	-	3.0 g/l Glu	MSE	Yes	Khlifi and Tremblay 1995
Pistacia vera	ESM	MS	4.0 mg/l BA	-	Ger	Yes	Onay et al. 2000
Prunus avium	ZE	MS	4.0 mg/l 2,4-D 2.0 mg/l KN	-	SE	-	March et al. 1993
P. avium	ZE	MS	0.2 mg/l KN 0.2 mg/l BA 0.1 mg/l NAA	-	SE	Yes	Garin et al. 1997
P. avium	ZE	MS	0.1 mg/l NAA 0.1 mg/l KN	0.25 g/l Glu 94.76 g/l maltose	EC	-	Talleux et al. 1999
	EC	MS	2.6 mg/l ABA	-	SE		
	SE	WPM	-	15.0 g/l Suc 2.0 mg/l Glu 2.0 mg/l Gly	Ger	Yes	
Prosopis tamarugo	Cot, H	MS	2.0 mg/l NAA 0.2 mg/lBA	-	C	-	Nandwani and Ramawat 1992
	EA	MS	5.0 mg/l BA 1–2.5 mg/l BA	- -	C, Sho Sho	- -	
	Sho	MS	3.0 mg/l IBA		R	Yes	
Quercus robur	L	MS	4.0 mg/l NAA, 0.5–1.0 mg/l BA	-	C	-	Cuenca et al. 1999
	C	MS	0.1 mg/l BA, 0.1 mg/l NAA	-	C	-	
	C	MS	-	-	SE	-	
	SE	1/2 MS	-	-	Ger	Yes	
Sapindus mukorossi	L	MS	0.5 mg/l KN	15%CW	C		Sinha et al. 2000
	C	mMS	2.0 mg/l KN	10% CW	SE	Yes	
Simarouba glauca	ImCot	mMS	2.5 mg/l BA, 1.5 mg/l NAA	600.0 mg/l Glu or +600.0 mg/l Pro	SE	-	Rout and Das 1994b
	SE	1/2 MS	0.5 mg/l ABA	2% Su	-	Yes	
S. glauca	Cot	mMS	2.5 mg/l BA 1.5 mg/l NAA	0.25 mg/lAA	C	Yes	Rout and Das 1994c
	C	MS	2.5 mg/l BA, 1.5 mg/l NAA	0.25 mg/lAA	SE		
	SE	1/2 MS		2% Su	Ger	Yes	
Sesbania spp.	H	MS	0.05–1.0 mg/l IBA 1.0–2.0 mg/l BA	-	C, Sho	-	Yan-Xiu et al. 1993
	Sho	MS	1.0 mg/l IBA	-	R	Yes	
S. album	NS	MS	0.2 mg/l TDZ, 1.05 mg/l IAA 0.2 mg/l KN	-	SE	-	Ragkhla and Jones 1998
	SE	MS	2.07 mg/l GA3	-	SE	Yes	
S. spicatum	L	MS	0.2 mg/l TDZ, 3 2.07 mg/l GA3	0.5 g/l Glu 150.0 mg/l CH	SE	Yes	Ragkhla and Jones 1998
	SE	MS	2.07 mg/l	5%CW	Ger	Yes	
Terminalia arjuna	L	MS	5.0 mg/l 2,4-D 0.01 mg/l Kn	-	C, GS	-	Kumari et al. 1998
	C, GS	MS	-	-	SE	Yes	
Thevetia peruviana	L	MS	2.0 mg/l 2,4-D 0.1 mg/l KN	-	C	-	Sharma and Kumar 1994
	C	MS	1.0 mg/l 2,4-D 0.1 mg/l KN	-	Sus	-	
	Cellag	MS	0.1 mg/l 2,4-D 2.0 mg/l 2IP	-	DE	-	
	DE	MS	-	-	SE	-	
	SE	1/2 MS	-	-	-	Yes	
Tectona grandis	Apib	MS	1.0 mg/l BA 0.01 mg/l NAA	-	C, SE	-	Kushalkar and Sharon 1996
	C	MS	0.1 mg/l BA 0.1 mg/l NAA	-	SE	-	
	Axib	1/2 lMS	1.0 mg/l BA 1.5 mg/l 2iP	-	SE	-	

Table 3 Genetic transformation using selectable marker and reporter genes in tree species. (*Ag Agrobacterium* -mediated, *ALS* Acetolactate synthase, *Bt* Endotoxin of *Bacillus thuringiensis*, *C* Callus, *Cat* Chloramphenicol acetyle transferase, *Cp* Coat protein, *Ct* Cotyledon, *E* Embryo, *EC* Embryogenic callus, *ESM* Embryonal suspensor masses, *Et* Embryogenic tissue, *H* Hypocotyle, *hpt* Hygromycine phosphotransferase, *ImE* Immature embryo, *IN* Internode, *ISE* Immature somatic embryo, *IZE* Immature zygotic embryo, *L* Leaf, *LDM* Leaf disc method, *LPt* Leaf protoplast, *ME* Mature embryo, *MPB* Microprojectile bombardment, *MSE* Mature somatic embryo, *MZE* Mature zygotic embryo, *N* nodule, *nptII* Neomycin phosphotransferase II, *P* Petiole, *Pat* Phosphinothricin acetyle transferase, *PEG* Polyethyleneglycol, *Pot* Pollen tube, *Pt* Protoplast, *rol-A* Root inducing loci A, *RSV-F* Human respiratory syncytial virus fusion protein, *S* Stem, *SAMase* S-adenocyl methionine hydrolase, *SE* Somatic embryo, *SL* Seedlings, *TC* Transgeniccallus, *Tcot* Transgenic cotyledon, *TEC* Transgenic embryogenic callus, *TPt* Transgenic pollen tube, *TS* Transgenic shoot, *TSE* Transgenic somatic embryo, *UidA* UidA-β-glucorinidase)

Plant species	Nature of tissue	Transformation method	Genes transferred	Transgenic plants	References
Acacia mangium	S	Ag	*UidA*, *nptII*	TS	Xie and Hong 2002
Allocasurina Verticillata	H	Ag	*UidA*, *nptII*	Yes	Franche et al. 1999
Betula platiphylla	L	Ag	*nptII*, *UidA*	Yes	Mohri et al. 1997
Castenia sativa	H	Ag	*nptII*, *UidA*	Yes	Seabra and Pais 1998
Coffea canefphora	L	Ag	*UidA*, *hpt*	Yes	Hatanaka et al. 1999
Eucalyptus camaldulensis	L	Ag	*nptII*, *UidA*	C	Mullins et al. 1997
E. camaldulensis	H	Ag	*nptII*, *UidA*	Yes	Ho et al. 1998
Fragaria × *ananassa*	L	Ag	*UidA*, *nptII*	Yes	Barcelo et al. 1998
Hevea braziliensis	C	MPB	*UidA*, *nptII*	TSE	Arokiaraj et al. 1994
			Cat	TEC	
Larix kaempferix, *L. decidua*	MSE	Ag	*nptII*	Yes	Levee et al. 1997
Malus × *domestica*	L	Ag	*UidA*	TC	James et al. 1993
Malus × *domestica*	L, Cot	Ag	*UidA*	TC	Bondt et al. 1994
Malus x domestica	Cot	Ag	*UidA*, *nptII*	TC	Bondt et al. 1996
Malus × *domestica*	L	Ag	*rol A*	Yes	Holefors et al. 1998
Malus × *domestica*	LPt	PEG	*RSV-F*	Yes	Sandhu et al. 1999
Malus × *domestica*	Pot	Ag	*UidA*, *nptII*	Yes	Yao et al. 1999
Malus × *domestica*	L	Ag	*MB39*	Yes	Liu et al. 2001
Picea glauca	SE	MPB	*nptII*, *UidA*	SE	Bommineni et al. 1993
P. mariana	ImE	MPB	*nptII*, *UidA*	TSE	Bommineni et al. 1994
P. mariana	SE	MPB	*hpt*, *UidA*	Yes	Tian et al. 2000
P.sitchensis	ImE, ME	Ag	*UidA*	ESM	Drake et al. 1997a
P. abies	ImE, ME	Ag	*bar*, *nptII*	ESM	Bishop-Harley et al. 2001
Pinus sylvestris	Cot, EC	MPB	*nptII*, *UidA*	Yes	Haggman and Aronen 1998
P. pinea	E	Ag	*UidA*	TC	Humara et al. 1999
P. pinea	E, Cot	Ag	*UidA*	TC	Humara et al. 1999
P.aristata, *P. griffithii*, *P. monticola*	Pt	MPB	*UidA*	TPT	Fernando et al. 2000
P. radiata	Et	MPB	*Bar*, *nptII*, *UidA*	Yes	Bishop-Harley et al. 2001
Populus nigra	L	Ag	*UidA*, *nptII*	Yes	Confalonieri et al. 1994
P. deltoides × *P.* × *euramericana*	L	Ag	*UidA*, *nptII*	Yes	Confalonieri et al. 1997
Prunus amygdalus	L	Ag	*UidA*, *nptII*	TC	Archilletti et al. 1995
P. subhirtella	SE	Ag	*UidA*	Yes	Machado et al. 1995
P. dulcis	L	Ag	*nptII*, *UidA*, *ALS*	Yes	Yao et al. 1999
P. dulcis	L	Ag	*nptII*, *UidA*	Yes	Miguel and Oliveira 1999
Rubus ideaus	L, P	Ag	*SAMase*	Yes	Mathews et al. 1995
Ulmus comprestres	IN	Ag	*T-DNA*	Yes	Dorion et al. 1995

Genetic transformation studies

Recent advances in the genetic transformation of forest trees have made it possible to transfer chimeric genes of academic and agronomic importance to the genome of recipient species. It is anticipated that this technology may help to circumvent some of the limitations of classical breeding programmes associated with forest tree improvement. The pace at which literature on genetic transformation is being generated makes it difficult to summarize. Research progress towards standardized genetic transformation protocols and transgenic tree production is outlined in Table 3.

Until recently trees were considered to be recalcitrant material for genetic transformation studies involving molecular techniques. The main obstacle for genetic transformation of trees is the regeneration of transformed plantlets. In addition to the research progress assessment, it has been found that *Agrobacterium* based genetic transformation is the main method used for developing transgenic trees. Further regeneration of plants from single cells is a requisite for Ag-mediated gene transfer to achieve homogenetically transformed plants.

Choice of explants having competence for transformation and regeneration is a crucial factor. At this point in time efficient tissue culture techniques become the foundation for genetic transformation studies. In addition to regeneration through organogenesis, somatic embryogenesis definitely offers the advantage of single cell regeneration and currently appears to be the most promising approach to introduce new genes into woody tree species.

Some of the recent novel approaches for genetic improvement of tree

The long generation period of tree species is one of the limiting factors for genetic improvement and study of mature traits. However, by taking advantage of recent developments in genetic engineering the time required to produce a new tree variety could be reduced significantly. Some of the novel biotechnological approaches used recently for the genetic improvement of trees are as follows.

Reduction in the reproductive cycle of tree

The 30–40 years period prior to the onset of reproductive phase in some trees is a constraint to plant breeding. If traits could be identified and manipulated to enable flowering to be induced at will, the fixing of beneficial recessive mutations, and introgression/back crossing to increase rare alleles in breeding populations could become realities. Several *Arabidopsis* homeotic genes that are involved in flower initiation induce early flowering when expressed ectopically in transgenic plants. It has been well established that the homeotic genes are involved in flower initiation. Recently, it has been demonstrated that early flowering can be induced when homeotic genes are expressed in transgenic plants (Mandel and Yonofsky 1995). Flowering in aspen is generally observed after 8–20 years. One of these genes (LFY) has been expressed in transgenic aspen and was able to produce flowers after 7 months of vegetative growth (Weigel and Nilsson 1995; Pena and Seguin 2001). A study of the diverse effects of over expression of the LFY gene has also been reported (Rottmann et al. 2000). Recently some of the promising findings have opened up the possibility of a reduction in generation time thus accelerating the genetic improvement programme in citrus (Pena et al. 2001). This approach can be extended to other economically important trees. A reduction of generation time in trees will open up several benefits with multiple applications; benefits include early fruit production, reduction of economic loss due to graft transmissible diseases, accelerated evaluation of mature agronomic traits and genetic improvement programmes using modern molecular methods of marker assisted selection. In addition six new genes that play key roles in flower development have recently been isolated and tested in transgenic plants. They will be useful for engineering male and female sterile trees, facilitating regulatory and public approval of transgenic plantations (Strauss and Scott 2002).

Plant hormone modification to change tree architecture and performance

Recently there have been attempts to use hormone biosynthetic genes to alter tree size, morphology and performance. Plant growth regulators play an important role in growth and development and they also affect formation of wood (Little and Savidge 1987). Thus the manipulation of plant hormone synthesis in trees looks very interesting. Some progress has been made using T-DNA auxin and cytokinin biosynthetic genes from *A. tumefaciens* and *A. rhizogenes*. It has been demonstrated that the manipulation IAA levels by the over expression of iaaM and iaaH *A. tumefaciens* T-DNA in *Populus* can change growth, development and wood formation (Tuominen et al. 1995). Over expression of these genes in the transgenic plants brings about alterations in growth pattern and wood properties (Tzfira et al. 1998). Leaf explants were transformed with *Agrobacterium* containing rol A gene and transgenic tissues developed into transgenic plants in apple root stock M26 (Holefors et al.1998). It would be of commercial interest to introduce genes to make M26 dwarf without affecting rooting ability. This study examines if the introduction of the rol A gene in the apple rootstock M26 could reduce the shoot growth without changing rooting performance. The over expression of GA-20 oxidase gene from *Arabidopsis* in hybrid aspen has shown altered phenotype such as faster growth in height, girth, larger leaves, larger xylem fibbers and increased biomass. These findings not only open up tree architecture but also wood quality opportunities (Eriksson et al. 2000). This approach may have a direct application in fruit crops. Down regulation of GA-20-oxidase in transgenic fruit trees could generate plant types with reduced height. This will allow higher planting density, mechanical fruit harvesting and easier management with reduced labour costs.

Manipulation of lignin and cellulose content

In the genetic improvement of trees, increased volume of wood and enhancement of the wood properties are obvious targets. The transgenic approach has recently been used to manipulate wood quantity and quality in trees. These studies involve alteration of the chemical composition of cell walls affecting the quantity or quality of lignins in the wood. Xylem cells, which make up wood, have highly lignified cell walls. Lignin functions as an inter- and intra-molecular glue, which interlinks various cell wall components (Campbell and Sederoff 1996). Tree material with reduced lignin content used for pulp and paper making is highly desirable from both an economic and an environmental point of view. As predicted the lignins from these plants were more readily removed and the plants could be useful in the pulping process resulting in the removal of more lignins under less harsh conditions (Franke et al. 2000). Recently field trails using transgenic poplar with antisense CAD transgene shows that the benefits could be realised with no apparent environmental impact (Pilate et al. 2002). Genetic manipulation has also been used to enhance wood properties other than that related to wood chemistry. Separation of lignin from wood fibers is a costly affair during pulp and paper

production and also generates paper industry related pollutants. Lignin, an abundantly available organic compound, constitutes 15–35% of the dry weight of trees. Lignins are an aromatic polymer that originate from the oxidative polymerization of three precursors. Due to the existence of different precursors chemical diversity is evident in angiosperm and gymnosperm lignin.

Despite the complexity of the lignin biosynthetic pathway transgenic trees with reduced or modified lignin content have been produced. Antisense regulation of 4-coumarate-CoA ligase (4CL) and cynamyl alcohol dehydrogenase (CAD) are involved. Reduction of lignin content of up to 45% was observed with increased growth rates (Hu et al. 1999). Down-regulation of O-methyl transferase (OMT) in hybrid poplar has revealed the possibility of modified lignin and increased pulp yield (Jouanin et al. 2000). Changes in lignin quality/quantity are becoming important aspects of forest tree breeding programmes. Recently molecular studies on QTLs in *Eucalyptus* have been developed with PCR-based markers for characterizing wood quality, rooting ability and vigour (Gion et al. 2000). The combination of genetic engineering and molecular marker techniques will accelerate the process for the genetic modification of trees for wood and timber quality. Recently, the isolation and characterization of a *Pinus radiata* lignin biosynthesis-related O-methyl transferase promoter has indicated that the control of lignin-related gene expression is conserved and can be compared to distantly related species such as tobacco and pine (Moyle et al. 2002).

Further identification of the genes that control wood formation could be a prerequisite for future transgenic tree strategies aimed at altering the rate of wood biogenesis and properties of wood. Towards this objective, the characterization of "wood transcriptome" is now in progress for several tree species, with expressed sequence tags (EST) available in the late 1990s in *Populus* (Sterky et al. 1998) and *Pinus* (Allona et al. 1998). Preliminary reports indicate that between 1,000 and 3,000 ESTs have been generated by randomly sequencing clones from cDNA libraries constructed from mRNA of differentiating xylem of these species. Currently more than 100,000 ESTs have been generated for *Populus* and 70,000 ESTs for *Pinus* and *Quercus* species; *Eucalyptus* has also been included in the research programme (Campbell et al. 2003).

Development of transgenic tree resistance to insect and diseases

Trees grow in most harsh environments. Due to their distribution in diverse climatic ecosystems many are attacked by pests and diseases. Significant loss caused by defoliating insects occurs in various tree species. This damage leads to reduction of growth and even death. In the long run it affects tree shape and fruit quality. The Bt endotoxin from *Bacillus thurigiensis* has been used successfully in commercial crop production for controlling insect pests. One of the limitations with trees is that vast areas have to be covered for the control of the insect attack. As with crop plants there is a need to express the Bt endotoxin in trees. *Populus* plants have been transformed using electric discharge particle acceleration of protoplasts and 18.7 kb plasmid containing nptII, gus and Bt gene have been transferred (McCown et al.1991). Transformed plants containing all three genes were recovered and analyzed. The incorporation of Bt type pest resistance into *Populus* germplasm may be a powerful adjunct to poplar where other lepidopteran pests are potential threats. Further, Bt toxin gene has been expressed in apple, poplar, spruce, and larch and reduction in the larval feeding on transgenic material was observed (Schuler et al. 1998; Tzfira et al. 1998). Increased larval mortality was observed with transgenic walnut (Dandekar et al. 1998).

Manipulation of production levels of natural products for increasing resistance to insect pests through genetic engineering to study tree–pest interactions has been undertaken. One of the candidate natural products is a proteinase inhibitor that affects the digestive enzymes of insects and can be an alternative to the use of Bt toxin genes. Over expression of proteinase inhibitor gene in transgenic poplar has been demonstrated. A novel *Arabidopsis thaliana* cystine protease inhibitor gene over expressed and pest resistance was studied (Delldonne et al. 2001). Transgenic plants of *Prunus americana* conferring coat protein gene of plum pox virus have been developed. The plum pox virus (ppv) is one of the most important pathogens of stone fruit trees in Europe and the Mediterranean area. This is the first report where the coat protein gene of ppv has been successfully integrated into the fruit tree species genome and this finding has opened up avenues for the control of this disease. Recently high levels of resistance against the ppv through, the post -transcriptional coat protein gene silencing has been found (Scorza et al. 2001). Woody internodes have been transformed using *Agrobacterium* mediated gene transfer in elm (Dorion et al. 1995). Four wild strains of *A. tumefaciens* were either inoculated on shoots or co-cultivated with internodes. This study was a preliminary attempt to assess the susceptibility of elm to *A. tumefaciens*. Keeping in view the failure of developing resistant elm to Dutch Elm Disease (DED) by conventional breeding methods the transgenic approach may be helpful for transferring agronomically useful traits.

Recently, the world's first genetically modified elm tree resistant to Dutch elm disease has been developed by a team from the University of Albertay Dundee. This finding could lead to the reintroduction into their natural habitat of elm trees resistant to the Dutch elm disease. The English elm, *Ulmus procera* , is highly valued both for its environmental benefits, especially in urban situations, and timber quality. This valuable plant has been the victim of a highly virulent fungal pathogen. The fungus, *Ophiostoma ulmi* , has caused the death of around 25 million elms in Britain alone. Traditional breeding programmes and antifungal chemical activities met with

only limited success due to the complex disease cycle. The University of Albertay workers have transferred an anti-fungal gene into the elm genome that could resist the killer DED fungus. Some of the transgenic elms have been grown in field conditions. There have been attempts to engineer trees conferring resistance to fungal and bacterial diseases by the expression of antifungal and anti bacterial proteins of distant plant origin. Fire blight disease in trees caused by the bacteria can now be controlled by developing transgenics (Reynoird et al. 1999). Recently transgenic apple conferring resistance to apple scab has been developed by expressing antifungal endochitinase isolated from mycoparasitic fungus (Bolar et al. 2000). Increased fungal resistance has been obtained in hybrid poplar leaves by expressing a wheat oxalate oxidase gene (Liang et al. 2001). MB39, a modified cecropin SB 37 gene derived from barley α-amylase placed under the control of a wound inducible promoter from tobacco, has been transferred to apple (Liu et al. 2000). Recently, a research group at the Tree Genetic Engineering Research Cooperative (TGERC), Oregon State University, generated 100 lines of transgenic aspens and cottonwoods that contain a synthetic gene from the *cry3a* strain of *B. thuringiensis*. All the lines showed strong resistance to the cottonwood leaf beetle, a devastating pest of poplars, and also enhanced growth rate (Strauss and Scott 2002).

Leaf protoplasts were transformed with RSV-F gene by the PEG method and transgenic plants were developed in apple (Sandhu et al.1999). Expression of the human respiratory syncytical virus fusion protein (F) is a step towards the exploitation of plants for expression of recombinant proteins of pharmaceutical value. This may lead to the development of transgenic plants able to deliver antigens in an edible vaccine form. Somatic embryos were used for micro projectile bombardment mediated transformation and transgenic somatic embryos were developed into transgenic plants in *Picea mariana* (Tian et al. 2000). The expression of gus gene in the needles of regenerated seedlings support the potential for long term transgene expression in spruce. In the conifer genetic engineering programme a gene conferring resistance to the herbicide buster has been transferred to *Pinus radiata* and *Picea abies* using the biolistic transformation procedure (Bishop-Harley et al. 2001). Genetically engineered herbicide resistance in conifers may help as a feasible option for plantation forestry. The TGERC group have reported 200 lines of transgenic aspen and cottonwoods showing high tolerance to the herbicide Roundup.

Genetically engineered decaffeinated coffee plants are becoming a reality, after the researchers cloned the gene that makes caffeine. The candidate gene is caffeine synthase that catalyzes the final step in the biosynthesis of caffeine. The next step is to switch off this gene and make the coffee plant naturally deficient in caffeine (Kato et al. 2000). Recently, another key enzyme in caffeine biosynthesis, 7-methylxanthine methyltransferase, has been isolated (Ogawa et al. 2001). Researchers at the University of Hawaii have created transgenic coffee plants that make less caffeine. The new coffee plant has an antisense gene for the enzyme xanthosine-N7-methyl transferase, the first enzyme in the pathway of caffeine biosynthesis.

The use of plants to repair polluted environments is known as phytoremediation. Lands that are unused due to the presence of high concentrations of heavy metals due to urban and industrial activities can be improved by planting trees which can detoxify the heavy metals. Phytoremediation potentially allows the possibility of inexpensive clean up of polluted environments. These can be both aqueous and soil based and the pollutants can range from complex organic molecules to heavy metals such as lead, cadmium, and zinc. Trees are an ideal tool for phytoremediation because of their large biomass and longevity. Transgenic yellow poplar trees have been developed for mercury phytoremediation (Rugh et al. 1998). Several options can now be envisaged to use tree genetic engineering with diverse agronomic, economic and environmental benefits (Pena and Seguin 2001). Herbicide resistant crops have been one of the major products of the first generation of agricultural biotechnology. A number of reports are available in the literature concerning the transfer of herbicide resistant genes in tree species. The first transgenic woody species was reported in *Populus* was for herbicide resistance (Fillatti et al. 1987). Transgenic hybrid poplar with a reduced sensitivity to glyphosate was produced. Strauss et al. (1997) reported on the generation of transgenic poplar harbouring glyphosate degradation genes and EPSP synthase. Recently 2 years of field trails of aspen and hybrid cottonwoods have shown stable high levels of tolerance, and an absence of any growth penalty in any that are used commercially (Meilan et al. 2000). Recently, methods for characterization of transgenic trees have been developed. Multiple PCR combining transgene and S-allele control primers to simultaneously confirm cultivar identity and production of transgenic plants in apple has been reported (Broothaerts et al. 2001). This is particularly useful to overcome the problem of mislabelling of cultivars during sub-culturing and other laboratory and greenhouse operations.

Conclusion

In woody tree species there has been tremendous advancement in in vitro culture research. In the last decade several protocols have been developed for in vitro micropropagation of woody tree species. The use of tissue culture methods as a micropropagation system for tree species is an established technology compared to traditional propagation systems. In these reports it is evident that it is possible to overcome some of the inherent problems of establishment of aseptic cultures and induction of axillary multiple shoots. However, very few protocols are available for micropropagation by using explants from elite mature trees in their natural habitat. More refinement in the protocols is essential in understanding the requirements for efficient regeneration

without the callus pathway that ensures genetic fidelity. The success obtained in micropropagation involving callus phase particularly via somatic embryogenesis has been encouraging. However, the basic understanding of the physiological mechanism involved has often been lacking. Recently, increased knowledge in the area of tree physiology has allowed propagation systems to be improved and for the main bottlenecks in particular ontogenetic aging and low conversion frequencies in somatic embryo systems to be overcome. Induction of somatic embryogenesis and their conversion to plants has been reported in a large number of tree species. Low induction frequency of somatic embryogenesis and the quick loss of embryogenic competence are two inherent problems with somatic embryogenesis. However, reports on the induction of somatic embryogenesis from mature tree explants are not yet available in sufficient numbers.

A method for the induction of somatic embryogenesis in adult trees would be a fundamental break-through. A method to induce somatic embryogenesis in adult material may arise with the help of molecular biology and new cell culture techniques. Nuclear transfer from adult cells to an embryonal environment, or the transformation with genes that induce embryogenesis could be two possible approaches. A candidate gene might be LEC2 from *Arabidopsis thaliana* that could be exploited (Stone et al. 2001). This aspect needs to be addressed. True to type plants via direct or indirect somatic embryogenesis may boost forest tree micropropagation. In recent times the genetic fidelity and variation of somatic embryo-derived seedlings and micropropagated plants have been tested using molecular markers (Shyama et al. 1997; Palombi and Damiano 2002).

In the past decade a number of research findings on evaluating transformation techniques in tree species have been reported. It has been found that *Agrobacterium* mediated transformation is used in most cases and marker genes have been transferred and a genetic transformation protocol has been standardized. Recent developments in transgenic trees can have multidirectional benefits. The benefits range from manipulating generation time, plant protection, wood quality, production of compounds of pharmaceutical value and improvements to polluted soils. These successes have shown avenues to include more agronomically useful genes for transfer to tree species as has been demonstrated in crop plants (Giri and Vijaya Laxmi 2000). From the global trend forestry biotechnology joint venture information there are now at least 24 tree species that have been subject to transgenic modification and released into the environment through field trials. Extensive lists of transgenic trees are being added day by day (WWF 2002). Recently, it has been emphasized that genetically modified trees can be excellent tools for physiological research (Herschbach and Kopriva 2002). The research work completed so far amply demonstrates the potential of these techniques in the improvement of forest tree species. *Arabidopsis* can be used as a model system in this molecular age of genetics and is exploited as a "proxy tree" for developing genetically modified trees. Trees have tremendous economic and ecological value, as well as unique biological properties of basic scientific interest. The inherent difficulties of experimenting on very large long-lived organisms motivate the development of model systems for forest trees. *Populus* trees (poplars, cottonwoods, aspens) have several advantages as a model system, including rapid growth, prolific sexual reproduction, ease of cloning, small genome, facile transgenesis, and tight coupling between physiological traits and biomass productivity. The poplar is the most worked on tree species in vitro. A combination of genetics and physiology is being used to understand the detailed mechanisms of forest tree growth and development.

One of the most important aspects of transgenic trees is the integration and expression of a gene. For long-lived tree species, new questions arise regarding the stability of integration and expression of foreign genes. Biosafety considerations, including the impact of transgene dispersion through pollen and unexpected effects on non target organisms, are now receiving attention. With recent research developments, molecular genetics provides tools that may allow genetic improvement to make up lost ground. Forest biotechnology has made a first phase impact. If current progress in tissue culture and genetic transformation combined with biotechnological applications continues the future may witness super tree species tailored for special agronomic and economic characteristics.

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