韓國林學會誌 77(4) : 467-483. 1988
Jour. Korean For. Soc. 77(4) : 467-483. 1988

〈Review〉

Biotechnological Applications in *Populus* Species[1*]

Young Woo Chun[2] · Ned B. Klopfenstein[3,4] · Harold S. McNabb Jr.[3,4]
Richard B. Hall[3,4]

포플러類의 Biotechnology 應用[1*]

全瑛宇[2] · Ned B. Klopfenstein[3,4] · Harold S. McNabb, Jr.[3,4]
Richard B. Hall[3,4]

ABSTRACT

The development of tissue culture systems for *Populus* species, and the utilization of tissue culture biotechnology will be reviewed and discussed. Special emphasis will be placed on prospects for genetic transformation by *Agrobacterium*-mediated gene transfer methods.

要 約

포플러類에 對한 組織培養法과 *Agrobacterium* 에 의한 遺傳子 插入法등의 開發 및 利用 現況과 그 가능성에 對하여 調査 分析하였으며, 이들 새로운 技術을 포플러類에 適用할 때 예상되는 問題點과 그 利用展望에 對하여 記述 하였다.

INTRODUCTION

It was first reported in 1983 that a plant gene was transferred to and expressed in a plant of a different species using an *Agrobacterium*-mediated gene transfer method (Hoekema *et al.*, 1983 ; Murai *et al.*, 1983). Since then, gene transfer methods have been developed for several important forest tree species such as *Populus alba* X *P. grandidentata* (Fillatti *et al.*, 1987), *Pinus taeda* (Sederoff *et al.*, 1986) and *Pseudotsuga menziesii* (Dandekar *et al.*, 1987). In addition, major applications of tissue culture techniques for forest tree improvement were utilized for *Populus* species (Frohich and Weisgerber, 1985), *Pinus taeda* (Mckeand and Weir, 1984), and *Pinus radiata* (Aitken-Christie and Gleed, 1984). Because of rapid progress in developing biotechnological applications with *Populus* species, this genus is well-suited as a model system for deciduous forest tree species.

FOREST BIOTECHNOLOGY

There are several different difinitions of biotechnology. Biotechnology can be defined broadly as a technology that uses living organisms

[1] 接受 5月 31日 Received on May 31, 1988.
[2] Department of Forestry, College of Forestry, Kookmin Univ., Seoul, Korea.
[3] Department of Plant Pathology and [4] Department of Forestry, Iowa State Univ., Ames, Iowa 50011 USA.
* This paper was presented at IUFRO Subject Group S. 2.05 Workshop, "Genetic and Molecular Biologly of Interactions Between Harmful Agents and Trees", 16-21 Oct. 1988. Ames, IA, USA.

(or parts of organisms) to make or modify products, to improve plants or animals, or to develop micro-organisms for specific uses (Sederoff and Ledig, 1984). Torrey (1985) defined plant biotechnology as "the application of existing techniques of plant organ, tissue, and cell culture, plant molecular biology, and genetic engineering to the improvement of plants and of plant productivity for the benefit of man". Recently, Nelson and Haissig (1984) defined forest biotechnology as micropropagation and pollen suppression as well as genetic engineering including DNA recombination and protoplast fusion. Gupta and Durzan (1987) and Haissig et al. (1987) also used the biotechnology term in the area of tissue culture as tissue culture biotechnology. Chun (1985b) defined forest biotechnology as shown in Figure 1. For other discussions of the potential applications of biotechnology to forest tree species, the reader is referred to other reviews by Sommers and Brown (1979), Karnosky (1981), Farnum *et al.* (1983), Faltonson et al. (1984), Sederoff and Ledig (1984), Chun (1985a), Ahuja (1986) and Haissig et al. (1987).

One of the most valuable features of forest biotechnology is that it allows rapid incorporation of specific improvements within a short period of time. Nelson and Haissig (1984) and Riemenschneider et al. (1987) have shown that biotechnology can reduce the long time periods required for tree improvement by conventional tree breeding technology alone.

POPULUS AS A MODEL SYSTEM FOR BIOTECHNOLOGICAL APPLICATIONS WITH DECIDUOUS FOREST TREE SPECIES

With regard to silvicultural aspects, *Populus* species are among the fastest growing deciduous forest tree species and are distributed throughout temperate forests of the northern hemisphere (Dickmann and Stuart, 1983). Because of their rapid growth, ease of establishment through stem or root cuttings, apparent ease of coppice

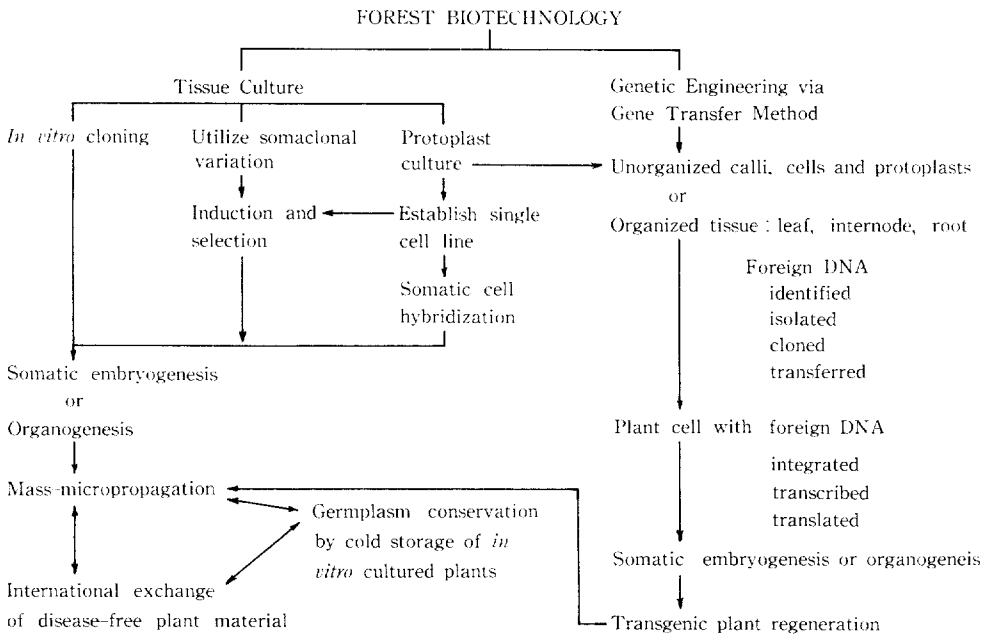

Fig. 1. Forest biotechnology by tissue culture and genetic engineering. On the left, established and developing *in vitro* techniques : on the right, prospective genetic engineering developments.

Table 1. Tissue culture research with *Populus* species.

Year	Species	Culture[a]	Regen.[b]	Reference
1964	*P. tremuloides*	C	S R	Mathes a, b
1968	*P. tremuloides*	C	S R W	Winton a, b
	P. tremuloides	C	S R	Wolter
1970	*P. tremuloides*	C	S R W	Winton
1971	*P. tremula*	C	S R W	Winton
1972	*P. deltoides* X *P. nigra*	C	S R W	Berbee *et al.*
	P. trichocarpa	Cat	S	Bawa and Stettle
1973	*P. nigra 'Italica'*	C	S R	Venverloo
1974	*P. euramericana*	C	S R	Chalupa
	P. tremula	C	S R	Chalupa
	P. deltoides	A	S R	Sato
	P. Sieboldii X *P. grandidentata*	A	S R	Sato
	P. maximowiczii	A	S R	Sato
1975	*P. nigra*	A	S R	Wang *et al.*
	P. tremuloides	C		Wolter and Gordon
	P. ussuriensis	A	S R	Heilungkiang Inst.
	P. simonii X *P. nigra*	A	S R	Heilungkiang Inst.
1977	*P. euramericana*	C	S R	Lester and Berbee
	P. tristis X *P. balsamifera*	A, M	S R W	Thompson and Gordon
	P. nigra 'Italica'	S	S R W	Whitehead and Giles
	P. deltoides X *P. nigra*	S	S R W	Whitehead and Giles
	P. yunnanensis	S	S R W	Whitehead and Giles
	P. berolinensis	A	S R W	North East For. Acad.
	P. harbinensis X *P. pyramidalis*	A	S R W	North East For. Acad.
	P. canadensis X *P. koreana*	A	S R W	North East For. Acad.
1978	*P. alba* ; *P. canescens*	S	S R W	Christie
	P. tremula ; *P. alba*	S	S R W	Christie
	P. alba X *P. tremula*	S	S R W	Christie
	P. tremuloides	S	S R W	Christie
	P. alba X *P. glandulosa*	S	S R W	Christie
1980	*P.* 14 *species*	A	S R W	Zhu *et al.*
	P. euramericana I-214	C	S	Saito a
	P. euramericana I-45/51	C	S	Saito b
	P. deltoides	C	S	Saito b
1981	*P. Sieboldii* X *P. grandidentata*	C	S R	Saito and Kawanobe
	P. alba X *P. glandulosa*	C	S R W	Kim *et al.*
1982	*P. maximowiczii* X *P. deltoides*	A	S R	Ho
1983	*P. tremula*	S	S R W	Ahuja a
	P. tremuloides	S	S R W	Ahuja a
	P. glandulosa	A	S R W	Kim et al.

[a] Culture=type of tissue culture. C : callus culture ; S : shoot tip (axillary bud) culture ; Cat : catkin culture ; AM : apical meristem culture ; A : anther culture ; I : internode culture ; IC : immature embryo culture ; EP : cultured explant culture ; CS : *in vitro* cold storage ; ER : immature embryo rescue ; T : transformation ; SV : induction and selection of somaclonal variation ; IE : *in vitro* culture for international exchange.

[b] Regen.=S : shoot regeneration ; R : root regeneration ; W : whole plant regeneration.

Table 1. Continued

Year	Species	Culture	Regen	Reference
1984	*P. alba* X			
	P. grandidentata	S	S R W	Chun and Hall
	P. trichocarpa X			
	P. tacamahaca	I	S R W	Douglas
	P. deltoides	IC	S R	Kouider *et al.*
1985	*P. heterophlla*	IC	S R	Savka and Dawson
1986	*P. alba* X			
	P. grandidentata	S, CS	S R W	Chun and Hall
	P. tremuloides	C	S R W	Noh and Minocha
	P. tremuloides	EP	S R W	Wann and Einspahr
	P. maximowiczii	A	S R W	Kim *et al.*
	P. alba X *P. glandulosa*	C	S R W	Kim *et al.*
	P. davidiana X			
	P. deltoides	ER	S R W	Noh *et al.*
	P. maximowiczii X			
	P. glandulosa	ER	S R W	Noh *et al.*
	P. nigra X *P. trichocarpa*	SV	S R W	Ettinger *et al.*
	P. trichocarpa	IE	S R W	McNabb *et al.*
	P. deltoides X			
	P. trichocarpa	IE	S R W	McNabb *et al.*
	P. nigra X			
	P. trichocarpa	IE	S R W	McNabb *et al.*
	P. deltoides X			
	P. maximowiczii	IE	S R W	McNabb *et al.*
1987	*P. nigra* X *P. trichocarpa*	SV	S R W	Ostry and Skilling
	P. maximowiczii X			
	P. trichocarpa	SV	S R W	Michler and Bauer
	P. alba X			
	P. grandidentata	T	S R W	Fillatti *et al.*
	P. alba X			
	P. grandidentata	EP	S R W	Chun

regeneration, *Populus* species have been considered ideal species for pulpwood and lumber production (Schreiner, 1974 ; Hall *et al.*, 1982). In addition, species and hybrids of *Populus* have been studies extensively for use in short-rotation, intensively cultured plantation systems for woody biomass production (Hall, 1985). Trees of this genus also represent wide genetic diversity, exhibit a wide range of site requirements, and respond well to cultural inputs (Schreiner, 1974).

There is a firm basis to support the use of *Populus* species as a model system for biotechnological applications with deciduous forest tree species. Most biotechnological applications with plants hinge on the ability to manipulate the morphogenetic potential of cells and tissue in culture.

Among the forest tree species, *Populus* species are the most well-studied in the area of tissue culture (Table 1). Tissue of *Populus* exhibits a high degree of developmental plasticity, similar to that of tobacco in the herbaceous species. *Populus* species are the only forest tree species for which *in vitro* systems have been defined to date for regeneration from protoplasts of leaf mesophyll tissue, sporophytic calli, gametophytic (anther) calli, leaf discs, internodes, root segments, axillary buds, and apical meristems. *Populus* species are the only woody species that have been transformed with a silviculturally-useful gene by

Table 2. Range of 1C nuclear DNA content per cell values for selected plants.

species	DNA content (picogram)	Reference
Soybena	1.2	Grierson and Covey, 1984
Tobacco	2.0	Grierson and Covey, 1984
Populus deltoides	0.535	Dhillon, 1987
Pinus taeda	12.0	Sederoff er al., 1986
Pinus lambertiana	43.8	Rake et al., 1980
Picea glauca	9.65	Rake et al., 1980
Pinus banksiana	14.9	Rake et al., 1980
Pinus resinosa	21.55	Rake et al., 1980
Pinus strobus	19.4	Kriebel, 1985
Pinus caribaea	12.5	Berlyn et al., 1987

an *Agrobacterium*-mediated gene transfer method (Fillatti *et al.*, 1987). There is also one report in which embryo rescue techniques were utilized to circumbent incompatibility of pollination between two different *Populus* species (Noh *et al.*, 1986).

In relation to other aspects of molecular biology, *Populus* species have a relatively small genome size which greatly facilitates the production and screening of genomic libraries (Parsons *et al.*, 1986) (Table 2). *Populus* species also are promising candidates as recipients for Ti plasmid -mediated gene transfer. Earlier work reports the susceptibility of various *Populus* species to infection by naturally-occurring *Agrobacterium* tumefaciens (De Cleene and Deley, 1976). Phytohormone independent callus growth has been obtained from stem and shoot segments of *Populus trichocarpa* x *P. deltoides* (*P.* x *interamericana* Brockh.) following transformation by wild type *Agrobacterium* tumefaciens strains A281 and A348 Parrsons *et al.*, 1986).

TISSUE CULTURE BIOTECHNOLOGY OF *POPULUS*

1) MICROPROPAGATION : Tissue culture systems for woody plant species have been developed primarily during the past two decades (Hartmann and Kester, 1983). For a diverse woody species, the application of tissue culture techniques for mass-propagation has become a primary alternative to more conventional propagation procedures. Economic factors currently restrict the large-scale utilization of tissue culture for mass propagation of poplar species (Haissig *et al.*, 1987) ; however, two routine methods, axillary bud production also termed axillary shoot production) and adventitious shoot initiation, have been developed for experimental use with these woody species.

Several *Populus* species have been successfully micropropagated in tissue culture systems using axillary bud culture and adventitious shoot initiation from cultured explants. Whitehead and Giles (1977) and Christie (1978) have shown that *Populus* species can be mass-propagated by axillary bud culture.

The advantages of using *in vitro* bud-cultured plantlets as a source material for biotechnological application are based on the following : 1) *in vitro* bud culture provides stable genotype sources (Lawrence, 1981 ; Karp and Bright, 1985), 2) a juvenile growth condition can be maintained under *in vitro* conditions (Scorza and Janick, 1980), 3) it provides a high morphogenetic potential for organogenesis (Wann and Einspahr, 1986 ; Chun, 1987), and 4) it also can provide a year-round plant source with a cyclical growth phase, which can be contained within a small space. There are also several reports that demonstrate the versatility of *in vitro* cultured poplar plantlets as a source material for biotechnological applications in manners such as : 1) direct morphogenesis from culture explants (Wann and Einspahr, 1986 ; Chun, 1987), 2) induction and selection of somaclonal variation for disease resistance (Ettinger *et al.*, 1986 ; Ostry and Skilling, 1987)

or herbicide tolerance (Nelson and Haissig, 1986 ; Michler and Bauer, 1987), 3) protoplast isolation and culture (Chun, 1985b ; Russell and McCown, 1986 ; Park and Son, 1988), 4) ploidy level manipulation (Chun, 1987), 5) international exchange of disease-free plant materials (McNabb *et al.*, 1986), 6) germplasm preservation through cold storage (Chun and Hall, 1986 ; Chun, 1987), and 7) regeneration of shoots transformed via co-culture with a genetically engineered *Agrobacterium* Ti plasmid binary vector system (Fillatti *et al.*, 1987, Chun *et al.*, 1988).

2) SOMACLONAL VARIATION : The technology of introducing and (or) selecting genetic variation by using tissue culture has been termed somaclonal variation (Larkin and Scowcroft, 1981 ; Evans *et al.*, 1984). Somaclonal variation has been observed for several phenotypic characteristics (Skirvin, 1978 ; Karp and Bright, 1985). The phenotypic characteristics of somaclonal variation can be caused by a range of genotypic or epigenetic changes. One of the most promising features of somaclonal variation is that it can involve agronomically-useful traits such as yield or disease resistance. The potential of somaclonal variation for improvement for woody species and crops has been reviewed by several scientists (Larkin and Scowcroft, 1981 ; Evans, 1986 ; Haissig *et al.*, 1987). Somaclonal variation has been utilized with a range of plant species including cereals (maize, oats, wheat, rice), seed crops (tobacco, tomato, alfalfa), vegetatively propagated crops (sugarcane, potato) and woody species (poplar) (Lester and Berbee, 1977). It is evident that the tools of somaclonal variation are technologies that can permit short-term accomplishment of breeding objectives (Haissig *et al.*, 1987). Somaclonal variation cannot replace conventional plant breeding, but it can generate or select desired variation to facilitate conventional breeding processes (Haissig *et al.*, 1987).

With regared to *Populus* species, Lester and Berbee (1977) demonstrated that callus culture-derived hybrid poplar (*P. deltoides* X *P. nigra*) exhibited somaclonal variation in height, number of branches, and leaf traits after one growing season. There are other reports in which somaclonal variation was utilized to select septoria leafspot-canker tolerant, hybrid poplar (Ettinger *et al.*, 1986 ; Ostry *et al.*, 1986 ; Ostry and Skilling, 1987) and herbicide tolerant, hybrid poplar (Nelson and Haissig, 1986 ; Michler and Bauer, 1987). Haissig *et al.* (1987) predict that applications of somaclonal and gametoclonal variation in tissue culture biotechnology of woody species will be the primary strategies for specific tree improvements of a monogenic or oligogenic nature.

3) PROTOPLAST CULTURE : One of the most significant developments in tissue culture techniques within recent years has been the isolation and culture of protoplasts. Such techniques have several potential applications, including : 1) selection of useful somaclonal variation in plants regenerated from protoplasts (Shepard et al., 1980 ; Kemble and Shepard, 1984), 2) insertion of silviculturally-important genes by micro-injection, electroporation, or *Agrobacterium* Ti plasmid-derived system (Ahuja, 1985 ; Cocking and Davey, 1987), 3) producing somatic cellular hybridization (Gleba and Sytnik, 1984), 4) uptake of nuclei, organelles, chromosomes or macro-molecules such as DNA and RNA Saxena *et al.*, 1986), and 5) physiological studies Vasil, 1976). In recent years, protoplasts have been routinely isolated, cultured, and regenerated with a number of plant species (Ahuja, 1982 ; 1984). Most woody plants, however, remain among the recalcitrant species that are difficult to regenerate from the protoplast cultures. Nevertheless, successful protoplast isolations or cultures have been regenerated with a few species of *Populus* (Table 3). Recently, Russell and McCown (1986) regenerated whole plants from single protoplasts of *Populus alba* X *P. grandidentata*. Since protoplasts from forest tree species remain difficult to grow and differentiate *in vitro*, it has not yet been possible to effectively utilize somatic hybridi-

Table 3. Protoplast research in Populus species

Species	Reference
P. euramericana cv. I-45/51	Saito, 1976, 1980c
P. trichocarpa X *P. tacamahaca*	Douglas, 1982
P. tremuloides	Verma and Wann, 1983
	Ahuja, 1983b
P. alba X *P. grandidentata*	Chun, 1985b
	Russell and McCown, 1986
P. tremula X *P. tremuloides*	Ahuja, 1984
P. tremula	Ahuja, 1984
P. nigra X *P. laurifolia*	Russell and McCown, 1986
P. tacatricho	Butt, 1985
P. euramericana	Park and Son, 1986
P. alba X *P. glandulosa*	Park and Han, 1986
	Park and Son, 1988
P. nigra	Lee *et al.*, 1987

zation techniques to produce new genotypes.

4) LIMITATION OF TISSUE CULTURE BIOTECHNOLOGY IN *POPULUS* SPECIES : *Populus* species are promising candidetes as the target species for tissue culture biotechnology applications because recent work has demonstrated rapid progress in specific areas such as micro-propagation, protoplast culture, and induction of beneficial somaclonal variation. This recent progress should stimulate more rapid incorporation of specific improvements with conventional tree breeding programs within a short period of time. There are still many basic problems, however, to overcome for utilization of biotechnology in *Populus* species. These problems include the limitations in 1) somatic embryogenesis, 2) generalized regeneration technique for protoplast culture, 3) uncontrolled somaclonal variation leading to a failure of true-to-type propagation in tissue culture.

GENETIC ENGINEERING OF *POPULUS*

To utilize genetic engineering in *Populus* species, there are four general considerations : 1) What genes and regulatory regions are available for transfer into target species ? 2) What delivery systems can be used to transfer the specific gene ? 3) Are systems established for regeneration from tissue culture ? and 4) Are selection methods available to screen for transformed tissue or whole plants ?

1) POSSIBLE GENES FOR TRANSFORMATION : Recently, several research groups have isolated and transferred a few agriculturally-useful genes into plants. These include genes for insect resistance such as proteinase inhibitor II gene from potato (Thornburg *et al.*, 1987a : b), trypsin inhibitor gene from cowpea (Klausner, 1987), and insecticidal protein genes from *Bacillus thuringiensis* (Adang *et al.*, 1986 ; Fischhoff *et al.*, 1987), disease resistance such as coat protein genes from tobacco mosaic virus (Abel *et al.*, 1986) and alfalfa mosaic virus (Tumer *et al.*, 1987), and herbicide tolerance from bacteria (Comai *et al.*, 1984) or from a plant (Shah *et al.*, 1986). Sederoff and Ledig (1984) reported some possible gene systems with potential for tree improvement. These single dominant genes include a blister rust resistance gene in white pine, an apical dominance gene in Scots pine, and a cedar leaf blight-resistance gene in Western red cedar and Japanese red cedar. Like other tree species, *Populus* species have the same difficulties in identifying and manipulating genes responsible for expression of traits that are under single or oligo-genic control. So far, the promoter region of proteinase inhibitor gene is the only identified one in *Populus* (Parsons at Department of Biochemistry, Univ. of Washington : personal communica-

tion). In addition, a bacterial 5-enolpyruvyl-shikimate 3-phosphate (EPSP) synthase gene was used to transform poplar and expressed to provide glyphosate tolerance (Fillatti *et al.*, 1987).

2) GENE TRANSFER METHOD : There presently are several non-sexual gene transfer methods available for introducing DNA sequences into plant cells (Goodman *et al.*, 1987 ; Perani *et al.*, 1986 ; Wilki-Douglas *et al.*, 1986 ; Konez *et al.*, 1987). These methods include : 1) protoplast (cell) fusion, 2) gene transfer by manipulating DNA directly, 3) *Agrobacterium*-mediated gene transfer, 4) microinjection, 5) virus-mediated gene transfer, and 6) transposon-mediated gene transfer. Among these different gene transfer methods, *Agrobacterium*-mediated gene transfer method is, apparently, the only method reportedly used thus far for woody species (Fillatti *et al.*, 1987). Direct DNA uptake methods of plant gene transfer require plant cell protoplasts, which are difficult

Table 4. Major events toward developing *Agrobacterium*-mediated gene transfer methods

Year	Major events
1974	-Identification of Ti plasmid (Zaenen *et al.*)
1977	Stable transformation of T-DNA into higher plant cells (Chilton *et al.*)
1980	-T-DNA integration into plant nuclear genome by insertion of a foreign gene into the T-DNA region of a Ti plasmid (Chilton *et al.* ; Willmitzer *et al.*)
1981	-Functional map of T-DNA delineated (Garfinkel *et al.*)
1982	Determination that virulence (*vir*) genes of Ti plasmid are required for T-DNA integration into plant nulear genome (Iyer *et al.*)
1983	-Selective marker genes conferring resistance to antibiotics were inserted into a Ti plasmid for selection of transgenic plant tissue (Herrera-Estrella *et al.*, b) -Development of transformation techniques by homologous recombination of hybrid and wild type Ti plasmid in *Agrobacterium tumefaciens* (Barton *et al.* ; Zambryski *et al.*) First plant gene (bean phaseolin gene) expressed in a plant of a different species (sunflower) by Ti plasmid (Murai *et al.*) -Development of binary T-DNA vectors for the transfer of a foreign gene (pea small subunit gene of ribulose 1,5-bisphos-phate carboxylase) into a plant (Hoekema *et al.*)
1984	Determined that T-DNA border sequences (right and left borders) are essential for transformation (Wang *et al.* ; Shaw *et al.*) Obtained expression of foreign genes in regenerated plants and their progeny (Herrera-Estrella *et al.*)
1985	Utilized a binary vector system of Agrobacterium for transformation of leaf discs (An *et al.* ; Klee *et al.*)
1986	Transformation of hybrid poplar and loblolly pine (Pinus taeda) by Ti plasmid (Parsons *et al.* ; Sederoff *et al.*)
1987	-Regeneration of glyphosate-tolerant hybrid poplar produced by *Agrobacterium* binary vector system (Fillatti *et al.*) -A kanamycin resistance marker gene was transferred to Douglas-fir by *Agrobacterium* (Dandekar *et al.*)

to regenerate for most woody species. This barrier can be circumvented with an *Agrobacterium*-mediated gene transfer system, because transformed plant material can be produced and regenerated with this gene transfer method without protoplast isolation and culture. As a result, such tissue culture-related limitations of woody species are diminished with *Agrobacterium*-mediated gene transfer methods.

3) *AGROBACTERIUM*-MEDIATED GENE TRANSFORMATION : *Agrobacterium*-mediated gene transfer utilizes the natural ability of *Agrobacterium tumefaciens* to infect many dicotyledonous and gymnospermous plants. *Agrobacterium* infection causes tumorous crown gall formation by introducing DNA into the plant cells at a wound site. Major events in the development of *Agrobacterium*-mediated gene transfer methods are summarized in Table 4. The recent discovery that the T-DNA and the *vir* region of *Agrobacterium* tumefaciens Ti plasmid could be separated into two different plasmids, without loss of the transfer capability of the T-DNA, promoted the construction of *Agrobacterium* binary vector systems. These binary vector plant transformation systems consist of two plasmids in *Agrobacterium* : the binary vector plasmid and a helper plasmid (Hoekema et al., 1983 ; An *et al*., 1985). A typical binary T-DNA plasmid vector contains several useful characteristics such as 1) T-DNA border sequences which are required for successful transfer of DNA from the Agrobacterium cell into the plant genome, 2) A wide host range replicon which can replicate in both *Escherichia coli* and *Agrobacterium* cell systems. The desired foreign gene is cloned into the engineered binary T-DNA plasmid between the border sequences, 3) A selectable marker gene that usually confers antibiotic resistance to allow selection of transformed plant material. The typical helper Ti plasmid is an intact wild type or a disarmed (T-DNA deleted) Ti plasmid that usually contain supervirulent (broad host range) *vir* genes.

The *vir* genes on a helper Ti plasmid can act in *cis* to promote transfer of the T-DNA of its own plasmid or in *trans*, to transfer the T-DNA on the binary plasmid (Hille *et al*., 1984).

4) SELECTABLE MARKERS FOR PLANT TRANSFORMATION : Several marker genes for plant transformation have been developed (Fraley *et al*., 1986). These markers for selection of plant transformation have two categories ; selectable markers and scorable-assayable markers. The selectable markers include neomycin resistance, methotrexate resistance, hygromycin resistance, chloramphenicol resistance, and aminoethyl cysteine resistance. The scorable-assayable markers include opine synthesis, neomycin phosphotransferase activity, chloramphenicol acetyl transferase activity and luciferase activity.

LEAF DISC TRANSFORMATION : Because *Populus* species demonstrate high degree of developmental plasticity through tissue culture manipulation, we can regenerate whole plants through explant culture of leaf, internode, and root segments of *Populus* species. This adventitious shoot formation from cultured explants allows the utilization of the *Agrobacterium* binary vector system to transfer specific genes into *Populus* species through co-cultivation of *Agrobacterium* binary vectors with leaf discs. There are two co-cultivation mathods which have become routine in some herbaceous plant species such as tobacco, tomato, petunia, cotton, and *Arabidopsis* (McCormick *et al*., 1986 ; Wei *et al*., 1986 ; An *et al*., 1986). One employs a nurse culture within feeder plates (Horsch *et al*., 1985 ; Lloyd *et al*., 1986 ; Fillatti *et al*., 1987). The use of feeder plates has some effects toward reducing the bacterial damage to the leaf pieces and stimulating transformation events. Another co-cultivation method is direct selection after co-cultivation of *Agrobacterium* with leaf segments without placing on feeder plates (An *et al*., 1985 ; An *et al*., 1986 ; Umbeck *et al*., 1987 ; Dandekar *et al*., 1987).

CONCLUSION

Populus biotechnology can offer the following potential in the near future : 1) greatly shortened tree improvement cycles for specific traits that are controlled by a mono- or oligogenes ; 2) incorporation of new gene combinations by recombinant DNA methods ; 3) mass micropropagation of plants with specific gene combinations through *in vitro* culture ; 4) induction and selection of somaclonal variation for improved pest tolerance or resistance.

LITERATURE CITED

1. Abel, P.P., R.S. Nelson, De Barun, N. Hoffmann, S.G. Rogers, R.T. Fraley, and R.N. Beachy. 1986. Delay of disease development in transgenic plants that express the Tobacco Mosaic Virus coat protein gene. Science 232 : 738-743.
2. Adang, M.J., E. Firoozabady, D.L. Deboer, J. Klein, D.J. Merlo, E. Murray, T. Rochelean, K. Rashka, G. Staffeld, and C. Stock. 1986. Expression of a *Bacillus thuringiensis* crystal protein gene in *Nicotiana tabacum*. Page 404 *in* D.N. Sommers, B.G. Gengenbach, B.G. Biesboer, W.P. Hackett, and C.E. Green, eds. Proc. Sixth International Cong. of Plant Tissue and Cell Culture. Univ. of Minn., Minneapolis, MN.
3. Ahuja, M.R. 1982. Isolation, culture and fusion of protoplasts : problems and prospects. Silvae Genetica 33 : 66-67.
4. Ahuja, M.R. 1983a. Somatic cell differentiation and rapid clonal propagation of aspen. Silvae Genetica 32 : 131-135.
5. Ahuja, M.R. 1983b. Isolation and culture of mega and normal protoplasts in aspen. Silvae Genetica 32 : 5-6.
6. Ahuja, M.R. 1984. Protoplast research in woody plant. Silvae Genetica 33 : 32-36.
7. Ahuja, M.R. 1985. Ti genes in forest tree protoplast regeneration. Page 5 *in* H. B. Kriebel, ed. Molecular Genetics of Forest Tree-Research Techniques and Strategies. Working party s2.04.06-IUFRO. The Ohio State Univ., Wooster, Ohio.
8. Ahuja, M.R. 1986. Perspective in plant biotechnology. Current Science 55 : 217-224.
9. Aitken-Christie, J., and J.A. Gleed. 1984. Uses for micropropagation of juvenile radiata pine in New Zealand. Pages 47-57 *in* Proc. International Symp. of Recent Advances of Forest Biotechnology, Traverse City, Michigan.
10. An, G., B.D. Watson, S. Stachel, M.P. Gordon, and E.W. Nester. 1985. New cloning vehicles for transformation of higher plants. EMBO J. 4 : 277-284.
11. An, G., B.D. Watson, and C.C. Chiang. 1986. Transformation of tobacco, tomato, potato, and *Arabidopsis thaliana* using binary Ti vector system. Plant Physiol. 81 : 301-305.
12. Barton, K.A., A.N. Binns, A.J.M. Marzke, and M.D. Chilton. 1983. Regeneration on intact tobacco plants containing full length copies of genetically engineered T-DNA, and transmission of T-DNA to R1 progeny. Cell 32 : 1033-1043.
13. Bawa, K.S., and R.F. Stettle. 1972. Organ culture with black cottonwood : morphogenetic responses of female catkin primordia. Can. J. Bot. 50 : 1627-1631.
14. Berbee, F.M., J.G. Berbee, and A.C. Hildebrandt. 1972. Induction of callus and trees from stem tip culture of a hybrid poplar. In *Vitro* 7 : 269. (Abstr.)
15. Berlyn, G.P., A.O. Anomo, R.C. Beck, and J. Cheng. 1987. DNA content polymorphism and tissue culture regeneration in Caribbean pine. Can. J. Bot. 65 : 954-961.
16. Butt, A.D. 1985. A general method for the high-yield isolation of mesophyll protoplasts from deciduous species. Plant Science 42 : 55-59.

17. Chalupa, V. 1974. Control of root and shoot formation and production of trees from poplar callus. Biol. Plant. 16 : 316-320.
18. Chilton, M.D., M.H. Drummond, D.J. Merlo, D. Sciaky, A.L. Montoya, M.P. Gordon, and E.W. Nester. 1977. Stable incorporation of plasmid DNA into higher plant cells : The molecular basis of crown gall tumorigenesis. Cell 11 : 263-271.
19. Chilton, M.D., R.K. Saiki, N. Yadav, M. P. Gordon, and F. Quetier. 1980. T-DNA from *Agrobacterium* Ti plasmid is in the nuclear fraction of crown gall tumor cells. Proc. Natl. Acad. Sci. USA 77 : 4060-4064.
20. Christie, C.B. 1978. Rapid propagation of aspen and silver poplars using tissue culture techniques. Proc. Int. Plant Prop. Soc. 28 : 255-260.
21. Chun, Y.W. 1985a. Prospects in forest biotechnology. J. Korean For. Soc. 68 : 99-110.
22. Chun, Y.W. 1985b. Isolation and culture of in vitro cultured *Populus alba* X *P. grandidentata* protoplasts. J. Korean For. Soc. 71 : 45-49.
23. Chun, Y.W. 1987. Biotechnology applications of *Populus* micropropagation. Ph. D. Dissertation. Iowa State University, Ames, Iowa. 149pp.
24. Chun, Y.W., and R.B. Hall. 1984. Survival and early growth of *Populus alba* X *P. grandidentata in vitro* cultured plantlets in soil. J. Korean For. Soc. 66 : 1-7.
25. Chun, Y.W., Ned B. Klopfenstein, Harold S. McNabb, Jr., and Richard B. Hall. 1988. Transformation of *Populus* species by an *Agrobacterium* binary vector system. J. Korean For. Soc. 77 : 199-207.
26. Chun, Y.W., and R.B. Hall. 1986. Low temperature storage of *in vitro* cultured hybrid poplar, *Populus alba* X *P. grandidentata* plantlets. Page 13 *in* D.A. Sommers, B.G. Gengenbach, D.D. Biesboer, W.P. Hackett, and C.E. Green, eds. Proc. Sixth International Congress of Plant Tissue and Cell Culture. Univl of Minn., Minneapolis, MN.
27. Chun, Y.W., R.B. Hall, and L.C. Stephens. 1986. Influences of medium consistency and shoot density on *in vitro* proliferation of *Populus alba* X *P. grandidentata*. Plant Cell Tissue Organ Culture 5 : 179-185.
28. Cocking, E.C., and M.R. Davey. 1987. Gene transfer in cereals. Science 236 : 1259-1262.
29. Comai, L., d. Facciotti, W.R. Hiatt, G. Thompson, R. Rose, and D. Stalker. 1985. Expression in plants of a mutant aro A gene from *Salmonella typhimurium* confers tolerance to glyphosate. Nature 317 : 741-744.
30. Dandekar, A.M., P.L. Gupta, D.J. Durzan, and V.Knauf. 1987. Transformation and foreign gene expression in micropropagated Douglas-fir (*Pseudotsuga menziesii*). Bio/Technology 5 : 587-590.
31. De Cleene, M., and J. Delay. 1976. The host range of crown gall. The Botanical Review 42 : 389-466.
32. Dhillon, S.S. 1987. DNA analysis during growth and development. *in* J.W. Hanover and D.E. Keathley eds. Genetic manipulation of Woody Plants. Plenum Press, New York.
33. Dickmann, D.I. and K.W. Stuart. 1983. The culture of poplars in Eastern North America. Michigan State University, East Lansing. 169 pp.
34. Douglas, G.C. 1982. Protoplast isolation from totipotent cell-cultures of *Populus* hybrid TT32. Pages 605-606 *in* A. Fujiwara, ed. Plant Tissue Culture 1982. Japanese Association of Plant Tissue Culture, Tokyo, Japan.
35. Douglas, G.C. 1984. Formation of adventitious buds in stem internodes of Populus spp. cultured *in vitro* on basal medium : influence of endogenous properties of explants. *Plant* Physiol. 116 : 313-321.

36. Ettinger, T.L., M.E. Ostry, W.P. Hackett, P.E. Read, and D.D. Skilling. 1986. Utilization of somaclonal variation in the development of Septoria musiva-resistance hybrid *Populus*. Page 214 *in* D.A. Sommers, B.G. Gengenbach, D.D. Biesboer, W.P. Hackett, and C.E. Green, eds. Proc. Sixth International Congress of Plant Tissue and Cell Culture. Univ. of Minn., Minneapolis, MN.

37. Evans, D.A., W.R. Sharp, and H.P. Medina-Filho. 1984. Somaclonal and gametoclonal variation. Am. J. Bot. 71 : 759-774.

38. Evans, E.E. 1986. Somaclonal and gametoclonal variation. Pages 63-96 *in* P.C. Augustine, H.D. Danforth, M.R. Bakst, eds. Biotechnology for solving agricultural problems. Martinus Nijhoff Publisher, The Hague, Netherlands.

39. Faltonson, R.R., Y.W. Chun, T.L. Robison, and R.B. Hall. 1984. The role of vegetative propagation in tree improvement. Proc. of Forestry Comm. Great Plains Agric. Counc. Publ. No. 112 : 36 : 202-213.

40. Farnum, P., T. Roger, and J.L. Kulp. 1983. Biotechnology of forest yield. Science 219 : 694-702.

41. Fillatti, J.J., J. Sellmer, B. McCown, B. Haissig, and L. Comai. 1987. *Agrobacterium* mediated transformation and regeneration of *Populus*. Mol. Gen. Genet. 206 : 192-199.

42. Fischhoff, D.A., K.S. Bowdish, F.J. Perlak, P.G. Marrone, S.M. McCormick, J.G. Niedermeyer, D.A. Dean, K. Kusano-Kretzmer, E.J. Mayer, D.E. Rochester, S.G. Rogers, and R.T. Fraley, 1987. Insect tolerant transgenic tomato plants. Bio/Technology 5 : 807-813.

43. Fraley, R.T., S.G. Rogers, and R.B. Horsch. 1986. Genetic transformation in higher plants. CRC Critical Reviews in Plant Science 4 : 1-46.

44. Frohlich, H.J., and H. Weisgerber. 1985. Research on *in vitro* techniques within the frame work of poplar breeding results and future trends. Silvae Genetica 34 : 132-137.

45. Garfinkel, D.J., R.B. Simpson, L.W. Ream, F.F. White, M.P. Gordon, and E.W. Nester. 1981. Genetic analysis of crown gall : fine structure map of the T-DNA by site-directed mutagenesis. Cell 27 : 143-153.

46. Gleba, Y.Y., and K.M. Sytnik. 1984. Protoplast fusion : Genetic engineering in higher plants. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo. 220 pp.

47. Goodman, R.M., H. Hauptli, A. Crossway, and V.C. Knauf. 1987. Gene transfer in crop improvement. Science 236 : 48-54.

48. Grierson, D., and S. Covey. 1984. Plant molecular biology. Blackie and son Limited, London. 176 pp.

49. Gupta, P.K., and D.J. Durzan. 1987. Biotechnology of somatic polyembryogenesis and plantlet regeneration in loblolly pine. Bio/Technology 5 : 147-151.

50. Haissig, B.E., N.D. Nelson, and G.H. Kidd. 1987. Trends in the use of tissue culture in forest imporvement. Bio/Technology 5 : 52-59.

51. Hall, R.B. 1985. Breeding strategy for *Alnus*, *Populus*, and *Salix*. Pages 75-95 *in* C.P. Mitchell, P.O. Nilsson, and L. Zsuffa, eds. Proc. Joint IEA Forestry Energy Program and FAO/Cooperative Network on Rural Forest Energy Conf. and Workshops on Research in Forestry for Energy. Rungstedgaard, Denmark. Oct. 1985. Swedish Univ. of Ag. Sci. nr. 49 1986.

52. Hall, R.B., G.D. Hilton, and C.A. Maynard. 1982. Construction lumber from hybrid aspen plantation in the Central States. J. of Forestry 80 : 291-294.

53. Hartmann, H.T., and D.E. Kester. 1983. Plant propagation-principles and practices. Fourth edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 727pp.

54. Heilungkiang Inst. For. Tree Improv. Lab. 1975. Induction of haploid poplar plants from

anther culture *in vitro*. Sci. Sinica 18 : 669-677.

55. Herrera-Estrella, L., A. Depicker, M. Van Montagu, and J. Schell. 1983a. Expression of chimeric genes transferred into plant cells using a Ti-plasmid-dervied vector. Nature 303 : 209-213.
56. Herrera-Estrella, L., M. De Block, E. Messens, J.P. Hernalsteens, M.Van Montagu, and J. Schell. 1983b. Chimeric genes as dominant selectable markers in plant cells. EMBO J. 2 : 987-995.
57. Herrera-Estrella, L., G. Van den Broeck, R. Maenhaut, M. Van Montagu, and J. Schell. 1984. Light-inducible and chloroplast-associated expression of a chimeric gene introduced into *Nicotiana tabacum* using a Ti plasmid vector. Nature 310 : 115-120.
58. Hille, J., J. van Kan, and R.A. Schilperoort. 1984. *trans*-Acting virulence functions of the octopine Ti plasmid from *Agrobacterium tumefaciens*. J. Bacteriol. 158 : 754-756.
59. Ho, R.H. 1982. Haploid plant culture in poplar. Pages 210-213 *in* Proc. 7th North American Forest Biology Workshop, July 26-28, 1982. Unov. of Kentucky, Lexington, Kentucky.
60. Hoekema, A., P.R. Hirsch, R.J.J. Hooykaas, and R.A. Schilperoort. 1983. A binary plant vector strategy based on separation of *vir* and T-region of the *Agrobacterium tumefaciens* Ti plasmid. Nature 303 : 179-180.
61. Horsch, R.B., J.B. Fry, N.L. Hoffmann, M. Wallroth, D. Eichholtz, S.G. Rogers, and R.T. Fraley. 1985. A simple and general method for transferring genes into plants. Science 227 : 1229-1231.
62. Iyer, V.N., H.J. Klee, and E.W. Nester. 1982. Units of genetic expression in the virulence region of a plant-tumor-inducing plasmid of *Agrobacterium tumefaciens*. Mol. Gen. Genet. 188 : 418-424.
63. Karnosky, D.F. 1981. Potential for forest tree improvement via tissue culture. BioScience 31 : 114-120.
64. Karp, A.S., and W.J. Bright. 1985. On the causes and origins of somaclonal variation. Oxford Surveys Surveys of Plant Molecular and Cell Biology 2 : 199-234
65. Kemble, R.J., and J.F. Shepard. 1984. Cytoplasmic DNA variation in a potato protoclone population. Theor. Appl. Genet. 69 : 211-216.
66. Kim, J.H., S.K. Lee, and Y.W. Chun. 1981. Mass propagation of tree species through *in vitro* culture. I. Bud culture of *Populus alba* X *P. glandulosa* F1. Inst. For. Genet. Res. Rep. 17 : 57-63.
67. Kim, J.H., E.W. Noh, and J.I. Park. 1983. Haploid plantlet formation through anther culture of *Populus glandulosa*. Inst. For. Genet. Res. Rep. 19 : 93-98.
68. Kim, J.H., H.K. Moon, and J.I. Park. 1986. Haploid plantlet induction through anther culture of *Populus maximowiczii*. Inst. For. Genet. Res. Rep. 22 : 116-121.
69. Kim, J.H., H.K. Moon, and J.I. Park. 1986. Plantlet regeneration on callus derived from internodal tissue of *Populus alba* X *P. glandulosa*. Inst. For. Genet. Res. Rep. 22 : 122-127.
70. Klausner, A. 1987. Trypsin inhibitor confers pest resistance. Bio/Technology 5 : 426.
71. Klee, H.J., M.F. Yanofsky, and E.W. Nester. 1985. Vectors for transformation of higher plants. Bio/Technology 3 : 637-642.
72. Konez, C., Z. Konez-Kalman, and J. Schell. 1987. Transposon Tn5 mediated gene transfer into plants. Mol. Gen. Genet. 207 : 99-105.
73. Kouider, M., R.M. Skirvin, P.K. Saladín, J.O. Dawson, and J.J. Jokela. 1984. A method to culture immature embryos of *Populus deltoides in vitro*. Can. J. For. Res. 14 : 956-958.
74. Kriebel, H.B. 1985. DNA sequence components of the *Pinus strobus* nuclear genome. Can. J. For. Res. 15 : 1-4.

75. Larkin, P.J., and W.R. Scowcroft. 1981. Somaclonal variation-a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60 : 197-214.

76. Lawrence, R.H., Jr. 1981. *In vitro* plant cloning system. Environ. and Exp. Botany 21 : 289-300.

77. Lee, J.S., S.K. Lee, S.S. Jang, and J.J. Lee. 1987. Plantlets regeneration from callus protoplasts of *Populus nigra*. Inst. For. Genet. Res. Rep. 23 : 143-148.

78. Lester, D.T., and J.G. Berbee. 1977. Within-clone variation among black poplar trees derived from callus culture. For. Sci. 23 : 122-131.

79. Lloyd, A.M., A.R. Barnason, S.G. Rogers, M.C. Byrne, R.T. Fraley, and R. B. Horsch. 1986. Transformation of *Arabidopsis thaliana* with *Agrobacterium tumefaciens*. Science 234 : 464-466.

80. Mathes, M.C. 1964a. The culture of isolated triploid aspen tissue. For. Sci. 10 : 35-38.

81. Mathes, M.C. 1964b. The *in vitro* formation of plantlets from isolated aspen tissue. Phyton 21 : 137-141.

82. McCormick, S., J. Niedermeyer, J. Fry, A. Barnason, R. Horsch, and R. Fraley. 1986. Leaf disc transformation of cultivated tomato (*L. esculentum*) using *Agrobacterium tumefaciens*. Plant Cell Reports 5 : 81-84.

83. Mckeand, S.E., and R.J. Weir. 1984. Tissue culture and forest productivity. J. of Forestry 82 : 212-218.

84. McNabb, H.S., Jr., R.A. Serres, M.M. Cram, and N.B. Klopfenstein. 1986. Biological constraints in the exchange of planting material for energy production. Pages 96-97 *in* Proceedings of the FAO/CNRE/E1 3-Forestry Biomass for Energy, IEA/Forestry Energy Agreement, IUFRO sponsored conference and workshop entitled "Research in Forestry for Energy", Rungstedgaard, Denmark ; 28 Oct.-1 Nov. 1985. Dept. of Operational Efficiency, Swedish University of Agricultural Sciences, Uppsala, Sweden.

85. Michler, C.H., and E.Bauer. 1987. Selection for herbicide tolerance in *Populus* to sultometuron methyl *in vitro*. *in* J.W. Hanover and D.E. Keathley eds. Genetic manipulation of Woody Plants. Plenum Press, New York.

86. Murai, N., D.W. Sutton, M.G. Murray, J. L. Slightom, D.J. Merlo, N.A. Reichert, C. Sengupta-Gopalan, C.A. Stock, R.F. Barker, J.D. Kemp, and T.C. Hall. 1983. Phaseolin gene from bean is expressed after transfer to sunflower via tumor-inducing plasmid vectors. Science 222 : 476-482.

87. Nelson, N.D., and B.E. Haissig. 1984. Biotechnology in the Forest Service's North Central Forest Experimental Station. Pages 139-154 *in* Proc. International Symp. of Ricent Advances in Forest Biotechnology. Traverse City, Michigan.

88. Nelson, N.D., and B.E. Haissig. 1986. Herbicide stress : use of biotechnology to confer herbicide resistance to selected woody plants. Pages 195-215 *in* T.C. Hennessey, P.M. Dougherty, S.V. Kossuth and J.D. Johnson, eds. Stress physiology and forest productivity. Martinus Nijhoff Publishers, The Hague, Netherlands.

89. Noh, E.R., Y.B. Koo, and S.K. Lee. 1986. Hybridization between incompatible poplar species through ovary and embryo culture. Inst. For. Genet. Res. Rep. 22 : 9-14.

90. Noh, E.W., and S.C. Minocha. 1986. High efficiency shoot regeneration from callus of quaking aspen (*Populus tremuloides M.*). Plant Cell Reports 5 : 464-467.

91. North East For. Acad., Gr. Tree Breed. 1977. Induction of haploid poplar plantlets from pollen. Acta Genet. Sinica 4 : 49-54.

92. Ostry, M.E., and D.D. Skilling. 1987. Somaclonal variation for disease resistance in forest trees. *in* J.W. Hanover

and D.E. Keathley eds. Genetic manipulation of Woody Plants. Plenum Press, New York.

93. Ostry, M.E., T.L. Ettinger, W.P.P. Hackett, P.E. Read, and D.D. Skilling. 1986. Development of bioassays to identify variant *Populus* cells and regenerated plants resistant to Septoria musiva. Page 303 *in* D. A. Sommers, B.G. Gengenbach, D.D. Biesboer, W.P. Hackett, and C.E. Green, eds. Proc. Sixth International Cong. of Plant Tissue and Cell Culture. Univ. of Minn., Minneapolis, MN.
94. Park, Y.G., and K.H. Han. 1986. Isolation and culture of mesophyll protoplasts from *in vitro* cultured *Populus alba* X *P. glandulosa*. J. Korean For. Soc. 73 : 33-42.
95. Park, Y.G., and S.H. Son. 1986. Factors affecting the isolation of mesophyll protoplasts from *Populus euramericana* cv. I-214. J. Korean For. Soc. 74 : 29-36.
96. Park, Y.G., and S.H. Son. 1988. Culture and regeneration of *Populus alba* X *glandulosa* leaf protoplasts isolated from *in vitro* cultured explant. J. Korean For. Soc. 77 : 208-215.
97. Parsons, T.J., V.P. Sinkar, R.F. Stettler, E.W. Nester, and M.P. Gordon. 1986. Transformation of poplar by *Agrobacterium tumefaciens*. Bio/Technology 4 : 533-536.
98. Perani, L., S. Radke, M. Wilke-Douglas, and M. Bossert. 1986. Gene transfer methods for crop improvement : Introduction of foreign DNA into plants. Physiol. Plant. 68 : 566-570.
99. Rake, A.V., J.P. Miksche, R.B. Hall, and K.M. Hansen. 1980. DNA reassociation kinetics of four conifers. Can. J. of Genetics and Cytology 22 : 69-79.
100. Riemenschneider, D.E., B.E. Haissig, and E.T. Bingham. 1987. Integrating biotechnology into plant breeding programs. *In* Proc. International Conference on the Genetic Manipulation of Woody Plants. 21-25. June, 1987. Michigan State University, East Lansing, MI. in press.
101. Russell, J.A., and B.H. McCown. 1986. Culture and regeneration of *Populus* leaf protoplasts isolated from non-seedling tissue. Plant Science 46 : 133-142.
102. Saito, A. 1976. Isolation of protoplasts from mesophyll cells of *Paulownia fortunei* Homsl. and *Populus euramericana* cv. I-45/51. J. Jpn. For. Soc. 58 : 301-305.
103. Saito, A. 1980a. Effects of inorganic elements in the medium on shoot differentiation from *Populus* callus. J. Jpn. For. Soc. 62 : 147-149.
104. Saito, A. 1980b. Medium for shoot formation from somatic callus tissue in *Populus*. J. Jpn. For. Soc. 62 : 270-272.
105. Saito, A. 1980c. Isolation of protoplasts from mesophyll cells of *Paulownia taiwaniana* and *Populus* X *euramericana*. Bull. For. Prod. Res. Inst. 309 : 1-6.
106. Saito, A., and K. Kawanobe. 1981. Shoot formation in *Populus* callus tissues precultured *in vitro* on some different media. Bull. For. and For. Prod. Res. Inst. 316 : 117-123.
107. Sato, T. 1974. Callus induction and organ differentiation in anther culture of *Populus*. J. Jpn. For. Soc. 56 : 55-62.
108. Savka, M.A., and J.O. Dawson. 1985. Culture of ovules containing immature embryos of *Populus heterophylla* L. *in vitro*. Univ. of Ill. Agric. Exp. Station, Forestry Research Report No 85-1.
109. Saxena, P.K., M. Mii, W.L. Crosby, L.C. Fowkem, and J. King. 1986. Transplantation of isolated nuclei into plant protoplasts. Planta 168 : 29-35.
110. Schreiner, E.J. 1974. *Populus* L., poplar. Pages 645-655 *in* Seeds of Woody Plants in the United States. US Forest Service Agriculture Handbook No 450.
111. Scorza, R., and J. Janick. 1980. *In vitro* flowering of *Passiflora suberse* L. J. Am. Soc. Hortic. Sic. 105 : 829-897.

112. Sederoff, R.R., and F.T. Ledig. 1984. Increasing forest productivity and value through biotechnology. Pages 253-276 *in* Proceedings of Weyerhaeuser Science Symposium Forest Potential Productivity and Value, Aug. 20-24, 1984. Tacoma, Washington.

113. Sederoff, R.R., A.-M. Stomp, W.S. Chilton, and L.W. Moore. 1986. Gene transfer into loblolly pine by *Agrobacterium tumefaciens*. Bio/Technology 4 : 647-649.

114. Shah, D.M., R.B. Horsch, H.J. Klee, G. M. Kishore, J.A. Winter, N.E. Tumer, C. M. Hironaka, P.R. Sander, C.S. Gasser, S. Aykent, N.R. Siegel, S.G. Rogers, and R.T. Fraley. 1986. Engineering herbicide tolerance in transgenic plants. Science 233 : 478-481.

115. Shaw, C.H., M.D. Watson, G.H. Cater, and C.H. Shaw. 1984. The right hand copy of the nopaline Ti-plasmid 25 bp repeat is required for tumor formation. Nucleic Acids Res. 12 : 6031-6043.

116. Shepard, J.F., D. Bidney, and E. Shakin. 1980. Potato protoplasts in crop improvement. Science 208 : 17-24.

117. Skirvin, R.M. 1978. Natural and induced variation in tissue culture. Euphitica 27 : 241-266.

118. Sommers, H.E., and C.L. Brown. 1979. Application of the tissue culture to forest tree improvement. Pages 460-491 *in* W.R. Sharp, P.O. Larsen, E.F. Paddock, and V. Raghaven, eds. Plant cell and tissue culture -principles and application. Ohio State University Press, Columbus, Ohio.

119. Thompson, D.G., and J.G. Gordon. 1977. Propagation of poplar by shoot apex culture and nutrient film technique. Pages 77-82 *in* TAPPI Conference Papers ; Forest Biology Wood Chemistry Conference 1977.

120. Thornburg, R.W., G. An, T.E. Cleveland, and C.A. Ryan. 1987a. Characterization and expression of a wound-inducible proteinase inhibitor II gene from potato. *In* T. Kosuge and G. Bruening, eds. Tailoring Plant Genes for Crop Improvement. Academic Press, New York. in press.

121. Thornburg, R.W., G. An, T.E. Cleveland, R. Johnson, and C.A. Ryan. 1987b. Wound -inducible expression of a potato inhibitor II -CAT gene fusion in transgenic tobacco plants. Proc. Natl. Acad. Sci. USA 84 : 744-748.

122. Torrey, J.G. 1985. The development of plant biotechnology. American Science 73 : 354-363.

123. Tumer, N.E., K.M. O'Connel, R.S. Nelson, P.R. Sanders, R.N. Beachy, R.T. Fraley, and D.M. Shah. 1987. Expression of alfalfa mosaic virus coat protein gene confers cross-protection in transgenic tobacco and tomato plants. EMBO J. 6 : 1181-1188.

124. Umbeck, P., G. Johnson, K. Barton, and W. Swain. 1987. Genetically transformed cotton (*Gossypium hirsutum* L.) plants. Bio/Technology 5 : 263-266.

125. Vasil, I.K. 1976. The progress, problems, and prospects of plant protoplast research. Adv. in Agron. 28 : 119-160.

126. Venverloo, C.J. 1973. The formation of adventitious organs. I. Cytokinin-induced formation of leaves and shoots in callus cultures of *Populus nigra* L. 'Italica'. Acta Bot. Neerl. 22 : 390-398.

127. Verma, D.C., and S.R. Wann. 1983. Isolation of high yields of viable protoplasts from quaking aspen seedlings and cultured loblolly pine cell suspensions. *In* 6th Int'l. Protoplasts symposium. I. Potykus et al., eds. Experientia Suppl. 45 : 10-11.

128. Wang, C.C. Z.C. Chu, and C.S. Sun. 1975. The induction of *Populus* pollen plants. Acta Botanica Sinica 17 : 56-59.

129. Wang, K., L. Herrera-Estrella, M. Van Montagu, and P. Zambryski. 1984. Right 25 bp terminus sequence of the nopaline T-DNA is essential for and determines direction of

DNA transfer from *Agrobacterium* to the plant genome. Cell 38 : 455-462.

130. Wann, S.R., and D.W. Einspahr. 1986. Reliable plantlet formation from seedling explants of *Populus tremuloides*. Silvae Genetica 35 : 19-24.

131. Wei, Z.M., H. Kamada, and H. Harada. 1986. Transformation of *Solanum nigrum* L. protoplasts by *Agrobacterium rhizogenes*. Plant Cell Reports 5 : 93-96.

132. Whitehead, M.C.M., and K.L. Giles. 1977. Rapid propagation of poplars by tissue culture method. N.Z.J. For. Sci. 7 : 40-43.

133. Wilke-Douglas, M., L. Perani, S. Radke, and M. Bossert. 1986. Gene transfer methods for crop improvement : Introduction of foreign DNA into plants. Physiol. Plant. 68 : 566-570.

134. Willmitzer, L., M. De Beuckeleer, M. Lemmers, M. Van Montagu, and J. Schell. 1980. DNA from Ti plasmid present in nucleus and absent from plastids of crown gall plant cells. Nature 287 : 359-361.

135. Winton, L. 1968a. The rooting of liquid-grown aspen callus. Am. J. Bot. 55 : 159-167.

136. Winton, L. 1968b. Plantlets from aspen tissue culture. Science 160 : 1234-1235.

137. Winton, L. 1970. Shoot and tree production from aspen tissue cultures. Am. J. Bot. 57 : 904-909.

138. Winton, L. 1971. Tissue culture propagation of European aspen. For. Sci. 17 : 348-350.

139. Wolter, K.E. 1968. Root and shoot initiation in aspen callus culture. Nature 219 : 509-510.

140. Wolter, K.E., and J.C. Gordon. 1975. Peroxidase as indicators of growth and differentiation in aspen callus culture. Physiol. Plant. 33 : 219-223.

141. Zaenen, I., N. Van Larebeke, H. Teuchy, M. Van Montagu, and J. Schell. 1974. Supercoiled circular DNA in crown gall-inducing *Agrobacterium* strains. J. Mol. Biol. 86 : 109-127.

142. Zambryski, P., H. Joos, C. Genetello, J. Leemans, M. Van Montagu, and J. Schell. 1983. Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity. EMBO J. 2 : 2143-2150.

143. Zhu, X. Y., R.L. Wang, and Y. Liang. 1980. Induction of poplar pollen plantlets. Sci. Silvae Sinica 16 : 1191-197.