10. Genetic Engineering of *Pinus radiata* for Production Forestry

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Introduction

Over the past 40-50 years, considerable effort has been made to genetically improve *Pinus radiata* through conventional breeding techniques including controlled pollination and clonal propagation using tissue culture protocols. However, a range of highly desirable traits, such as herbicide and pathogen resistance, or lignin composition, are not readily available in the breeding population. Genetic engineering as a tool to introduce new traits or influence existing genes in this species is considered as a complement to conventional breeding, and we have developed the first transformation protocol for the genus *Pinus*.

Embryogenic tissue culture as a source for transformation

A protocol for the clonal propagation of conifers using embryogenesis was developed at FRI. The process involves the production of dedifferentiated embryo initials (1-4 cells)which can be induced to form mature embryos and subsequently plants. Tissue at the single cell stage is transformed using a biolistic technique and foreign genes of interest, including the *gus* reporter gene. Stably transformed cotyledonary stage embryos can routinely be obtained at a frequency of one transgenic line per bombarded plate. Transgenic embryos are germinated and subsequently regenerated to plants.

Molecular analysis of transgenic tissue and plants

Transformed tissue was analyzed using fluorometric assays (Table 1) and PCR with primers for the *gus* and *npt* 11 gene. Results confirmed the presence of these genes in tissue and needles of transgenic plants (Fig. l). Non-transformed controls were negative. Southern and Northern analysis also confirmed the integration and expression of the foreign genes in transgenic tissue and regenerated plants.

Regeneration of plants from transgenic tissue of *P. radiata*

Transgenic tissue, selected on geneticin-containing media, and transformed with different genes, was subsequently regenerated to plants. More than 150 transgenic plants are currently growing in the GMO greenhouse, awaiting field release in late 1997.

Discussion

A reliable and cost-efficient genetic transformation protocol has been developed for

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Pinus radiata and transgenic plants were produced which exhibit a range of different genes integrated into their genome. The transformation technique is also applicable to *Pinus taeda* and a range of other conifers including non-pine species (*Pseudotsuga menziesii*). Transgenic *Pinus radiata* plants will be released into the FRI nursery for further studies including seasonal variation in gene expression. Commercial applications of this technology will include the transfer of herbicide, pathogen and insect resistance, acceleration or abolition of flowering, and reduction or change of lignin composition and content.

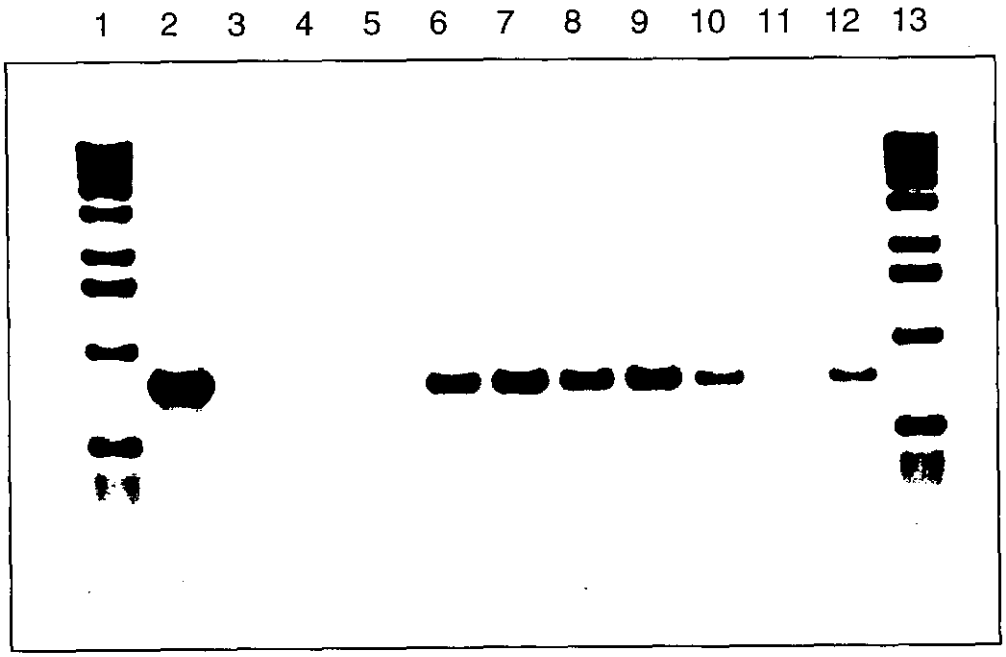

Fig.1 PCR analysis of transgenic *P. radiata* tissue and a plant. Amplification of the *npt* II gene. Lanes 1 ands 13: DNA ladder; lane 2: plasmid-control; lane 3: water control; lane 4: non-transformed *P. radiata* tissue; lanes 5-10: different transformed *P. radiata* lines; lane 11: non-tranformed needless; lane 12: transgenic needles.

Tissue clone	*gus* activity [nmol MU/min* mg protein]
D93-199 (control)	0.08
94/3-1	4.80
94/6-1	7.10
94/6-3	13.00
94/8-3	4.00
94/8-4	0.90
94/10-3	0.03
94/12-1	43.60
94/15-1	36.60
94/16-4	62.50

Table 1 Fluorometric analysis of *Pinus radiata* tissue transformed with the *gus* reporter gene.