High-frequency transformation of cottonwoods (genus *Populus*) by *Agrobacterium rhizogenes*

Kyung-Hwan Han, Milton P. Gordon, and Steven H. Strauss

**Abstract:** Many species of *Populus*, particularly cottonwoods of sections Aigeiros and Tacamahaca, remain recalcitrant to genetic transformation. We demonstrate that transgenic trees can be readily produced in several poplar genotypes using wild-type *Agrobacterium rhizogenes*. Hairy roots were produced in a variety of clones that included *Populus trichocarpa* Torr. & A. Gray, *Populus deltoides* Bartr. ex Marsh., *P. trichocarpa* × *P. deltoides* hybrids, and *Populus alba* L. × *Populus grandidentata* Michx., some of which were otherwise recalcitrant to regeneration or transformation. The frequency of hairy roots ranged from 17 to 92%; nearly all hairy roots could be regenerated into transgenic plants. Among 18 transgenic lines that were confirmed by Southern analysis and grown into small trees, 5 showed severe dwarfism and other phenotypic abnormalities, while the majority grew normally. *Agrobacterium rhizogenes* appears to be an effective means for transformation of diverse poplar genotypes and may be useful in genotypes where disarmed strains of *Agrobacterium* or regeneration protocols are inadequate to produce transgenic plants.

**Résumé** : Plusieurs espèces du genre *Populus*, et plus particulièrement celles des sections Aigeiros et Tacamahaca, demeurent réfractaires à la transformation génétique. Les auteurs démontrent que des arbres transgéniques peuvent être obtenus facilement pour plusieurs génotypes de peuplier à l’aide d’une souche sauvage de *Agrobacterium rhizogenes*. Des racines avec poils absorbants ont été obtenues pour un nombre important de clones représentatifs des espèces *Populus trichocarpa* Torr. & A. Gray et *Populus deltoides* Bartr. ex Marsh., ainsi que des hybrides de *P. trichocarpa* × *P. deltoides* et de *Populus alba* L. × *Populus grandidentata* Michx., dont certains étaient réfractaires à la régénération ou à la transformation. La fréquence à laquelle des racines avec poils absorbants furent obtenues variait de 17 à 92% et presque toutes les lignées de racines avec poils absorbants pouvaient être régénérées en plantes transgéniques. Parmi les 18 lignées transgéniques qui furent confirmées par analyse de type Southern et qui se sont développées jusqu’au stade de petit arbre, 5 ont affiché un nanisme sévére ainsi que d’autres anormalités phénotypiques, alors que la majorité des lignées se sont développées normalement. L’utilisation de *A. rhizogenes* apparaît donc être un moyen efficace pour transformer divers génotypes de peuplier. Cette stratégie pourrait être utile pour la production de plantes transgéniques dans le cas des génotypes pour lesquels la transformation par des souches désarmées de *Agrobacterium* ou les protocoles de régénération s’avèrent inadéquats.

**Introduction**

Poplar species are important for biomass, pulp and paper, and solid wood products, and commercial plantation area is growing rapidly in the United States. Genetic engineering can complement conventional breeding efforts by providing means for insertion of genes that confer desired traits not readily available in sexually accessible gene pools. In contrast with sexual breeding, it allows new genes to be added while the desired genotypes of clones are preserved and can therefore reduce the time required to produce an elite line. For genetic engineering to be commercially viable, transformation systems are needed that can efficiently produce transgenic trees from diverse genotypes.

Poplars have been transformed in many laboratories. However, the majority of work has been confined to a few model hybrids of aspen (reviewed in Jouanin et al. 1993). Cottonwoods of sections Aigeiros and Tacamahaca are usually recalcitrant to regeneration and transformation. There has been only one published report of transgenic cottonwoods (*Populus trichocarpa* Torr. & A. Gray × *Populus deltoides* Bartr. ex Marsh.) established in soil (De Block 1990). One possible explanation for its recalcitrance to transformation is the lack of an efficient regeneration system (De Block 1990), which often requires customizing regeneration conditions to specific genotypes (Han et al. 1995) to promote cell competence for regeneration (Coleman and Ernst 1990). An alternative to genotype-specific manipulation may be to take advantage of the morphogenetic effects of genes transferred by *Agrobacterium rhizogenes*.

The soil bacteria *Agrobacterium tumefaciens* and *A. rhizogenes* are the two species widely used to transform higher plants, including poplars. Both systems rely on a similar transformation mechanism. However, an important difference between the two species is that transgenic plants can be regenerated from roots induced by wild-type *A. rhizogenes*, while plant cells transformed with *A. tumefaciens* carrying wild-type Ti (tumor-inducing) plasmids cannot be regenerated into plants. Plants regenerated from Ri (root-inducing)-transformed roots,
however, display the “hairy-root syndrome” (described in Tepfer 1984) caused by the T-DNA (transferred DNA)-borne genes (Schmulling et al. 1988; Gaudin et al. 1994). Transformation with a disarmed \textit{A. tumefaciens} strain produces transgenic plants without such abnormal phenotypes; however, with disarmed \textit{A. tumefaciens} species a precise regeneration–selection system must be developed for each plant species or genotype. Although efficiency varies widely, \textit{A. rhizogenes} selection system must be developed for each plant species or genotype.

Four bacterial strains were used. \textit{Bacterial strains} however, display the “hairy-root syndrome” (described in Tepfer 1984) caused by the T-DNA (transferred DNA)-borne genes (Schmulling et al. 1988; Gaudin et al. 1994). Transformation with a disarmed \textit{A. tumefaciens} strain produces transgenic plants without such abnormal phenotypes; however, with disarmed \textit{A. tumefaciens} species a precise regeneration–selection system must be developed for each plant species or genotype. Although efficiency varies widely, \textit{A. rhizogenes} selection system must be developed for each plant species or genotype.

The abnormal growth often observed in plants transformed with \textit{A. rhizogenes} may be used to advantage in some instances. Careful phenotypic testing of transgenic trees may identify transformants with increased growth rates and larger root masses (Han et al. 1993; Tepfer 1984). The adaptive significance of extensive root development may include improved anchorage, increased interactions with soil microorganisms, and stronger drought tolerance. Apple trees infected with \textit{A. rhizogenes} withstood drought better than uninfected trees (Moore et al. 1979). The greater production of root exudates and water uptake expected from large root systems may increase soil remediation by direct uptake and plant and microbial degradation.

In the present study, we describe a successful transformation system for a recalcitrant but economically important kind of hybrid cottonwood (\textit{P. trichocarpa} × \textit{P. deltoides}). The \textit{Ri}-transformed poplar trees were regenerated and established in soil, stable incorporation of T-DNA genes into the poplar genome is demonstrated by Southern blot analysis, and phenotypic effects of the expression of Ri T-DNA genes in the transgenic trees are documented.

Materials and methods

Bacterial strains

Four bacterial strains were used. \textit{Agrobacterium rhizogenes} R1000 has the chromosomal background of \textit{A. tumefaciens} strain C58 into which the Ri plasmid of pRI4A4b has been conjugated (White et al. 1985). The strain R1240 is disarmed by removal of the core TL- and TR-DNA (Macrae and van Staden 1993), \textit{Larix} (Huang et al. 1991; Shin et al. 1994), \textit{Malus} (Lambert and Tepfer 1992), \textit{Populus} (Brasileiro et al. 1991; Charest et al. 1992; Pythoud et al. 1987), and \textit{Robinia} (Han et al. 1993). Pythoud et al. (1987) reported genetic transformation of hybrid cottonwood (\textit{P. trichocarpa} × \textit{P. deltoides}) clone H11 using \textit{A. rhizogenes}. They were able to regenerate shoots from callus tissues derived from the hairy roots, but did not propagate the shoots in soil or report on shoot morphology.

The protocols described for transformation (Han et al. 1993, 1994) and for tissue culture (Han et al. 1995) were used in this study, with minor modifications. \textit{Agrobacterium rhizogenes} strains used as transformation agents are listed in Table 1. Greenwood stems (about 5 mm in diameter) were sterilized in 30% Clorox solution for 15 min followed by five washes with sterile distilled water. Internodal stem discs (5 mm thick) were cut and soaked for several minutes in an overnight culture of \textit{A. rhizogenes} (\textit{A. rhizogenes} medium (Murashige and Skoog (MS) medium (Murashige and Skoog 1962) without growth regulators (MSO). After 3 days of co-cultivation, the explants were washed in sterile distilled water three times and transferred to either MSO or MS supplemented with 1 µM indolebutyric acid (IBA) containing 300 mg/L Timentin® (SmithKline Beecham Pharmaceuticals, Philadelphia, Pa.) to kill residual bacteria. The tissue was maintained on the same medium for at least 3 weakly subculture periods for hairy root induction. For shoot regeneration, hairy roots from the clone 53-246 induced by the strain R1601 were cultured on shoot induction medium (MS + 5 µM 6-benzylaminopurine + 10 µM α-naphthaleneacetic acid) with 100 µg/L kanamycin. Shoots regenerated from stem inter-nodes of clone 53-246 that were not treated with \textit{Agrobacterium} were used as negative controls throughout the experiments.

Growth of transgenic poplars

Shoots regenerated from hairy roots were multiplied and rooted in a Magenta G-7 box (Sigma Co.) containing 50 mL of shoot induction medium with kanamycin. Four ramets per independent transgenic clone were potted (25 cm diameter pot) as in Han et al. (1990) and grown in a greenhouse. Measurements were taken after 5 months of growth; trees were then pruned.

Southern blot analysis

Total plant DNA was isolated from young leaves harvested from potted plants, as in Bradshaw and Stettler (1993). Isolated DNA (3 µg/lane) was digested with HindIII and transferred to a Zeta-probe blotting membrane by DNA alkaline blotting as specified by the manufacturer (Bio-Rad). The blots were hybridized in rollor bottles (Robins Scientific) with approximately 5 × 10⁶ cpm/mL (1 cpm (count per minute) = 0.0167 Bq) 32P-labeled probe in 0.5 M Na₂HPO₄, pH 7.2, 7% SDS, 10 mM EDTA, and 0.5% nonfat dry milk at 65°C overnight and washed according to Church and Gilbert (1984). Autoradiography was done at –70°C with a single intensifying screen. For probes we used either the HindIII fragment 16 (T₅-DNA right junction fragment; Han et al. 1993), T₅-DNA (pfW302; White et al. 1985), or T₅-DNA (pfW41; Huffman et al. 1984).

Statistical analysis

All data were processed using analysis of variance (ANOVA) from the GLM procedure in SAS (SAS Institute Inc. 1990). The data for each clone were separated by Duncan’s multiple range test where ANOVA indicated statistical significance.

Results

Transformation

Hairy roots started to form on the cut surface of stem discs 7 days after inoculation with \textit{A. rhizogenes}. These roots differed from normal roots in the following ways: (1) roots were initiated in the presence of little or no auxin in the culture medium; (2) hairy roots arose on the cut surface from cells that were transformed with \textit{A. rhizogenes}, while normal roots were induced on the periphery of the stem segments in response to exogenously supplied auxin that diffused into the stem tissue from the medium (Figs. 1A, 1B, and 1C); and (3) hairy roots developed a large number of root hairs and lateral branches (Figs. 1A and 1B).

Hairy root formation frequency varied from 17 to 92%, depending on plant genotype (Table 1). Infection with the wild-type strain R1000 transformed the hybrid cottonwood clone...
24-305 (17%), but the infection with the supervirulent strain R1600 substantially increased transformation frequency (92%). The disarmed strain R1240 produced no hairy roots after infection. *Populus deltoides* clone ILL129, which did not respond to ordinary tissue culture manipulations for regeneration, produced hairy roots from 19% of explants. Spontaneous shoot regeneration (7%) was also observed with this clone when inoculated with R1601.

The hairy root tissues (longer than 2 cm) induced by R1601 from the clone 53-246 were transferred to shoot induction medium. Shoot regeneration was observed on roots 20–35 days later (Fig. 1D). Nineteen of 75 hairy roots (25%) produced transgenic shoots in the presence of 100 mg/L kanamycin. All of the resulting shoots were successfully rooted in either MSO or MS medium supplemented with 1 µM IBA in the presence of 100 mg/L kanamycin.

**Phenotypic consequences of transformation**

Hairy root regenerants easily produced aerial roots in tissue culture (Fig. 1E), and their roots showed plagiotropism in soil. They tended to form densely packed, horizontally radiating roots near the surface of the soil. About 10% of all transgenic
Eight transgenic lines carrying both TL- and TR-DNA sequences (clones 6D9 and R20) showed the greatest height growth. DNA as a probe showed that all four transgenic trees analyzed, however, showed stunted growth. These two clones (6D9 and R20) had only TR-DNA sequences. These two strains had stunted growth. These dwarfed trees had short internodes and abundant lateral branches. Some of the lateral branches were vinelike, tended to grow horizontally, and grew faster than the apical leader. Applications of 2.5 mM GA$_3$ resulted in increased shoot growth. Two-thirds of the hairy root regenerants showed apical dominance and were morphologically indistinguishable from the normal plant (Fig. 1G). One regenerant (clone 6D9, carrying only TR-DNA) was significantly taller than normal. All transgenics had fewer branches than did normal plants with the exception of one, whose number was not significantly different from normal (Table 2). In the summer of 1995 we planted these transgenic trees in the field for study of transgene expression over time.

### Table 1. Hairy root formation frequency.

<table>
<thead>
<tr>
<th>Bacterial strains*</th>
<th>Plant genotype*</th>
<th>No. of explants</th>
<th>Explants producing hairy roots (%)*</th>
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<tr>
<td>R1601</td>
<td>AG: NC-5339</td>
<td>48</td>
<td>69</td>
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*R1000, A. tumefaciens strain C58 + pRlA4b; R1240, disarmed R1000; R1600, R1000 + pTVK291 (carries the virulence region of a supervirulent plasmid pTiBo542); R1601, R1600 + nptII gene.  
*T. P. trichocarpa; D, P. deltoides; TD, P. trichocarpa × P. deltoides; AG, Populus alba L. × P. grandidentata Michx. Clones 53-246, 93-968, and ILL129 were obtained from Dr. Toby Bradshaw at University of Washington, Seattle, Wash., U.S.A.; H11 and NC-5339, from Brent McCown at University of Wisconsin, Madison, Wis., U.S.A.; and 24-105, from Dr. Brian Stanton at the James River Corporation, Camas, Wash.

†Results from single experiment.

Dwarfism was also observed among transgenic tobacco plants overexpressing the roleA gene (Dehio et al. 1993) and potato plants overexpressing the roleC gene (Schmulling et al. 1993). Leaves of those plants contained 20–60% less GA$_3$ than control plants. The GA$_3$ application reduced the severity of phenotypic alteration induced by the roleA transgene but not by the roleC transgene (Schmuling et al. 1993). Promoter activities of both roleB and roleC differed from that of roleA in transgenic tobacco (Schmulling et al. 1988). In all trees tested, the dwarfism of our cottonwood clones appeared to be reversed by GA$_3$ application. This suggests that initially they had low gibberellic acid concentrations because of the overexpression of the roleA gene. The lack of dwarfism among most of our transformants, even when they contained both T$_{1}$- and T$_{2}$-DNA sequences, may have resulted from differential transfer or expression of individual role genes among independent transformants.

We observed dark green, wrinkled leaves in about 10% of the transgenic poplars. This phenotype varied in expression during development, with no obvious environmental cue, when the trees were maintained in a greenhouse. The wrinkled leaves tended to appear in the young leaves on the leader but not on the branches. Expression of the roleA transgene was responsible for dark green, wrinkled leaves in transgenic potato (Schmulling et al. 1993).

Transgenic trees with normal phenotypes suggest that the role gene may have been silenced in those trees after the hairy root formation–regeneration process. Sinkar et al. (1988b) observed frequent reversion of hairy root phenotypes in tobacco. Their Southern and Northern blot analyses suggested that the revertant phenotypes were due to the transcripional inactivation of T-DNA genes. Gene silencing in transgenic plants is widely known (Finnegan and McElroy 1994). Brandle et al. (1995) reported that co-suppression of a transgene was triggered by the common agronomic practice of seedling transplantation in field-grown transgenic tobacco. Several different genetic mechanisms appear to be involved (Matzke and Matzke 1995), often associated with multiple copies of transgenes (Flavell 1994) and DNA methylation.

We report high transformation efficiency for hybrid cottonwood, which has shown recalcitrance in our transformation experiments with A. tumefaciens. The main obstacle in transformation appears to be targeting and isolating regenerable cells. Actively dividing cells are more susceptible to Agrobacterium infection (Akama et al. 1992), and competence for transformation is associated with the timing of cell division at the wound site (Braun and Mandle 1948). Therefore, transformation frequency may be improved by increasing the number of regenerable cells that are in active cell division. Agrobacterium rhizogenes may “naturally” condition the plant cells for transformation–regeneration by providing plant hormones to promote cell division in cells surrounding the wound site. Conditioning may also result from morphogenic potential in hairy root tissues. For example, inoculation of P. deltoides clone ILL129 with A. rhizogenes strain R1601 resulted in high root

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Zeta-probe membrane (Bio-Rad). The blots were hybridized with DNA was digested with Agrobacterium rhizogenes (Han et al. 1995).

Fig. 2. Southern blot analysis of transgenic poplars. Total poplar DNA was digested with HindIII, size-fractionated (in kilobases (kb)) on agarose gel by electrophoresis, and transferred to Zeta-probe membrane (Bio-Rad). The blots were hybridized with 32P-labeled HindIII border fragment (H-16) of T2-DNA of A. rhizogenes R1601 (White et al. 1985). Autoradiograms of blots are shown: lane 1, total DNA (10 ng) of A. rhizogenes R1601; lane 2, normal poplar (3 µg); lanes 3–6, transformants 6D1, 6D8, 6D11, and 6R24 (3 µg each), respectively. Each hybridizing band indicates the incorporation of a copy of T2-DNA.

There are at least three possible means of taking advantage of A. rhizogenes for transformation of recalcitrant species without encountering phenotypic abnormalities. One is to place the rol genes under an inducible gene expression system and have them expressed only during the transformation process. A number of systems for inducible gene expression have now been described in plants (Roder et al. 1994; Mett et al. 1996). Another option is to use co-transformation, where two separate T-DNAs contained within a single bacterium are transferred into plant genomes. Binary vectors derived from A. tumefaciens can be efficiently transferred into plant cells when they are placed in a virulent strain of A. rhizogenes. A high frequency of co-transfer of wild-type and binary vector T-DNAs is generally obtained: 43–60% in alfalfa (Simpson et al. 1986; Sukhapinda et al. 1987), 39–85 % in Brassica (De Block and Debrouwer 1991), 86 % in Stylosanthes (Manners and Way 1989), and 70–80% in tobacco (Hamill et al. 1987; Hamamoto et al. 1990). As demonstrated in the present study, these hairy roots can often be induced to regenerate into transgenic plants. Under some conditions, the T-DNAs from the binary and wild-type plasmids integrate into unlinked sites and can be segregated during sexual reproduction. Moreover, in a population of co-cultivated cells, not all will be transformed with both the wild-type and binary T-DNA, potentiating their separation during regeneration. For example, when co-transformed tomato tissues were cultured on a medium supplemented with appropriate growth regulators and antibiotics, they produced callus instead of hairy roots, from which phenotypically normal transgenic plants could sometimes be obtained (Shahin et al. 1986). Finally, our results suggest that a useful proportion of transgenic poplar lines produced with A. rhizogenes transformation show no discernible phenotypic effect; whatever the mechanism, it may be possible to simply screen

<table>
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<th>Transgenic clones*</th>
<th>Height (cm)</th>
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<th>Leaf length (mm)</th>
<th>Leaf width (mm)</th>
<th>No. of leaves</th>
<th>Branch length (mm)</th>
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<td>277a</td>
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Note: Means with different letters are significantly different at the 0.01 probability level.

*All transgenic lines were derived from P. trichocarpa × P. deltoides clone 53-246, and control was nontransgenic shoot regenerated from stem internode of the same clone.

and shoot regeneration. Without inoculation this particular clone was incapable of regeneration in our tissue culture studies (Han et al. 1995). Agrobacterium rhizogenes has been the only vector able to give rise to transformed plants in some recalcitrant tree species (Han et al. 1993; Shin et al. 1994).
a large number of transgenic lines for those that remain free of abnormalities.

Acknowledgements

We thank Ronglin Wu for his help on the measurement and statistical analyses of growth data, Priscilla Licht for editing the manuscript, and the Tree Genetic Engineering Research Cooperative at Oregon State University and the Occidental Chemical Corporation for financial support.

References


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