

Ten lessons from 15 years of transgenic *Populus* research

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Summary

Based on extensive experience with transgenic poplars in laboratory and field environments, we have found that transformation is an extremely useful tool for research in biotechnology and functional genomics. The key lessons from our experience are: (1) stable gene expression is the rule in vegetatively propagated transgenic poplars; (2) somaclonal variation is modest and manageable; (3) transformation and field tests are extraordinary functional genomics methods; (4) there are many social and technical motivations for transformation centres; (5) regulations may choke biotechnology without scientist involvement; (6) the value of transgenic traits look high, but await careful, broad evaluation; (7) public-sector scientists need to play a serious, free role in value studies; (8) gene flow is complex and needs careful consideration; (9) sterility systems can be developed via diverse means; and (10) domestication transgenes can provide new avenues to promote biosafety. In short, transformation in poplar is extremely reliable and there are diverse and promising means for improving biosafety, but considerable time, institutional commitments and public-private partnerships are required to deliver them to society.

Our system and perspective

The goal of this paper is to describe the diverse kinds of research and interactions with the private and public sectors we have had while creating and field-testing many different kinds of transgenic poplars (genus *Populus*, aspens and cottonwoods) over the last ~15 years. [We use 'transgenic' to refer to any trees produced using asexual gene transfer, regardless of the origin of the genes.] Our laboratory has generated more than 6500 independent gene-transfer events in 17

different genotypes of *Populus*, and field-tested more than 1600 of these lines. This has given us extensive experience with how transformation affects the behaviour of trees grown on a moderately large scale. It has also given us a different perspective about the reliability of transformation than seems to be common in most academic laboratories that study transgenic plants for basic research purposes.

We have benefited from excellent growing conditions for poplars in the Pacific Northwestern USA, where trees grow in the order of 3 m in height

per year under natural or fertigated (fertilized during irrigation) conditions (Figure 1). In 1–3 years, we produce transgenic trees of a size that foresters will pay attention to. Although the large majority of funding in our laboratories has been from public sector competitive grants, we have also benefited from private-sector support through an academic–private–government consortium formerly called the Tree Genetic Engineering Research Cooperative (TGERC; now named the Tree Biosafety and Genomics Research Cooperative, TBGRC; <http://www.data.forestry.oregon-state.edu/tgbb/>). The focus of the consortium has been on genetic engineering methods to mitigate gene flow to address environmental concerns over the deployment of transgenic trees, using poplar as a model taxon almost exclusively. Despite this

private sector support, our research projects and publications are under the control of Oregon State University, and none of the views we express below have been reviewed, edited, or directly influenced by any private sector companies. Moreover, none of the authors of this paper have a financial conflict of interest with respect to commercialization of transgenic poplars we have produced or studied.

Although ‘biosafety traits’ (e.g. modified flowering and stature) have been our area of emphasis, we have also engaged in several field studies of potential commercial traits, especially herbicide tolerance and insect resistance (Meilan *et al.*, 2000), and conducted basic research on poplar functional genomics using transformation as a tool (e.g. Busov *et al.*, 2003; Groover *et al.*,

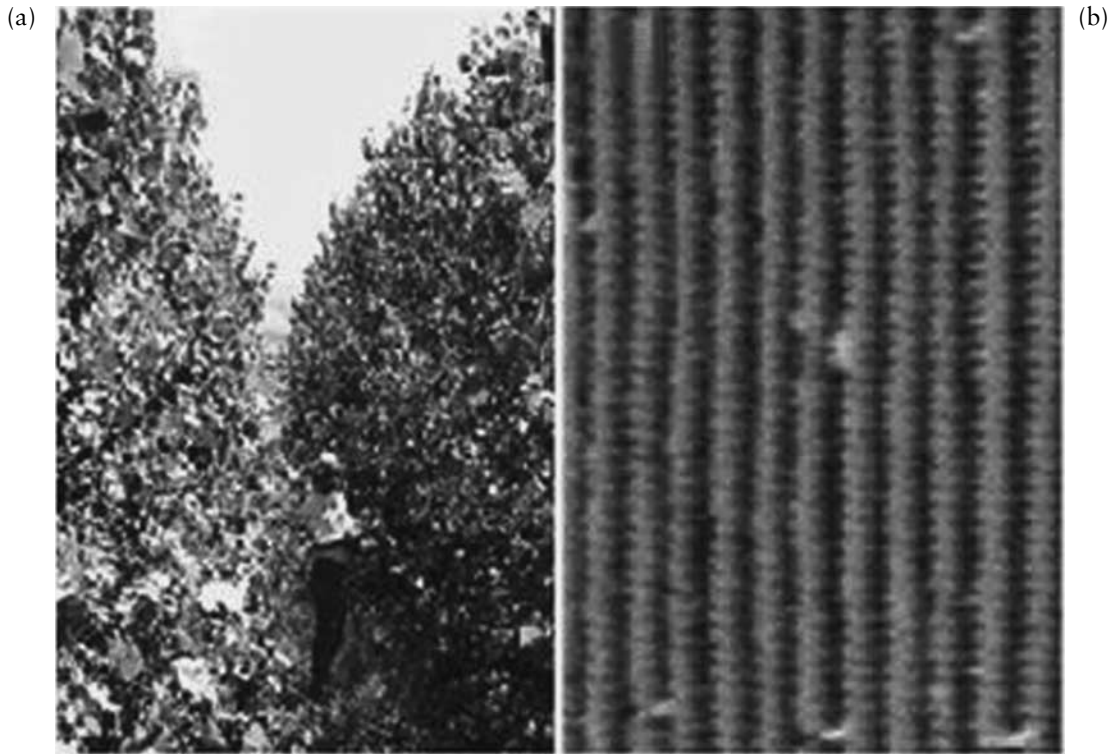


Figure 1. Field trial of transgenic poplars (*Populus deltoides* × *P. nigra*) in their second growing season. Several ramets of nine independent transgenic lines, and one non-transgenic control line of the same parent clone, are planted randomly within alternating rows; all of the trees in alternate rows contain only the non-transgenic line. (a) View between adjacent rows (the man is 1.8 m in height). (b) Infrared aerial photograph of a section of the plantation, showing uniformity. Reprinted from *Trends in Plant Science*, Vol. 9, Brunner *et al.*, ‘Poplar genome sequence . . .’, pp. 49–56, ©2004, with permission from Elsevier.

2004). The transformability, clonability, rapid growth, small genome, and extensive genomic resources, including a publicly available genome sequence (reviewed in Brunner *et al.*, 2004), make poplar an extraordinary system in which to investigate the potential for transgenic biotechnology, and to make real contributions to tree domestication (Bradshaw and Strauss, 2000; Strauss and Brunner, 2004).

We are motivated by a strong ethical imperative to move forward with genetic engineering (GE) research based on the expectations that: (1) we can help to improve productivity and economic value of plantations for social benefit; (2) increased productivity in established plantations provides society with the option of protecting and restoring more land for environmental purposes; (3) new methods for improving biosafety of transgenic trees, the focus of our work, can help to alleviate current environmental problems, including the spread of exotic and highly bred organisms into the wild; (4) many products of GE, such as trees with modified wood, improved bioremediation activity, and resistance to exotic pests, can provide major and direct environmental benefits; and (5) by connecting genes to traits via genomics and biotechnology, we are advancing the science of breeding, ultimately allowing trees to be bred in a more directed, predictable and safe manner.

We comment on what we see as the 10 most important lessons about transformation, biosafety, and associated issues regarding commercial deployment. Although strongly influenced by the transformability and other facilitating aspects of poplar biotechnology research, similar results concerning the performance of transgenic trees has been observed in other tree species, including conifers (Pena and Seguin, 2001; Meilan *et al.*, 2004b).

Ten lessons

Transformation is powerful and reliable

(1) Stable gene expression is the rule

Perhaps because most transgenic crops and model organisms are sexually propagated, and because scientists focus on exceptions to study

mechanisms, there appear to have been many more papers reporting unstable transgene expression or gene silencing compared with those reporting stable transgene expression (e.g. Finnegan and McElroy, 1994; Pawlowski *et al.*, 1998). Because of their high heterozygosity and the commercial preference for well-characterized, and often heterotic, clones, it is stability of vegetative propagules that is most relevant to deployment of transgenic poplars. Likewise, in contrast to studies of sexually propagated crops, we are aware of no reports of associations between transgene structure or copy number and gene silencing under vegetative propagation, even in well-studied taxa such as potato (D. Duncan, Monsanto, personal communication) and poplar (Meilan *et al.*, 2004b). Instability of gene expression has been reported in studies of transgenic poplar that employ transgenes whose variation in expression is dramatically amplified by its effects on plant development (e.g. *rolB*; Kumar and Fladung, 2001). However, there is as yet no evidence that expression of transgenes under vegetative propagation is more variable than expression of most endogenes. Normal variation in expression might be dramatically amplified by linkage to potent growth regulatory protein.

We have studied stability of gene expression extensively in transgenic poplars using easily visualized marker genes (β -glucuronidase (GUS), herbicide tolerance, and/or insect resistance) and, in some cases, native poplar genes (Rottman *et al.*, 2000). In more than 1000 independent events that we produced containing one or more of these genes we have yet to see a single case of obvious post-regeneration gene silencing despite many rounds of vegetative propagation, annual cycles of growth, and diverse environments to which they have been subjected (Meilan *et al.*, 2002, 2004b). Similar results have been reported for other field studies of transgenic poplars that used a similar transformation method and included some of the same genotypes that we often employ (Pilate *et al.*, 1997). Finally, most academic studies do not make use of a large number of gene transfer events, nor high intensities of selection, both of which are common in breeding programmes; over 95 per cent of events are typically discarded early in the screening process in molecular breeding. Although few data on

stability are available for a biosafety trait such as sterility, we expect that once key target genes are identified, effective transgenes based on them are configured, and the most desirable events selected, they should impart highly stable sterility. The quantitative adequacy of sterility will, of course, vary widely depending on the transgene concerned, its environmental benefits and risks, and the extent of wild or non-GE flowering plantations that buffer transgene spread (DiFazio *et al.*, 2004; Slavov *et al.*, 2004). For example, for a lignin modification gene that is expected to have a negative or neutral effect on fitness of wild trees, and if grown where wild populations are common, even a very modest level of sterility should be adequate (i.e. if sterility is required at all).

(2) Somaclonal variation is modest and manageable

The large majority of poplars that we produced have shown normal growth and form. We have observed obvious morphological abnormalities that were not induced by transgene expression in only three events (~0.06 per cent) of the thousands we have studied. Interestingly, in all cases the aberrant phenotypes were not observed until after trees had gone through dormancy in the field. However, when they occurred, they affected all ramets of the transgenic events, showing that it was a transgenic event-associated phenomenon. A dwarf phenotype of a hybrid cottonwood seen after vegetative propagation and release from dormancy in all propagated ramets is shown in Figure 2. These results suggest that accelerated dormancy cycles may be an effective means to rapidly screen out aberrant events. Other laboratories appear to have observed higher levels of somaclonal variation than we have (Wang *et al.*, 1996; Kumar and Fladung, 2001). However, none appear to be so high as to pose a significant constraint on commercial programmes (where events undergo intensive selection) or on functional genomics studies (where several events are studied for each experimental treatment).

(3) Transformation and field tests are extraordinary functional genomics methods

Given the health of transgenic trees and stability of imparted traits, it is no surprise that trans-



Figure 2. Dwarf somaclonal variant first observed after release from dormancy in a field trial of fertigated transgenic poplars. The photograph was taken near the end of their first growing season after being planted as 40-cm dormant 'sticks.' A normal transgenic tree and 1.8-m man is in the background.

formation can be used to study gene function with high efficiency. This capability has been employed both in reverse genetics, where specific genes are chosen based on sequence and then mutants generated by altering expression of those genes, and in forward genetics, where mutants are first generated and the affected genes then identified. In gene-tagging and promoter/genetrapping methods, phenotypes are generated in a

