Critical Reviews in Plant Sciences, 30:415–434, 2011

ISSN: 0735-2689 print / 1549-7836 online
DOI: 10.1080/07352689.2011.605737

Transgenic *Populus* Trees for Forest Products, Bioenergy, and Functional Genomics

Xia Ye,[1,2] Victor Busov,[3] Nan Zhao,[2] Rick Meilan,[4] Lisa M. McDonnell,[5] Heather D. Coleman,[5] Shawn D. Mansfield,[5] Feng Chen,[2] Yi Li,[6] and (Max) Zong-Ming Cheng[2,7]

[1]*College of Horticulture, Henan Agricultural University, Zhengzhou, Henan, P.R. China*
[2]*Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996*
[3]*School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931*
[4]*Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907*
[5]*Department of Wood Science, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4*
[6]*Department of Plant Sciences, University of Connecticut, Storrs, CT 06269*
[7]*Bioenergy Science Center, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831-6342*

Table of Contents

Address correspondence to (Max) Zong-Ming Cheng, Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996. E-mail: zcheng@utk.edu

Species within the genus *Populus* are among the fastest growing trees in regions with a temperate climate. Not only are they an integral component of ecosystems, but they are also grown commercially for fuel, fiber, and forest products in rural areas of the world. In the late 1970s, they were designated as a bioenergy crop by the U.S. Department of Energy, as a result of research following the oil embargo. *Populus* species also serve as model trees for plant molecular biology research. In this article, we will review recent progress in the genetic improvement of *Populus*, considering both classical breeding and genetic engineering for bioenergy, as well as in using transgenics to elucidate gene functionality. A perspective for future improvement of *Populus* via functional genomics will also be presented.

Keywords Bioenergy, biotechnology, forestry, Functional genomics, *Populus*

I. INTRODUCTION

The genus *Populus*, in the family Salicaceae, consists of some 40 species of poplar, cottonwood, and aspen, and various hybrids within and among sections. The genus is divided into six sections: Abaso, Turanga, Leucoides, Aigeros, Tacamahaca, and Populus (Eckenwalder, 1996; Dickmann, 2001) (unless specified, hereafter collectively referred to as *Populus*, used as a plural term in this review). They are widely distributed across the northern hemisphere, are generally pioneer species, and when mineral soil is exposed, their seeds germinate. *Populus* species play significant roles in global ecosystems, and provide raw materials for forest industry and fuels for rural areas of developing countries (IPCC, 2008). All *Populus* species are dioecious (i.e., male and female flowers borne on separate trees), and have been shown to be paleopolyploids, or ancient polyploids (Sterck *et al.*, 2005; Tuskan *et al.*, 2006). Furthermore, the whole genome has undergone massive rearrangement and diploidization after each whole-genome duplication. All extant *Populus* species have a haploid chromosome number of 19 (Tuskan *et al.*, 2006).

Conventional breeding of *Populus* species has made enormous progress in improving traits such as growth rate, pest resistance, and environmental adaptability (Li *et al.*, 1993; Subramaniam *et al.*, 1993; Bradshaw *et al.*, 1994). However, traditional improvement processes are time consuming because of relatively long generation times, and expensive because of the significant land requirement and labor investment. In addition, because most *Populus* genotypes are highly heterozygous, it is difficult to precisely determine the genetic control of specific traits (Bradshaw *et al.*, 1994; Wu and Stettler, 1994). Inbreeding depression contributes to the challenge of studying the mode of inheritance and to revealing recessive mutants; as a result, it may take 15–20 years to release a commercially acceptable variety (Stettler *et al.*, 1980). However, once an elite genotype is developed, genetic gains can be maintained by vegetative propagation (hardwood or greenwood cuttings and/or tissue culture). Due to the high cost of maintaining materials in the field, underperforming trees are generally discarded early on in breeding programs. As such, very few of the inferior breeding populations are available for evaluation, even though they may have value for genetic studies, as is done with other model species. Genetic engineering can overcome the disadvantages of traditional breeding by introducing known genes of plant or non-plant origin into a specific genotype, which may include an already "superior" tree developed through conventional breeding. This is particularly important for trees because it allows further improvement of the elite genotype by altering mono- or polygenic

traits in the absence of genetic recombination. Furthermore, various strategies can be used to genetically engineer different types of mutants and tag genes for identification and cloning (Groover *et al.*, 2004; Busov *et al.*, 2005).

Populus trees were among the first plants to be stably transformed (Fillatti *et al.*, 1987b). Over the last 20+ years, progress has been made in many aspects of *Populus* transformation. Here, we will only briefly review the history and progress that has been made, including developing protocols for diverse genotypes and the introduction of genes controlling traits such pest resistance, herbicide tolerance, and wood properties. We will also review the progresses made in *Populus* functional genomics. Finally, we will discuss issues related to environmental stewardship of transgenic *Populus* and offer some perspectives for their future.

II. BRIEF HISTORY OF *POPULUS* TRANSFORMATION AND DEVELOPMENT OF TRANSFORMATION SYSTEMS

In the mid-1980s, work began on developing particle bombardment and *Agrobacterium*-mediated transformation technologies for *Populus* (Fillatti *et al.*, 1987b). To date, there are more reports on transgenic *Populus* than on all other tree species combined (Taylor, 2002). Because *Populus* species are natural hosts of *Agrobacterium tumefaciens* (the causative agent of crown gall disease) and *A. rhizogenes* (cause of hairy root disease) (Parsons *et al.*, 1986; Pythoud *et al.*, 1987), it was not surprising that *Populus* is amenable to *Agrobacterium*-mediated transformation. Few reports have been published with other transformation methods, which will not be discussed in this review. Table 1 shows a general *Agrobacterium*-mediated transformation protocol and the factors that should be tested when attempting to improve the efficiency of each step in the process, which may respond in a genotype-specific fashion. The factors that affect recovery of transgenic plants are both genetic and non-genetic. The genetic factors mainly are the effect of *Populus* species and within-species genotypes. Because genotype-specific variation is great, numerous transformation systems have been published (Han *et al.*, 2000; Dai *et al.*, 2003; Song *et al.*, 2006; Cseke *et al.*, 2007; and references therein). Within the genus *Populus*, transformation protocols are generally more efficient (require less time, are not as complicated, and yield higher numbers of stable transformation events per unit of effort) for species within the section Populus (aspens and white poplars), and those in sections of Tachamahaca (poplars and cottonwoods) are more recalcitrant (De Block, 1990; Han *et al.*, 2000; Dai *et al.*, 2003; Ma *et al.*, 2004; Cseke *et al.*, 2007). Han *et al.* (2000) compared 14 genotypes of *Populus* for transformation efficiencies and found considerable variation with respect to transformability and efficiency. Dai *et al.* (2003) have developed a transformation system for two elite hybrid aspen with single steps for callus induction and shoot regeneration, requiring four to five months to recover transgenic plants, with frequencies of up to 50%. Song *et al.* (2006) developed a protocol for 'Nisqually-1,' the sequenced genotype of *P. trichocarpa*, that requires multiple transfers to new media and a total time of eight to nine months with a transformation frequency of about 6%.

Several nongenetic factors also significantly affect the *Populus* transformation efficiency. With 'Nisqually-1,' greenhouse-grown materials were more amendable to transformation (Song *et al.*, 2006) than *in vitro*–grown tissues (Ma *et al.*, 2004). Callus-induction medium (De Block, 1990; Han *et al.*, 2000; Dai *et al.*, 2003; Cseke *et al.*, 2007), shoot-induction medium, *Agrobacterium* strain (Han *et al.*, 2000), and matrix attachment

TABLE 1
Transgenic *Populus*: factors influencing transformation and regeneration

Transformation process	Factors influencing transformation and regeneration	Example references
Populus material	Greenhouse/ *in vitro* plants/ field-grown mature trees genotypes	Tsai *et al.* (1994), Han *et al.* (2000), Fladung *et al.* (2000)
Explants	Hypocotyls, stem, leaf, midrib	Cseke *et al.* (2007), Song *et al.* (2006)
↓		
Infection by *Agrobacterium*	*Agrobacterium* strain,	Fillatti *et al.* (1987), Han *et al.* (2000), Block (1990), Fladung *et al.* (2000), Tzfira *et al.* (1997)
↓	Vector	Han *et al.* (1997)
Callus induction	Medium compositions	Block (1990), Dai *et al.* (2003)
↓		
Shoot formation	Medium compositions, culture condition	Howe *et al.* (1994), Han *et al.* (2000), Dai *et al.* (2003)
↓		
Rooting formation	Antibiotics	Block (1990)

regions (Han *et al.*, 1997) all affected transformation efficiency. If producing transgenic plants is part of a genetic improvement strategy, a transformation protocol will need to be developed for the target genotype. It is possible to compensate for low transformation efficiency, to a certain degree, by co-cultivating a larger number of explants. Generally, somaclonal variation associated with *in vitro* culture is quite low, although unstable events can be identified (Li *et al.*, 2009a). Once a gene is integrated into the *Populus* genome, transgene expression has been found to be highly stable throughout the complete lifespan and through propagation events (Li *et al.*, 2008a, 2009a). However, if transformation is to be used for the generation of genome-wide mutants, a highly efficient protocol must be developed for a key genotype, such as sequenced genotype 'Nisqually,' whose transformation efficiency is currently very low (Ma *et al.*, 2004; Song *et al.*, 2006).

III. TRANSGENIC *POPULUS* FOR BIOTIC AND ABIOTIC RESISTANCE

Populus species are grown worldwide for forest products, such as pulp fiber, dimension lumber, furniture components, flakes for oriented strand board (OSB), and veneer, and are now being proposed as a bioenergy crop. To meet the huge demand without deforesting natural stands, dedicated plantations will be needed (Wullschleger *et al.*, 2002; Davis, 2008). Improvement of growth and production traits would greatly boost the biomass availability, while reducing production costs (Table 2).

A. Transgenic *Populus* for Insect Resistance

Insect pests are one of the major factors limiting productivity in *Populus* plantations worldwide. In North America, only a small number of the over 150 species of *Populus*-feeding insects are regarded as serious pests. However, those few can severely hamper establishment, reduce growth, and increase mortality in managed stands (Dickmann and Stuart, 1983; Coyle *et al.*, 2005; Nordman *et al.*, 2005). The cottonwood leaf beetle (CLB, *Chrysomela scripta*) is considered the most widespread and important defoliator of *Populus* plantations in North America (Harrell *et al.*, 1981, 1982; Coyle *et al.*, 2005). It is a multivoltine insect that can result in outbreaks causing severe defoliation, particularly in young plantations (Hart *et al.*, 1996). By contrast, in China, damage to hybrid *Populus* plantations by the poplar lopper (*Apochemia cinerarua*) and the gypsy moth (*Lymantria dispar*) has resulted in substantial (up to 40%) stand loss (Hu *et al.*, 2001). In addition to defoliating insects, fungal, bacterial, and viral pathogens can affect plantation health and productivity. The following sections highlight the results of genetic modifications aimed at improving defenses against insect pests in *Populus*.

1. Bt Toxins

There are two major classes of *Populus* insect pests, chrysomelid beetles and lepidopteran caterpillars and both are susceptible to insecticidal proteins derived from different strains of *Bacillus thuringiensis* (Bt). This bacterium synthesizes polypeptides that are activated within the gut of certain insects, causing lesions and eventually insect death (Knowles and Dow, 1993). These Bt toxins have been used safely for many years as microbial pesticides in wide variety of plant species (Carozzi and Koziel, 1997), both exogenously and endogenously. These toxins are relatively selective insecticides that have very few non-target effects (James *et al.*, 1999). Numerous Bt strains have been identified, each affecting a select group of insects that are usually closely related phylogenetically (Thompson *et al.*, 1995). Genetically modifying trees, which are able to produce forms of Bt toxin can offer an appealing alternative for establishing plantations that are resistant to damage from a broad range of insect pests.

Trees expressing Bt transgenes may be preferable to exogenous applications for several reasons. First, vegetation, soil, and water surrounding the crop are not exposed to spray drift. Insects present in areas adjacent to the transgenic trees would, therefore, not be exposed, reducing the potential for them to develop resistance to Bt toxins. Second, spray applications quickly degrade, persisting on leaves for, at most, a few days (Thompson *et al.*, 1995; James *et al.*, 1999). Genetically engineered trees, however, can produce the toxin continuously, thereby avoiding sensitivities to application timing and the costs associated with repeated applications. Finally, because transgenic trees produce the toxin within their tissues, it is possible to control insects residing in the plant, such as wood borers and leaf folders. For many of these pests, no insecticides are currently available for targeting the insect life stage(s) most responsible for damage.

McCown *et al.* (1991) were the first to report on *Populus* that were stably transformed with a Bt toxin gene. One transgenic line in particular showed high levels of resistance to both the forest tent caterpillar (*Malacosoma disstria*) and the gypsy moth. A protein encoded by the *Cry3A* gene was shown to be highly effective against the CLB (James *et al.*, 1999). In a related study, a binary vector containing the *Cry3A* gene under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter was used to produce 51 insect-resistant lines (i.e., independent transgenic events) in four genotypes of *Populus*, which were field-tested in eastern Washington state. This trial relied on insect pressure from the surrounding, commercial stands to evaluate insect resistance. Trees were evaluated for damage, basal diameter, and height at various stages during the growing season. Virtually all of the Bt transgenics showed very low feeding damage, whereas the non-transgenic lines sustained significantly higher levels of defoliation. Moreover, in most cases, the mean growth for transgenic lines was greater than that of the non-transgenic controls within each genotype (Meilan *et al.*, 2000).

Leaves of transgenic *P. tremula* × *P. tremuloides* expressing a *Cry3Aa* toxin were also shown to be highly resistant to damage by the phytophagous beetle *Chrysomela tremulae* (Génissel *et al.*, 2003). This synthetic Bt gene had modified codon usage and lacked AT-rich regions. Beetles feeding on leaves of high- and low-expressing *Cry3Aa* lines died within days of exposure.

TABLE 2
Transgenic *Populus* for stress resistance

Category of stress	Category of resistance	Transgene	Reference
Insect resistance	Bt	Bt toxins	McCown *et al.*, 1991
	Alternatives to Bt	Cry3A	James *et al.*, 1999; Génissel *et al.*, 2003
	Alternatives to Bt	Trypsin proteinase inhibitor: KTi3	Confalonieri *et al.*, 1998
	Alternatives to Bt	Arabidopsis cysteine proteinase inhibitor (Atcys)	Delledonne *et al.*, 2001
	Alternatives to Bt	Scorpion neurotoxin AaIT	Lin *et al.*, 2006
Disease resistance	Fungal pathogens	Bacterio-opsin gene (bO)	Mittler *et al.*, 1995; Mohamed *et al.*, 2001
	Fungal pathogens	Stilbene synthase (StSy)	Giorcelli *et al.*, 2004
	Fungal pathogens	Defensin (NP-1)	Zhao *et al.*, 1999
	Fungal pathogens	Chitinase gene (CH5B)	Lin *et al.*, 2006
	Fungal pathogens	Germin-like oxalate oxidase gene	Liang *et al.*, 2001
	Fungal pathogens	Anti-microbial peptides	Liang *et al.*, 2002; Powell *et al.*, 2006
	Bacterial pathogens	Antimicrobial peptide D4E1	Mentag *et al.*, 2003
Herbicide resistance	Glyphosate	AroA:5-enolpyruvyl-3-phosphoshikimate synthase (EPSPS)	Fillatti *et al.*, 1987a; 1987b; Riemenschneider *et al.*, 1988; Donahue *et al.*, 1994; Donahue *et al.*, 1994; Karnosky *et al.*, 1997
	Glyphosate	CP4	Meilan *et al.*, 2000; Meilan *et al.*, 2002b; Li *et al.*, 2008
	Chlorsulfuron	Acetolactate synthase gene: crs1-1	Brasileiro *et al.*, 1992
	Chloroacetanilide	γ-glutamylcysteine synthetase (γ-ECS)	Gullner *et al.*, 2001
	Glufosinate	BAR	Chupeau *et al.*, 1994
	Glufosinate	Glutamine synthetase (GS)	Pascual *et al.*, 2008
Abiotic resistance	Drought and salinity	Mannitol-1-phsphate dehydrogenase (mtlD)	Hu *et al.*, 2005
	Drought and salinity	Jasmonic acid responsive AP2/ERF-domain transcription factor (JERF)	Li *et al.*, 2009b
	Low nitrogen	glutamine synthase (GS)	Gallardo *et al.*, 1999; El-Khatib *et al.*, 2004; Pascual *et al.*, 2008; Man *et al.*, 2005
	Low temperature	C-repeat binding factor (CBF1)	Benedict *et al.*, 2006
	Low temperature	Isoprene synthase	Behnke *et al.*, 2007
	Small volatile hydrocarbons	Cytochrome P450 2E1	Doty *et al.*, 2007
	Tolerance to mercury	Mercuric ion reductase (merA), organomercury lyase (merB)	Lyyra *et al.*, 2007
	Heavy metal like zinc	Glutamylcysteine synthetase	Bittsászky *et al.*, 2005

Although verification of resistance in both short- and long-term field trials is needed, stable expression of synthetic Bt toxin appears to be an effective method to reduce damage and, hence, minimize mortality caused by various insect pests.

The potential for insects to develop resistance to genetically engineered crops is a major concern (DiCosty and Whalon, 1997; James, 1997; Roush, 1997; Roush and Shelton, 1997). Before insect-resistant transgenics can be commercialized, government regulators require the development of a resistance management plan. Many management strategies have been proposed based on prior experiences with pesticide resistance (Roush, 1997; Gould, 1998; McGaughey *et al.*, 1998). Stacking resistance genes is one way of reducing the risk of insects becoming resistant to Bt proteins (Roush, 1997). This approach has proven to be an effective strategy for resistance management with many insects, including the cotton bollworm (*Helicoverpa armigera*) (Zhao *et al.*, 1997).

2. Alternatives to Bt

A variety of other genes have been evaluated for their effect on insect pests of *Populus*. For example, Confalonieri *et al.* (1998) generated black poplar (*P. nigra*) expressing a soybean (*Glycine max*) trypsin proteinase inhibitor (KTi3). Although the transgenic protein inhibited digestive proteinases of the polyphagous moths such as the gypsy moth and black-back prominent moth (*Clostera anastomosis*) *in vitro*, leaf feeding bioassays showed no increase in larval mortality. Perhaps higher expression levels would have improved resistance or a different proteinase inhibitor would have been more detrimental to these larvae. Greater success was achieved in white poplar (*P. alba*) expressing an *Arabidopsis* cysteine proteinase inhibitor (*Atcys*), which resulted in up to 100% mortality of chrysomelid beetle (*Chrysomela populi*) larvae after 16 days of feeding on transgenic leaf tissue (Delledonne *et al.*, 2001). Moreover, expression of the scorpion neurotoxin *AaIT* in hybrid *Populus* led to resistance against the gypsy moth (Lin *et al.*, 2006).

B. Transgenic *Populus* for Disease Resistance

1. Fungal Pathogens

Fungal infections also can severely damage *Populus*. Several plants genes have been tested for their ability to impart fungal resistance with varying levels of success. Expression of the bacterio-opsin gene (*bO*) from *Halobacterium halobium* in tobacco (*Nicotiana tabaccum*) resulted in a hypersensitive-like response (Mittler *et al.*, 1995). However, the expression of *bO* in hybrid *Populus* did not elicit a significant increase in defense-response against a variety of fungal pathogens, such as leaf rust, leaf and shoot blight, and stem canker (Mohamed *et al.*, 2001). Similarly, white poplar expressing grapevine (*Vitis vinifera* L.) stilbene synthase (*StSy*), which has been implicated in the production of resveratrol compounds, did not significantly impact the efficacy of resistance against the rust disease *Melampsora pulcherrima* (Giorcelli *et al.*, 2004). In contrast, transgenic *Populus* expressing a rabbit defensin (*NP-1*) (Zhao *et al.*, 1999) or chitinase gene (*CH5B*) appear to have increased resistance to a wide range of fungal pathogens (Lin *et al.*, 2006). Hybrid *Populus* expressing a wheat (*Triticum aestivum*) germin-like oxalate oxidase gene, able to metabolize the oxalic acid produced by fungal pathogens, showed signs of delayed infection by *Septoria musiva* (Liang *et al.*, 2001). Liang *et al.* (2002) have also studied transgenic *Populus* expressing anti-microbial peptides, in an effort to alter resistance to *S. musiva*. Two-year-old transgenic trees expressing a combination of antimicrobial peptides did, indeed, show greater resistance in leaf disc assays, and initial field trials show less frequent *Septoria* cankers on transgenic trees (Powell *et al.*, 2006). Based on recent results, it is apparent that increasing resistance to fungal pathogens will likely require a combination of transgenic products.

2. Bacterial Pathogens

Efforts to genetically engineer *Populus* for increased resistance to bacterial pathogens are less common. Although severe bacterial damage is rare, serious infections of *Xanthomonas* spp. have been reported (De Kam, 1984; Haworth and Spiers, 1988). Transgenic *Populus* expressing the antimicrobial peptide, D4E1, showed mixed resistance against *Agrobacterium* and *Xanthomonas* infection (Mentag *et al.*, 2003). A line producing the highest amount of transgene transcript exhibited a significant increase in resistance, as defined by reduced tumor formation following *Agrobacterium* inoculation or the development of smaller cankers after *Xanthomonas* infection. However, resistance against one strain of *Agrobacterium,* C58, was not improved, suggesting that D4E1 has limited specificity. Moreover, D4E1-containing transformants did not show improved resistance against fungal pathogens (Mentag *et al.*, 2003).

C. Transgenic *Populus* for Herbicide Resistance

In addition to insect and disease resistance, another major objective of genetic engineering in trees is herbicide tolerance (Chupeau *et al.*, 1994). Improvements in this area should lead to lower total herbicide use, as well as reliance on more environmentally benign active ingredients and more flexibility with regard to the timing of application.

1. Glyphosate

Fillatti *et al.* (1987a; 1987b) were first to report on genetically engineered herbicide tolerance in *Populus* (*P. alba* × *P. grandidentata*); they used the *Salmonella typhimurium aroA* gene. A mutant of this gene encodes a 5-enolpyruvyl-3-phosphoshikimate synthase (EPSPS) that allows plants to tolerate glyphosate, the active ingredient in the herbicide Roundup® (Comai *et al.*, 1983). Transformants containing *aroA* driven by the mannopine synthase promoter (MAS_{prom}) resulted in unexpectedly low levels of herbicide tolerance (Riemenschneider *et al.*, 1988), which was thought to be the result of low cytosolic expression of the gene. When *Populus* was later transformed with *aroA* under the control of the 35S promoter and a

chloroplast-transit peptide, the resulting plants showed higher levels of glyphosate tolerance (Donahue *et al.*, 1994).

Although use of the 35S promoter led to higher expression levels than MAS_{prom}, and the transit peptide directed transgene product to the chloroplast, performance of the *aroA*-containing lines was still disappointing (Karnosky *et al.*, 1997). During greenhouse trials, chlorophyll content in all transgenic lines was inversely correlated to glyphosate concentration, and height growth was arrested following herbicide treatment, where only one line retained live leaves six weeks following treatment (Donahue *et al.*, 1994).

More recently, Meilan *et al.* (2002a) tested a construct containing *CP4*, another gene that imparts glyphosate tolerance in plants. *CP4* is an alternative form of EPSPS that originates from *Agrobacterium tumefaciens* strain CP4; the enzyme it encodes has a low affinity for glyphosate. Using an *Agrobacterium*-mediated transformation protocol (Leple *et al.*, 1992; Han *et al.*, 2000), Meilan *et al.* (2000) generated transgenic plants in 12 genotypes of hybrid *Populus*. The resulting transgenics were field-tested for two years in Oregon. Growth of the lines expressing *CP4* was significantly better than controls or lines expressing both the *CP4* and *GOX*, which is another gene used to impart glyphosate tolerance. In addition, the lines expressing only *CP4*, had less damage in response to glyphosate treatment. This was the first report of transgenic *Populus* exhibiting high levels of glyphosate tolerance when grown under field conditions (Meilan *et al.*, 2002b). Moreover, herbicide tolerance remained stable for over eight years in trees grown under field conditions (Li *et al.*, 2008b).

2. Chlorsulfuron

The sulfonylurea herbicide Chlorsulfuron acts on acetolactate synthase, blocking the biosynthesis of valine and isoleucine (Ray, 1984). A mutant acetolactate synthase gene (*crs1-1*) from *Arabidopsis*, which confers tolerance to Chlorsulfuron, has been expressed in hybrid poplar (*P. tremula* × *P. alba*) under the control of either the native or 2×35S promoter. Both promoters led to transgenic lines that were completely resistant to higher-than-normal field application rates of Chlorsulfuron in greenhouse tests, while the associated control trees died within two to three weeks of treatment. Despite initially delayed growth and root development, the transgenic plants exhibited normal growth following the treatment (Brasileiro *et al.*, 1992).

3. Chloroacetanilide

Acetochlor and metochlor are active ingredients in chloroacetanilide herbicides, which are detoxified by glutathione (GSH)-dependent reactions (Gullner *et al.*, 2001). Glutathione S-transferase (GST) plays a crucial role in the degradation of several herbicides. GSTs are able to catalyze conjugation reactions between GSH and a number of xenobiotics, and the ensuing conjugates are less toxic, and more water soluble than the herbicide molecules alone (Edwards *et al.*, 2000). When *Populus* expressing γ-glutamylcysteine synthetase (γ-ECS) in the chloroplast or cytosol were exposed to acetochlor and metochlor dispersed in soil, the growth and biomass of all lines was markedly reduced. However, the reduction was less dramatic in the transgenic lines relative to the non-transformed control trees, and the growth rate of cytosol-expressing lines was less affected than the chloroplast expressers (Gullner *et al.*, 2001).

4. Glufosinate

Glufosinate (phosphinothricin, PPT) is the active ingredient in herbicides known as Basta and Buster, and is a structural analog of glutamate. It inhibits glutamine synthetase (GS), causing ammonium to accumulate, which is lethal at elevated levels (Bishop-Hurley *et al.*, 2001). This interaction causes irreversible inactivation of GS, which also blocks photorespiration, and results in the depletion of amino-acid pools (Pascual *et al.*, 2008). Plants respond to PPT by developing necrosis, beginning at or near the apical meristem and spreading throughout the plant (Pascual *et al.*, 2008). The *BAR* gene encodes phosphinothricin acetyltransferase (PAT), which inactivates glufosinate by acetylating its free ammonium group (Thompson *et al.*, 1987). *Populus* explants transformed with *BAR* were able to survive and grow on glufosinate-containing medium at the callus phase, proving it to be an effective selectable marker gene (Chupeau *et al.*, 1994).

Populus overexpressing the pine GS gene showed increased resistance to PPT. Resistance was measured at 5, 25, and 100 μM PPT. At 5 μM, there was limited effect on all plants, whereas 75% of control plants died and 50–100% of the transgenic plants remained viable when exposed 25 μM PPT. At 100 μM, all of the wild-type trees died within eight days, whereas 20–45% of the transgenics survived (Pascual *et al.*, 2008).

D. Transgenic *Populus* for Abiotic Resistance

Abiotic stresses (e.g., drought, salinity, temperature extremes, pollutants) are environmental factors that limit the growth and development of an organism (Wang *et al.*, 2003). Climate change, expansion of cultivated land into marginal environments for food, feed and bioenergy production (Perlack *et al.*, 2005; Ragauskas *et al.*, 2006; Jordan *et al.*, 2007; Robertson *et al.*, 2008), and increase in the prevalence of man-made pollutants, have led to interest in developing crop varieties that can be productive under a range of such stresses (Altman, 2003; Good *et al.*, 2004; Hirel *et al.*, 2007). Modifications of abiotic stress properties through transgenic approaches provides the shortest path from gene discovery to the field (Altman, 2003). This is particularly true for trees that are notoriously difficult to improve through conventional breeding approaches due to inherently long generation cycles. Moreover, genes controlling some desirable traits, such as phytoremediation of pollutants, are rare or do not exist in the plant kingdom and, therefore, require transgenic approaches (Van Aken, 2008).

1. Resistance to Drought and Salinity

Drought and soil salinity are rapidly emerging as a major impediment to plant growth and development in many parts of the world, and projections indicate that by 2050 as much as 50% of total arable land will be under such pressure (Wang *et al.*, 2003). For example, approximately half of China's land mass is classified as arid, semi-arid, or saline-alkali (Li *et al.*, 2009b). Drought and salinity are often interconnected because under both, plants experience significant osmotic stress (Wang *et al.*, 2003). Therefore, some of the approaches to increasing tolerance to either employ a targeted production of compatible osmolytes (e.g., amino acids, quaternary amines, and sugars) that alleviate stress via maintaining cell turgor, which drives water uptake. For example, transgenic *Populus* expressing bacterial mannitol-1-phosphate dehydrogenase (mtlD), which converts fructose to mannitol, showed increased tolerance to salt in the soil (Hu *et al.*, 2005). This modification, known as Balizhuang poplar, showed survival rates of up to 67% in soils containing 0.5% salt. Due to its exceptional performance, and economic and environmental importance, the Chinese government has granted a license for this transgenic variety to be grown operationally. Because of the complexity and multiplicity of responses to abiotic stress, it is difficult to simultaneously enhance all associated functions. Therefore, there has been recent interest in an approach known as regulon engineering (Umezawa *et al.*, 2006). This is done by regulating the expression of several genes or triggering a cascade effect, ultimately activating/repressing a downstream process or an entire pathway, utilizing transcription factors, which control many aspects of a plant's growth, development, and response to the environment. Hence, targeting key "master" regulators has the potential to engineer much more robust protection against abiotic stresses. Using this approach, a tomato jasmonic acid responsive AP2/ERF-domain transcription factor (JERF) that previously was shown to provide protection against abiotic stress in tobacco was transformed in a hybrid *Populus* (*P. alba* x *P. berolinensis*) (Li *et al.*, 2009b). Expression of JERF in *Populus* increased productivity under high salt concentrations and affected several factors that influence stress tolerance in general (e.g., concentration of proline and chlorophyll). A field trial on soils containing 0.3% salt showed up to 33.6% greater height growth of the transgenics relative to control plants.

2. Enhancement of Growth in Low Nitrogen Soil

Nitrogen is a macronutrient that frequently limits growth and productivity (Hodge, 2006). Nitrogen fertilization has been shown to effectively increased crop yields but at the same time, has had negative environmental effects because the excess nitrogen leaches into freshwater and marine ecosystems, resulting in eutrophication and elevated prevalence of disease vectors (Jordan *et al.*, 2007). As such, there has been increased interest in developing crop varieties that can maintain high yields under low-N regimes (Hirel *et al.*, 2007). Plants that are incapable of nitrogen fixation predominantly take up inorganic nitrogen in the form of ammonium and nitrate, which is then converted into glutamate and glutamine, which are the main nitrogen donors for production of various nitrogenous organic compounds (e.g., amino acids, nucleic acids, polyamines) (Suarez *et al.*, 2002). The first step in the conversion is catalyzed by glutamine synthase (GS) (Suarez *et al.*, 2002). Transgenic *Populus* that expressed the pine cytosolic GS showed higher early vegetative growth, increased concentration of total extractable protein, and increased tolerance to drought (Gallardo *et al.*, 1999; El-Khatib *et al.*, 2004; Pascual *et al.*, 2008). Furthermore, enrichment with ^{15}N showed that transgenic plants incorporated 27% more nitrogen into organic compounds and displayed increased growth in a low-nitrogen environment (Man *et al.*, 2005).

3. Resistance to Low Temperature

Temperatures extremes significantly impact plant survival, negatively impact productivity, and increase the incidence of pest outbreaks. Trees suffer most cold injuries as a result of late-spring frosts. Because of a general trend of warmer winters, associated with precocious bud flush, the incidence of frost damage in forest and fruit trees has increased dramatically (Gu *et al.*, 2007). Transgenic approaches have provided opportunities for protection to both high and low temperatures. The C-repeat binding factor is an AP2/ERF transcription factor, which binds to a conserved *cis*-element, and is involved in low-temperature signaling pathway in *Arabidopsis* (Jaglo-Ottosen *et al.*, 1998). Overexpression of CBF1 in *Populus* plants resulted in increased freezing tolerance in non-acclimated leaves and stems (Benedict *et al.*, 2006). Isoprene is thought to play a role in protection against temperature extremes. Downregulation of isoprene synthase in transgenic *Populus* led to decreased isoprene production and greater damage from high temperatures, compared to the associated control plants. In contrast, overexpression of the gene did not lead to increased isoprene production, which is likely due to post-transcriptional co-suppression (Behnke *et al.*, 2007). However, ectopic expression of the *Populus* enzyme in *Arabidopsis* did lead to increased thermo-tolerance and elevated levels of isoprene synthesis (Sasaki *et al.*, 2007).

4. Resistance to Environmental Pollutions

Industrialization over the last century has led to the release of several anthropogenic pollutants into the environmental. Removal of these pollutants using plants that take up, metabolize, sequester, and/or volatilize these compounds is known as phytoremediation (Van Aken, 2008). Plants provide a highly efficient system for environmental clean-up because they are autotrophic and inexpensive to deploy (Van Aken, 2008). Furthermore, phytoremediation can lead to carbon sequestration, soil stabilization, and the development of lignocellulosic feedstocks for bioenergy production. Trees are even more attractive because they are perennial, so their effects are compounded and long-lived. Transgenic approaches provide a convenient method to transfer the innate capacity common in heterologous sources (e.g., bacteria and mammals) to plants, and effectively combat the pollutants produced by industrial processes. For

example, recent studies have shown that overexpression of a mammalian cytochrome P450 2E1 in transgenic *Populus* plants increased removal rates of hydrocarbons that pose significant health risk from both hydroponic solutions and the atmosphere (Doty *et al.*, 2007). In addition, the bacterial mercuric ion reductase (merA) and organomercury lyase (merB) genes were simultaneously transformed in eastern cottonwood (*P. deltoides*). The merA/merB transgenic plants showed increased tolerance to mercury and were able to detoxify organic mercury compounds 3- to 4-fold faster than controls (Lyyra *et al.*, 2007). Overexpression of bacterial glutamylcysteine synthetase in transgenic *P. canescens* plants increased glutathione levels, resulting in increased tolerance and uptake of heavy metals, such as zinc (Bittsászky *et al.*, 2005). Clearly, the success of using transgenic approaches to remediate pollutants is substantial.

IV. TRANSGENIC *POPULUS* FOR ALTERING GROWTH AND DEVELOPMENTAL TRAITS

A. Transgenic *Populus* for Rooting Enhancement

Populus species can be divided into two groups, depending on the ease with which their hardwood cuttings can be rooted, which is the simplest and most economical propagation method (Dickmann and Stuart, 1983). In general, species within the sections Turanga, Leucoides, Tacamahaca, and Aigeiros are easy to root, while species in the section Populus, are recalcitrant. Dai *et al.* (2004) engineered a hybrid aspen with the *rol*B gene under control of both the CaMV35S and heat shock (HS) promoters, and when exposed to heat shock, the cuttings taken from transgenic plants displayed rooting frequencies of 70–80%, while the rootability of the control plants was 15–20%. The transgenic plants appeared to morphologically normal (Dai *et al.*, 2003). Cheng *et al.* (2005) also used the auxin biosynthetic gene, *iaa*M, to improve the rooting of aspen hardwood cuttings. Although the initial results seem promising, additional evaluation is required (Table 3).

B. Growth Rate

One of the most important traits for the forest-product and bioenergy industries is growth rate. Faster growing trees will lead to shorter rotations, which can minimize the land mass required to produce a given amount of biomass. One way to increase productivity is to manipulate levels of endogenous plant hormones. For example, auxin is a critical factor for stimulating vascular differentiation and wood development (Eklund and Little, 1994; Avsian-Kretchmer *et al.*, 2002). Increasing endogenous auxin levels can be accomplished by misregulation of *iaa*M and *iaa*H, the auxin biosynthetic genes from *Agrobacterium tumefaciens* T-DNA (Klee and Lanahan, 1995, Guilfyole *et al.*, 2003). The *iaa*M gene encodes the tryptophan monooxygenase, which catalyzes the conversion of tryptophan into indole-3-acetamide, while *iaa*H encodes the indole acetamide hydrolase, which converts indole-3-acetamide into indole-3-acetic acid (IAA) (Follin *et al.*, 1985). Cambial activity, secondary xylem development, wood composition, and ultimately wood production can be altered by manipulating auxin level or signaling in transgenic plants (Tuominen *et al.*, 1995; Grünward *et al.*, 2000; Labb *et al.*, 2005; Nieminen *et al.*, 2008).

Gibberellins (GAs) are another group of endogenous hormone that influence many aspects of plant growth and development, including shoot elongation and root development (Davies, 1995; Sun, 2000; Olszewski *et al.*, 2002). Previous studies have shown that exogenous applications of GAs can impact differentiation of xylem fibers (Wareing, 1958; Digby and Wareing, 1966), and alter both longitudinal and radial growth in angiosperms (Little and Pharis, 1995) and gymnosperms (Wang *et al.*, 1995). Recent studies have shown that it is possible to manipulate GA levels by modifying GA 20-oxidase levels. For instance, Eriksson *et al.* (2000) reported that ectopic overexpression of a GA 20-oxidase gene in hybrid aspen results in increased GA content, which translated into improvements in stem height and diameter growth, and increases in xylem fiber and biomass production (Table 3).

TABLE 3
Transgenic *Populus* for altering growth and developmental traits

Category of traits	Transgene	Reference
Rooting enhancement	*Rol*B	Dai *et al.*, 2003; Dai *et al.*, 2004
	Agrobacterial auxin biosynthesisis (*iaa*M)	Cheng *et al.*, 2005
Growth rate	GA 20-oxidase	Eriksson *et al.*, 2000
Early flowering	*LEAFY (LFY)*	Nilsson *et al.*, 1998; Rottmann *et al.*, 2000
	FLOWERING LOCUS T2 (FT2)	Hsu *et al.*, 2006; Bohlenius *et al.*, 2006
Tree stature (dwarfism)	Gibberellin (GA) 2-oxidase	Busov *et al.*, 2003
	DELLA-less versions of GAI (GA1-5 Insensitive)	Busov *et al.*, 2006
	Phytochrome A (PHYA)	Olsen and Junttila, 2002

1. Early Flowering

Acquiring the competence to produce flowers is a key aspect of plant growth and development, but it can take decades in some tree species. A reduction in the juvenile period is particularly important for trees because it affects the length of breeding cycles, and directly impacts the productivity of fruit and nut crops. Acceleration of flowering in *Populus* has also been explored through genetic engineering (Meilan *et al.*, 2001). The *P. trichocarpa* ortholog (*PTLF*) of the *Arabidopsis* flowering-time gene *LEAFY* was over-expressed in *Populus* (Nilsson *et al.*, 1998; Rottmann *et al.*, 2000), but Rottmann *et al.* (2000) found that the *PTLF* was ineffective in moderating the flowering time in many genotypes and the flowers produced were largely abnormal and infertile.

More recently, Hsu *et al.* (2006) reported that the *Populus* ortholog of the *Arabidopsis* flowering-time gene *FT*, *FLOWERING LOCUS T2* (*FT2*), controls first-time and seasonal flowering in *Populus*. When *FT2* was ectopically expressed in juvenile *Populus***,** flowering occurred within one year. They also found that during the transition from vegetative to reproductive growth, *FT2* transcripts are abundant under long days. These results suggest that *FT2* is not only a part of the flower initiation pathway, it also plays a role in regulating seasonal flower initiation and its integration with the perennial growth habit of *Populus*. Bohlenius *et al.* (2006) also overexpressed a *Populus* ortholog of *FT*, which they called *PtFT1*, in hybrid aspen, and showed that the *CO*/*FT* regulatory module, which is known to control flowering time in annual plants in response to variations in day length, also controls flowering in *Populus*. In addition, they found that the *CO*/*FT* regulatory module regulates the short day-induced growth cessation and bud set in the fall, a common phenomenon in trees growing in temperate zones and across a wide latitudinal gradient (Bohlenius *et al.* 2006).

A *Populus* ortholog of *CENTRORADIALIS* (*PopCEN1*), a gene that is involved in maintaining juvenility, was downregulated using RNA interference (RNAi) (Mohamed *et al.* 2010) and shown to exhibit early onset of maturation-related characteristics, such as age of first flowering, number of inflorescences, and the proportion of short shoots when grown under field conditions. The most strongly silenced lines produced inflorescence buds within two years of planting, whereas wild-type plants usually flower several years later. In contrast, overexpression of *PopCEN1* led to an almost complete absence of flowering; however, it also was detrimental to shoot development and crown architecture. The authors suggested that *PopCEN1* might be useful for preventing the transition to flowering if driven by floral- or inflorescence meristem-predominant promoters.

2. Tree Stature

Busov *et al.* (2003) produced dwarf *Populus* trees by overexpressing gibberellin (GA) 2-oxidase, a key GA-catabolizing enzyme, leading to a GA deficiency. Apart from short stature, the transgenic trees displayed no obvious abnormalities. Heterologous expression *Arabidopsis* DELLA-less versions of *GA1-5 Insensitive* (*GAI*) and *RGA-Like1* (*RGL1*) in *Populus*, which encode strong repressors of GA signaling, also elicited dwarfism, along with a proliferation of roots and complex changes in leaf and root metabolism (Busov *et al.*, 2006). Overexpression of the oat phytochrome A (PHYA) in a hybrid aspen (*P. tremula* × *P. tremuloides*) prevented growth cessation in response to a short photoperiod, and plants exhibited dwarfism, which was related to reduced cell numbers and lower gibberellin content (Table 3).

V. TRANSGENIC *POPULUS* FOR BIOMASS TRAITS FOR BIOENERGY

Despite the inherent rapid growth rate of *Populus* and its extensive range, the utility of it as feedstock for bioenergy production is limited by the chemical composition of the secondary walls (Mansfield, 2009). In this section, we will emphasize traits that pertain to the chemical and physical properties of the *Populus* feedstock, focusing on transgenic studies that affected lignin and/or increase cellulose content (Table 4).

Phenylalanine is the entry point of phenylpropanoid biosynthetic pathway. This compound is first deaminated by phenylalanine ammonia lyase (PAL), and is then subsequently hydroxylated and methylated, by several unique enzymes culminating in the production of the various monolignols, *p*-hydroxyphenyl, and coniferyl and sinapyl alcohol. These monomers give rise to p-hydroxylphenyl (H), guaiacyl (G), and syringyl (S) units of the lignin polymer, respectively. The various enzymes implicated in lignin biosynthesis include cinnamic acid 4-hydroxylase (C4H), 4-(hydroxy)cinnamoyl CoA ligase (4CL), hydroxycinnamoyl CoA:shikimate hydroxycinnamoyl transferase (HCT), p-coumaroylshikimate 3′-hydroxylase (C3′H), caffeoyl CoA O-methyltransferase (CCoAOMT), (hydroxy)cinnamoyl CoA reductase (CCR), ferulic acid 5-hydroxylase (F5H), caffeic acid O-methyltransferase (COMT), and (hydroxy)cinnamyl alcohol dehydrogenase (CAD) (Boerjan *et al.*, 2003). Following the synthesis of the monolignol precursors, they are transported to the cell wall and polymerized into the lignin macromolecule. Although the exact mechanisms of transport and polymerization are unknown, it is apparent the polymerization is a function of random coupling.

Conversion of plant biomass to ethanol entails breakdown of cell-wall carbohydrates to simple sugars, which are, in turn, fermented to ethanol (Gray *et al.*, 2006). Therefore, one desirable trait of bioenergy crops destined for producing liquid biofuels, is a greater polysaccharide content and access, which can be efficiently and cost-effectively converted to ethanol (Demirbas, 2005). Toward this end, either lignin content needs to be reduced or its composition altered. As an integral part of the secondary plant cell wall, lignin forms a matrix that anchors the cellulose and hemicellulose components (Chabannes *et al.*, 2001; Jones *et al.*, 2001). Despite its important biological functions, lignin negatively impacts cellulosic biofuel production (Palmqvist and Hahn-Hagerdal, 2000). In biomass conversion, the presence of the lignin interferes with access of hydrolytic enzymes to the

TABLE 4
Transgenic *Populus* for biomass traits for bioenergy

Transgene	Approach	Results	Reference
4CL	Antisense	50% reduction in lignin content	Li *et al.*, 2003
C3H	RNAi	A significant decrease in total lignin contents, and Increase in S/G ratio	Coleman *et al.*, 2008
CCoAOMT	Antisense	Reduction in lignin content	Zhong *et al.*, 2000; Meyermans *et al.*, 2000
COMT	Sense	17% reduction of lignin levels	Jouanin *et al.*, 2000
CCR	Sense and antisense	50% reduction of lignin content with a compensatory increase in cellulose content	Leple *et al.*, 2007
CAD	Antisense	No change in lignin content and S/G ratio	Baucher *et al.*,1996; Lapierre *et al.*, 1999
COMT	Antisense	Reductions in syringyl (S) / guaiacyl (G) ratio	Van Doorsselaere *et al.*, 1995; Lapierre *et al.*, 1999; Tuskan *et al.*, 2006
F5H	Overexpression	A dramatic increase in S/G ratio, no change in lignin content	Franke *et al.*, 2000; Li *et al.*, 2003
4CL and F5H	Cotransfer antisense 4CL and sense F5H	52% less lignin, a 64% higher S/G ratio, and 30% more cellulose	Li *et al.*, 2003
Xyloglucanase	Overexpression	Increased growth and cellulose accumulation	Park *et al.*, 2004
UDP-glucose pyrophosphorylase	Overexpression	Increases in cellulose contents	Coleman *et al.*, 2007

Note: 4CL: 4-hydroxycinnamoyl-CoA ligase; C3H: *p*-coumarate 3-hydroxylase; CCoAOMT: caffeoyl-CoA *O*-methyltransferase; COMT: caffeic acid *O*-methyltransferase; CCR: cinnamoyl-CoA reductase; CAD: cinnamyl alcohol dehydrogenase; F5H: ferulate 5-hydroxylase.

cellulose polymer. Lignin can also adsorb hydrolytic enzymes that are involved in saccharification, and some lignin degradation products strongly inhibit subsequent fermentation performance (Cullis and Mansfield, 2010). Davison *et al.* (2006) analyzed the relationship between the lignin content and the xylose release by dilute acid hydrolysis. Results indicated that low lignin content resulted in dramatic improvement in the rate of dilute acid hydrolysis (Ragauskas *et al.*, 2006). Chen and Dixon (2007) analyzed biomass digestibility after both acid pre-treatment and enzymatic hydrolysis for six transgenic alfalfa lines in which the lignin biosynthetic pathway had been disrupted. They showed both acid pretreatment and enzymatic digestion is directly proportional to lignin content (Chen and Dixon, 2007). Therefore, reducing lignin content is a major objective for improving the utility of bioenergy feedstocks.

Lignin content has been reduced to 50% of its natural abundance by down-regulating 4CL expression, using an antisense approach (Li *et al.*, 2003). Similarly, RNA-interference (RNAi), downregulation of C3′H was used to generate transgenic *Populus* (*P. grandidentata* x *P. alba*) with about a 55% reduction in lignin in the most strongly suppressed line (Coleman *et al.*, 2008). In addition, the C3′H RNAi downregulated transgenics showed a change in lignin monomer composition. The proportion of G subunits decreased while the concentration of the S subunits remained unchanged (Coleman *et al.*, 2008). A 13.5% increase in cell-wall carbohydrates, which was accounted for primarily by elevated levels of glucose, xylose, and arabinose, was also observed in the C3′H RNAi lines (Coleman *et al.*, 2008).

CCoAOMT is a bifunctional enzyme that uses S-adenosyl-L-methionine (SAM) as methyl donor, catalyzing the conversion of caffeoyl CoA to feruloyl-CoA and 5-hydroxyferuloyl-CoA to sinapoyl-CoA in monolignol biosynthesis. Suppression of CCoAOMT expression via antisense regulation caused a significant decrease in total lignin content in *Populus*, which resulted in a reduction in both the G and S subunits (Zhong *et al.*, 2000). Furthermore, the reduction in lignin content resulted in a less condensed and less cross-linked lignin macromolecule. Using a sense suppression approach, Meyermans *et al.* (2000) generated transgenic *Populus* with a 90% reduction in CCoAOMT activity, where the trees showed a 12% reduction in lignin content and a higher S/G ratio, when compared to wild-type plants (Meyermans *et al.*, 2000). In both studies, repression of CCoAOMT expression led to the incorporation of *p*-hydroxybenzoic acid

into the cell wall (Meyermans *et al.*, 2000; Zhong *et al.*, 2000).

COMT is thought to catalyze the ortho-methylation of caffeate to ferulate and 5-hydroxyferulate to sinapate, and may also be involved in methylation of other intermediates. Downregulation of COMT activity has been achieved using either antisense or sense transgenic approaches (Van Doorsselaere *et al.*, 1995; Tsai *et al.*, 1998; Lapierre *et al.*, 1999; Jouanin *et al.*, 2000; Tuskan *et al.*, 2006). All these studies resulted in dramatic reductions in S/G ratio. Suppression of COMT by an antisense strategy did not result in significant changes in lignin content (Van Doorsselaere *et al.*, 1995; Lapierre *et al.*, 1999; Tuskan *et al.*, 2006). Different results were obtained from sense cosuppression studies, however. In quaking aspen, no significant changes in lignin content were observed, although transgenic plants exhibited novel phenotypes with either mottled or complete red-brown coloration in their woody stems (Tsai *et al.*, 1998). In contrast, sense suppression of COMT in *Populus* resulted in a 17% reduction in lignin levels (Jouanin *et al.*, 2000).

CCR participates in the reduction of hydroxycinnamoyl-CoA thioesters to the corresponding aldehydes, a reaction considered to be a potential control point for regulating lignin biosynthesis (Lacombe *et al.*, 1997). Transgenic *Populus* with reduced CCR expression was generated by sense and antisense strategies. Downregulation of CCR expression in transgenic *Populus* resulted in a 50% reduction of lignin content (Leple *et al.*, 2007). An orange-brown coloration was observed in the xylem of transgenic plants, which was thought to be the result of the evaluated levels of ferulic acid during lignification.

F5H is a cytochrome P450-dependent monooxygenase that catalyzes the hydroxylation of ferulic acid, coniferaldehyde, and coniferyl alcohol leading to the synthesis of syringyl lignin. To determine the regulatory role of F5H in woody plants, Franke *et al.* (2000) generated the *Arabidopsis* C4H::*F5H* transgenic *Populus* and reported a dramatic increase in S/G ratio, with no change in lignin content (Huntley *et al.*, 2003). A similar result was also reported in transgenic aspen, where a sweetgum (*Liquidambar styraciflua*) *F5H* ortholog under the control of a xylem-specific promoter (*Pt4CL1P*) was established in transgenic aspen. The resulting transgenic plants displayed a 2.5-fold increase in the S/G ratio, and no changes in lignin content (Li *et al.*, 2003).

Recently, Li *et al.* (2010) reported on the conversion of *Arabidopsis* lines with altered S/G ratios. One was a *fah1-2* mutant that was defective in *F5H* and did not deposit S lignin; in the other, *F5H* was overexpressed. When liquid hot water pretreatment was included before enzyme hydrolysis, the S lignin–rich plants gave a much higher glucose yield than either the wild-type or plants rich in G lignin.

CAD catalyzes the last step in the biosynthesis of monolignols. Baucher *et al.* (1996) downregulated CAD expression in transgenic *P. tremula* x *P. alba* by both antisense and cosuppression strategies. Severa1 antisense and sense CAD transgenic *Populus* had an approximate 70% reduction in CAD activity. But neither the lignin content nor the lignin monomeric composition was significantly altered. Later, Lapierre *et al.* (1999) evaluated two-year-old *Populus* that had been engineered to suppress *CAD* via antisense. Lignin content of transgenic plants was only slightly lower than that of controls. In addition, the monomer composition of the transgenics was similar to those of the control, but the lignin was enriched in free phenolic groups, and displayed a red-brown coloration (Lapierre *et al.*, 1999).

In contrast to these single-gene studies, Li *et al.* (2003) used *Agrobacterium* to simultaneously transfer antisense *4CL* and sense F5H constructs into *P. tremuloides*. The transgenic plants had 52% less lignin and a 64% higher S/G ratio, thus exhibiting an additive effect (Li *et al.*, 2003). This study suggests that a multigene, co-transformation approach may be effective for genetically engineered lignin.

In contrast to lignin formation, which is now relatively well understood, cellulose synthesis in woody species remains poorly characterized (Joshi and Mansfield, 2007). Only a few studies have reported on genetic manipulation of cell-wall carbohydrates in *Populus*. Cellulose microfibrils are cross-linked by xyloglucans, so degradation of xyloglucan may contribute to cell-wall loosening and stimulate cell expansion. Park *et al.* (2004) reported that overexpression of xyloglucanase in *Populus* which led to increased growth and cellulose accumulation. In another study, transgenic *Populus* with elevated levels of UDP-glucose pyrophosphorylase, an enzyme that catalyzes the production of UDP-glucose, the immediate pre-cursor for cellulose production (Coleman *et al.*, 2007), resulted in altered cell-wall chemistry, but at the expense of tree growth. More recently, it has been shown that by altering sucrose metabolism it is possible to influence carbon partitioning to cellulose production, by specifically targeting sucrose synthase (SuSy) (Coleman *et al.*, 2009) and sucrose phosphate synthase (SPS) (Park *et al.*, 2009).

In addition to their role in controlling plant growth and development, lignin and cellulose composition play important roles in regulating plant-environment interactions. Lignin affects cell-wall permeability, enabling transport of water and solutes through the vascular system (Sarkanen, 1971). Moreover, lignin provides protection against microbial pathogens (Moerschbacher *et al.*, 1990). How transgenic *Populus* with altered lignin perform in the field has not been extensively studied. However, such information is vital before growth of *Populus* as a biofuel crop will be feasible from both economic and regulatory perspectives. While the cell wall–related traits can be improved using genetic engineering, it must be shown that these manipulations don't lead to unintended consequences. This can only be accomplished through extensive, multiyear field tests.

VI. TRANSGENIC *POPULUS* FOR FUNCTIONAL GENOMICS

In contrast to *Arabidopsis* and rice, there are unique challenges associated with doing genetic studies with *Populus*, such

as long generation times, large physical size, and the labor involved in maintaining genetically altered stocks either through *in vitro* culture or field planting. Genome-wide duplication also makes functional genomics studies difficult because of gene redundancy. Nevertheless, a group of labs have attempted to create various types of genome-wide mutations, including activating tagging, gene and enhance trapping, RNAi, and transposon tagging.

A. Activation Tagging

The value of forward-genetic approaches employing mutagenesis is well established (Alonso *et al.*, 2003), but practically impossible to apply to trees because of their long generation cycles and predominantly out-crossing mating systems. Only dominant approaches, such as activation tagging, can be used to identify mutations in the first generation (Busov *et al.*, 2005). Activation tagging uses a T-DNA vector that contains strong enhancers positioned near the left- or right-hand T-DNA borders. Insertion of such strong enhancers in a location near a gene tends to cause its upregulation and a gain-of-function. Because the mutations are dominant, activation tagging is feasible in *Populus*, and results from small, pilot populations are very encouraging (Busov *et al.*, 2003, 2010; Bush *et al.*, 2007). Activation tagging offers a number of other advantages. For example, many of the genes identified through activation tagging in *Populus* belong to gene families (e.g., GA 2-oxidase) (Busov *et al.*, 2003); gain-of-function mutations provide an alternative for functional characterization of these highly redundant gene families (Nakazawa *et al.*, 2003), which represent a significant problem in the duplicated genome of *Populus* (Tuskan *et al.*, 2006). Activation tagging also offers facile characterization of genes adjacent to the insertion site using the tag sequence (Liu *et al.*, 1995), preferential insertion in gene-rich genome sectors (Kim *et al.*, 2007), and discovery of poorly annotated or non-protein-coding loci (Palatnik *et al.*, 2003). The availability of annotated genomic sequence enables activation tagging approaches that aim to saturate the *Populus* genome. Such endeavors will likely involve tens of thousands of independent transformation events, which will require substantial investment for production and maintenance. Recent interest in trees as biofuel crops may generate the support that will be needed to fund such efforts (Ragauskas *et al.*, 2006).

B. RNAi/Antisense

Loss-of-function mutation is the 'gold standard' for dissecting gene function. However, most loss-of-function lesions are recessive and, therefore, require successive rounds of selfing to expose the mutation. As mentioned above, due to long generation cycles, this approach is not feasible in trees because it requires decades to accomplish. Knock-down dominant transgenic modifications are typically achieved via post-transcriptional gene silencing (PTGS), targeting specific mRNAs for degradation (Brodersen and Voinnet, 2006). Antisense technology was one of the first techniques used to trigger PTGS in transgenic plants. This approach was successfully used in *Populus* to study several key enzymes in the lignin biosynthetic pathway (Baucher *et al.*, 1996) and genes involved in control of dormancy (Rohde *et al.*, 2002).

Because of the relatively low efficiency and inability to discriminate between closely related paralogs, antisense-mediated gene suppression is rapidly being replaced by RNAi (Matthew, 2004), a more potent inducer of gene silencing. Studies in plants and other eukaryotic organisms have shown that inverted-repeat transgenes (especially if they are separated by an intron) provide a reliable and highly efficient means of gene suppression (Chuang and Meyerowitz, 2000; Smith *et al.*, 2000). Studies on efficiency and stability of gene suppression in *Populus* are still scarce. In a recent study, RNAi-induced gene suppression was found to be stable over two years and during the annual growth cycle, despite the highly variable RNAi transgene expression driven by a Rubisco promoter (Li *et al.*, 2007). Antisense gene suppression appeared to be stable over time as demonstrated in transgenic *Populus* with silenced cinnamyl alcohol dehydrogenase (CAD) or caffeate/5-hydroxy-ferulate O-methyltransferase (COMT) genes (Pilate *et al.*, 2002). In a different study, Li *et al.* (2008a) used RNAi to target the *Arabidopsis rbc*S promoter, which was used to drive expression of the *BAR* gene. The degree of gene suppression in each event was highly stable over two years in the field, and was not related to insert copy number.

C. Gene and Enhancer Trapping

Gene disruption-based knockout or knockdown mutations can lead to a recognizable phenotype and this has been quite successful for gene identification (see above). However, the function of many *Populus* genes cannot be revealed by mutagenesis because it naturally possesses functional redundancy in many genes resulting from whole-genome, segmental, and tandem duplications, which have overlapping functions (Tuskan *et al.*, 2006). Because whole or partial function can be compensated for by one or more other members in the same or even a different family, mutation of one of the functionally redundant genes may not cause an obvious phenotype (Springer, 2000). In addition, many genes are regulated or expressed developmentally in response to specific environmental cues. Therefore, a recognizable phenotype may not be exhibited in non-expressing tissues, at various developmental stages, or under certain conditions (spatially and temporally regulated). Other gene mutations may also escape detection due to lethality, an alteration in the pathway for a secondary metabolite, or expression in specific tissues (i.e., roots) that requires specialized equipment for detection.

Gene and enhancer trapping are alternative insertion-based strategies that are based on detecting gene expression, instead of detecting an aberrant phenotype (Springer, 2000; Groover *et al.*, 2004). A gene-trap vector carries a reporter gene lacking a functional promoter, while an enhancer-trap vector carries a reporter gene with a minimally functional promoter. When the reporter gene is inserted at a site where it will be under the control of a

functional native promoter, it will be expressed in a manner that mimics the normal expression pattern of the endogene. Therefore, the number of genes that can be functionally classified is limited only by the number of transgenic lines produced. The genomic region flanking the insertion site can be easily amplified via PCR or through rapid amplification of cDNA ends (RACE) polymerase chain reaction (PCR), and the amplified fragment can be sequenced. The cloned gene can be further characterized for functionality by overexpression and/or suppression in mutant and/or wild-type plants. Groover *et al.* (2004) demonstrated the potential of gene and enhancer traps as a new resource for novel gene discovery in *Populus*. Using the gene- and enhancer-trap vectors carrying the β-glucuronidase (GUS) reporter gene, they generated 55 GUS-expressing gene-trap and 455 enhancer-trap lines. Many inserts led to GUS expression in primary and secondary vascular tissues, although other expression patterns were also seen (Groover *et al.*, 2004). They also found significant overlap in genes responsible for the development and function of secondary vascular tissues of stems and primary vascular tissues in other organs of the plant. Using the thermal asymmetric interlaced (TAIL) PCR and sequencing, Groover *et al.* (2004) amplified and identified the chromosomal DNA at the insertion sites by reference to the *P. trichocarpa* genome sequence. They further isolated full-length cDNAs for five genes of interest, including a new type of vascularly expressed gene tagged by enhancer-trap line cET-1-pop1-145. These gene- and enhancer-trap lines have been maintained and are available for screening (http://www.fs.fed.us/psw/programs/ifg/genetraps.shtml). However, there have been no reports of this strategy being used to generate trap lines that exhibit expression patterns in response to biotic or abiotic stresses.

D. Transposon-Insertional Mutagenesis in *Populus*

In addition to T-DNA-based insertional mutagenesis, naturally occurring plant transposable elements have been engineered into heterologous transposon vectors for insertional mutagenesis (Nishal *et al.*, 2005). Adaption for use with *Populus* has largely involved the maize *Ds* transposon system (Fladung and Ahuja, 1997; Fladung *et al.*, 1997; Kumar and Fladung, 2003; Fladung *et al.*, 2004). Fladung and Ahuja (1997) have also used the maize Ac transposable element in combination with the phenotypic selectable marker gene, *rolC*, to generate a number of transgenic hybrid aspen clones showing light-green sectors on its leaves. However, only one out of 385 regenerated plants exhibited this phenotype. They confirmed *Ac* excision and restoration of *rolC* expression by both PCR and northern analysis. Their Southern blots suggested that *Ac* excision in the non-green L1-epidermal layer, leading to periclinal chimerism in this plant. Kumar and Fladung (2003) further characterized the genomic sequences flanking the *Ac* insertions and showed that about one-third (22/75) of them were highly similar to sequences represented in public databases, suggesting these inserting sites might correspond to genes. The frequency of *Ac* landing into coding regions was about two-fold higher when compared to the frequency of T-DNA landing in predicted genes (5/32) in the aspen genome. These results suggest that *Ac* is a potentially useful heterologous transposon tag in *Populus* for obtaining both dominant gain-of-function mutants and recessive loss-of-function mutants (Kumar and Fladung, 2003).

VII. TRANSGENIC *POPULUS* AND THE ENVIRONMENT

Before genetically engineered trees can be deployed commercially, federal regulators will likely require a robust method for mitigating the risk of transgene spread and persistence in the environment (Meilan, 2006). To achieve transgene confinement, researchers are attempting to genetically engineer trees that do not produce functional flowers (Meilan *et al.*, 2001; Lemmetyinen and Sopanen, 2004). Different strategies are being tested, including cell ablation, which involves floral-specific expression of a cytotoxin gene; RNAi to down-regulate the expression of an endogene necessary for normal floral development; and dominant negative mutations that lead to the production of dysfunctional versions of gene products (e.g., transcription factors, necessary for the expression of genes required for proper floral development) (Meilan *et al.*, 2004). The approaches being used to manipulate flower development and recent progress with each has recently been reviewed by Brunner *et al.* (2007).

Genetically engineering trees that are reproductively sterile may help alleviate public and regulatory concerns over the commercialization of transgenic trees. In addition, sterility can reduce genetic pollution from non-transgenic plantations and eliminate nuisance tissues (e.g., pollen, seed pods). Flowering control may also lead to abbreviated juvenile periods, resulting in shorter breeding cycles. Moreover, it is assumed that trees engineered for reproductive sterility will re-direct carbohydrate to harvestable products while, at the same time, avoiding gene flow to wild populations. Different types and degrees of sterility also may be obtained via polyploidy (e.g., triploids or aneuploids), by genes specifically controlling male/female floral development, or by genes controlling the onset of maturation. Ideally, flowering control should be reversible, so that with appropriate stimulus, the tree can be used for conventional breeding.

Despite indications that one or more of the strategies involving flowering control can be successfully employed to engineer transgene confinement, no single method fulfills the essential requirements for long-term commercial use. Researchers are continuing to determine whether sterility can be complete, successfully identified in juvenile trees, and lack negative growth impacts. Finally, in order to engineer durable sterility, it must be shown that transgene expression is stable after multiple rounds of propagation, under a variety of conditions, and over several growing seasons.

The recently reported gene deletor technology, based on a novel combination of the phage CRE/loxP and yeast FLP/FRT DNA recombination systems, may provide an alternative tool to address the gene-flow problems with transgenic trees (Li *et al.*, 2007; Luo *et al.*, 2007). As demonstrated in a model plant, the gene deletor technology is highly efficient at eliminating

all transgenes from both pollen and seed; none of more than 30,000 progeny examined from self-pollination of T_0 transgenic plants contained transgenes. The gene deletor technology, if successfully used in woody plants, could offer an additional advantage when compared to sterility. Large-scale (e.g., thousands of acres) plantings of sterile trees with no flower, pollen, or seed production may negatively impact insects and other animals that depend on these tissues for nourishment, which could reduce biodiversity in forests or push insect populations into nearby farmland, parks, and wilderness. By using the gene deletor technology, though, transgenic trees will produce normal pollen and seeds but these organs will be non-transgenic.

VIII. FUTURE PERSPECTIVES

Although there has been tremendous progress in making *Populus* transgenics and employing them for genetic improvement, functional genomics in *Populus* remains far behind that of *Arabidopsis* and rice. Numerous challenges are listed below.

1. The transformation efficiency for the sequenced genotype remain low and the protocol remains very cumbersome (Ma *et al.*, 2004; Song *et al.*, 2006). Before it can be realistically employed for large-scale functional genomics studies, considerable improvement of transformation efficiency is needed. The recent developments in discovery of regeneration-promoting genes (Umezawa *et al.*, 2006; Garces *et al.*, 2007; Matsuo and Banno, 2008) may be helpful. Currently, few studies have employed the sequenced genotype when generating mutants or in validating gene function, due to its low transformation efficiency. Although the genetic homology between the sequenced genotype and other *Populus* species is very high, redundant and paralogous genes make it challenging for determining true orthologs in the reference genome. New types of mutagenesis strategies, such as more efficient, high throughput RNAi 'knockout' systems, especially coupled with sterility or gene deletor technologies (Luo *et al.*, 2007) and in the reference genotype would greatly facilitate long-term trials of transgenic mutants in the field, and it appears to be surmountable with a coordinated, global effort. As the power of genome sequencing increases, an alternative strategy may be to sequence, annotate, and assemble a highly regenerable and transformable genotype using the currently sequenced genome as a reference.
2. One of the challenging issues in *Populus* transgenics and functional genomics is identifying recessive traits, because *Populus* trees are genetically heterozygous, suffer from inbreeding depression, and have long juvenile periods. The recently duplicated genome contributes to this difficulty. It is possible to employ haploid trees for detection of recessive mutations (Busov *et al.*, 2005), and haploid *Populus* have been generated in various ways (Kiss *et al.*, 2001; Deutsch *et al.*, 2004). However, the very low viability, early spontaneous chromosome doubling, and low vigor and survivability make it difficult for practical large-scale, functional genomics applications (Deutsch *et al.*, 2004). Recently, Qu *et al.* (2007) have developed a method for transforming a haploid *Populus*. Whether this genotype can be used as starting material for generation of large number of recessive mutations, which will survive long enough for trait evaluation, remains to be determined. Another approach may be to employ a site-specific recombinase system, which can direct the exchange of specific sequences.
3. Although several different mutagenesis strategies have been employed in various labs in generating various types of mutants, there is no coordinated effort to saturate the *Populus* genome. This approach is particularly important because a huge amount of resources are required. Moreover, none of the existing mutants is in the sequenced genotype, which adds to the challenge of follow-up functional characterization of these mutants.
4. As suggested by Busov *et al.* (2005), the *Populus* community also needs to establish a stock center that maintains propagules harboring mutations, similar to what has been done with *Arabidopsis* (Alonso *et al.*, 2003) (http://www.arabidopsis.org). Because generating and storing seeds for transgenic *Populus* is impractical due to: extended juvenility; the need for permits to generate transgenic seeds (because trees would have to be grown in the field); genetic heterozygosity; low seed viability (preventing long-term storage); and high cost, the most feasible model for storage and dissemination of *Populus* genetic material is via vegetative propagules. This center, if established, could also co-exist with a transformation center at which transgenic plants can be produced and confirmed, and from which they can be ordered/distributed at low cost. Given the constraints of a physical center, it may be more practical to establish a virtual one. At the same time, the *Populus* community should make a coordinated effort to preserve and utilize the existing activation tagged and enhancer/gene-trap lines (Busov *et al.*, 2005).
5. Another area of research in *Populus* transgenics is to improve methods for directly inducing mutations and detecting them. Current insertion methods are generally random, but zinc-finger site-specific mutagenesis, which has been demonstrated in *Arabidopsis* (Kumar *et al.*, 2006) and maize (Shukla *et al.*, 2009), may be employed to improve directed mutagenesis, but this needs to demonstrated in *Populus*. Large-scale detection of mutants through technologies, such as RootViz® for root architecture (www.phenotypescreening.com) and metabolomics for secondary metabolites, will also improve the efficiency with which transgenics can be characterized (Chen *et al.*, 2009). Finally, because many of the desired traits, specifically for industrial use (pulp and paper, and bioenergy), there is a pressing need for standardized, low-cost, and high-throughput technologies for wood cell-wall characterization.

6. In the last few years, great advances have been made in sequencing technologies and, as a result, sequencing costs have come down drastically. Consequently, the amount of genomic and transcriptomic sequence data available for *Populus* has increased dramatically. Much of these data are replacing, updating, or complementing available genetic resources, such as high-density genetic maps (Yin *et al.*, 2004), large expressed sequenced tag databases (Annegret *et al.*, 2003; Sterky *et al.*, 2004), and microarrays (Drost *et al.*, 2009). Examples include: resequencing the *Populus* genome using different species, which is underway in several institutes worldwide, including Nanjing Forestry University (Tongming Yin, personal communication) and University of British Columbia, Canada (Shawn Mansfield, personal communication); whole-genome association of different ecotypes of *P. trichocarpa* for cell wall-related traits that is occurring at Oak Ridge National Laboratory (Gerald A. Tuskan, personal communication); and various transcriptomic databases that are being generated for plants responding to treatments such as drought (Xiaohan Yang, personal communication). Using advanced bioinformatic tools to work on whole genomes, transcriptomes, specific gene families (e.g., Yang *et al.*, 2006; Ye *et al.*, 2009a; 2009b), lineage-specific genes (Yang *et al.*, 2009), and gene networks, and in combination with high-throughput transformation systems for large-scale demonstration of functionality, we can be sure that many more candidate genes will be validated in the near future. These will undoubtedly lead to an improvement in our understanding of *Populus* growth and development, and allow us to more precisely manipulate *Populus* genetically to yield better products for forestry, bioenergy, and the environment.

ACKNOWLEDGMENTS

We would like to thank two anonymous reviewers for their helpful comments on this manuscript. We also apologize for being unable to cite other important research papers but, alas, we were constrained by space. The research in Cheng's lab has been supported by the Consortium for Plant Biotechnology Research, Inc., the BioEnergy Science Center, which is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science, and by Tennessee Agricultural Experiment Station.

REFERENCES

Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., Stevenson, D. K., *et al.* 2003. Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301:** 653–657.

Altman, A. 2003. From plant tissue culture to biotechnology: Scientific revolutions, abiotic stress tolerance, and forestry. *In Vitro Cell. Dev. Biol.-Plant.* **39:** 75–84.

Annegret, K., Christine, D., David, M., Nathalie, E., and Francis, M. 2003. The poplar root transcriptome: analysis of 7000 expressed sequence tags. *FEBS Lett.* **542:** 37–41.

Baucher, M., Chabbert, B., Pilate, G., Van Doorsselaere, J., Tollier, M. T., PetitConil, M., Cornu, D., *et al.* 1996. Red xylem and higher lignin extractability by down-regulating a cinnamyl alcohol dehydrogenase in poplar. *Plant Physiol.* **112:** 1479–1490.

Behnke, K., Ehlting, B., Teuber, M., Bauerfeind, M., Louis, S., Häsch, R., Polle, A., *et al.* 2007. Transgenic, non-isoprene emitting poplars don't like it hot. *Plant J.* **51:** 485–499.

Benedict, C., Skinner, J. S., Meng, R., Chang, Y., Bhalerao, R., Huner, N. P. A., Finn, C. E., *et al.* 2006. The CBF1-dependent low temperature signalling pathway, regulon and increase in freeze tolerance are conserved in *Populus* spp. *Plant, Cell Environ.* **29:** 1259–1272.

Bishop-Hurley, S. L., Zabkiewicz, R. J., Grace, L., Gardner, R. C., Wagner, A., and Walter, C. 2001. Conifer genetic engineering: transgenic *Pinus radiata* (D. Don) and *Picea abies* (Karst) plants are resistant to the herbicide Buster. *Plant Cell Rep.* **20:** 235–243.

Bittsászky, A., Kömives, T., Gullner, G., Gyulai, G., Kiss, J., Heszky, L., Radimszky, L., and Rennenberg, H. 2005. Ability of transgenic poplars with elevated glutathione content to tolerate zinc(2+) stress. *Environ. International* **31:** 251–254.

Boerjan, W. 2005. Biotechnology and the domestication of forest trees. *Curr. Opin. Biotechnol.* **16:** 159–166.

Boerjan, W., Ralph, J., and Baucher, M. 2003. Lignin biosynthesis. *Annu. Rev. Plant Biol.* **54:** 519–546.

Bohlenius, H., Huang, T., Charbonnel-Campaa, L., Brunner, A.M., Jansson, S., Strauss, S. H., and Nilsson, O. 2006. CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. *Science* **312:** 1040–1043.

Bradshaw, H. D., Ceulemans, R., Davis, J., and Stettler, R. 2000. Emerging model systems in plant biology: Poplar (*Populus*) as a model forest tree. *J. Plant Growth Regul.* **19:** 306–313.

Bradshaw, H. D., Villar, M., Watson, B. D., Otto, K. G., Stewart, S., and Stettler, R. F. 1994. Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. *Theor. Applied Genet.* **89:** 167–178.

Brasileiro, A., Tourneur, C., Leple, J.-C., Combes, V., and Jouanin, L. 1992. Expression of the mutant *Arabidopsis thaliana* acetolactate synthase gene confers chlorsulfuron resistance to transgenic poplar plants. *Trans. Research* **1:** 133–141.

Brunner, A., Li, J., DiFazio, S., Shevchenko, O., Montgomery, B., Mohamed, R., Wei, H., *et al.* 2007. Genetic containment of forest plantations. *Tree Genet. Gen.* **3:** 75–100.

Bush, M., Plett, J. M., McPhee, D. P., Vitez, R., O'Malley, B., Sharma, V., Bosnich, W., *et al.* 2007. Diverse developmental mutants revealed in an activation-tagged population of poplar. *Can. J. Bot.* **85:** 1071–1081.

Busov, V., Fladung, M., Groover, A., and Strauss, S. 2005. Insertional mutagenesis in *Populus*: relevance and feasibility. *Tree Genet. Gen.* **1:** 135–142.

Busov, V. B., Meilan, R., Pearce, D. W., Ma, C., Rood, S. B., and Strauss, S. H. 2003. Activation tagging of a dominant gibberellin catabolism gene (GA 2-oxidase) from poplar that regulates tree stature. *Plant Physiol.* **132:** 1283–1291.

Busov, V., Yordanov, Y., Gou, J., Meilan, R., Ma, C., Regan, S., and Strauss, S. 2010. Activation tagging is an effective gene tagging system in *Populus*. *Tree Genet.Gen.* In press.

Carozzi, N., and Koziel, M. 1997. *Advances in Insect Control: the Role of Transgenic Plants*. Taylor and Francis, London, UK.

Chabannes, M., Ruel, K., Yoshinaga, A., Chabbert, B., Jauneau, A., Joseleau, J. P., and Boudet, A. M. 2001. In situ analysis of lignins in transgenic tobacco reveals a differential impact of individual transformations on the spatial patterns of lignin deposition at the cellular and subcellular levels. *Plant J.* **28:** 271–282.

Chen, F. and Dixon, R. A. 2007. Lignin modification improves fermentable sugar yields for biofuel production. *Nature Biotechnol.* **25:** 759–761.

Chen, F., Liu, C.-J., Tschaplinski, T. J., and Zhao, N. 2009. Genomics of Secondary Metabolism in Populus: Interactions with Biotic and Abiotic Environments. *Cri. Rev. Plant Sci.* **28:** 375–392.

Cheng, Z.-M., Dai, W. H., Bosela, M. J., and Osburn, L. D. 2005. Genetic engineering approach to enhance adventitious root formation of hardwood cuttings. *J. Crop Improvement* **17:** 211–225.

Chuang, C.-F. and Meyerowitz, E. M. 2000. Specific and heritable genetic interference by doublestranded RNA in *Arabidopsis thalian*a. *Proc. Natl. Acad. Sci. USA* **97:** 4985–4990.

Chupeau, M.-C., Pautot, V., and Chupeau, Y. 1994. Recovery of transgenic trees after electroporation of poplar protoplasts. *Trans. Res.* **3:** 13–19.

Coleman, H. D., Canam, T., Kang, K. Y., Ellis, D. D., and Mansfield, S. D. 2007. Over-expression of UDP-gluclose pyrophosphorylase in hybrid poplar affects carbon allocation. *J. Exp. Bot.* **58:** 4257–4268.

Coleman, H. D., Park, J. Y., Nair, R., Chapple, C., and Mansfield, S. D. 2008. RNAi-mediated suppression of p-coumaroyl-CoA 3 '-hydroxylase in hybrid poplar impacts lignin deposition and soluble secondary metabolism. *Proc. Natl. Acad. Sci. USA* **105:** 4501–4506.

Coleman, H. D., Yan, J., and Mansfield, S. D. 2009. Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure. *Proc. Natl. Acad. Sci. USA* **106:** 13118–13123.

Comai, L., Sen, L. C., and Stalker, D. M. 1983. An altered aroA gene product confers resistance to the herbicide glyphosate. *Science* **221:** 370–371.

Confalonieri, M., Allegro, G., Balestrazzi, A., Fogher, C., and Delledonne, M. 1998. Regeneration of *Populus* nigra transgenic plants expressing a Kunitz proteinase inhibitor (KTi3) gene. *Mol. Breed.* **4:** 137–145.

Coyle, D., Nebeker, T., Hart, E., and Mattson, W. 2005. Biology and management of insect pests in North American intensively managed hardwood forest systems. *Annu. Rev. Entomol.* **50:** 1–29.

Cseke, L., Cseke, S., and Podila, G. 2007. High efficiency poplar transformation. *Plant Cell Rep.* **26:** 1529–1538.

Cullis, I. F. and Mansfield S. D. 2010. Optimized delignification of wood-derived lignocellulosics for improved enzymatic hydrolysis performance. *Biotechnol. Bioengineering* **106**(6): 884–893.

Dai, W. H., Cheng, Z.-M., and Sargent, W. A. 2003. Regeneration and Agrobacterium-mediated transformation of two elite aspen hybrid clones from in vitro leaf tissues. *In Vitro Cell. Dev. Biol.-Plant.* **39:** 6–11.

Davis, J. M. 2008. Genetic improvement of poplar (*Populus* spp.) as a bioenergy crop. **In:** *Genetic Improvement of Bioenergy Crops*, pp. 397–419. Vermerris W., Ed., Springer-Verlag, New York.

De Block, M. 1990. Factors influencing the tissue culture and the *Agrobacterium tumefaciens*-mediated transformation of hybrid aspen and poplar clones. *Plant Physiol.* **93:** 1110–1116.

De Kam, M. 1984. *Xanthomonas campestris* pv. populi, the causal agent of bark necrosis in poplar. *European J. Plant Pathology* **90:** 13–22.

DeBell, D.S. 1990. Populus trichocarpa Torr. & Gray. Black cottonwood. **In:** *Silvics of North America. Vol. 2*, pp. 570–576. Burns, R. M., and Honkala, B. H., Eds., USDA, Forest Service, Washington, D.C.

Delledonne, M., Allegro, G., Belenghi, B., Balestrazzi, A., Picco, F., Levine, A., Zelasco, S., *et al.* 2001. Transformation of white poplar (*Populus alba* L.) with a novel *Arabidopsis thaliana* cysteine proteinase inhibitor and analysis of insect pest resistance. *Mol. Breed.* **7:** 35–42.

Demirbas, A. 2005. Bioethanol from cellulosic materials: A renewable motor fuel from biomass. *Energy Sources* **27:** 327–337.

Deutsch, F., Kumlehn, J., Ziegenhagen, B., and Fladung, M. 2004. Stable haploid poplar callus lines from immature pollen culture. *Physiolo. Plantarum* **120:** 613–622.

Dickmann, D. I. and Stuart, K. W. 1983. *The Culture of Poplars in Eastern North America.* Michigan State University Press, East Lansing, MI.

Dickmann, D. I. *et al.* 2001. *Poplar Culture in North America*. NRC-Research Press, Ottawa, Canada. 397 pp.

Dickmann, D. L. 2006. Silviculture and biology of short-rotation woody crops in temperate regions: then and now. *Biomass and Bioenergy* **30:** 696–705.

DiCosty, U. and Whalon, M. 1997. Selection of Colorado potato beetle resistant to CryIIIA on transgenic potato plants. *Resistant Pest Management Newsletter* **9:** 33–34.

Dinus, R. J. 2000. *Genetic Modification of Short Rotation Poplar Biomass Feedstock for Efficient Conversion to Ethanol*. Environmental Sciences Division, Oak Ridge National Laboratory, TN.

Donahue, R., Davis, T., Michler, C., Riemenschneider, D., Carter, D., Marquardt, P., Sankhla, N., *et al.* 1994. Growth, photosynthesis, and herbicide tolerance of genetically modified hybrid poplar. *Can. J. Forest Res.* **24:** 2377–2383.

Doty, S. L., James, C. A., Moore, A. L., Vajzovic, A., Singleton, G. L., Ma, C., Khan, Z., Xin, G., Kang, J. W., *et al.* 2007. Enhanced phytoremediation of volatile environmental pollutants with transgenic trees. *Proc. Natl. Acad. Sci. USA* **104:** 16816–16821.

Drost, D. R., Novaes, E., Boaventura-Novaes, C., Benedict, C. I., Brown, R. S., Yin, T., Tuskan, G. A., and Kirst, M. 2009. A microarray-based genotyping and genetic mapping approach for highly heterozygous outcrossing species enables localization of a large fraction of the unassembled *Populus trichocarpa* genome sequence. *Plant J.* **58:** 1054–1067.

Eckenwalder, J. E. 1996. Systematics and evolution of *Populus*. **In:** *Biology of Populus and its implications for management and conservation*, pp. 7–32. Stettler, R. F., Bradshaw, H. D., Heilman, Jr. P. E., and Hinckley, T. M., Eds., NRC Research Press, National Research Council of Canada, Ottawa, Ont.

Edwards, R., Dixon, D. P., and Walbot, V. 2000. Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health. *Trends Plant Sci.* **5:** 193–198.

El-Khatib, R. T., Hamerlynck, E. P., Gallardo, F., and Kirby, E. G. 2004. Transgenic poplar characterized by ectopic expression of a pine cytosolic glutamine synthetase gene exhibits enhanced tolerance to water stress. *Tree Physiol.* **24:** 729–736.

Fillatti, J. J., Kiser, J., Rose, R., and Comai, L. 1987a. Efficient transfer of a glyphosate tolerance gene into tomato using a binary *Agrobacterium tumefaciens* vector. *Nature Biotechnol.* **5:** 726–730.

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

Fladung, M. and Ahuja, M. R. 1997. Excision of the maize transposable element Ac in periclinal chimeric leaves of 35S-Ac-rolC transgenic aspen-Populus. *Plant Mol. Biol.* **33:** 1097–1103.

Fladung, M., Deutsch, F., Honicka, H., and Kumar, S. 2004. T-DNA and transposon tagging in aspen. *Plant Biol. (Stuttg)* **7:** 5–11.

Fladung, M., Kumar, S., and Raj Ahuja, M. 1997. Genetic transformation of Populus genotypes with different chimaeric gene constructs: transformation efficiency and molecular analysis. *Transgenic Res.* **6:** 111–121.

Franke, R., McMichael, C. M., Meyer, K., Shirley, A. M., Cusumano, J. C., and Chapple, C. 2000. Modified lignin in tobacco and poplar plants overexpressing the Arabidopsis gene encoding ferulate 5-hydroxylase. *Plant J.* **22:** 223–234.

Gallardo, F., Fu, J., Cantón, F. R., García-Gutiérrez, A., Cánovas, F. M., and Kirby, E. G. 1999. Expression of a conifer glutamine synthetase gene in transgenic poplar. *Planta* **210:** 19–26.

Garces, H.M.P., Champagne, C.E.M., Townsley, B. T., Park, S., Malho, R., Pedroso, M. C., Harada, J. J., and Sinha, N. R. 2007. Evolution of asexual reproduction in leaves of the genus *Kalanchoe*. *Proc. Natl. Acad. Sci. USA* **104:** 15578–15583.

Génissel, A., Leplé, J.-C., Millet, N., Augustin, S., Jouanin, L., and Pilate, G. 2003. High tolerance against *Chrysomela tremulae* of transgenic poplar plants expressing a synthetic cry3Aa gene from *Bacillus thuringiensis* ssp. tenebrionis. *Mol. Breed.* **11:** 103–110.

Giorcelli, A., Sparvoli, F., Mattivi, F., Tava, A., Balestrazzi, A., Vrhovsek, U., Calligari, P., *et al.* 2004. Expression of the Stilbene Synthase (StSy) gene from grapevine in transgenic white poplar results in high accumulation of the antioxidant resveratrol glucosides. *Transgenic Research* **13:** 203–214.

Good, A. G., Shrawat, A. K., and Muench, D. G. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends Plant Sci.* **9:** 597–605.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. *Annu. Rev. Entomol.* **43:** 701–726.

Gray, K. A., Zhao, L. S., and Emptage, M. 2006. Bioethanol. *Curr. Opin. Chem. Biol.* **10:** 141–146.

Groover, A. T. 2005. What genes make a tree a tree? *Trends Plant Sci.* **10:** 210–214.

Groover, A., Fontana, J. R., Dupper, G., Ma, C., Martienssen, R., Strauss, S., and Meilan, R. 2004. Gene and enhancer trap tagging of vascular-expressed genes in poplar trees. *Plant Physiol.* **134:** 1742–1751.

Gu, L., Hanson, P. J., Post, W. M., Kaiser D. P., Yang, B., Nemani, R., Pallardy, S. G., and Meyers, T. 2008. The 2007 Eastern US spring freeze: Increased cold damage in a warming world? *BioScience* **58:** 253–262.

Gullner, G., Komives, T., and Rennenberg, H. 2001. Enhanced tolerance of transgenic poplar plants overexpressing gamma-glutamylcysteine synthetase towards chloroacetanilide herbicides. *J. Exp. Bot.* **52:** 971–979.

Han, K.-H., Ma, C., and Strauss, S. H. 1997. Matrix attachment regions (MARs) enhance transformation frequency and transgene expression in poplar. *Transgenic Research* **6:** 415–420.

Han, K. H., Meilan, R., Ma, C., and Strauss, S. H. 2000. An *Agrobacterium tumefaciens* transformation protocol effective on a variety of cottonwood hybrids (genus *Populus*). *Plant Cell Rep.* **19:** 315–320.

Harrell, M., Benjamin, D., Berbee, J., and Burkot, T. 1981. Evaluation of adult cottonwood leaf beetle, *Chrysomela scripta* (Coleoptera: Chrysomelidae), feeding preference for hybrid poplars. *Great Lakes Entomol.* **14:** 181–184.

Harrell, M., Benjamin, D., Berbee, J., and Burkot, T. 1982. Consumption and utilization of leaf tissue of tissue-cultured *Populus x euramericana* by the cottonwood leaf beetle, *Chrysomela scripta* (Coleoptera, Chrysomelidae). *Can. Entomol* **114:** 743–749.

Hart, E., James, R., Nebeker, T., Robison, D., Raffa, K., and Wagner, M. 1996. Entomological research in North American *Populus* and *Salix*: an overview. **In:** *Proc. Intl. Poplar Comm*. pp. 1–4. Budapest, Hungary.

Haworth, R. and Spiers, A. 1988. Characterisation of bacteria from poplars and willows exhibiting leaf spotting and stem cankering in New Zealand. *Forest Pathol.* **18:** 426–436.

Heilman, P. E. 1999. *Planted Forests: Poplars*. pp. 89–93. Kluwer Academic Publishers, Boston, MA.

Hirel, B., Le Gouis, J., Ney, B., and Gallais, A. 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Exp. Bot.* **58:** 2369–2387.

Hodge, A. 2006. Plastic plants and patchy soils. *J. Exp. Bot.* **57:** 401–411.

Hsu, C.-Y., Liu, Y., Luthe, D. S., and Yuceer, C. 2006. Poplar FT2 Shortens the juvenile phase and promotes seasonal flowering. *Plant Cell* **18:** 1846–1861.

Hu, J. J., Tian, Y. C., Han, Y. F., Li, L., and Zhang, B. E. 2001. Field evaluation of insect-resistant transgenic *Populus nigra* trees. *Euphytica* **121:** 123–127.

Hu, L., Lu, H., Liu, Q., Chen, X., and Jiang, X. 2005. Overexpression of mtlD gene in transgenic *Populus tomentosa* improves salt tolerance through accumulation of mannitol. *Tree Physiology* **25:** 1273–1281.

Huntley, S. K., Ellis, D., Gilbert, M., Chapple, C. and Mansfield, S. D. 2003. Improved pulping efficiency in C4H-F5H transformed poplar. *J. Agricultural Food Chemistry*, **51:** 6178–6183.

IPCC. 2008. *Synthesis of country progress reports*. FAO, Rome, Italy.

Isebrands, J. G. and Karnosky, D. F. 2002. Environmental benefits of poplar culture. **In:** *Poplar Culture in North America*. pp. 207–218. Dickmann, D. I., Isebrands, J. G., Eckenwalder, J. E., and Richardson, J., Eds., National Research Council Press, Ottawa, Canada.

Jaglo-Ottosen, K. R., Gilmour, S. J., Zarka, D. G., Schabenberger, O., and Thomashow, M. F. 1998. Arabidopsis CBF1 overexpression induces COR genes and enhances freezing tolerance. *Science* **280:** 104–106.

James, R. R. 1997. Utilizing a social ethic toward the environment in assessing genetically engineered insect-resistance in trees. *Agriculture and Human Values* **14:** 237–249.

James, R., Croft, B., and Strauss, S. 1999. Susceptibility of the cottonwood leaf beetle (Coleoptera: Chrysomelidae) to different strains and transgenic toxins of *Bacillus thuringiensis*. *Environ. Entomol.* **28:** 108–109.

Jones, L., Ennos, A. R., and Turner, S. R. 2001. Cloning and characterization of irregular xylem4 (irx4): a severely lignin-deficient mutant of Arabidopsis. *Plant J.* **26:** 205–216.

Jordan, N., Boody, G., Broussard, W., Glover, J. D., Keeney, D., McCown, B. H., McIsaac, G., *et al.* 2007. ENVIRONMENT: Sustainable development of the agricultural bio-economy. *Science* **316:** 1570–1571.

Joshi, C. P. and Mansfield, S. D. 2007. The cellulose paradox - simple molecule, complex biosynthesis. *Curr. Opin. Plant Biol.* **10:** 220–226.

Jouanin, L., Goujon, T., De Nadai, V., Martin, M. T., Mila, I., Vallet, C., Pollet, B., *et al.* 2000. Lignification in transgenic poplars with extremely reduced caffeic acid O-methyltransferase activity. *Plant Physiol.* **123:** 1363–1373.

Karnosky, D., Podila, G., Shin, D., and Riemenschneider, D. 1997. Differential expression of aroA gene in transgenic poplar: influence of promoter and ozone stress. **In:** *Micropropagation, Genetic Engineering, and Molecular Biology of Populus*, pp. 70–73. Klopfenstein, N., Chun,Y. W., Kim, M. S., and Ahuja, M., Eds., USDA, Forest Service, Rocky Mountain Forest and Range Experimental Station, Fort Collins, CO.

Kim, S.-I., Veena, and Gelvin, S. 2007. Genome-wide analysis of *Agrobacterium* T-DNA integration sites in the Arabidopsis genome generated under non-selective conditions. *Plant J.* **51:** 779–791.

Kiss, J., Kondrák, M., Törjék, O., Kiss, E., Gyulai, G., Mázik-Tökei, K., and Heszky, L. E. 2001. Morphological and RAPD analysis of poplar trees of anther culture origin. *Euphytica* **118:** 213–221.

Knowles, B. and Dow, J. 1993. The crystal ß-endotoxins of *Bacillus thuringiensis*: Models for their mechanism of action on the insect gut. *BioEssays* **15:** 469–476.

Kumar, S. and Fladung, M. 2003. Somatic mobility of the maize element Ac and its utility for gene tagging in aspen. *Plant Mol. Biol.* **51:** 643–650.

Kumar, S., Allen, G. C., and Thompson, W. F. 2006. Gene targeting in plants: fingers on the move. *Trends Plant Sci.* **11:** 159–161.

Labbé, N., Rials, T. G., Kelley, S. S., Cheng, Z.-M., Kim, J.-Y., and Li, Y. 2005. FT-IR imaging and pyrolysis-molecular beam mass spectrometry: new tools to investigate wood tissues. *Wood Sci. Technol.* **39:** 61–76.

Lacombe, E., Hawkins, S., Van Doorsselaere, J., Piquemal, J., Goffner, D., Poeydomenge, O., Boudet, A. M., and GrimaPettenati, J. 1997. Cinnamoyl CoA reductase, the first committed enzyme of the lignin branch biosynthetic pathway: Cloning, expression and phylogenetic relationships. *Plant J.* **11:** 429–441.

Lapierre, C., Pollet, B., Petit-Conil, M., Toval, G., Romero, J., Pilate, G., Leple, J. C., *et al.* 1999. Structural alterations of lignins in transgenic poplars with depressed cinnamyl alcohol dehydrogenase or caffeic acid O-methyltransferase activity have an opposite impact on the efficiency of industrial kraft pulping. *Plant Physiol.* **119:** 153–163.

Lemmetyinen, J. and Sopanen, T. 2004. Modification of flowering in forest trees. **In:** *Genetics Breeding Forest Trees*, pp. 263–291. Kumar, S., and Fladung, M., Eds., Molecular Haworth Press, Inc., New York.

Leple, J., Brasileiro, A., Michel, M., Delmotte, F., and Jouanin, L. 1992. Transgenic poplars: expression of chimeric genes using four different constructs. *Plant Cell Rep.* **11:** 137–141.

Leple, J. C., Dauwe, R., Morreel, K., Storme, V., Lapierre, C., Pollet, B., Naumann, A., *et al.* 2007. Downregulation of cinnamoyl-coenzyme a reductase in poplar: Multiple-level phenotyping reveals effects on cell wall polymer metabolism and structure. *Plant Cell* **19:** 3669–3691.

Li, B., Wyckoff, G. W., and Einspahr, D. W. 1993. Hybrid aspen performance and genetic gains. *Northern J. Appl. Forestry* **10:** 117–122.

Li, J., Brunner, A. M., Meilan, R., and Strauss, S. H. 2009a. Stability of transgenes in trees: expression of two reporter genes in poplar over three field seasons. *Tree Physiol.* **29:** 299–312.

Li, J., Brunner, A.M., Shevchenko, O., Meilan, R., Ma, C., Skinner, J. S., and Strauss, S. H. 2008a. Efficient and stable transgene suppression via RNAi in field-grown poplars. *Transgenic Research* **17:** 679–694.

Li, J., Meilan, R., Ma, C., Barish, M., and Strauss, S. 2008b. Stability of herbicide resistance over 8 years of coppice in field-grown, genetically engineered poplars. *Western J. Appl. Forestry* **23:** 89–93.

Li, L., Zhou, Y. H., Cheng, X. F., Sun, J. Y., Marita, J. M., Ralph, J., and Chiang, V. L. 2003. Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. *Proc. Natl. Acad. Sci. USA* **100:** 4939–4944.

Li, X., Ximenes, E., Kim, Y., Slininger, M., Meilan, R., Ladisch, M. R., and Chapple, C.C.S. 2010. Lignin monomer composition affects *Arabidopsis* cell-wall degradability after liquid hot water pretreatment. *Biotechnol. Biofuels* **3:** 27.

Li, Y., Duan, H., and Smith, W. 2007. *Gene-deletor.a new tool to address concerns over GE crops. ISB News Report.* http://www.isb.vt.edu/news/2007/news07.jun.htm

Li, Y., Su, X., Zhang, B., Huang, Q., Zhang, X., and Huang, R. 2009b. Expression of jasmonic ethylene responsive factor gene in transgenic poplar tree leads to increased salt tolerance. *Tree Physiology* **29:** 273–279.

Liang, H., Catranis, C. M., Maynard, C. A., and Powell, W. A. 2002. Enhanced resistance to the poplar fungal pathogen, *Septoria musiva*, in hybrid poplar clones transformed with genes encoding antimicrobial peptides. *Biotechnol. Lett.* **24:** 383–389.

Liang, H., Maynard, C. A., Allen, R. D., and Powell, W. A. 2001. Increased *Septoria musiva* resistance in transgenic hybrid poplar leaves expressing a wheat oxalate oxidase gene. *Plant Mol. Biol.* **45:** 619–629.

Lin, S.-Z., Zhang, Z.-Y., Zhang, Q., and Lin, Y.-Z. 2006. Progress in the study of molecular genetic improvements of poplar in China. *J. Integr. Plant Biol.* **48:** 1001–1007.

Liu, Y., Mitsukawa, N., Oosumi, T., and Whittier, R. 1995. Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. *Plant J.* **8:** 457–463.

Luo, K., Duan, H., Zhao, D., Zheng, X., Deng, W., Chen, Y., Stewart, C. N. J., *et al.* 2007. 'GM-gene-deletor': fused loxP-FRT recognition sequences dramatically improve the efficiency of FLP or CRE recombinase on transgene excision from pollen and seed of tobacco plants. *Plant Biotechnol J.* **5:** 263–274.

Lyyra, S., Meagher, R. B., Kim, T., Heaton, A., Montello, P., Balish, R. S., and Merkle, S. A. 2007. Coupling two mercury resistance genes in Eastern cottonwood enhances the processing of organomercury. *Plant Biotechnol. J.* **5:** 254–262.

Ma, C., Strauss, S., and Meilan, R. 2004. Agrobacterium -mediated transformation of the genome-sequenced poplar clone, nisqually-1 (*Populus trichocarpa*). *Plant Mol. Biol. Rep.* **22:** 311–312.

Man, H.-M., Boriel, R., El-Khatib, R., and Kirby, E. G. 2005. Characterization of transgenic poplar with ectopic expression of pine cytosolic glutamine synthetase under conditions of varying nitrogen availability. *New Phytol.* **167:** 31–39.

Mansfield, S. D. 2009. Solutions for dissolution – Engineering cell walls for deconstruction. *Curr. Opin. Biotechnol.* **20:** 286–294.

Matsuo, N. and Banno, H. 2008. The Arabidopsis transcription factor ESR1 induces in vitro shoot regeneration through transcriptional activation. *Plant Physiol. Biochem.* **46:** 1045–1050.

Matthew, L. 2004. RNAi for plant functional genomics. *Comp Funct Genomics.* **5:** 240–244.

McCown, B. H., McCabe, D. E., Russell, D. R., Robison, D. J., Barton, K. A., and Raffa, K. F. 1991. Stable transformation of *Populus* and incorporation of pest resistance by electric discharge particle acceleration. *Plant Cell Rep.* **9:** 590–594.

McGaughey, W., Gould, F., and Gelernter, W. 1998. Bt resistance management. *Nat. Biotechnol.* **16:** 144–146.

Meilan, R. 2006. Challenges to commercial use of transgenic plants. *J. Crop Improvement* **18:** 433–450.

Meilan, R., Auerbach, D., Ma, C., DiFazio, S., and Strauss, S. 2002a. Stability of herbicide resistance and GUS expression in transgenic hybrid poplars (Populus sp.) during four years of field trials and vegetative propagation. *Hort.* **37:** 277–280.

Meilan, R., Brunner, A. M., Skinner, J. S., and Strauss, S. H. 2001. Modification of flowering in transgenic trees. **In:** *Molecular Breeding of Woody Plants. Progress in Biotechnology Series*. pp. 247–256. Morohoshi, N., and Komamine, A., Eds., Elsevier Science BV, Amsterdam.

Meilan, R., Han, K., Ma, C., DiFazio, S., Eaton, J., Hoien, E., Stanton, B., *et al.* 2002b. The CP4 transgene provides high levels of tolerance to Roundup® herbicide in field-grown hybrid poplars.. *Can. J. Forest Research* **32:** 967–976.

Meilan, R., Ma, C., Cheng, S., Eaton, J., Miller, L., Crockett, R., DiFazio, S., and Strauss, S. 2000. High levels of Roundup® and leaf-beetle resistance in genetically engineered hybrid cottonwoods. **In:** *Hybrid Poplars in the Pacific Northwest: Culture, Commerce and Capability*, pp. 29–38. Blatner, K., Johnson, J., and Baumgartner, D., Eds., Washington State University Cooperative Extension Bulletin MISC0272, Pullman, WA.

Meilan, R., Sabatti, M., Ma, C., and Kuzminsk, E. 2004. An early-flowering genotype of *Populus*. *J Plant Biol.* **47:** 52–56.

Mentag, R., Luckevich, M., Morency, M. J., and Seguin, A. 2003. Bacterial disease resistance of transgenic hybrid poplar expressing the synthetic antimicrobial peptide D4E1. *Tree Physiology* **23:** 405–411.

Meyermans, H., Morreel, K., Lapierre, C., Pollet, B., De Bruyn, A., Busson, R., Herdewijn, P., *et al.* 2000. Modifications in lignin and accumulation of phenolic glucosides in poplar xylem upon down-regulation of caffeoyl-coenzyme A O-methyltransferase, an enzyme involved in lignin biosynthesis. *J. Biol. Chem.* **275:** 36899–36909.

Mittler, R., Shulaev, V., and Lam, E. 1995. Coordinated activation of programmed cell death and defense mechanisms in transgenic tobacco plants expressing a bacterial proton pump. *Plant Cell* **7:** 29–42.

Moerschbacher, B. M., Noll, U., Gorrichon, L., and Reisener, H. J. 1990. Specific-inhibition of lignification breaks hypersensitive resistance of wheat to stem rust. *Plant Physiol.* **93:** 465–470.

Mohamed, R., Meilan, R., Ostry, M., Michler, C., and Strauss, S. 2001. Bacterioopsin gene overexpression fails to elevate fungal disease resistance in transgenic poplar (*Populus*). *Can. J. Forest Research* **31:** 1–8.

Mohamed, R., Wang, C.-T., Ma, C., Shevchenko, O., Dye, S., Puzey, J., Etherington, E., *et al.* 2010. *Populus CEN/TFL1* regulates first onset of flowering, axillary meristem identity and dormancy release in *Populus*. *Plant J.* **62:** 674–688.

Nakazawa, M., Ichikawa, T., Ishikawa, A., Kobayashi, H., Tsuhara, Y., Kawashima, M., Suzuki, *et al.* 2003. Activation tagging, a novel tool to dissect the functions of a gene family. *Plant J.* **34:** 741–750.

Nilsson, O., Lee, I., Blazquez, M. A., and Weigel, D. 1998. Flowering-time genes modulate the response to LEAFY activity. *Genet.* **150:** 403–410.

Nishal, B., Tantikanjana, T., and Sundaresan, V. 2005. An inducible targeted tagging system for localized saturation mutagenesis in Arabidopsis. *Plant Physiol.* **137:** 3–12.

Nordman, E. E., Robison, D. J., Abrahamson, L. P., and Volk, T. A. 2005. Relative resistance of willow and poplar biomass production clones across a continuum of herbivorous insect specialization: Univariate and multivariate approaches. *Forest Ecol. Management* **217:** 307–318.

Olsen, J. E. and Junttila, O. 2002. Far red end-of-day treatment restores wild type-like plant length in hybrid aspen overexpressing phytochrome A. *Physiol. Plantarum* **115:** 448–457.

Palatnik, J. F., Allen, E., Wu, X., Schommer, C., Schwab, R., Carrington, J. C., and Weigel, D. 2003. Control of leaf morphogenesis by microRNAs. *Nature* **425:** 257–263.

Palmqvist, E. and Hahn-Hagerdal, B. 2000. Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. *Bioresour. Technol.* **74:** 17–24.

Park, J.-Y., Canam, T., Kang, K. Y., Unda, F., and Mansfield, S. D. 2009. Sucrose phosphate synthase (SPS) expression influences phenology in poplar. *Tree Physiology*. **29:** 937–946.

Park, Y. W., Baba, K., Furuta, Y., Iida, I., Sameshima, K., Arai, M., and Hayashi, T. 2004. Enhancement of growth and cellulose accumulation by overexpression of xyloglucanase in poplar. *FEBS Lett.* **564:** 183–187.

Parsons, T. J., Sinkar, V. P., Stettler, R. F., Nester, E. W., and Gordon, M. P. 1986. Transformation of poplar by *Agrobacterium tumefaciens*. *Bio/Technology* **4:** 533–536.

Pascual, M. B., Jing, Z. P., Kirby, E. G., Covas, F. M., and Gallardo, F. 2008. Response of transgenic poplar overexpressing cytosolic glutamine synthetase to phosphinothricin. *Phytochem.* **69:** 382–389.

Perlack, R., Wright, L., Turhollow, A., and Graham, R. 2005. *Biomass as Feedstock for Bioenergy and Bioproducts Industry: The Technical Feasbility*

of a Billion-Ton Supply. Oak Ridge National Laboratory, Oak Ridge, TN. ORNL/TM-2005/66.

Pincon, G., Maury, S., Hoffmann, L., Geoffroy, P., Lapierre, C., Pollet, B., and Legrand, M. 2001. Repression of O-methyltransferase genes in transgenic tobacco affects lignin synthesis and plant growth. *Phytochemistry* **57:** 1167–1176.

Powell, W., Maynard, C., Boyle, B., and Seguin, A. 2006. Fungal and bacterial resistance in transgenic trees. **In:** *Tree Transgenesis*, pp. 235–252. Fladung, M., and Ewald, D., Eds., Springer, New York.

Pythoud, F., Sinkar, V. P., Nester, E. W., and Gordon, M. P. 1987. Increased Virulence of agrobacterium rhizogenes conferred by the vir region of pTiBo542: Application to genetic engineering of poplar. *Bio/Technology* **5:** 1323–1327.

Qu, G. Z., Liu, G. F., Wang, Y. C., Jiang, J., and Wang, M. H. 2007. Efficient Tissue culture and agrobacterium-mediated transformation of haploid poplar derived from anthers *Russian J. Plant Physiol.* **54:** 559–563.

Ragauskas, A. J., Williams, C. K., Davison, B. H., Britovsek, G., Cairney, J., Eckert, C. A., Frederick, W. J., Jr., *et al.* 2006. The path forward for biofuels and biomaterials. *Science* **311:** 484–489.

Ray, T. B. 1984. Site of action of chlorsulfuron: inhibition of valine and isoleucine biosynthesis in plants. *Plant Physiol.* **75:** 827–831.

Riemenschneider, D., Haissig, B., Sellmer, J., and Fillatti, J. 1988. Expression of an herbicide tolerance gene in young plants of a transgenic hybrid poplar clone. **In:** *Somatic Cell Genetics of Woody Plants*, pp. 73–80. Ahuja, M., Ed., Kluwer Academic Publishers, Boston, MA.

Robertson, G. P., Dale, V. H., Doering, O. C., Hamburg, S. P., Melillo, J. M., Wander, M. M., Parton, W. J., *et al.* 2008. AGRICULTURE: Sustainable Biofuels Redux. *Science* **322:** 49–50.

Rohde, A., Prinsen, E., De Rycke, R., Engler, G., Van Montagu, M., and Boerjan, W. 2002. PtABI3 impinges on the growth and differentiation of embryonic leaves during bud set in poplar. *Plant Cell* **14:** 1885–1901.

Rottmann, W. H., Meilan, R., Sheppard, L. A., Brunner, A.M., Skinner, J. S., Ma, C., Cheng, S., *et al.* 2000. Diverse effects of overexpression of *LEAFY* and *PTLF*, a poplar (*Populus*) homolog of *LEAFY/FLORICAULA*, in transgenic poplar and Arabidopsis. *Plant J.* **22:** 235–245.

Roush, R. 1997. Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? *Pestic. Sci.* **51:** 328–334.

Roush, R. and Shelton, A. 1997. Assessing the odds: The emergence of resistance to Bt transgenic plants. *Nature Biotechnol.* **15:** 816–817.

Sarkanen, K. V. and Ludwig, C. H. 1971. Classification and distribution. **In:** *Lignins: Occurrence, Formation, Structure, and Reactions*, pp. 43–94. Sarkanen, K. V., and Ludwig, C. H., Eds., Wiley-Intersciences, New York.

Sasaki, K., Saito, T., Lamsa, M., Oksman-Caldentey, K.-M., Suzuki, M., Ohyama, K., Muranaka, T., *et al.* 2007. Plants utilize isoprene emission as a thermotolerance mechanism. *Plant Cell Physiol.* **48:** 1254–1262.

Shukla, V. K., Doyon, Y., Miller, J. C., DeKelver, R. C., Moehle, E. A., Worden, S. E., Mitchell, J. C., *et al.* 2009. Precise genome modification in the crop species Zea mays using zinc-finger nucleases. *Nature* **459:** 437–441.

Smith, N. A., Singh, S. P., Wang, M.-B., Stoutjesdijk, P. A., Green, A. G., and Waterhouse, P. M. 2000. Gene expression: Total silencing by intron-spliced hairpin RNAs. *Nature* **407:** 319–320.

Song, J., Lu, S., Chen, Z.-Z., Lourenco, R., and Chiang, V. L. 2006. Genetic transformation of *Populus trichocarpa* genotype Nisqually-1: A functional genomic tool for woody plants. *Plant Cell Physiol.* **47:** 1582–1589.

Springer, P. S. 2000. Gene traps: Tools for plant development and genomics. *Plant Cell* **12:** 1007–1020.

Sterck, L., Rombauts, S., Jansson, S., Sterky, F., Rouzé, P., and Van de Peer, Y. 2005. EST data suggest that poplar is an ancient polyploid. *New Phytol.* **167:** 165–170.

Sterky, F., Bhalerao, R. R., Unneberg, P., Segerman, B., Nilsson, P., Brunner, A. M., Charbonnel-Campaa, L., *et al.* 2004. A *Populus* EST resource for plant functional genomics. *Proc. Natl. Acad. Sci. USA* **101:** 13951–13956.

Stettler, R. F., Koster, R., and Steenackers, V. 1980. Interspecific crossability studies in poplars. *Theo. Appl. Genet.* **58:** 273–282.

Suarez, M. F., Avila, C., Gallardo, F., Canton, F. R., Garcia-Gutierrez, A., Claros, M. G., and Canovas, F. M. 2002. Molecular and enzymatic analysis of ammonium assimilation in woody plants. *J. Exp. Bot.* **53:** 891–904.

Subramaniam, R., Reinold, S., Molitor, E. K., and Douglas, C. J. 1993. Structure, inheritance, and expression of hybrid poplar (*Populus trichocarpa* x *Populus deltoides*) phenylalanine ammonia-lyase genes. *Plant Physiol.* **102:** 71–83.

Taylor, G. 2002. Populus: Arabidopsis for forestry. Do we need a model tree? *Ann. Bot.* **90:** 681–689.

Thompson, C., Movva, N., Tizard, R., Crameri, R., Davies, J., Lauwereys, M., and Botterman, J. 1987. Characterization of the herbicide-resistance gene bar from Streptomyces hygroscopicus. *Eur. Mol. Biol. Organization J.* **6:** 2519–2523.

Thompson, M., Schnepf, H., and Feitelson, J. 1995. Structure, function and engineering of Bacillus thuringiensis toxins. **In:** *Genetic Engineering,Vol. 17*, pp. 99–117. Setlow, J., Ed., Plenum Press, New York.

Tsai, C. J., Popko, J. L., Mielke, M. R., Hu, W. J., Podila, G. K., and Chiang, V. L. 1998. Suppression of O-methyltransferase gene by homologous sense transgene in quaking aspen causes red-brown wood phenotypes. *Plant Physiol.* **117:** 101–112.

Tuskan, G. A., DiFazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., Putnam, N., *et al.* 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313:** 1596–1604.

Umezawa, T., Fujita, M., Fujita, Y., Yamaguchi-Shinozaki, K., and Shinozaki, K. 2006. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Curr. Opin. Biotechnol.* **17:** 113–122.

Van Aken, B. 2008. Transgenic plants for phytoremediation: helping nature to clean up environmental pollution. *Trends Biotechnol.* **26:** 225–227.

Van Doorsselaere, J., Baucher, M., Chognot, E., Chabbert, B., Tollier, M. T., PetitConil, M., Leple, J. C., *et al.* 1995. A novel lignin in poplar trees with a reduced caffeic acid 5-hydroxyferulic acid O-methyltransferase activity. *Plant J.* **8:** 855–864.

Wang, W., Vinocur, B., and Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* **218:** 1–14.

Wu, R. and Stettler, R. F. 1994. Quantitative genetics of growth and development in Populus. I. A three-generation comparison of tree architecture during the first 2 years of growth. *Theo. Appl. Genet.* **89:** 1046–1054.

Wullschleger, S. D., Jansson, S., and Taylor, G. 2002. Genomics and forest biology: *Populus* emerges as the perennial favorite. *Plant Cell* **14:** 2651–2655.

Yang, X., Tuskan, G. A., and Cheng, Z.-M. 2006. Divergence of the Dof gene families in poplar, Arabidopsis, and rice suggests multiple modes of gene evolution after duplication. *Plant Physiol.* **142:** 820–830.

Yang, X., Jawdy, S., Tschaplinski, T. J., and Tuskan, G. A. 2009. Genome-wide identification of lineage-specific genes in *Arabidopsis*, *Oryza* and *Populus*. *Genomics* **93**: 473–480.

Ye, X., Kang, B.-G., Osburn, L. D., and Cheng, Z.-M. 2009a. The COBRA gene family in *Populus* and gene expression in vegetative organs and in response to hormones and environmental stresses. *Plant Growth Regul.* **58:** 211–223.

Ye, X., Kang, B.-G., Osburn, L., Li, Y., and Zong-Ming, C. 2009b. Identification of the flavin-dependent monooxygenase-encoding YUCCA gene family in *Populus trichocarpa* and their expression in vegetative tissues and in response to hormone and environmental stresses. *Plant Cell, Tiss. Org. Cult.* **97:** 271–283.

Yin, T., DiFazio, S., Gunter, L., Riemenschneider, D., and Tuskan, G. 2004. Large-scale heterospecific segregation distortion in *Populus* revealed by a dense genetic map. *Theo. Appl. Genet.* **109:** 451–463.

Zhao, J., Fan, X., Shi, X., Zhao, R., and Fan, Y. 1997. Gene pyramiding: an effective strategy of resistance management for *Helicoverpa armigera* and *Bacillus thuringiensis*. *Resistance and Pest Management* **9:** 19–21.

Zhao, S., Zu, G., Liu, G., Huang, M., Xu, J., and Sun, Y. 1999. Introduction of rabbit defensin NP-1 gene into poplar (*P. tomentosa*) by *Agrobacterium*-mediated transformation. *Acta Genetic Sinica* **26:** 11–14.

Zhong, R., Morrison, W. H., III, Himmelsbach, D. S., Poole, F. L., II, and Ye, Z.-H. 2000. Essential role of caffeoyl coenzyme A O-methyltransferase in lignin biosynthesis in woody poplar plants. *Plant Physiol.* **124:** 563–578.