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Chapter 4

Production of Traditional and Novel Biopolymers in Transgenic Woody Plants

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Abstract Recent advances in plant biotechnology are expanding the potential for woody plants to provide industrially useful biopolymers. Transgenic approaches can enable plants to produce novel compounds that are not normally present (e.g., bioplastics such as polyhydroxybutyrate). This chapter summarizes the strategies that have been used to produce biopolymers in plants, with emphasis on bioplastics from transgenic poplar. So far, the yields of bioplastic in plants have been accompanied by unfavorable metabolic expenses associated with the diversion of carbon resources, but it may be possible to obtain improvements through careful control of expression of the three genes for biosynthesis of polyhydroxybutyrate. This chapter also discusses the potential for transgenic technology to improve the yields and qualities of traditional biopolymers including cellulose (wood), latex, and oil. A major emphasis with wood has been the modification of lignin content and structure to facilitate pulp and biofuel production. Other ongoing projects involving biopolymers may lead to improved production of latex from guayule (*Parthenium argentatum*) and Russian dandelion (*Taraxacum kok-saghyz*) and of fuel oil from *Jatropha* (*Jatropha curcas*). We believe that substantial improvements in these traditional plant products are likely with additional research on control of gene expression and if regulatory concerns about field research and commercial deployment can be adequately addressed.

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4.1 Introduction

Woody plants have long been vital sources of industrial biopolymers, most notably wood and rubber. Indeed, the importance of these materials as socioeconomic driving forces can scarcely be understated. The advent of genetic engineering is currently providing opportunities to enhance the production of these compounds as well as to develop new woody plant-based production systems for novel compounds. The potential advantages of such innovations are substantial and include the reduction of petroleum use, CO₂ emissions, and pressure on natural resources. Bioplastics and biofuels have the potential to be biodegradable, close to carbon neutral, and thus truly sustainable on a long-term basis. Furthermore, transgenics offers great potential for improving traditional biopolymers from woody plants, such as wood, rubber, and oils. Numerous research efforts have recently been initiated to fulfill the promise of engineering of transgenes in woody plants, but no new products or systems have yet reached commercial feasibility. This review will cover these developments with respect to biopolymers in transgenic woody plants. We will not cover improvements specifically in nonwoody plants, even though some of those concepts (e.g., pharmaceuticals, proteins, vitamins) are certainly applicable to woody plants. Nor will we cover biopolymers that have been produced only in nonwoody transgenic plants (e.g., cyanophycin; [1]). Research into transgenic woody plants is very much an emerging field. Since actual applications have yet to appear, we will also discuss likely scenarios for future development as well as impediments towards their fulfillment.

4.2 The Advantages of Woody Plants

Woody plants offer many advantages over nonwoody plants with regard to the production of compounds for human use. Perhaps the most compelling of these advantages is simply the issue of scale. Most plant-made industrial products would be needed in large amounts, and trees have sufficient biomass to provide larger yields. For instance, poplar (*Populus* spp.) and eucalyptus (*Eucalyptus* spp.) can accumulate biomass at rates over 25 Mg ha⁻¹ yr⁻¹, which is roughly twice the biomass yield expected for switchgrass (*Panicum virgatum*) [2, 3]. Even if the target compounds are present at low concentrations, the recoverable yields per hectare could still be substantial, and the residual biomass could serve a beneficial unrelated use. Indeed, one of the most compelling arguments for engineering these products in woody plants is that the compound would be a secondary or coproduct, with the bulk of the plant serving as biofuel or a more traditional product such as timber or fiber for paper. Metabolic pathways for synthesis of phenylpropanoids (e.g., lignin), terpenoids, and cellulose are well characterized and generally highly active in woody plants, thus providing opportunities for production of many novel compounds.

Poplar (*Populus* spp.) is by far the leading model for genetic studies in woody plants. The moniker of poplar as "Arabidopsis for forestry" is a valid reflection of its dominance in the field [4]. Poplar shares many of the advantages of *Arabidopsis*

including ease of *Agrobacterium*-based transformation, reliable regeneration from tissue culture, availability of the complete genome physical map and sequence (<http://www.upsc.se/Technology/Populus-Genome-Programme/index.html>), a large EST database, cDNA microarrays, and corresponding expression analyses that can be accessed on the World Wide Web (<http://popgenie.org/tool/efp-browser>). Furthermore, substantial genetic resources have been developed over many years in the form of hybrids consisting of interspecific crosses and backcrosses. These hybrids complement the natural genetic and ecological diversity of poplar that arises from the large number of species (~35) that are widely distributed throughout a range of habitats in the northern hemisphere [5, 6].

Unlike *Arabidopsis*, poplar allows for genetic studies related to secondary growth (wood), mycorrhizal associations, and seasonality (dormancy). Poplars are among the fastest growing of any tree species, and silvicultural practices are well developed. Poplars have substantial commercial value, and this has led to a corresponding influx of resources towards its further development as a crop.

Other tree species besides poplar have proven tractable with respect to transgenic technology. Some of these species are important conventional providers of fiber and timber such as eucalypts (*Eucalyptus*), ash (*Fraxinus*), birch (*Betula*), black locust (*Robinia*), chestnut (*Castanea*), conifers (especially *Pinus*), elm (*Ulmus*), European oaks (*Quercus* spp.), sweetgum (*Liquidambar*), yellow poplar (*Liriodendron*), and walnut (*Juglans*) [7–9]. Techniques have also been developed to genetically transform other tree species with specialized uses, such as latex from the rubber tree (*Hevea*) and oil from *Jatropha* (*Jatropha*; see later sections); however, the transgenic technology for these other species lags behind that of poplar, though genomic resources are rapidly being developed, especially for *Eucalyptus* and *Jatropha* [10–12].

Despite these success stories, not all woody plants are amenable to GE (genetic engineering) technology. For instance, American oaks and alder (*Alnus rubra*) can be transformed with standard *Agrobacterium*-based techniques but are difficult to regenerate into plants [9, 13]. Douglas-fir (*Pseudotsuga*), one of the top timber-producing species in the world, has not been a target for GE technology in part because it is difficult and costly to transform and there is no obvious need for transgenic manipulation. Many other tree species, especially those from the tropics, have simply not been investigated for their capacity for GE technology. Future technological improvements have great promise for extending the range of tree species amenable to GE technology and for the production of unique or enhanced products.

4.3 Bioplastics

Biotechnology offers the potential to develop transgenic plants that produce bioplastics, especially poly(3-hydroxyalkanoates) or PHAs. These are structurally simple natural polymers synthesized by most genera of eubacteria to serve as carbon and energy storage compounds that are osmotically inert and easily reclaimed for

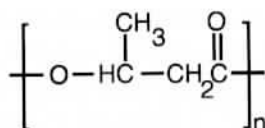


Fig. 4.1 Chemical structure of (PHB) polyhydroxybutyrate. Other PHAs of commercial interest contain longer chains at the methyl position of PHB

metabolism [14, 15]. PHAs are typically synthesized during periods of stress and can make up to 90% of the bacterial cell dry weight. The simplest PHA is polyhydroxybutyrate (PHB, Fig. 4.1). PHAs have the potential to be truly transformative products since they are a biodegradable plastic that is not based on petroleum.

The biosynthesis of PHB involves three enzymes (*phbA*, *phbB*, and *phbC*) that convert acetyl CoA into PHB in a pathway that has been well described in other reviews [16–20]. The production of PHB through bacterial fermentation is routine and already a commercial success. Although no plants produce PHAs naturally, there are at least 12 plant species that have been engineered to do so through the introduction of bacterial transgenes. Private industry is actively pursuing the development of PHB production in various crop plants, especially switchgrass [21]. Other types of PHAs with longer side chains (compared to the methyl group of PHB) are also being developed in transgenic plants [22].

The recent report of PHB production in transgenic poplar is the first example of PHB production in a woody plant (Fig. 4.2) [23]. Yields of up to 2% (w/w leaf dry weight) were reported following application of an ecdysone-based inducing agent. A number of critical issues need to be resolved before such ventures become practical.

4.4 Subcellular Location

Early work with PHB production in *Arabidopsis* established that localization to chloroplasts led to higher yields and lower impact on plant health [24]. The generally accepted explanation for this is that chloroplasts have a high flux of acetyl CoA, which is used for biosynthesis of fatty acids and is also the precursor for biosynthesis of PHB. Consequently, most subsequent work with transgenic PHB-producing plants has incorporated a plastid-targeting sequence into the appropriate genes for PHB biosynthesis (*phbA*, *phbB*, and *phbC*; Fig. 4.3). The presence of PHB granules in chloroplasts could lead to a physical disruption of the internal organization of chloroplasts and a subsequent decrease in photosynthesis. In poplar, the granules nearly fill the entire volume of chloroplasts, suggesting that this might be a barrier to achieving high yields [23]. There has been some interest in targeting the PHB biosynthetic enzymes to peroxisomes. This strategy has proven successful in yeast, *Arabidopsis*, and sugarcane with plant yields up to 1.6–1.8% dry weight [25–27].

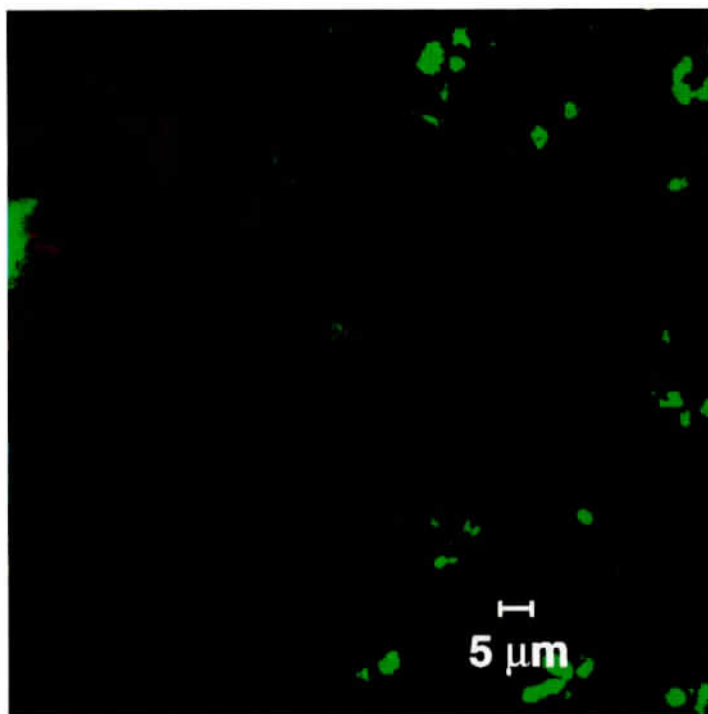


Fig. 4.2 Confocal microscopy showing *red* and *green* fluorescent agglomerations of the bioplastic polyhydroxybutyrate (PHB) in leaf palisade mesophyll cells of transgenic poplar. Separate images were merged such that the *red* color shows a section through a plane that is 8 mm below the *green* section (Photo by Peter Kitin)

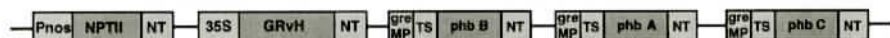


Fig. 4.3 T-DNA region of PHB expression cassette used to transform poplar. *Pnos* promoter for nopaline synthase, *NPTII* kanamycin resistance gene, *NT* terminator for nopaline synthase gene, *35S* promoter for 35 S cauliflower mosaic virus, *GRvH* glucocorticoid response element, *greMP* minimal promoter with glucocorticoid response element binding site, *TS* plastid-targeting sequence, *phb ABC* genes for biosynthesis of PHB

Woody plants offer possibilities for locating PHB biosynthesis outside of chloroplasts or even outside of leaves. For instance, production could be directed to the vascular cambium or ray parenchyma using appropriate promoters such as that for the bark storage protein (BSP) [28]. It might even be possible to incorporate bioplastic production into the cell walls of secondary xylem, perhaps by indirect association of PHB with cell walls from cells in which PHB has been synthesized intracellularly and then released following cell death. This could create unique

materials that could be used directly without complicated extraction or processing. Such concepts are highly speculative at this time, although similar successful modifications have been achieved with fibers of cotton and flax [29, 30].

4.5 The Metabolic Cost (Trade-Off) of PHB Production

A key problem with PHB production in plants is controlling expression in such a manner that the overall growth of the plant is not impacted too heavily. In extreme cases, it has been possible to achieve PHB yields as high as 40% (dry weight) in *Arabidopsis*, but the concomitant diversion of carbon resources to PHB results in severe stunting or other gross defects [31]. Metabolic profiling of PHB-producing plants has indicated substantial decreases in isocitrate and fumarate, suggesting an impairment of the tricarboxylic acid cycle due to diversion of acetyl CoA [31]. Surprisingly, no changes were detected in fatty acids, and increases were observed in proline, mannitol, and several sugars. In terms of growth and overall plant health, the most thorough examination of the metabolic trade-off costs of PHB production in any plant was recently reported for poplar [23]. This study included a chemically inducible promoter based on an analog of ecdysone in order to minimize negative impacts. After induction, leaves contained variable amounts of PHB (between 0 and 2% of leaf dry weight; Fig. 4.4). A greenhouse study indicated that there were no negative consequences of PHB production on growth unless the PHB content exceeded 1%. At PHB levels above 1%, significant decreases were observed in all growth parameters except for wood density (Table 4.1, Fig. 4.5). Chlorophyll fluorescence (Fv/Fm) also declined significantly, indicating that the plants were under stress.

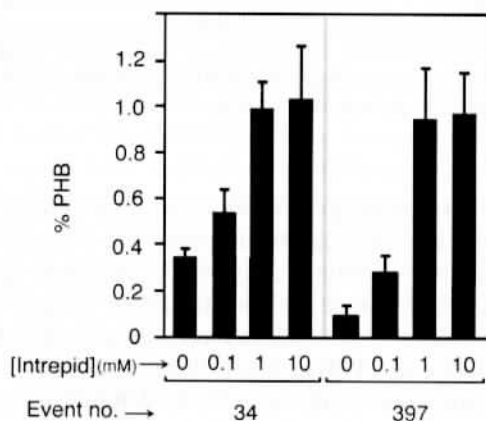


Fig. 4.4 PHB concentrations (% by dry weight) in leaves of transgenic poplar following 6 weeks of biweekly application of the inducing chemical Intrepid at concentrations from 0 to 10 mM (From [23])

Table 4.1 Effects of PHB content on growth parameters in transgenic poplar. Data are based on 144 6-month old plants that received various concentrations (0–10 mM) of the inducing chemical Intrepid. Intrepid alone had no significant effect on plant health (Modified from [23])

| Parameter | Mean for all plants | Mean for plants with <1% PHB | Mean for plants with >1% PHB | % decrease if PHB >1% |
|-----------------------------------|---------------------|------------------------------|------------------------------|-----------------------|
| Height (cm) | 62.51 | 63.84 | 57.38 | 10.1* |
| Diameter (mm) | 6.69 | 6.87 | 6.06 | 11.8* |
| Volume index (mm ³) | 30459 | 32635 | 24147 | 26.0* |
| Leaf mass (g) | 6.44 | 6.71 | 5.35 | 20.3* |
| Stem mass (g) | 4.57 | 4.88 | 3.35 | 31.4* |
| Root mass (g) | 3.07 | 3.29 | 2.16 | 34.2* |
| Shoot (leaf + stem) mass (g) | 9.679 | 10.893 | 8.089 | 25.7* |
| Total mass (g) | 14.11 | 15.22 | 10.42 | 31.5* |
| Chlorophyll fluorescence | 0.733 | 0.736 | 0.713 | 3.1*** |
| Wood density (g/cm ³) | 0.327 | 0.328 | 0.321 | 2.1 |
| PHB % | 0.659 | 0.394 | 1.495 | – |

**P* value < 0.05

***P* value < 0.001

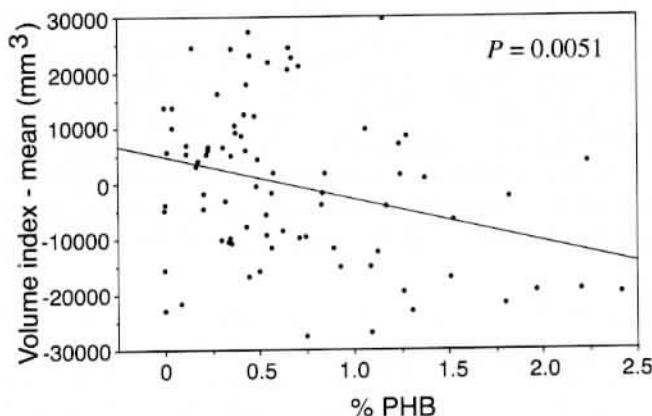


Fig. 4.5 Decreases in volume index (height × diameter squared) as a function of PHB content in leaves of transgenic poplar. Values are for individual plants and are corrected for the means of separate transformation events (From [23])

4.6 Regulation of Expression of the Genes for PHB Production

Most transgenic PHB-producing plants have been based on the common, constitutive 35 S cauliflower mosaic virus (CaMV) or an enhanced version. Some studies have attempted to use genetic constructs incorporating inducible promoters in an attempt to increase PHB yield while minimizing impacts on plant health. The goal is to allow plants to reach maturity before resources are diverted to PHB production.

The first such inducing study was the use of salicylic acid to control expression of *phbA*. In this case, the transformation efficiency was improved, but the yield of PHB was still rather low [32]. Lössl et al. used a transplastomic *phb* operon that was inducible with ethanol, which led to slightly higher yields [33]. The use of the *cab-m5* light-inducible promoter in switchgrass was successful with respectable though highly variable yields of up to 3.7% [21]. The most successful inducible system developed to date was based on conventional nuclear transformation of *Arabidopsis* and a commercially available nonsteroidal ecdysone analog [34]. PHB yields of up to 14% were observed. When used in transgenic poplar, this same inducible system allowed for yields of up to ~2%, although plant health was impaired at levels above 1% as described above [23]. The advantages of this ecdysone-analog-based system include the availability of commercial inducing chemicals such as Intrepid® and Mimec® that are already licensed for field use, the absence of negative effects on nontarget organisms (including the plant to which it is applied), efficient transport throughout the plant, and full induction at low concentrations (1 mM for poplar). The study of inducible promoters in plants is an emerging field, and the development of improved systems holds considerable promise (reviewed by [35]).

Most recently, a transplastomic approach has been used in tobacco to include a polycistronic synthetic operon that was driven by the native *psbA* promoter (the *psbA* gene codes for the DI subunit of photosystem II [36]). The transgenes for PHB biosynthesis were thus inserted into the plastome as an extension of the *psbA* operon in such a manner that no foreign promoter was required. This system had the double advantage of providing some of the highest yields (18.8%) of PHB yet observed as well as a high level of gene containment due to the maternal inheritance of plastid DNA. This development is highly encouraging and may in fact be the breakthrough that leads to a viable commercial system, especially considering that the goal for such feasibility has been set at a PHB yield of >7.5% by Metabolix [21].

4.7 Extraction and Recovery

Historically, most of the research and commercial efforts for PHB production and extraction have been focused on the microbial fermentation, which generally yields PHB concentrations much higher (up to 90% of cell dry weight) than those obtained in transgenic plants [37, 38]. Several extraction methods for PHA recovery have been developed. These typically involve centrifugation, filtration, extraction with organic solvents chloroform and methanol, bleaching with sodium hypochlorite, and digestion with enzymes [37–40]. These methods are suitable for extraction of PHB in bacterial cells; they are not suitable for plant biomass. In particular, chlorophyll in the plant biomass interferes with the organic solvent extraction; sodium hypochlorite and enzymatic digestion are not severe enough to degrade the recalcitrant plant cell walls. Recently, a modified solvent sequential extraction method was used to extract the PHB from genetically modified hybrid poplar leaves [41]. There is a need for additional research into extraction of PHB, especially from the higher

plants, as none of the current extraction processes meet all the requirements of economics, safety, and scalability.

Primary challenges in economically viable commercial production of microbial PHB are in the high feedstock and the extraction costs. Presently many companies produce PHA (primarily PHB) via the fermentation route in the price range of \$2.15–\$5.31/kg, and current world production capacity of PHA is estimated to be 100,000 tons/year [42]. While the technical feasibility of PHB production and extraction from higher plants has been demonstrated, there is little information on the techno-economic feasibility of PHB extraction from higher plants. However, based on preliminary techno-economic analysis, it is known that the PHB content of the plant biomass is a significant factor in the commercial feasibility of PHB production from plants [41].

Life cycle assessments (LCA) have demonstrated the advantages of microbially produced PHAs over petrochemical plastics [18, 43–45]. While one of the advantages attributed to production of PHB in higher plants is the direct conversion of sunlight and CO₂ into biodegradable plastics, relatively few LCA studies exist for the production of PHA from higher plants. In one such study, Kurdiker et al. demonstrated that life cycle reductions in greenhouse gas profiles of plant-derived PHA are better than those of polyethylene produced from fossil resources [46]. However, the LCA studies for higher plants need to be performed in conjunction with techno-economic feasibility of the PHA production in higher plants. Given the present state of research and development, as well as the significant technical challenges to be addressed, it may be reasonable to expect large-scale commercial production of PHA from plants in about 10 years.

4.8 Biopolymers in Wood

Wood is the most prominent plant-produced biopolymer and has long been the target of scientific improvements based on conventional genetic breeding and selection. Efforts based on transgenics have focused so far primarily on reducing the content of lignin so as to facilitate the production of paper. Lignin biosynthesis proceeds via the shikimate and phenylpropanoid pathways and is thus complex with a large range of potential targets for genetic manipulation. Furthermore, many essential compounds besides lignin are produced in these pathways, so any metabolic tinkering has the potential to introduce unintended disruptions. One early example involved the antisense inhibition of the lignin biosynthetic pathway gene *Pt4CL1* encoding 4-coumarate:coenzyme A ligase (4CL) in aspen (*Populus tremuloides*) [47]. This resulted in up to a 45% reduction in lignin and a surprising 15% increase in cellulose content. Leaf, root, and stem growth were all enhanced. Co-downregulation of *4CL* and *CAld5H* (another gene in required for lignin biosynthesis) resulted in an additive effect with even further reductions in lignin and increases in cellulose [48]. Additional successes in reducing lignin content and/or increasing the potential efficiency of pulping have involved transgenes that alter the

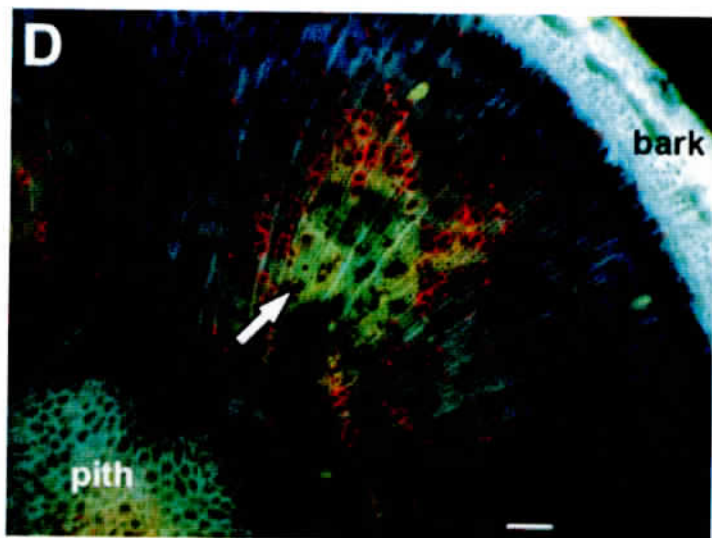


Fig. 4.6 Cross section of low-lignin, transgenic poplar stem visualized by cryofluorescence. Conducting vessels are stained red. The *arrow* indicates nonconductive tissue that is a result of phenolic deposition within a low-lignin zone. *Bar* = 200 μ m (From [51])

relative ratios of the components of lignin. These studies have been at least partially successful and have recently been reviewed by Li et al. [49]. It is also possible to downregulate lignin production in poplar by transforming with the transcription factor EgMYB1 from *Eucalyptus* [50].

Further studies involving downregulation of *4CL* in transgenic poplar have revealed cautionary flaws in the general strategy. The decrease in lignin impairs wood structure and strength, xylem conductivity, growth efficiency, saccharification potential, and survival [51–53]. Adequate lignification is required for mechanical support of the stem, water transport, and general health of the tree (Fig. 4.6), so it appears that the goal of reduced lignin content has unintended, possibly unavoidable, negative consequences. Reports of the apparent lack of negative stem effects in reduced-lignin, nonwoody plants (specifically *Arabidopsis*) appear to be of limited relevance to woody plants [54]. An alternative approach to modifying woody cell walls for enhanced properties involves engineering of pathways for synthesis of hemicelluloses or pectins [55, 56]. These studies are still in the very early stages but offer considerable promise.

4.9 Latex

Rubber ranks behind only wood in terms of plant industrial products with commercial value. Rubber tree (*Hevea brasiliensis*) is readily transformed with *Agrobacterium*-based techniques [57], but there are no reports of transgenic

modifications in which the products are modified. *Hevea* does have potential in this regard, as the pathway by which latex is produced (the isoprene or terpenoid pathway) naturally leads to hundreds of valuable compounds such as essential oils, carotenoids, sterols, phytohormones, miscellaneous pharmaceuticals (e.g., artemisinin and taxol), and many others. Since latex from *Hevea* naturally contains proteins such as heveins that are dangerously allergenic to some humans, it might prove feasible to eliminate these problematic by-products using transgenics. A hevein promoter that restricts gene expression to the latex-producing cells (laticifers) has recently been used successfully to allow for expression of a GUS reporter in *Hevea* [58].

There is currently considerable interest in the USA in developing latex-producing crops that can be grown in temperate regions. Two plant species from the Asteraceae are the focus of these efforts: guayule (*Parthenium argentatum*) and Russian dandelion (*Taraxacum kok-saghyz*) [59–61]. Guayule, a shrub that is native to the southwestern USA and northern Mexico, was used successfully during World War II to provide a domestic source of rubber, but the practice was abandoned at the conclusion of the war. The revival of guayule is being led by various companies such as Yulex (Maricopa, AZ) and Mendel Biotechnology (Hayward, CA), the Agricultural Research Service of the US Department of Agriculture, and consortia such as PENRA (<http://oardc.osu.edu/penra/>) and EU-PEARLS (<http://www.eu-pearls.eu/UK/>). Conventional breeding and selection of guayule has been used to improve rubber yield by as much as 250% [61]. Guayule can be transformed with *Agrobacterium* [62], but attempts to improve the yield or quality by introducing genes for allylic diphosphate synthases led to inconsistent results [63]. A further advantage of latex from guayule is that it lacks the allergens that are problematic with latex from *Hevea*.

Russian dandelion is a perennial plant that is native to Uzbekistan and Kazakhstan. The history of Russian dandelion parallels that of guayule in that it was cultivated extensively and successively in the Soviet Union (and, to a lesser extent, in the USA) during World War II as an emergency source of high-quality rubber, but the practice was mostly abandoned after the war. As with guayule, Russian dandelion is readily amenable to transformation with *Agrobacterium*. In a recent attempt to improve yields and quality, RNAi gene silencing of a gene for polyphenol oxidase led to decreased coagulation of latex and a 4–5-fold increase in latex expulsion, but it is uncertain if such an approach would be useful in other species [64].

Future improvements through transgenics of latex-bearing plants will likely require the development of molecular tools and genomic resources that are currently not available. Such progress may be especially critical for *Hevea*, which has an extremely low genetic diversity and is threatened by a fungal pathogen (South American leaf blight, *Microcyclus ulei*) that is endemic to the Amazon basin. Despite repeated attempts, breeders have not been able to develop blight-resistant lines of *Hevea* using conventional methods [59, 65]. Due to this fungal pathogen, it is no longer possible to grow *Hevea* on a plantation scale in Central or South America, even in the Amazon basin where *Hevea* is native. World rubber supplies would be decimated if the fungus were to become established in Southeast Asia where 80% of global rubber production occurs. Transgenics might provide a means

for saving *Hevea* from future epidemics and perhaps even allow for its reintroduction into South America, a scenario that would have huge social and economic benefits for that region.

Finally, we note that the synthesis of latex through the mevalonate pathway begins with the same precursor (acetyl CoA) as does the synthesis of PHB. Latex-producing plants have a naturally high flux of acetyl CoA and a proven ability to provide products on a long-term nondestructive basis. Would these plants make good candidates for genetic engineering of bioplastic production? Specifically, could the expression of *phb* transgenes be controlled by the laticifer-specific hevein promoters mentioned above? These questions are, to our knowledge, beyond the realm of current research and will likely remain unanswered in the near term. Major resources would be required, and these are unlikely to be committed unless other GE tree programs currently in development prove successful and provide compelling incentives.

4.10 Oils for Biofuels

Woody plants also have great potential to provide oils suitable for use as biodiesel (reviewed by [12, 66]). Despite the recent, huge influx of resources into biofuels, little attention has yet been paid to genetic engineering of woody crops for oil production. Gressel has argued that transgenics are imperative for the development of these crops [12]. Most current crops are poorly suited for this purpose in part because they have been domesticated for millennia for another goal—food production. At present, biodiesel is produced from oil from palm, soybean, and rape, but several other underexploited oilseed-bearing shrubs offer potential for future development. These shrubs include most notably *Jatropha* (*Jatropha curcas*), but also castor bean (*Ricinus communis*), Jojoba (*Simmondsia chinensis*), *Pongamia pinnata*, and *Calophyllum inophyllum* [12].

Of these potential new oil crops, *Jatropha*, a nonfood shrub that requires low agricultural input, has recently risen to the center of attention. Seeds of *Jatropha* contain 30–40% oil, and the resultant biodiesel has properties comparable to petroleum-derived diesel [67]. This plant, which is native to tropical America, is cultivated in tropical and subtropical regions around the world. Numerous countries, most prominently India, are pushing hard for its development as a means of energy independence and income for impoverished, rural populations. Several NGOs and private companies are vigorously promoting *Jatropha* as a source of biofuels that are considerably cheaper than petroleum-based fuels. The resources being applied are substantial. For example, the Syngenta Foundation is developing a germplasm bank and conducting field trials (<http://www.syngentafoundation.org/index.cfm?pageID=554>), and Synthetic Genomics Inc., in collaboration with the Asiatic Centre for Genome Technology, recently completed the full genome sequence for *Jatropha* (<http://www.syntheticgenomics.com/media/press/52009.html>). Similar efforts are also being directed to develop genetic resources for palm (*Elaeis guineensis*) oil [12].

The situation as outlined above seems highly encouraging, but a deeper examination reveals some serious impediments to the successful development of oilseed crops such as *Jatropha*. These hindrances are often overlooked in the glow of the eco-friendly appeal of biodiesel. The most pressing restraint has to do with the extreme toxicity of *Jatropha* [12]. The oil (commonly called "hell oil") and the seed proteins are extremely poisonous to humans, either by contact, ingestion, or inhalation. Seeds contain toxic alkaloids and, more importantly, a type of toxalbumin (curcin) that is similar in structure and effect to ricin—the notorious deadly protein from castor bean that has long been feared for its potential as a bioweapon. Furthermore, the oil contains phorbol esters (diterpenoids) that are a strong irritant and a potential carcinogen [68].

Existing cultivars vary widely with respect to content of toxins [69]. Conventional breeding and selection has resulted in some varieties with reduced toxicity but progress has been slow. Transgenics may be the best option to accelerate the development of novel crops such as *Jatropha*. Techniques for transformation of *Jatropha* based on *Agrobacterium* and biolistics have recently been developed [70, 71]. These techniques could be used to eliminate toxins, increase yield, and design crops with traits better suited to standard agricultural practices. Vega-Sánchez and Ronald have suggested a number of specific genes for enzymes and transcription factors that could be targeted to improve either the quality or quantity of plant oils, though none of these have yet been used in transgenic studies of a woody species [66]. Yields in *Arabidopsis* and rape have been increased up to 40% with such strategies.

4.11 Regulatory and Market Obstacles to Developmental Research and Commercial Applications

Transgenic trees modified for changes in feedstock quality or engineered to produce new industrial products face substantial regulatory and market hurdles. We have recently described in detail the applicable laws and derived regulations that pertain to trees and other perennial crops in the USA [72, 73] and the importance of field trials for study of transgenic traits [74], and earlier described the restrictions to research and commercial use of transgenic trees in forestry programs that are "green certified" by the Forest Stewardship Certification (FSC) system in the USA and elsewhere in the world [75, 76]. In brief, transgenic modifications to wood quality, such as modifications to lignin, will in most cases have significant impacts on tree physiology, adaptation, and productivity that are often invisible, or much less expressed, in greenhouse or laboratory environments. For example, Voelker et al., in a recent series of papers [52, 53, 77], showed how the much heralded fast-growth, low-lignin trees described a decade earlier [47, 48] completely failed to display improved growth or desirable changes to wood characteristics in a field environment. Thus, it is essential that new kinds of transgenic genotypes are field tested very early in their development. As discussed above, it is well known that PHB, when expressed to high levels, has deleterious effects on plant health and

biomass yields. Thus, field trials are required to establish whether PHB and other biopolymers can be produced to commercial levels without excessive impact on general plant health.

Unfortunately, field trials are intensively regulated in the USA and most other countries in the world, making them costly and risky to undertake [78]. As a result, very few academic research laboratories and companies are engaged in transgenic field research. Any modifications to wood quality, even when using native genes (often called cisgenes [79]), are regulated as though they are hazards to the environment. Thus, serious federal penalties would be imposed for the same small releases to the environment as commonly occur during conventional tree-breeding research. Transgenic trees that produce bioindustrial compounds such as PHB are regulated to an even higher level of scrutiny, requiring separate harvesting equipment, special treatment of biomass, multiple inspections per year, and other costly management procedures [78]. Because companies that are FSC certified are not allowed to plant any transgenic trees, even for short-term, contained research trials [76], poplar-growing companies such as GreenWood Resources and Potlatch in the USA, who once collaborated extensively with transgenic biotechnology researchers, can no longer host any transgenic research on their lands. Thus, all the costs of field trials must be borne by public sector researchers, who often do not have the land, funds, or means to do so. In addition, even if the technology was successful in providing economic and environmental benefits, such companies could not grow or market products from the trees commercially without losing their certification. This constellation of obstacles provides a strong disincentive to companies and to public sector granting agencies, to invest in research and development.

Despite these impediments, some field trials with GM trees have been accomplished with an encouraging track record of safety. A recent survey of publicly accessible databases worldwide tallied >700 field trials with GM trees with not a single incidence of harm to biodiversity, human health, or the environment [80].

4.12 Prospects

The only transgenic trees for any products currently in commercial use in the world are transgenic papaya in Hawaii and Bt poplar in China [80]. GE papayas are a special case because there was strong support from growers and almost no risk of gene flow because of the cleistogamous flowers (i.e., little to no pollen released) and lack of wild relatives [81]. Without GE technology, papayas would have disappeared as a viable commercial crop in Hawaii due to an uncontrollable epidemic of ringspot virus. Conversely, Bt poplars have benefited from the much more lenient regulatory policies in China where over one million Bt poplars have been planted in the field [80, 82]. Many other transgenic tree projects are currently under development in China and will likely see field applications within the next decade. Thus, it seems likely that China may provide the testing grounds that ultimately establish the

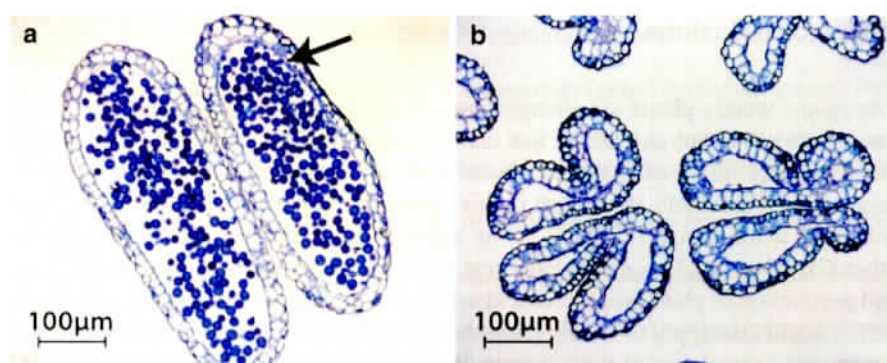


Fig. 4.7 Transverse sections of nearly mature anthers showing pollen grains (*arrow*) in nontransgenic control (**a**) and lack of pollen grains in male sterile anthers (**b**) of field-grown poplar. Sterility was produced by action of an RNase (*barnase*) whose expression was driven by a tapetal-specific promoter. Interruption of tapetal function prevented formation of normal pollen grains (From [83])

balance of safety and risks, as well as the economic incentives, for transgenic trees upon which western countries could ultimately base future policies.

The most pressing issue of true environmental concern regarding transgenic trees is the spread of transgenes into native populations, an issue particularly relevant to poplar with its many native species and long-distance pollen and seed movement. Thus, it is unlikely that any flowering transgenic poplars will be allowed for field use in the USA until absolute sterility can be assured, or there is substantive evidence from many years of careful study of an absence of significant environmental harms. For biofuels, coppice systems where harvest precedes flowering would obviate this issue. At least five strategies are currently being explored towards sterility technologies for trees grown through flowering (reviewed by [83]). Some of these techniques have been at least partly successful (Fig. 4.7), but not absolutely infallible. Consequently, they may fall short of meeting the “precautionary principle” (PP) in the judgment of some. The PP is interpreted by some environmental groups as the guiding rule that no policy or action should be undertaken unless the scientific proof is completely convincing that there is zero risk of harm to the public or environment from any action or policy. However, for many transgenes, the benefits may outweigh risks, such as for biofuel applications, and these risks may be lower than for use of exotic poplars, as are commonly used with no gene flow restriction. A case in point is the familiar Lombardy poplar, a bud sport mutant of *Populus nigra* that originated in Italy and has been widely planted in the USA for over two centuries with no adverse consequences. Thus, in addition to the market and regulatory factors discussed above, political and legal fights are likely to play a significant role in commercial prospects for transgenic trees—as they have for recently commercialized transgenic crops.

4.13 Conclusions

Transgenic woody plants are emerging as a potential source of numerous biopolymers, both novel and traditional, that are of great economic value to humans. Their development could greatly advance broad goals towards sustainability and independence from fossil fuels. Prominent in this regard are bioplastics, fuel oils, latex, and enhanced wood products. Many of the applications that have been proposed for other GE crops, such as enhanced stress tolerance, improved nutritional qualities, and production of pharmaceuticals and proteins (e.g., vaccines and spider silk polymers), could also apply to woody plants with the added advantage that the perennial nature and general size of trees suggest larger yields as well as substantial secondary uses, especially as biofuels. Technological and policy-based restrictions have slowed their development but are probably surmountable as long as policies are guided by sound science and not political rhetoric.

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