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27 Paulownia

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27.1 INTRODUCTION

Rising energy prices and environmental problems have led to increased interest in alcohol as a
fuel. Using corn, a human food resource, for ethanol production raises major ethical and moral
issues. Today, malnourished people in the world number approximately 3 billion (WHO 2000). The
current food shortages throughout the world call attention to the importance of using U.S. exports
of corn and other grains for human food and search for alternative sources for biofuel production.
For the production of cellulosic ethanol, residue including postharvest corn plants (stover) and
timber residues could be used. There is a growing awareness among farmers to establish and use
specialized high-biomass “energy crops” such as domesticated poplar trees, switchgrass, bamboo,
and Paulownia. The United States uses approximately 140 billion gal of gasoline per year, and there is a growing urgency to replace it with biological resources or biomass (US DOE 2006).

Biomass is a complex mixture of organic materials (such as carbohydrates, fats, and proteins) along with small amounts of minerals (such as sodium, phosphorus, calcium, and iron). The main components of plant biomass are carbohydrates (~75%, dry weight) and lignin (~25%) which can vary with plant type. The carbohydrates are mainly cellulose or hemicellulose fibers which impart strength to the plant structure, and lignin which holds the fibers together. A major advantage of using biomass as a source of fuels or chemicals is its renewability. The net annual production of biomass by photosynthesis has been estimated to be 10 times that of our current annual consumption of fossil fuels (Miyamoto 1997).

It is easier to produce ethanol from sugar feedstocks (e.g., sugarcane, sugarbeets, sweet sorghum, fruits, and other materials known as saccharides) or starchy feedstocks (e.g., corn, cereal grains, sweet potatoes or cassava). However, the cost of production is prohibitive because both of these groups are in the human food chain. For the production of cellulosic ethanol, residue including postharvest corn plants (stover) and timber residues could be used. Therefore, the scientific community is engaged in exploring other systems that are more efficient in breaking down the cellular structures for cellulosic ethanol production.

Paulownia species are highly suitable to revalidate agricultural set-aside areas, to reclaim mining areas, or to restore contaminated sites where major emphasis is on biomass production for chemical or thermophysical processing. Because of the low cost, plentiful supply, and amenability to biotechnology, carbohydrates appear likely to be the dominant source of feedstock for biocommodity processing. In the case of starch, the advantage of enzymatic compared with chemical hydrolysis has already been realized. In the case of cellulose, this has not yet been realized. Cellulose hydrolyzing enzymes can only act effectively after pretreatment to break up the very stable lignin, cellulose, or hemicellulose composites. These treatments are still mostly thermal, thermomechanical, or thermochemical and require a considerable input of energy.

With a deep root system that is fed by underground water at a level below 2 m, Paulownia does not compete with the roots of other crops. Intercropping with Paulownia improves microclimate by reducing the effects of drying winds by 20–50% on average and increasing air moisture. This can considerably increase the yield of some crops, such as ginger, winter wheat, and millet (Zhu et al. 1986). The tree benefits by recovering excess fertilizer that runs deep into the ground and the crops benefit from the nutrients put into the topsoil by fallen leaves. Further, large green leaves are rich in nitrogen and can be used for fodder and green manure. Therefore, the leaves are rich in protein (16.2%), carbohydrates (9.44%), and minerals (Song 1988), making them ideal for animal fodder and green fertilizer (a 10-year-old tree produces 80 kg of dry leaves/year). The fast growth rate of Paulownia may be capitalized upon for agroforestry (Wang and Shogren 1992; Jiang et al. 1994), biomass production (Song 1988), land reclamation (Carpenter 1977), and animal waste remediation systems (Bergmann et al. 1997). Sufficient variation among P. elongata clones was revealed for growth parameters and foliar nutrient concentrations to anticipate a benefit from the selection of genotypes that are the most efficient for the remediation of animal waste. The data show that P. elongata has potential for use as a swine waste utilization species (Bergmann et al. 1997). This particularly suits the southeastern region of the United States where there is high a concentration of swine and poultry industry. Swine industry effluents and chicken litter are available at comparatively lower prices, replacing the need for synthetic fertilizers.

### 27.2 HISTORY OF PAULOWNIA IN THE ORIENT AND THE UNITED STATES

Paulownia is known as kiri in Japan, specifically referring to P. tomentosa. The name kiri comes from the word kiru (to cut) because it was believed that the tree would grow better and more quickly when it was cut down regularly. It was once customary in Japan to plant a Paulownia tree when a baby girl was born and then make it into a dresser as a wedding present when she gets married.
According to Chinese legend, the mythical phoenix alights only in the branches of the Paulownia tree when it lands on earth (http://www.onmarkproductions.com/html/ho-oo-phoenix.shtml).

China has historically been the largest grower of Paulownia. Chinese people use its wood for making furniture, house construction, toys, plywood, musical instruments, and for packaging. For an ideal multipurpose tree, the Paulownia intercropping models have been applied and extended to 15 million ha in rural areas of the central plains of north China. The total number of Paulownia trees growing in China is approximately 1 billion, including those grown as the shelterbelts at the canal banks and road sides or for ornamental forests around houses and villages. For decades, Japanese craftsmen have used this revered wood in ceremonial furniture, musical instruments, decorative moldings, laminated structural beams, and shipping containers. The tree made its way to the United States in the mid-1800s. Paulownia seeds were used as packaging material for delicate porcelain dishes on their journey across the Pacific. Once unpacked, the tiny wind-blown seeds became naturalized throughout the eastern states.

Paulownia also has a unique biological character (i.e., a root system that grows deep and a crown that develops in loose structure) that makes it suitable for intercropping or in a mixed planting with other shade-enduring trees. The traditional monocropping models in many of these countries have been replaced by Paulownia-crop intercropping fields which have resulted in more reasonable use of sunlight, heat, water, and air resources so that the farmland productivity and the product diversity are raised. The adoption of Paulownia-crop intercropping is also a good solution to the land competition between the development of agriculture, forestry, and animal husbandry (Zhu et al. 1986).

### 27.3 BOTANY AND DISTRIBUTION

*Paulownia* species are commonly known as Empress tree, *Kiri* tree, Dragon tree, Royal Paulownia, Princess tree, and many other derivatives. *Paulownia* is a genus of between 6 and 17 species (depending on taxonomic authorities) of plants in the monogeneric family Paulowniaceae, related to and sometimes included in the family Scrophulariaceae. The Latin name *Paulownia* was given by the Swiss botanist Thunberg, and the taxonomic details were published in the “Japanese Flora” in 1781 (Zhao-Hua et al. 1985). They are native to much of China, southern to northern Laos and Vietnam, and have long been cultivated elsewhere in eastern Asia, notably in Japan and Korea. They are deciduous trees 40–50 ft tall, with large leaves 15–40 cm across arranged in opposite phyllotaxy on the stem. Surface studies of structures present on *P. tomentosa* suggest that there are three types of structures that protect young plant parts and/or reproductive organs from herbivores (Kobayashi et al. 2008). These structures have been named as bowl-shaped organs, glandular hairs, and dendritic trichomes. Glandular hairs on leaves, stems, and flowers secrete mucilage containing glycerides and flavonoids and trap small insects. These chemicals seem to play a major role in its antiherbivore property. The flowers are produced in early spring on panicles 10–30 cm long, with a tubular purple corolla resembling a foxglove flower. The fruit is a dry capsule containing thousands of minute winged seeds. The main species in the genus *Paulownia* are *P. tomentosa*, *P. fortunei*, *P. elongata*, *P. albiphloea*, *P. catalpifolia*, *P. australis*, *P. kawakamii*, and *P. fargesii* (Zhu et al. 1986). Molecular marker-based studies including random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) of chloroplast DNA have been used to establish the hybrid origin of *Paulownia taiwaniana* (Wang et al. 1994). This study provided molecular evidence suggesting that *P. taiwaniana* is the natural hybrid between *P. fortunei* and *P. kawakamii* and that the maternal parent is *P. kawakamii*.

*Paulownia* species grow on flat or mountainous land, in various types of soil including rich humus soil in temperate areas, dry poor soil, rich forest soil, and light clay soil in the subtropics, laterite soil in the tropics, and dry steppes (semiarid grass covered plains in southeast Europe, Siberia, central Asia, and Central America). It also adapts to various climates, from warm and temperate to tropical, and can even withstand temperatures as low as –20°C. Paulownia can survive between

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latitudes of 40° north and 40° south and at altitudes of up to 2000 m (El-Showk and El-Showk 2003). *P. elongata* grows faster and is suitable for intercropping with arable crops, whereas *P. catalpifolia* and *P. tomentosa* grow slower but have better wood quality. The annual mean temperature in the Paulownia growing areas varies a great deal, ranging from 10 to 22°C. The optimal temperature range for Paulownia growth is 24–29°C. The lowest temperature that Paulownia can resist is –20°C for *P. tomentosa*, –16°C for *P. elongata*, and –13°C for *P. fortunei*, suggesting that some of these fast-growing species can be grown in large, unused tracts of land in the United States (Zhu et al. 1986).

Geographical areas suitable for *P. elongata* cultivation are shown in the Figure 27.1a. Paulownia can adapt to a wide range of precipitation ranging from 500 to 2500 mm. The suitable edaphic conditions for Paulownia growth are fertile sandy loam to heavy loam soils with loose structure and salt content less than 0.05%. *Paulownia* species respond very well to fertilizer application but have little tolerance to water-logging. In a comparative study to assess nutrient requirements and stress response of *Populus simonii* and *P. tomentosa*, it was found that the maximum weight increase was 19% and over 25% per day, respectively. Further, the nitrogen retention in the above-ground parts of *P. tomentosa* was very high in comparison to *P. simonii* [0.26 g dry weight (gN)⁻¹ h⁻¹ vs. 0.16 g dry weight (gN)⁻¹ h⁻¹], establishing *Paulownia* sp. as one of the fastest growing tree species for biomass production (Hui-Jun and Ingestad 1984).

The leaf, flower, fruit, and bark of Paulownia have been extensively used in Chinese medicine to treat bronchitis, especially on relieving the cough and reducing phlegm, tonsillitis, and dysentery (Jiang et al. 2004). Further, chemical analysis of flowers revealed high quantity of bioactive flavonoid apigenin that has been found to show various pharmacological activities, including anti-inflammatory, antispasmodic, antiarrheal, vasorelaxant, and an antibacterial activities (Jiang et al., 2004). Antiviral furanoquinone and antimicrobial phenylpropanoid glycosides have been isolated from *P. tomentosa* (Kang et al. 1994, 1999).

Except for *P. tomentosa* (Miller 2004), most *Paulownia* species grown in the United States are noninvasive. Although there is little doubt that it is an exotic genus, the question of its invasiveness is open to conjecture. The prolific small seeds of Paulownia are windblown. However, the seeds do not germinate and survive unless they fall on soils with low pathogen load. Young Paulownia seedlings have a high rate of mortality because of damping-off disease caused by various soil fungi. Generally, Paulownia does not colonize in open areas. Requiring full sunlight for continued development, it is often overtopped by other species and succumbs. Paulownia is usually found on the edge of a forest where sunlight is more available rather than in the interior forest. Because of the strict sunlight and soil requirements, the number of Paulownia plants appears to have declined in the recent years. Seed dispersed from Paulownia plantings does not appear to establish and colonize outside of Paulownia plantations.

### 27.3.1 Reproduction and Early Growth

#### 27.3.1.1 Flowering and Fruiting

The perfect flowers of Paulownia are borne in terminal panicles up to 25 cm (10 in) long in April and May. Their violet, lavender, or blue appearance before the leaves emerge is quite striking (Figure 27.1e). The fruits are ovoid, pointed, woody capsules approximately 30–45 mm long. Immature green fruits from the current year and mature, dehisced, brown to black capsules from the previous year can be spotted on a tree at the same time (Figure 27.1g, h). Capsules turn brown as they mature in September or October and persist on the tree through the winter (Bonner and Burton 1974).

#### 27.3.1.2 Seed Production and Dissemination

Numerous seeds are borne in fruits called “capsules.” Each capsule contains up to 2000 seeds, and a large tree may produce as many as 20 million seeds a year. The tiny, flat, winged seeds (Figure 27.1i)
FIGURE 27.1 (See color insert) Various aspects of *P. elongata* biology. (a) Area falling under green line in the U.S. map is suitable for commercial cultivation of *P. elongate*. (b) A 4-year-old, well-managed plantation of *P. elongata* near Lenox, GA. (c) A 20-week-old *P. elongata* tree. (d) Many new shoots sprout from a coppiced tree. (e) Vigorously growing shoots photographed 1 year after coppicing. (f) *P. elongata* in bloom during early spring. Note that there are no leaves at this time of the year. (g) Immature green fruits. (h) Mature and dehisced fruits (capsules). (i) Winged seed. A cluster of seeds attached to the placenta is shown in the inset. (j) Seed germination on tissue culture medium. (k) Six-week-old planting of *P. elongata* at Fort Valley State University farm in Georgia.
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are light (1,70,000 seeds/oz). As the capsules break open on the trees throughout the winter and into the spring, wind dissemination occurs easily (Bonner and Burton 1974). Seed surface architecture and RAPD markers have been employed to profile and differentiate \( P. \) *fortunei*, \( P. \) *tomentosa*, and their hybrid. The surface patterns of winged seeds were examined by scanning electron microscopy. The patterns of reticulation on the wings and seed coat of \( P. \) *fortunei* and the hybrid were found to be comparable while that on \( P. \) *tomentosa* was different (Kumar et al. 1999).

27.3.1.3 Seedling Development

The seeds of the genus Paulownia germinate quickly and grow rapidly when conditions are favorable (Figure 27.1j). Seed germination is epigeal. Laboratory studies have found that light is required for germination (Bonner and Burton 1974). Cold storage (stratification) reduces the light requirement (Tang et al. 1980). \( P. \) *tomentosa* needs bare soil, sufficient moisture, and direct sunlight for good seedling establishment. Seedlings are very intolerant to shade.

27.3.1.4 Vegetative Reproduction

Paulownia roots sprout easily. In fact, lateral root cuttings of 1-year-old seedlings can be used for propagation directly in the field (Tang et al. 1980).

27.3.2 Plant Production

27.3.2.1 Root Cuttings

The propagation of Paulownia is simple and easy; for example, the root, stem, shoot or seed can all be used for propagation with facile methods. Current practice for large-scale production of Paulownia planting stocks is to use the roots of 1- to 2-year old Paulownia seedlings for rooted cutting development. With the combination of advanced propagation techniques and the intensive management in the nursery, a 1-year-old Paulownia tree could have the height of 4 m and root collar diameter of 6 cm.

27.3.2.2 Propagation by Seeds

Seedling and plant development using seed is simple and straightforward. The seeds should be collected from the selected desired pest-free trees that grow well and have not been damaged by artificial hybridization of parent trees. Seed treatment for stimulating the germination is needed by soaking seeds in water until they are fully imbibed. Soaking seeds for 15 min in water (40°C) results in good germination. Maintaining the moisture of seedlings by watering the beds regularly, timely weeding, and thinning are needed. When seedlings grow to 5–10 cm high, they should be transplanted into the nursery field for better development. The related management techniques for seedling development in the field are similar to those for the plants developed through rooted cuttings.

27.3.3 Physical Properties of Paulownia Wood

The wood of Paulownia is strong but light and does not crack or split when nails or screws are used. It is very stable dimensionally and is not subjected to warping, cupping, or splitting, even when exposed to an outdoor environment (http://www.worldpaulownia.com/html/tech.html). Paulownia wood is lightweight, 17–21 lb/ft\(^3\) as compared with the Appalachian Red Oak of 39–41 lb/ft\(^3\). Paulownia wood takes 30–60 days for air-drying, whereas kiln-drying takes 36–60 h depending on dry kiln configuration, horsepower, and dimension of lumber. The wood is resistant to decay and rotting if it is not in permanent contact with the ground. Paulownia species vary in porosity ranging from 75 to 88% in comparison to poplar with 70–72%. The density of Paulownia wood, at 10% moisture content, ranges from 17.8 to 23.2 depending on the species and growing conditions. The
thermal conductivity of Paulownia wood is very low, thus giving it excellent heat/cool insulation properties. Further, it has an ignition temperature of 420–430°C, as compared with the average hardwood of 220–225°C. *P. tomentosa* has excellent flame retardancy, and it was found that Paulownia wood contains less lignins in comparison to cedar wood (Li and Oda 2007). Paulownia wood generates very little combustible gas when heated. Because of this property, it has been used to make clothing wardrobes for decades in Japan. In other studies conducted to evaluate *P. elongata* as the raw material for paper production, it was revealed that pulp obtained from Paulownia is not of the highest quality but can be readily used for paper production when mixed with long fibrous material (Ates et al. 2008).

### 27.3.4 Diseases of Paulownia

The main diseases that damage the Paulownia at seedling stage are caused by *Gloeosporium kawkamii* and *Spliacelorna paulowniae*, which attack the juvenile leaves and shoots. No major insect pests are known for royal Paulownia in the United States. Minor damage from several foliage diseases has been reported on the species. Two powdery mildew-causing fungal species, *Phyllactinia guttata* and *Uncinula clintonii*, and another fungal species caused by *Phyllosticta paulowniae* producing small brown spots on the leaves have also been reported (Hepting 1971). No major disease problems have appeared yet in the United States.

Paulownia witch’s broom (PWB) is a serious disease affecting Paulownia production. The disease incidence rate of Paulownia at 5 years old was approximately 50–80%, which could reduce 25% of timber production. Primers based on the PI-like adhesion gene sequence of *Mycoplasma pneumoniae* have been successfully used to detect this disease (Zhong and Hiruki 1994). If PWB disease is found, the host seedlings should be immediately removed.

### 27.4 Development of High-Yielding Paulownia Farms

On the basis of investigation, within 10 years a well-managed Paulownia plantation can attain a mean height of approximately 16–20 m, a mean diameter at breast height (DBH) of 35–40 cm, and a standing volume of 0.5 m³. However, a species should be chosen depending on the growth characteristics and ecological requirements for planting on specific site conditions. The qualified planting stock for a high-yielding plantation should be 4 m in height and 6 cm in DBH. A well-managed 4-year-old *P. elongata* farm near Lenox, GA that was established for lumber is shown in the Figure 27.1b. Following the guidelines carefully and attending the needs of the growing plants, 15- to 16-ft-tall *P. elongata* trees can be easily obtained in the first season of growth within 4–5 months (Figure 27.1c). Whatever type of planting models is adopted, the intercropping of crops or other shade-enduring economic plants are always beneficial.

Paulownia lumber farming is an environmentally sound alternative to expensive, lightweight hardwoods grown in jungles and rain forests—thriving on marginal or even toxic land (Bergmann et al. 1997). Paulownia roots penetrate down as far as 40 ft, regulating the water table and removing soil salinity. Paulownia trees have been shown to be very effective in absorbing waste pollutants from hog, chicken, and dairy facilities as well as various other pollutants (Bergmann et al. 1997). Paulownia saves forests by producing sawn timber in 6–8 years and growing 2–4 times more lumber than most other commercial trees in the same time period [World Paulownia Institute (WPI) personal communication]. This is vital because the supply of exotic hardwoods rapidly diminishes. After harvesting, a new Paulownia tree grows back from the stump and uses the same well-established root system (Figure 27.1d,e). This saves postharvest clearing costs, land erosion, and runoff. A recently established 6-week-old *P. elongata* demonstration farm (spacing 12 × 12 ft) at the Fort Valley State University is shown in Figure 27.1k.
27.5 A TREE FOR LIGNOCELLULOSIC ETHANOL

Fossil fuels have been the dominant energy resource for the modern world. The major limitations of solid biomass fuels are the difficulty of handling and the lack of portability for mobile engines. To address these issues, research is being conducted to convert solid biomass into liquid and gaseous fuels. Biological (fermentation) and chemical means (pyrolysis and gasification) can be used to produce fluid biomass fuels. Ethanol for automotive fuels is currently produced from starch biomass in a two-step process: starch is enzymatically hydrolyzed into glucose, and then yeast is used to convert the glucose into ethanol. The first four aliphatic alcohols (methanol, ethanol, propanol, and butanol) are of interest as fuels because they can be synthesized biologically and they have characteristics that allow them to be used in current engines. Ethanol is nontoxic, water-soluble, and quickly biodegradable. Blending ethanol in gasoline dramatically reduces carbon monoxide tailpipe emissions. Carbon monoxide emissions are responsible for as much as 20% of smog formation.

27.5.1 BIOMASS PRODUCTION AND MOLECULAR BIOLOGY OF CELLULOSE

Trees constitute most lignocellulosic biomass existing on our planet. Trees also serve as important feedstock materials for various industrial products. Wood from forest trees modified for more cellulose or hemicelluloses could be a major feedstock for fuel ethanol. Xylan and glucomannan are the two major hemicelluloses in wood of angiosperms. However, little is known about the genes and gene products involved in the synthesis of these wood polysaccharides. Further, much research at present is being directed to understand regulatory mechanisms of cellulose synthase (CesA) genes of trees. In the production of cellulose fiber materials, it is highly desirable to engineer trees with more cellulose and controllable cellulose properties such as degree of polymerization and crystallinity. However, little is known about the genes controlling wood cellulose formation. The discovery of differential expression of three secondary cell-wall-related CesA genes in response to tension stress and the identification of an mechanical stress-responsive element (MSRE) containing a DNA fragment in the EgraCesA3 promoter provide an important clue for the future improvement of cellulosic material production in trees (Lu et al. 2008).

Genetic improvement of cellulose biosynthesis in woody trees is one of the major goals of tree biotechnology research. However, progress in this field has been slow because of (1) unavailability of key genes from tree genomes; (2) the inability to isolate active and intact CesA complexes; and (3) the limited understanding of the mechanistic processes involved in the wood cellulose development. Recent advances in molecular genetics of CesA from aspen trees suggest that two different types of CesA are involved in cellulose deposition in primary and secondary walls in xylem (Joshi 2003). The three distinct secondary CesA from aspen—PtrCesA1, PtrCesA2, and PtrCesA3—appear to be aspen homologs of Arabidopsis secondary CesAs, AtCesA8, AtCesA7, and AtCesA4, respectively, on the basis of their high identity/similarity (>80%). These aspen CesA proteins share the transmembrane domain (TMD) structure that is typical of all known “true” CesA proteins: two TMDs toward the N-terminal and six TMDs toward the C-terminal. The putative catalytic domain is present between TMDs 2 and 3. All signature motifs of processive glycosyltransferases are also present in this catalytic domain. In a phylogenetic tree based on various predicted CesA proteins from Arabidopsis and aspen, aspen CesAs fall into families similar to those seen with Arabidopsis CesAs, suggesting their functional similarity. The coordinate expression of three aspen secondary CesAs in xylem and phloem fibers and their simultaneous tension stress-responsive upregulation suggest that these three CesA may play a pivotal role in the biosynthesis of better quality cellulose in the secondary cell walls of plants. These results are likely to have a direct effect on the genetic manipulation of trees in the future.

Further, because lignin limits the use of wood for fiber, chemical, and energy production, strategies for its downregulation are of considerable interest. Transgenic aspen trees, in which expression of a lignin biosynthetic pathway gene Pt4CL1 encoding 4-coumarate:coenzyme A ligase...
Paulownia (4CL) was downregulated by antisense inhibition, exhibited up to a 45% reduction of lignin and it was further compensated for by a 15% increase in cellulose (Hu et al. 1999).

27.5.2 SUSTAINABLE AGRICULTURE AND ENERGY CROPS

Defining agricultural sustainability is subjective, but extractive agricultural practices are always ecologically unsound. The “highest use” for a farm’s land must take into account the farm’s economics, the environment of which it is a part, and the social value of enabling people to live on the land. The long-term view is important (e.g., for soil productivity and minimization of “boom and bust” economic fluctuations). Woody biomass can give farmers flexibility in fluctuating economic conditions and help to keep farms profitable and intact. Biomass crops can be planted on marginal lands that require vegetative cover for critical periods or that would otherwise provide very little or no income without significant ecological damage. To be profitable, the amount of energy that the biomass fuel provides must exceed that used to produce it. Harvest must be timed to protect important wildlife species. Leaving residues on the field and a root system in the soil will protect against soil erosion and preserve soil structure. A market and cash flow must be guaranteed to reduce the risk for the farmer and provide the profitability needed to think long-term.

Optimizing cellulose processing by refining biomass pretreatment and converting crop residues, first-generation energy crops, and other sources to liquid fuels will be the immediate focus. This will entail reducing cost, enhancing feedstock deconstruction, improving enzyme action and stability, and developing fermentation technologies to more efficiently use sugars resulting from cellulose breakdown. One goal is to decrease industrial risk from a first-of-a-kind technology, allowing more rapid deployment of improved methods. To achieve higher production goals, new energy crops with greater yield per acre and improved processibility are needed.

27.5.3 BIOMASS PRODUCTION POTENTIAL OF PAULOWNIA

The term “biomass” in the present context as a bioenergy feedstock is intended to refer to materials that do not directly go into foods or consumer products but may have alternative industrial uses. Common sources of biomass are (1) agricultural wastes, such as corn stalks, straw, seed hulls, sugarcane leavings, bagasse, nutsheells, and manure from cattle, poultry, and hogs; (2) wood materials, such as wood or bark, sawdust, timber slash, and mill scrap; (3) municipal waste, such as waste paper and yard clippings; and (4) energy crops, such as poplars, willows, switchgrass, alfalfa, prairie bluestem, corn (starch), and soybean (oil).

27.5.4 LIGNOCELLOUS BIOFUEL AND PAULOWNIA

Paulownia is one of the fastest-growing tree species capable of generating a large amount of biomass in a short period of time (~70–80 lb/tree per year in the first year and ~200 lb/tree per year from the second year onward; unpublished data from WPI), and it can be grown and harvested seasonally or annually in many states of the United States. Research conducted at WPI suggests that up to 68 wet tons of fiber per acre per year can be produced by establishing a Paulownia farm. All of our current micropropagation field trials for biomass yields at different spacing at the Fort Valley State University are being conducted on P. elongata.

Environmental concerns regarding the use of fossil fuels and production of carbon dioxide, coupled with increased energy costs, fostered the expansion of the fuel ethanol industry. The industry has become an important partner with U.S. agriculture. The U.S. Department of Agriculture estimates that 17,000 jobs are created for every billion gallons of ethanol produced. The most recent thrust in the United States has been the construction of new corn-based ethanol production facilities. Over the last decade, technology has improved and refined the process of conversion of grains
and grasses to alcohol. However, the high cost of feedstock and the cost of production have placed barriers for being widely accepted.

Assuming corn produces 80 gal of ethanol per ton, a 50-million gallon corn-fed facility would require 625,000 tons of feedstock annually, harvested at the rate of 4.29 tons/acre national average yield or the equivalent of 145,690 growing acres (7344 acres corn/day). Paulownia grown specifically for cellulosic ethanol production would be planted on a grid of 8- by 8-ft spacing or 680 plants/acre, and unlike the practices used for forestry (growing for lumber) the plants would be manipulated for multiple sprouts (coppicing). Approximately 8–10 sprouts emerge from each plant. The Paulownia can be harvested each year or left to grow for 2- to 3-year harvest cycles. Once harvested, the Paulownia will regenerate from the stump and the cycle is repeated. Another distinct advantage of tree-type cellulosic feedstock is that one can harvest year-round and supply fiber to the production facility on a daily basis. Corn and row crops must be harvested when the crop is ready because they are seasonal. The latter poses numerous problems—not only the large-scale harvesting, but also the transportation, drying, and storage of hundreds of thousands of tons of fiber, all delivered in one month to be used over the year.

We have evaluated Paulownia wood and found the composition to be 14.0% extractive, 50.55% cellulose, 21.36% lignin, 0.49% ashes, and 13.6% hemicellulose (N. Joshee et al. unpublished research). This material is an attractive candidate for thermochemical conversion to fuels and energy through pyrolysis or gasification. The lignin content may provide the opportunity to extract this lignin for higher-value uses before thermochemical conversion. The resulting syngas will also be lower in tars.

Plant design, bioprocess engineering, and biomass processing strategies are intimately linked. Plants have evolved complex mechanisms for resisting assault on their structural sugars (wall polymers) from the microbial and animal kingdom. Cell-wall polymer organization and interactions are formidable barriers to access by depolymerizing enzymes and must be deconstructed in the pretreatment step to obtain adequate rates of release and sugar yields.

27.5.5 Economic Justification

A biofuel industry would create jobs and ensure growing energy supplies to support national and global prosperity. In 2004, the ethanol industry created 147,000 jobs in all sectors of the economy and provided more than $2 billion of additional tax revenue (RFA 2005). Growing energy crops and harvesting agricultural residuals are projected to increase the value of farm crops, potentially eliminating the need for some agricultural subsidies. Finally, cellulosic ethanol provides positive environmental benefits in the form of reductions in greenhouse gas emissions and air pollution.

Another advantage of tree-type cellulosic feedstock is that one can harvest year-round and supply fiber to the production facility on a daily basis. According to the estimates of our collaborator, WPI (Lenox, GA), Paulownia producing 80 gal of ethanol per dry ton for a 50-million-gallon-per-year facility would require 1712 tens of fiber per day or the equivalent of 50 acres of fiber per day to be harvested. It would require 18,250 acres of Paulownia to sustain the supply to the facility year-round. Excluding the cost of land, Paulownia grown on land contiguous or close to the production facility could be supplied for less than $40.00 per dry ton, one-third of the cost predicted for corn.

The argument in favor of cellulosic ethanol as a replacement for gasoline is compelling. Cellulosic ethanol will reduce dependence on imported oil, increase energy security, and reduce the trade deficit for many nations. The benefit for rural economies will be in the form of increased incomes and jobs. The use of food grains for ethanol production has caused a steep rise in the price of corn- and wheat-based products and the related dairy, meat, and cattle feed industries.

27.6 PAULOWNIA BIOTECHNOLOGY

The wide use of forest-tree products and the progressive deterioration of natural forests mean that foresters can no longer rely on the exploitation of existing forests. Extensive accelerated breeding
programs are needed for reforestation and to improve existing forest tree species. Plant genetic transformation techniques and gene isolation and characterization are no longer serious problems; forest-tree species should be a major target for commercial genetic engineering and molecular breeding. The introduction of cloned genes into plant cells and the recovery of stable fertile transgenic plants can be used to make modifications in a plant and has created the potential for genetic engineering of plants for crop improvement.

27.6.1 Tissue Culture

The use of an in vitro propagation technique provides a supply of healthy, homogenous planting material. Micropropagation of tree species offers a rapid means of producing clonal planting stock for afforestation, woody biomass production, and an effective way to capture genetic gains. Generally Paulownia is propagated through seed or by root cuttings. A conventional method of propagation through seed is unreliable because of disease and pest problems, poor germination, altered growth habit, and slower growth than root cuttings (Bergmann 1998; Bergmann and Moon 1997). Most of the tissue culture work conducted on Paulownia has used MS (Murashige and Skoog 1962) and woody plant medium (Lloyd and McCown 1981) with considerable success. For optimal results, it is customary to optimize the composition of growth medium for the variety and the stage of growth.

27.6.1.1 Organogenesis

High-frequency plant regeneration and rapid multiplication are important aspects of plant tissue culture. Plantlet formation from cultured cells and tissues can occur via one of two routes: organogenesis or somatic embryogenesis. Micropropagation research on various species of Paulownia is available. Reproducible shoot and then root induction protocols have been perfected for *P. tomentosa* (Burger et al. 1985; Rao et al. 1996; Rout et al. 2001; Corredoira et al. 2008), *P. elongata* (Bergmann and Whetten 1998), and *P. fortunei* (Rao et al. 1993; Venkateswarlu et al. 2001; Khan et al. 2003). A preliminary study carried out on *P. kawakamii* reported the presence of a differentially expressed cDNA encoding a putative bZIP transcription factor (Low et al. 2001). A 6-fold increased expression of this gene in the shoot apex region suggests involvement of this gene during the adventitious shoot regeneration process in Paulownia. A very interesting research study to investigate the effect of magnetic field was carried out on *P. tomentosa* nodal cultures (Celik et al. 2008). The study revealed that the magnetic field strength and exposure duration are important factors for rapid multiplication, which is probably supported by a higher concentration of chlorophyll a and b and total chlorophyll in treated explants. Most of the micropropagation protocols that have been developed for various species of *Paulownia* to date have predominantly used nodal explants. Figure 27.2a presents a schematic depiction of a nodal explant-based multiple shoot regeneration protocol developed for *P. elongata*. Paulownia plants can be produced commercially and shipped to domestic and international destinations (Figure 27.2b). In general, rooting in Paulownia is fairly easy (Figure 27.2c). Another explant that has been used successfully to a lesser extent is petiole with cut leaf (Figure 27.2d). In our laboratory, we have achieved considerable success in multiple shoot regenerations using shoot tip explants (Figure 27.2e). To assist commercial production by rapid multiplication, we have tried a liquid-culture-based production system using a liquid laboratory rocker system (Caisson Labs, North Logan, UT). Insertion of a filter paper as a substratum in the culture box helps multiplication a great deal. In our experience, plants can be hardened in 7–10 days in a greenhouse under misting conditions to produce a uniform planting stock (Figure 27.2f). There have been reports of vitrification during in vitro propagation of many *Paulownia* species, but reduction of vitrified shoots was possible by adjusting culture medium conditions, especially gelling substance and concentration of sugar (Ho and Jacobs 1995).

27.6.1.2 Somatic Embryogenesis

One of the earliest reports of somatic embryogenesis and plantlets from callus cultures was in *P. tomentosa* (Radojevic 1979). The culture medium used was MS containing 0.7% agar, 200 mg/L
casein hydrolysate, 100 mg/L myo-inositol, 2 mg/L thiamine, 5 mg/L nicotinic acid, 2 mg/L adenine, and 10 mg/L pantothenic acid. Repeating this media formulation on another four species of Paulownia did not yield any positive results (Yang et al. 1996), suggesting that strong genotypic factors are in play. Callus induced on fertilized ovular explants showed a persistent embryogenic capacity, eventually differentiating into plantlets. Direct and indirect somatic embryogenesis have
been reported in *P. elongata* (Ipecki and Gozukirmizi 2003, 2004). Direct somatic embryogenesis was induced on leaf and internodal explants of *P. elongata* and synthetic seeds were also produced (Ipecki and Gozukirmizi 2003). For large-scale production of elite plants, this technology has great relevance because the process can be easily scaled-up using a bioreactor. Advances in somatic embryogenesis have brought mass clonal propagation of the top commercial trees closer to reality, and efficient gene transfer systems have been developed for a number of conifers and hardwoods.

### 27.6.2 Genetic Transformation

Advances in technology for in vitro propagation and genetic transformation have accelerated the development of genetically engineered trees during the past 15 years. Targeted traits include herbicide tolerance, pest resistance, abiotic stress tolerance, modified fiber quality and quantity, and altered growth and reproductive development. Commercial potential has been demonstrated in the field for a few traits, in particular herbicide tolerance, insect resistance, and altered lignin content. Now that commercial implementation is feasible, at least for the few genotypes that can be efficiently transformed and propagated, environmental concerns have become the main obstacle to public acceptance and regulatory approval. Ecological risks associated with commercial release range from transgene escape and introgression into wild gene pools, to the effect of transgene products on other organisms and ecosystem processes. Evaluation of those risks is confounded by the long life span of trees and by the limitations of extrapolating results from small-scale studies to larger-scale plantations.

Preliminary experiments using in vitro shoots to establish a transformation protocol were carried out using *Agrobacterium tumifaciens* (strains 542, A281, or C58) and *A. rhizogenes* (strain R1601). Opine analyses demonstrated the expression of the introduced gene in proliferating galls or hairy roots (Bergmann et al. 1999). In a separate study, *A. tumifaciens* (LBA4404) harboring binary vector pBI121 (Clontech Laboratories, Inc., Mountain View, CA) was used to transform *P. fortunei* using in vitro grown petiole segments (Mohri et al. 2003). Successful transformation was confirmed by histochemical analysis of β-glucuronidase (GUS) activity in kanamycin-resistant calli; however, the frequency of shoot regeneration was very low. Transformation studies coupled with molecular techniques helped in establishing the role of transcription factor *PkMADS1* isolated from *P. kawakamii* (Prakash and Kumar 2002). In this study, it was seen that the antisense suppression of *PkMADS1* resulted in gross morphological changes such as a change in phyllotaxy. Leaf explants obtained from the antisense *PkMADS1* transgenic plants showed an almost 10-fold decrease in adventitious shoot formation compared with the explants from the sense transgenic lines or the wild-type plants. In a recent study (Castellanos-Hernandez et al. 2009), a biolistic protocol for stable genetic transformation was developed using leaf explants. Regenerated plants exhibited the integration of the transgenes as stable expression was demonstrated by GUS assay, determination of NPTII activity, and polymerase chain reaction analysis.

PWB disease, caused by PWB phytoplasma, is one of the most devastating diseases of this genus. PWB seriously slows down tree growth and is even capable of causing seedling death. Introduction of the *shiva-1* gene that encodes an antibacterial peptide using *A. tumifaciens*-mediated gene transfer resulted in plants with fewer phytoplasma and less symptoms in plants (Du et al. 2005). Further analysis of transgenic plants suggested that breeding *shiva-1* Paulownia is an effective strategy to control PWB disease. Developing reproducible transformation systems will be of great help in developing fast-growing lignocellulosic feedstock in the future.

Radical alterations in the quantity and quality of lignin in wood have been shown to be possible in softwoods and hardwoods through identification of naturally occurring mutants and by engineering the lignin biosynthetic pathway with transgenes. The potential environmental and social impacts of the release of transgenic trees have become an increasingly contentious issue that will require more attention if we are to use these technologies to their full advantage.
Current regulations restricting field releases of all transgenes in time and space need to be replaced with regulations that recognize different levels of risk (as determined by the origin of the transgene, its impact on reproductive fitness, and nontarget impacts) and assign a commensurate level of confinement. The next step in determining the acceptability of transgene technology for forest tree improvement is the unconfined release of constructs that pose little risk in terms of gene escape and nontarget impacts (e.g., lignin-altered poplar or pine) to permit evaluation of ecological risks and environmental or agronomic benefits at relevant scales (van Frankenhuyzen and Beardmore 2004).

### 27.7 SUMMARY

It is clear from the literature that *Paulownia* species have been used as a multipurpose tree in many countries in the east. There has been a growing interest in Paulownia in the western world since the 1970s. Countries that lack large forested areas and must therefore import timber can use Paulownia to help establish a local supply program around it (El-Showk and El-Showk 2003). It is a highly sought after companion tree in many agroforestry models in China. Further, it is well known for its fast growth and high biomass accumulation. Because of this property, it will fit very well in the present scene of lignocellulosic feedstock production.

Genetic modifications by plant transformation allow for stable alterations in biochemical processes that direct traits such as increased yield, disease and pest resistance, increased vegetative biomass, herbicide tolerance, nutritional quality, drought and stress tolerance, and genes to improve the production of ethanol from lignocellulosic biomass. In these methods, foreign constructs are introduced into the plant cell, followed by isolation of cells containing the foreign DNA integrated into the cell’s DNA, to produce stably transformed plant cells. To date Paulownia has been selected primarily to enhance its growth rate and wood quality value. Thus, it has been managed primarily as a forest crop for which these traits are important. These targets are quite different from the criteria for biofuel crops for which high biomass yield, high cellulose, and traits involved with bioprocessing to ethanol are essential. Paulownia provides a great opportunity to develop transgenic Paulownia trees with desirable characteristics including higher cellulose and lower lignin content, disease resistance, and tolerance to a wide variety of biotic and abiotic stress conditions. Multipurpose attributes of the Paulownia tree justify thorough research for the deeper understanding of the plant biology related to medicinal properties, biomass production, and positive environmental and economic impact. In addition to this, a resource that has not been tapped to its full potential is marginal farmland, specifically its use for growing tree crops. More than 30 million acres of woodland and idle pasture and cropland exist in the Southeast, and much of this land could be producing valuable tree crops (Clatterbuck and Hodges 2004), with Paulownia being one of them.

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### REFERENCES


Carpenter SB. 1977. This “princess” heals disturbed land. *Amer For* 83:22–23


Radojevic L (1979) Somatic embryos and plantlets from callus cultures of *Paulownia tomentosa* Steud. *Z Pflanzenphysiol* 91:57–62


