Enhanced Energy Density in Plants: Scope and Prospects

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ABSTRACT

The advances in conversion technologies to make fuels from biomass have become more economically viable, although bioenergy production will primarily depend on biomass availability. However, as biomass availability becomes increasingly restricted, so too does the availability of biomass as a feedstock for bioenergy industry become questionable. Forest resources are the primary source of biomass that cannot be further exploited due to a need for environment preservation and sustainable growth. Therefore, R&D strategies to regenerate sufficient feed stock for the bioenergy industry are a priority to make bioenergy production viable. In this context, enhanced biomass production of tree species is a valid R&D proposition which could play key role for foreseeable bioenergy production. This R&D proposition entails an understanding of the regulation of plant radial growth that underlies wood development. Strengthening our knowledge is essential to strategically plan the enhancement of tree biomass production. Phytohormones play a significant role in the regulation of wood development. Auxin is required for cell proliferation and cell differentiation during cambial development. Besides auxin, several other phytohormones, including cytokinin, gibberellin and ethylene, play regulatory roles in the control of cambial activity bearing of their stimulatory effect on cell division. The potential exploitation of these hormones to enhance biomass production should be explored. This paper outlines R&D approaches to exploit biotechnological tools for manipulation of physiological responses of selected woody plants to enhance biomass production.

Keywords: phytohormones, wood formation, biotechnological approach, biomass production, bioenergy

Abbreviations: ABA, abscisic acid; ARF, auxin response factor; Aux/IAA, auxin-responsive target genes; GA, gibberellic acid; Glc, glucose; IAA, indole-3-acetic acid; iaaH, gene encoding amidohydrolase; iaaM, gene encoding tryptophan 2-monoxygenase; ipt, isopentenyl transferase gene; rolA, rolB, rolC, root loci gene A, B, C of Agrobacterium rhizogenes T-DNA; T-DNA, transfer DNA

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INTRODUCTION

Energy being one of the essential commodities in all times its demand will remain ever for sustenance of humanity. The increasing depletion of natural resources, on the other hand increasing energy demand to meet needs of the inflating human population and changing life style from manual to mechanized in all fronts, is adding burden on energy production. Fossil fuels being the easy sources of energy, its overexploitation has become one of the prime causes of climate change which has become an serious issue worldwide (Sneddon et al. 2006). The consequence of climate change has brought in serious environmental issues, like non-availability of forest biomass, scanty rainfall insufficient hydroelectric power generation adding to the energy constrain burden. In many parts of the world, forest resource are shrinking to alarming level truly, wood biomass harvest from forest resources is next to impossible. The present scenario of energy stock and foreseeable fuel constraint evoke to search intensely for renewable energy options and generate new technologies for sustained energy harvest. Following the concept of sustainable development (Brundland Commission WCED 1987), energy systems should be ecologically, socially, and economically affordable, so that the energy needs could be met in the present, immediate future and for the future generations for the sustenance of our planet. When we ponder on this issue, biological systems based bio-energy production is a prospective approach that fit in the ambit of Green Technology principles. Energy harvest from plant biomass and using
techno-economically feasible chemo-biochemical process could play substantial role to ease energy deficit in future time. It helps preserve non-renewable resources, improves energy security, mitigates the greenhouse effect, and promotes regional development, when established under favorable conditions (Ecosense 2007). Bioenergy has several advantages over fossil fuels; energy harvest at low net CO₂ emission levels, and contributes to sustain future energy supply and employment. The below ground biomass could play profound role in maintaining biodiversity (Borjesson 1999). However, critics pointing to the adverse sustainability balance of certain forms of bio-energy production with respect to; availability of land mass for biomass production in place of agricultural commodities and its whole life-cycle assay. Negative impacts concern for example acidification, human and ecological toxicity and eutrophication (von Blottnitz and Curran 2007).

Due to the advancement in conversion technologies from biomass to bio-energy production from biomass has emerged as a potential alternative to enhance energy security, spur the transition into a sustainable future energy system and could also contribute mitigate climate change (Offermann et al. 2010). Biomass could be one of the potential sources for the major global primary energy sources during the next century, and modernized bioenergy systems are suggested to be important contributors to future sustainable energy systems for industrialized as well as in developing countries (Kartha and Larson 2000; UNDP/WEC 2001). Since biomass is a renewable and carbon-neutral energy source, interest in such resources is increasing due to the global warming problem. Biomass has been a key player in energy generation even in the past (Gielien et al. 1998; Fearnside 1999) and sustainable development (van den Broek et al. 2002). Due to the dominance of coal and petroleum products in the industrial age, there was a fall in biomass usage. Anyway, in India, though the energy scenario indicates a growing dependence on the industrial forms of energy, about 32% of the total primary energy is still harvested from biomass and more than 70% of the country’s population depends upon it for its energy needs. Biomass used for bio-energy comprises wood, agricultural and forestry residues, energy crops, human and animal excrement as well as industrial and municipal bio-degradable waste (Allen et al. 1998). Biomass is used to meet a variety of energy needs, including generating electricity, heating homes, fueling vehicles and providing process heat for industrial facilities. Electric power production from woodchips, agriculture residues is increasingly seen as a part to fill the gap between dwindling fossil fuel reserves and increased demand. The absolute quantity of biomass contribution to human energy needs rose by 80% in the last three decades (Sagar and Kartha 2007).

Biomass-based fuels reduce the need for oil and gas imports as well as, facilitate to support growth of agriculture, forestry, and rural economies; and foster major new domestic industries – biofactories – making a variety of fuels, chemicals and other products. The main global and national policies for increased biomass include (FAO 2008): possibility of reduced carbon emissions and meeting climate change commitments through both sequestration of carbon during biomass growth, avoided emissions through reduction in fossil fuel consumption, rural development through employment and increased livelihood and market opportunities. Security of supply through local production and/or processing, technological development whereby biogas could be used to bridge the gap between current reliance on fossil fuels and future technologies. In addition to the many benefits common to renewable energy, biomass is particularly attractive because it is the only current renewable source of transportation fuel. This, of course, makes it invaluable in reducing oil imports — one of the most pressing energy needs. Secondary residues generated in the processing of forest products account for 50 percent of current biomass energy consumption. These materials are used by the forest products industry to manage residue streams, produce energy, and recover important chemicals. In the year 2001, the Japanese government officially defined biomass as one of the new energy resources in the “Law Concerning Special Measures for Promotion of the Use of New Energy” for the first time. Bioenergy can reveal the dependency of social systems on ecological systems as ruthlessly as it can be. The question is how to design bioenergy systems in a sustainable way: how is it possible to derive maximum social and economic benefits from the biomass while staying within ecological limits?

**WOOD: SUSTAINABLE AND RENEWABLE RESOURCE**

Wood is the most important abundant, easily available in vicinity and readily convertible bio-based renewable energy source in the environment. It has been used since ancient time as a fuel and construction material. It is an environmentally acceptable future alternative to fossil fuel resources (Lal 2009). Tree plantation is designated as energy plantation that has taken industrial status to harvest wood for energy production, besides, as a raw material for other industries. According to industrial aspects wood is the fifth highly derived raw material for world trade (Plomper et al. 2001; Berndes et al. 2003). It is a complex material comprise of cellulose, hemicelluloses, lignin, and pectin also makes it an ideal raw material for what could be a future “ligno-chemical” industry that could replace the petrochemical industry, in providing not only plastic and all kinds of chemical products, but also food and textile products. Wood biomass adds humus to the soil, reduce erosion effects and have positive impact on land quality (Borjesson 1999; Hoogwijk et al. 2003). Healthy ecosystems and environments play vital role in sustenance of the biota that include humans and other organisms, forests, wood and agricultural products are the only renewable item from this point of view.

**Where to find more wood in this millennium?**

The advances in conversion technologies to make fuels from plant biomass have become more economically viable; however, energy production will primarily depend on availability of biomass. Distribution of plant kingdom is spread worldwide, its abundant availability, chemical simplicity and economical value makes it a best alternative for bioenergy sources. Conversely, availability of biomass as feedstock for bioenergy industry remains a question (Fenning and Gershenson 2002). Biomass for bioenergy production is mostly obtained from forests which cannot be harnessed further due to their prime role in safeguarding global environment and sustainable growth. In view of this, R&D strategies need to be focused for alternative supply of biomass feedstock for bioenergy production.

The solution for this problem could be adopting wood agriculture as a crop like maize and rice. Tree farming is a number of patients of energy crop conversion; since saplings spawn completely at random. The impending availability of forest product industry depends on the management of forest. Biomass production is expected to intensify, resulting in an expected increase of land prices and at least a doubling of grain prices. The demand for land to supply the expanding urban fringe may result in a lower supply of market wood. Further challenges arise from competing land use between biomass production for food, material and energy use, which may have severe repercussions primarily in developing countries (Kerckow 2007). Water shortage as well may be severely exacerbated in certain regions by the cultivation of energy crops (Gerbens-Leenes et al. 2009). The demand for wood is expected to grow by 20% in the next decade, while the world’s forest cover declines at an annual rate of 9.4 million ha – a size comparable to that of Portugal (http://www.fao.org/FO/SOFO/). Contextually, in view of the foreseeable need of plant biomass for...
energy production and maintain the resource stock, the science of wood formation and enhance plant biomass production is a current R&D priority. To compensate for the ever-increasing demand for wood and to reduce pressure on native forests, more wood of higher quality will need to be produced on less land by planting highly productive trees. In addition to other agronomic practices for enhanced wood formation, the biotechnological approach is another prospective R&D area which could be instrumental to develop quality saplings as well as engineer plant species with inherent ability for rapid wood formation (Eriksson et al. 2006; Jessup 2009; Amezaga et al. 2010). Biotechnology has shown great promise for forest tree improvement and over the past 10 years this field has flourished. Not only has the potential of transgenic trees with optimized yield and quality traits been demonstrated in field trials, but progress in functional genomics and association genetics provides quantum leaps forward for tree improvement (Boerjan 2005). Enhanced growth rate of tree species has been proposed through altered ascorbate/dehydroascorbate levels that are thought to play an important role in cell division and elongation (Kawaoka et al. 2003). Genetic engineering in trees has also focused on pathogen and pest resistance (Mentag et al. 2003; Tang et al. 2003), bioremediation (Che et al. 2003), the acceleration and prevention of flowering (Brunner et al. 2004) and herbicide resistance (Meilan et al. 2002). But, noteworthy study have not been undertaken to introgress hormone biosynthesis genes into tree species for enhanced wood formation through programmed manipulation of cell division, elongation and lignification. This paper outlines R&D approaches to exploit biotechnological tools for manipulation of physiological responses of select woody plant to enhance biomass production, emphasizing on biomass accretion by induction of secondary wood formation through hormonal manipulation. The density of the wood has correlation with calories/volume of wood. Understanding the regulation of the radial growth that underlies wood development is of great importance for the future use of tree products as a renewable resource. To understand the role of various phytohormones in regulation of wood development, it is important to investigate the consequences of their reduced action, which will help to develop strategies for enhancing wood formation through judicious manipulations of phytohormones.

The physiology of wood formation in plants

Wood is produced as secondary xylem from the vascular tissues in the stems of trees. Development of vascular tissue in a plant because it is not uncommon (Kleine-Vehn and Friml 2008; Nieminen et al. 2008). During embryogenesis, a continuum of provascular tissue is evident between the shoot and root apical meristems. Soon after germination, a subset of these provascular cells differentiate into two conductive tissue types, xylem and phloem. Between the xylem and phloem, however, some meristematic cells persist through primary development. On initiation of secondary development, a lateral meristem, vascular cambium, is derived from these procambial cells, together with interfascicular cells in shoots and pericycle cells in root. Secondary vascular xylem and phloem are subsequently produced via cell divisions taking place in the cambium. The composition of wood varies from tree part (root, stem or branches), type of wood geographic location, climate and soil condition (Wareing 1958; Brown 1979; Zhong and Ye 2007). Wood is produced as secondary xylem from the vascular cambium. The physiology of wood formation in plants is the structural components of primary cell wall. The fundamental structure units are the microfibrils (MFs), which are the result of a strong association of inter- and intrachain hydrogen bonds between the different chains of linked Glc (glucose) residues in a wood (root, stem or branches). Cellulose is largely crystalline-derived from D-glucose units, which condense through β (1→4) glycosidic bonds. Hemicellulose is a polysaccharide related to cellulose that comprises 20% of the biomass of most plants. In contrast to cellulose, hemicellulose is derived from several sugars addition to glucose, especially xylose but also including mannose, galactose, rhamnose, and arabinose. Hemicellulose consists of shorter chains around 200 sugar units. Furthermore, hemicellulose is branched, whereas cellulose is unbranched. Lignin is other major component of wood (25–35%) is a phenolic polymer derived from three hydroxy-cinnamyl alcohols (monolignols): p-coumaryl alcohol, coniferyl alcohol, and syringyl alcohol, giving rise to H, G, and S units, which differ from each other only by their degree of methoxylation. However, it is somewhat surprising that recent attempts at synthesizing lignin have not yet succeeded. Further, current models of the pathway are incomplete (Grima-Pettenati and Goffner 1999; Scarpetta and Meijer 2004). There is evidence that wood formation is outcome of cambial activity in woody species which is correlated with active growth extension of the shoot (Wareing 1958; Digby and Wareing 1966). Wood is a product of the vascular cambium (cambium) of trees (Catesson 1994; Larson 1994). The formation of annual xylem rings occurs due to the production of cells by cambium, growth, expansion and secondary wall thickening of cambial derivatives. The former two processes determine the annual increment of width, the latter one add to the thickening of cell walls, and all of them in total give rise to wood biomass accumulation and wood increment. Most investigations on wood formation have been devoted to study the effects of environment on wood formation taking the width increment into account only, thereby, the different aspects of wood formation are examined as if only one. In such investigations the case of real productivity is ignored. The cambium consists of a thin layer of cells (Kitin et al. 2000). Cambial derivative procambial cells, develop into xylem cells and xylem formation is a concerted activity of four major steps; cell formation, cell expansion, deposition of secondary walls and lignification of cells and finally programmed cell death. Expansion of the procambial cells and the cambial daughter cells involves primary wall formation and modification followed by secondary wall formation, which includes the biosynthesis of polysaccharides (cellulose, hemicelluloses) and cell wall proteins and lignification. Finally, progammatic cell death occurs when the xylem cells are dead. The primary walls of secondary walls are made up of a different kind of a cell to perform different necessary functions such as; mechanical support, water transport and metabolism of the plant besides, it is the major source of organic biomass with many energy-rich molecules like lignin, oils and other secondary metabolites (Zhang et al. 2011). Wood cells are build up of an insoluble polymeric matrix of polysaccharides and lignin. Primary cell wall contain lower proportion of cellulose as compared to secondary walls. The mechanism regulating the sequen-
tional development of secondary xylem cell walls is not well understood, but the plant growth regulator, i.e., indole acetic acid (IAA)/auxin has been proposed to play a key role in it (Sundberg et al. 2000). Auxin plays a key role in regulating wood formation through its effects on cambial activity and xylem development (Sundberg et al. 2000). In recent years genetic approaches in the model plant Arabidopsis have considerably increased our knowledge about auxin action (Leyser 2001; Moyle et al. 2004; Nakashima et al. 2007; Yang et al. 2008). These findings imply that the GA/auxin balance is established by the expression of auxin transport and GA metabolism genes and that auxin indirectly controls cambial activity (Funada et al. 2008) required for elongation growth is dependent on the supply of polarly transported auxin.

Auxins positively influence cell enlargement, bud formation and root initiation, which is a key regulator of cambial growth activity in wood formation. The IAA (indole-3-acetic acid) status in the plant is critical for the amount and quality of the produced wood. Although other compounds with similar activity, such as indole-3-butyric acid, phenyl acetic acid, and 4-chloro-IAA, are also present in plants (Little and Savidge 1987; Lachaud 1989; Plomion et al. 2001), their physiological role in wood formation is known. Certain bacteria and plant cells transformed with Agrobacterium tumefaciens synthesize IAA via a unique pathway in which tryptophan is converted to IAA in two steps. First, tryptophan monoxygenase, converts tryptophan into indole-3-acetamide (IAA) by indole-3-acetamide hydrolase. The genes encoding these enzymes have been used to alter IAA levels in transgenic plants (Klee and Romano 1994). The evidences from past to present, indicates that a continuous supply of basipetally transported IAA is required to maintain fusiform cambial cell structure and to enable cambial growth to proceed, but IAA by itself is insufficient to promote mitosis, cytokinesis, and tracheid secondary wall differentiation, and to control the formation of cambial orientation. Evidence for a regulatory role of IAA level is consistent only for tracheid radial enlargement, supporting many observations implicating IAA in the control of primary-wall extension in non-forest dicots (Vans 1985; Cleland 1986). The level of IAA may also control the formation of compression wood. The cambial response to exogenous IAA varies with the season of application and decreases with time (Little and Bonga 1974), its transport (Zajaczkowski et al. 2008) and with increasing cambial age. The evidence can be interpreted as indicating that IAA indirectly controls cambial growth by directing the transport of substances that affect cambial growth more directly, the levels of which vary in time and space. These substances presumably include non-specific assimilates (Wodzicki et al. 1982; Patrick 1986) and presumptive substances affecting specific aspects of cambial growth, e.g. tracheid differentiation (Savidge and Wareing 1981; Savidge 1983). It is also possible that IAA regulates cambial growth through changes in the pattern of its transport (Zajaczkowski et al. 1984) or in the sensitivity of the cambial zone and differentiating derivative cells to its presence (Trewavas et al. 1983).

Both IAA and gibberellins (GAs) stimulate cell and organ growth (Björklund et al. 2007). It has been shown that auxin supplied to stem tissues via the polar transport pathway stimulates cambial growth in a dose-dependent. Apical feeding to decapitated stems has been extensively exploited to evaluate the role of auxin in cambial growth and xyleogenesis. Apical auxin treatment resulted in cambial growth and xylem development, and increases in IAA concentrations in the stem tissues. The experiment demonstrated dose-dependent, correlated responses in internal IAA levels and cambial growth. The results provide quantitative evidence that auxin is increased in the cambium and is transported to the cambial tissues by polar transport is a key regulator of secondary xylem (wood) production. The current knowledge, imply that the GA/auxin balance is established by intricate crosstalk and self-control mechanisms involving the expression of auxin transport and GA metabolism genes (Björklund et al. 2007; Mauriat and Moritz 2009; Zhang et al. 2011). Majority of identified transcripts can be attributed to GA/methylation and auxin. It is established that plant growth in length and cell elongation is conditioned by endogenously synthesized growth regulators similar to the nature of hormones. The plant hormones are involved in several stages of plant growth, differentiation and maturation such as; cell elongation, cell division, tissue differentiation (Torrey et al. 1971; Wang et al. 1997), apical dominance, flowering, maturation and senescence. Auxin, cytokinins, abscissic acid (ABA) and gibberellic acid (GA) are major growth regulating hormones. The evidence and accumulating evidence suggest how these hormones act in concert to produce the complex growth responses in plants. GA and auxin are known to interact at several points in the growth and development of the plant. Both hormones play important roles in the post-meristematic expansion of cambial derivatives and auxin dep-
Cytokinins are \( \text{N} \)-substituted adenine derivatives that have a crucial role in many aspects of plant growth and development (Howell et al. 2003). Cytokinin responses in plants are mediated by a signal transduction pathway. The growth of plants depends on continuous function of the meristems. Shoot meristems are responsible for all the post-embryonic aerial organs, such as leaves, stems and flowers. To address the mode of cytokinin function in the secondary meristem, vascular cambium, Nieminen et al. (2008) studied cytokinin signaling during cambial development in the trunks of 2 hardwood tree species, poplar (Populus trichocarpa) and silver birch (Betula pendula). The transgenic plant engineered for ectopic expression of cytokinin degrading enzyme for the purpose of repressing cambial cytokinin signalling indicated that cytokinins are major hormones that regulate the cell cycle switch for cambial development. It has been assumed that the phytohormone cytokinin has a positive role in shoot meristem function. A severe reduction in the size of meristems in a mutant that is defective in all of its cytokinin receptors has provided compelling evidence that cytokinin is required for meristem activity. Based on various genetic and molecular studies (Miyawaki et al. 2006; Kurakawa et al. 2007), including systemic over-expression of a catalytic cytokinin hydrodase (CKX) gene, it is become evident that in the shoot, apical meristem cytokinins appear to promote cell proliferation. In addition to higher plants, several bacteria, including Agrobacterium, produce cytokinins (Gaudin et al. 1994). Genetic manipulation of CKX gene may provide a strategy through which cytokinin levels can be modified. The plant hormone ethylene is another important signal in plant growth responses to environmental cues. In vegetative growth, ethylene is generally considered as a regulator of cell expansion. Role of ethylene in the control of meristem growth has also been suggested based on pharmacological experiments and ethylene-overproducing mutants. It have also been demonstrated that both applied and ectopically produced ethylene stimulates xylem growth by means of cambial cell division, and that endogenous ethylene mediates growth in the vascular cambium in response to leaning. Available evidence suggests that ethylene is involved in the control of cambial growth, particularly in the differentiation of compression wood tracheids. The application of etrel (2-chloroethylphosphonic acid), an ethylene-generating compound, stimulated radial growth in vertical stems of Pinus radiata (Barker 1979), Picea strobus (Brown and Leopold 1973), and Pinus taeda (Telewski et al. 1983), apparently by increasing the production of both xylem and phloem tissue (Barber 1979). Other observed effects included a decrease in tracheid wall thickness and a stimulation of the production of rays and vertical resin ducts (Telewski and Jaffe 1986a). They observed that ethylene production in Pinus taeda and Abies fraseri (Pursh) Pursh Poar shoots was enhanced by wind and by mechanically-induced flexing, which they found also increased tracheid production and decreased tracheid length (Telewski and Jaffe 1986b). Using ethylene-insensitive and ethylene-overproducing transgenic hybrid aspen (Populus tremula) in combination with ethylene perception inhibitor [1-methylcyclopropene (1-MCP)], it has been demonstrated (Lovea et al. 2009) that endogenous ethylene produced in response to leaning stimulates cell division in the cambial meristem. This ethylene controlled growth gives rise to the eccentricity of Populus stems that is formed in association with tension wood.

**Effect of environment on wood formation**

To understand the mechanism of the influence of ecological factors on wood formation it is important not only to evaluate the results of such influences (radial diameters and thickness of cell walls) but also to estimate the effects of the factors on the process responsible for these results (Cateson 1994; Borjesson 1999; Yang et al. 2004). Consequent stages of formation of the cells by cambium and their development in each of differentiation have been found to be independent processes and their reactions to environmental factors are different (Antonova and Shebeko 1981; Antonova et al. 1983; Antonova and Stasova 1997; Richet et al. 2011).

**BIOTECHNOLOGICAL APPROACHES TO ENHANCE WOOD FORMATION AND TREE IMORovement**

With a few exceptions, very little is known about the cellular, molecular, and developmental processes that underlie wood formation. Trees in general are difficult experimental organisms due to their large size and long generation times. The properties of wood, and the process of wood formation, are concert action of the genes and proteins in differentiating secondary xylem. These genes and proteins need to be studied to understand wood formation, and are potential targets for the directed modification of wood properties. Improvement of trees by conventional breeding is constrained by their long reproductive cycles, which usually include long juvenile periods, and by the complex reproductive characteristics of most of these species, including self-incompatibility and varying degree of heterozygosity. Genetic transformation offers an attractive alternative to breeding because it provides the potential to transfer specific traits into selected genotypes without affecting their desirable genetic background (Peña and Seguin 2001). Recent advances in the molecular study of these processes have revealed the highly regulated genetic control of wood formation (mainly at the transcriptional level). Many research programmes are being developed to track the genes and proteins involved in these processes (Demura and Fukuda 2007). Indeed, the composition and structure of the cell wall has a dramatic impact on the technological value of raw materials, not only wood fibres but also other plant products such as forage stocks (Boudet et al. 2003). Consequently, numerous strategies have been and are being developed to optimize the composition of plant cell walls for improved agroindustrial uses (Boudet et al. 2003). This will become more and more important as we enter an era in which plant-derived raw materials are increasingly used as renewable carbon source in the context of sustainable development. The modification of wall structure via genetic engineering or the use of microbial systems is now becoming a realistic goal. Technologies for measuring gene expression in plants have improved during the past decade (Meyers et al. 2004).

**Strategies for endogenous hormone biosynthesis for enhanced wood formation**

These finding provides molecular support to physiological experiments demonstrating that either hormone can induce growth if the other hormone is absent/deficient because of mutations or experimental treatments (Anterola and Lewis 2002; Aloni 2010). After gibberellin had been applied to the vertical xyleme of four species of angiosperm trees for approximately months, eccentric radial growth was observed as a result of enhanced growth rings on the sides of stems to which gibberellin had been applied. Moreover, the application of gibberelin resulted in the formation of wood fibers in which the thickness of inner layers of cell walls was enhanced (Koehler and Telewski 2006).

**Hormone biosynthesis genes**

Modification of plant hormone levels in trees has been achieved (Peña and Seguin 2001) mainly using genes from the T-DNA of A. tumefaciens and A. rhizogenes. The iaaM and iaaH auxin-biosynthetic genes, and the ipt cytokinin biosynthetic gene from A. tumefaciens, have been overexpressed in poplar, producing alterations in growth characteristics and wood properties. By modifying hormone metabolism, the rolA, rolB and rolC genes from A. rhizogenes...
Molecular biology advances to enhance wood synthesis in the tree

In lobolly pine genomic approach was used to identify genes and proteins involved in cell wall biosynthesis during xyleogenesis (Whetten et al. 2001). The expressed sequenced tags (ESTs) were obtained from wood forming tissues and studied related to identification of expressed genes based on metabolite production microarray analysis of relative expression levels of a subset of genes (Zhang et al. 2003; Pavy et al. 2008; Dharmawardhana et al. 2010). Among the most abundantly expressed genes in are many genes expected to be involved in the formation of the wood cell wall (Zhong and Ye 2007). Genes involved in monolignol precursor biosynthesis are found, including genes encoding 4-coumarate-CoA ligase (Boerjan et al. 2003; Ohad 2010) and caffeoyl-CoA O-methyltransferase (Meyermans et al. 2000; Widiez et al. 2011). In poplar also ESTs was constructed from cDNA libraries isolated from different zone such as cambial zone (CZ), differentiating xylem (DX) and mature xylem (MX). The proportion of EST involved in cell cycle (Sterky et al. 2004), DNA processing and protein synthesis is twice as high in CZ. On the other hand, the proportion of EST involved in metabolism and cellular organization is at least twice as high in DX than in CZ, mostly due to EST corresponding to cell wall synthesis enzymes and cell wall structural proteins. These studies reports the comparison between the expression level of different tissue from different Zone and give an idea of expression analysis in normal condition (Li et al. 2011).

In order to gain insight molecular expression, study of molecular expression profile was done across black locust (Robinia pseudoacacia L.) trees; 2,915 ESTs were generated and analyzed, out of which 55.3% showed no match to known sequences (Yang et al. 2003; Li et al. 2009). Cluster analysis of the ESTs identified a total of 2278 unigene sets, which were used to construct cDNA microarrays (Kirst et al. 2004). Microarray hybridization analyses have been performed to survey the changes in gene expression profiles of trunk wood (Li et al. 2011). The gene expression profiles of wood formation differ according to the region of trunk wood sampled, with highly expressed genes defining the metabolic and physiological processes characteristic of each region (Demura and Fukuda 2007). For example, the gene encoding sugar transport had the highest expression in the sapwood, while the structural genes for flavonoid biosynthesis were up-regulated in the sapwood-heartwood transition zone. This analysis also established the expression patterns of 341 genes, and their cDNA libraries of bark/cambial region (BCS), sapwood region (SWS), and transition zone (TZS) of trunk-disk of 10-year-old black locusts harvested in early summer and one cDNA library (TZF) from a black locust harvested at late fall. The comparison compared differentially expressed genes in the three zones. To visualize the inner-wood gene expression patterns that could potentially identify the wood cell wall composition related genes. Hierarchical clustering of the arrayed genes based on microarray results represents genes that show higher expression in inner wood (i.e., sapwood and transition zone) than in bark/cambial region (Beaulieu et al. 2011). The exhaustive sequencing of ESTs generated from large numbers of cDNA libraries isolated from specialized tissues and organs provides a useful tool for gene profiling (Harfouche et al. 2011). Production and analysis of ESTs required for forming tissues have increased our understanding of the gene regulation involved in wood formation in tree species including loblolly pine (Pinus taeda) (Hetten et al. 2001; Pavy et al. 2005; Bomal 2008; Li et al. 2011), poplar (Populus species) (Désjardin et al. 2004; Pavy et al. 2008; Arnaud et al. 2012), black locust (Yang et al. 2004; 2003), Eucalyptus (Krist et al. 2003; Pau et al. 2004, 2005; Poke et al. 2005; Rengel et al. 2009; Elissètche et al. 2011), and white spruce (Picea glauca) (Pavy et al. 2005; Beaulieu et al. 2011; Rigault et al. 2011).
Like the ESTs derived from the developing xylem cells of pine and popular, *R. pseudoacacia* library contained a higher concentration of those genes involved in cell wall synthesis, when compared to the other two libraries (Li et al. 2009). However, this library possessed only a few transcripts coding for enzymes involved in the synthesis of lignin. These results indicate that sapwood gene expression patterns more closely resemble the characteristics of inner wood gene expression than that of developing-xylem (Li et al. 2011).

The application of genetic engineering awaits two developments: better method of gene transfer and a fundamental understanding of the developmental process of wood formation (Whetten and Sederoff 1991; Beaulieu et al. 2011; Harfouche et al. 2011). To compensate for the ever-increasing demand for wood and to reduce pressure on native forests, more wood of higher quality will need to be produced on less land by planting highly productive trees. Biotechnology has shown great promise for forest tree improvement and over the past 10 years this field has flourished. Not only has the potential of transgenic trees with optimized yield and quality traits been demonstrated in field trials, but progress in genomics and association genetics promise quantum leaps forward for tree improvement (Koehler and Teleshki 2006; Lefebvre et al. 2010). Breeding of fast-growing trees is a slow process mainly because of the long generation intervals of most forest trees and because many traits can only be properly assessed at rotation age (Fenning and Gershenson 2002). One can distinguish two main avenues to accelerate domestication: one is through genetic modification, by introducing new genes into already existing elite genotypes, and the other is through the smart exploitation of genetic diversity in breeding programs. Both strategies largely benefit from a profound understanding of gene-function relationships. The identification of the genes that control traits relevant to tree domestication is a challenging task, especially as our knowledge on tree-specific processes is still scarce (Beaulieu et al. 2011). The wood forming tissue in trees as an experimental system to investigate auxin action, has several advantages in addition to the unique role of auxin in wood formation (Robert and Frits 2009; Aloni 2010). The establishment of micro techniques for measuring both auxin content and gene expression makes it feasible to investigate alterations in the endogenous auxin level, gene expression and the attendant developmental changes in an auxin-regulated phenomenon at almost cellular resolution (Tuominen et al. 1997; Hertzberg et al. 2001; Srivastava 2002; Bjorklund et al. 2007; Morris et al. 2010). These advantages of wood-forming tissues in trees have successfully used to demonstrate an overlap of an auxin gradient and a developmental gradient in the wood forming tissues, thereby suggesting a role for auxin as a positional signal in wood formation (Knox-Uggla et al. 1996; Ohashi-ito and Fukuda 2010).

Despite a critical role for auxin in regulating diverse aspects of wood formation as well as the obvious advantages in studying auxin action in wood formation, little is known about the cellular basis of these processes. One of the best-characterized auxin responses is the induction of gene expression by auxins. Among these auxin-induced genes, the Aux/IAA family encoding 19±36 kDa short-lived nuclear-localized proteins is probably the most well studied and several independent lines of evidence have suggested their role in mediating auxin responses (Reed 2001). AUX/IAAs and auxin response factors: mutation of the MONOP-TOURN/AUXIN RESPONSE FACTOR 5 (MP/ARF5) gene (Demura and Fukuda 2007), which encodes a transcription factor that belongs to a family of ARFs, disrupts the normal body organization along the apical–basal axis and results in discontinuous and reduced vascular formation (Moyle et al. 2002). Meristems of shoot and root are a source of cells that must be maintained in a proliferative state, often regarded as undifferentiated, so one approach has been to study mutants in which meristematic function has been compromised (Haecker and Lux 2001). Until now, transgenic research has focused on a handful of genes involved in secondary wall formation. Over the past few years, the post-genomic era should be in full swing. Indeed, a wealth of xylem-specific EST sequences is now available from woody species (Desjardins et al. 2004). Exploitation of the corresponding genes and of genes involved in the supramolecular organization of the cell wall (interactions between polymers) is a promising additional tool that can result in interesting applications (Pavy et al. 2005a; Han et al. 2007; Pavy et al. 2007). Our knowledge about genetic control of cambium development is very less.

Using a transgenic approach based on down-regulating auxin signaling, it was demonstrated that auxin is required for cell proliferation and cell differentiation during cambial development (Nilsson et al. 2008; Aloni 2010). Aside from auxin, several other hormones, including cytokinin (Loomis and Torrey 1964; Saks et al. 1984; Niinemets et al. 2008; Ohashi-Ito and Fukuda 2010), gibberellin (Dettmer et al. 2009; Zhang et al. 2011; Zhou et al. 2011), and ethylene (Junghans et al. 2004; Nilsson et al. 2008), have been implicated in control of cambial activity because of their stimulatory effect on cell division upon hormone treatment. In vitro systems have been reported as an effective tool for obtaining genetically uniform plants. Plant hormones affect gene expression and transcription levels, cellular division, and growth.

**CONCLUSION**

Enhanced secondary growth for wood formation and deposit of high energy molecule such as, fats, oil, terpenes and lignins in the wood biomass are viable R&D strategies to add calorific value of the wood biomass. These strategies for enhancing synthesis of biomolecules in wood biomass is in contradiction to minimize synthesis of lignin and other biomolecules, which is essential for easy processing of wood biomass for product formation. Enhanced synthesis of these high energy molecules; such as, oils, lignins, terpenes, etc. will increase calorific value, hence energy yield from the wood biomass for direct combustion, syngass or energy harvest through other such means. These strategies could be prospective alternatives for increased energy yield from wood biomass. Variability in stem diameter growth and wood quality arises as a result of variable subcellular processes. This is true whether the development is considered in terms of the degree of stem taper, the partitioning of photosynthetic among shoots, roots and branches, the formation of juvenile, normal, spiral-grained or reaction wood, the extent of fiber elongation, or the weight or chemical composition of wood. All of these developments involve a host of subcellular processes such as cell division, primary-wall expansion, development of pith or perforation plates, deposition of cellulose, hemicellulose, and lignin polymers, microfibril orientation, production of extractives, storage of reserve materials, and protoplasmic autolysis. However, each of these subcellular differentiation processes comprises many more minute biochemical and biophysical processes that are responsible for the environment, or both. Plant growth regulator (PGR) research can be done at any level from whole trees to cell-free biochemical systems, and necessarily must be done at all levels before it will become possible to model PGR regulation of whole tree stem growth and development. It is, however, at the molecular level that we ultimately must probe if the biochemical/biophysical processes under PGR regulation are to be understood. One of the best ways of achieving this is through the use of molecular genetics, which has largely benefited from the supramolecular understanding of gene expression. This is certainly true for the topic of cambial growth control in forest trees. A summary of the last 50 years of research into PGR regulation of stem growth and development must conclude that although there has been considerable research...
effort and numerous publications generated, nothing of practical use for forestry has yet emerged. Nevertheless, there can be little doubt that stem growth and development are under PGR regulation. Thus, continuing PGRs research for improved wood formation is one of the priority areas. Virtually all processes connected with growth, development and metabolism in plants are governed in one way or the other by hormones. This overview highlights the new possibilities regarding cell biology of the tree, specially cambium and wood formation, with emphasis on manipulation of plant growth regulators as the “driver” to enhance energy density in plant biomass to increase energy yield from wood biomass towards meeting the present and future energy requirement.

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