

Enhanced Energy Density in Plants: Scope and Prospects

Bijaya Ketan Sarangi^{1*} • Sarita Tiwari¹ • Dinesh Yadav² • Ram Avatar Pandey¹

¹ National Environmental Engineering Research Institute-CSIR, Nehru Marg, Nagpur-440020, India

² Department of Biotechnology, DDU Gorakhpur University, Gorakhpur-273009, India

Corresponding author: * bk_sarangi@neeri.res.in

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 because 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 select 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-monooxygenase; *ipt*, isopentenyl transferase gene; *rolA*, *rolB*, *rolC*, root loci gene A, B, C of *Agrobacterium rhizogenes* T-DNA; T-DNA, transfer DNA

CONTENTS

INTRODUCTION.....	22
WOOD: SUSTAINABLE AND RENEWABLE RESOURCE.....	23
Where to find more wood in this millennium?	23
The physiology of wood formation in plants.....	24
Outline of wood formation: role of phytohormones	24
Effect of environment on wood formation.....	26
BIOTECHNOLOGICAL APPROACHES TO ENHANCE WOOD FORMATION AND TREE IMPROVEMENT	26
Strategies for endogenous hormone biosynthesis for enhanced wood formation.....	26
Hormone biosynthesis genes	26
Increase biosynthesis of energy-rich molecules: lignin, terpenes and other fatty compounds and storage in wood	27
Molecular biology advances to enhance wood synthesis in the tree	27
CONCLUSION	28
ACKNOWLEDGEMENTS	29
REFERENCES.....	29

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 a 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 hydel 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 (Brundtland 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 supplies and development. The biomass plantations 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 resource, 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 (Gielen *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 wood-chips, agriculture residues is increasingly seen as a part to fill the gap between dwindling fossil fuel reserves and increasing global 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 – biorefineries – making a variety of fuels, chemicals and other products. The main global and national level drivers for increased use of energy from 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 bioenergy 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 prod-

ucts 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 it could be 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 demanded raw material for world trade (Plomion *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 bio-energy sources. Conversely, availability of biomass as feedstock for bioenergy industry remains a question (Fenning and Gershenzon 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 feed stock for bioenergy production.

The solution for this problem could be adopting wood agriculture as a crop like maize and rice. Tree farming is a matter of patience and luck more than resources or skill; 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 promise 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 tissues in plants is unique because of its dynamic nature (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 differentiates 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 mainly composed of two types of cells with secondary cell walls: (i) fibres, which mechanically support the plant bodies and (ii) tracheary elements composed of vessels (not found in gymnosperm wood) and tracheids (found in both angiosperm and gymnosperm). Woods transport water and solutes to other parts of plant. Two major chemical components of wood are lignin (35%) and carbohydrate (65-75%). The carbohydrate part of wood comprises of cellulose and hemicelluloses. Between 40% and 50% of wood consists of cellulose. Cellulose, the

world's most abundant biopolymer is an integral part of human society and fundamental to plant morphogenesis. Humans exploit its abundance and properties in the form of wood. Cellulose is the major constituents of plant cell wall. It 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 manner. Cellulose is largely crystalline-derived from D-glucose units, which condense through β (1 \rightarrow 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 hydroxycinnamyl alcohols (monolignols): *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl 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 engineering lignin biosynthesis have demonstrated that current models of the pathway are incomplete (Grima-Pettenati and Goffner 1999; Scarpella and Meijer 2004).

Outline of wood formation: role of phytohormones

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, programmed cell death occurs to form an empty tube with secondary walls. The division of cambial cells produces secondary phloem on the outside and secondary xylem on the inside. Since there is usually much more secondary xylem than secondary phloem (Larson 1994) mature secondary xylem are used as a wood. Wood is a complex and non uniform material, in respect to its both anatomical and its chemical properties. Meristem differentiates into secondary phloem (inner bark) and secondary xylem (wood). It is 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-

tial 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.* 2002; Koornneef *et al.* 2004; Kleine-Vehn and Friml 2008; Nilsson *et al.* 2008; Groover *et al.* 2010; Zhang *et al.* 2011); nevertheless, the picture of auxin regulation is far from complete. Auxin is required for maintaining the cambium in a meristematic state, as depleting cambium auxin leads to differentiation of cambial cells to axial parenchyma (Savidge 1983). The wood-forming tissue also provides an experimental system in which cellular sensitivity to auxin is strongly modulated by environmental signals. It has been shown that the sensitivity of the cambium to auxin is lost when trees enter dormancy at the end of the growing season (Little and Bonga 1974). This phase of dormancy, known as rest, can be overcome by giving a chilling treatment to the tree upon which the cambium makes the transition from rest to quiescence and regains responsiveness to IAA. Auxin has further been proposed to be an important regulator of xylem development (Knox Ugglä *et al.* 1996). Therefore, analyzing the role of auxin in wood formation will allow a better understanding of how auxin distribution regulates the sequence of developmental events and how environmental signals can modulate cellular sensitivity to auxin. Growth cessation during cambial transition into dormancy is accompanied by the loss of cambial sensitivity to auxin but the molecular mechanism of this phenomenon is not well understood (Little and Bonga 1974). Auxin promotes growth and is required for continued cell division in the cambium (Little and Bonga 1974; Savidge 1983). However, since auxin levels in the cambium do not undergo a reduction upon transition into dormancy (Knox Ugglä *et al.* 1996), it cannot be a lack of auxin that causes the cell division to cease. Hence, it has been proposed that the entry into dormancy involves a loss of sensitivity of the cambium to auxin (Little and Bonga 1974; Srivastava 2002). It is also known that applied GA (gibberellic acid) stimulates cambial activity (Bradley and Crane 1957; Wareing 1964; Biemelt *et al.* 2004). Interactions between auxin and GA have been intensively studied in the elongation growth of primary stems, a process that is stimulated by both substances (Ross *et al.* 2003; Cleland 2004). The hypothesis suggested that auxin was the active hormone in the elongation response, and that the observed GA effect was mediated by increases in auxin biosynthesis or auxin transport. These findings imply that biosynthesis of the endogenous GA (Funada *et al.* 2008) required for elongation growth is dependent on the supply of polarly transported auxin.

Interactions between auxin and GA have been intensively studied in the elongation growth of primary stems, a process that is stimulated by both substances (Ross *et al.* 2003; Cleland 2004; Björklund *et al.* 2007). 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, cytokinin, gibberellins, abscisic acid (ABA) and ethylene are considered as the principal types of phytohormones. It is known that hormones induced affect in an organ of a plant is determined by the combined rates of its biosynthesis, breakdown, import, and export (Srivastava 2002).

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. The first enzyme, tryptophan monooxygenase, converts tryptophan to indole-3-acetamide, which in turn is converted to 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 fusiform cambial cell 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 basipetally from the application point 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 xylogenesis. 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 data to support the concept that the amount of IAA supplied 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 late-response genes maintained by the increase in respective hormone, and reflect biological processes that are affected by them. The results indicate that auxin supplied through the polar transport pathway affects not only the expression of GA biosynthesis genes, but also more than a thousand other genes of the 9700 unique gene models represented on the microarray (Yang *et al.* 2003a). This gives that auxin stimulates both cambial cell division and xylogenesis. GA treatment, which stimulated division and expansion of cambial zone, has been demonstrated that GA stimulates polar transport of IAA in poplar stems. We have also shown that GA has a common transcriptome with auxin, including many transcripts related to cell growth. Interpretation of available information from feeding experiments and endogenous hormone distributions in poplar suggests that, whereas auxin is a key regulator of cambial cell division, both hormones play important roles in the post-meristematic expansion of cambial derivatives and auxin dep-

letion results in delayed fiber lignification.

Cytokinins are N^6 -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 hormonal regulators required 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 catabolic cytokinin oxidase (*CKX*) gene, it has 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 has 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 (Barker 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 fraser* (Pursh) Poir. 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 zones 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 IMPROVEMENT

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 high 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 Séguin 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 agro-industrial 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 stems 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 gibberellin 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 Séguin 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*

alter growth and development, and rooting ability when overexpressed in transgenic poplar and aspen plants. Phenotypic modifications of *rolC*-overexpressing plants were associated with reduced levels of auxins, modified gibberellin biosynthesis and increased cytokinin levels (Tzfira *et al.* 1998; Grünwald *et al.* 2000). The *rolA* and *rolB* genes were introduced into apple and *rolC* was also incorporated in trifoliolate orange and pear (Bell *et al.* 1999; Kaneyoshi and Kobayashi 1999; Zhu *et al.* 2001; Krist *et al.* 2003) with the aim of producing dwarf rootstocks. Transgenic plants were characterized by a reduction in plant height associated with shortened internodes and smaller leaves, and, in general, transformants possessed greater rooting abilities. Poplar trees transformed with a homeobox gene from rice showed morphological abnormalities accompanied by a suppression of gibberellin (GA) 20-oxidase gene expression (Mohri *et al.* 1999; Groover *et al.* 2010), suggesting that homeobox genes could regulate hormone biosynthesis. A more promising approach to the modification of tree size is the expression in sense or antisense of the GA 20-oxidase and other genes encoding GA-catabolizing enzymes in transgenic trees. It has been shown that constitutive overexpression of GA 20-oxidase in *Arabidopsis* resembles the effects of repeated application of GA3 to wild-type plants (Coles *et al.* 1999). Furthermore, ectopic overexpression of the GA 20-oxidase gene from *Arabidopsis* in hybrid aspen has resulted in trees with faster growth in height and diameter, larger leaves, more numerous and longer xylem fibres, and increased biomass, opening up ways of modifying not only tree size but also wood quality (Eriksson *et al.* 2000).

Increase biosynthesis of energy-rich molecules: lignin, terpenes and other fatty compounds and storage in wood

Lignin is made of aromatic polymers that originate and an integral part of the secondary cell walls of plants. It is one of the most abundant organic polymers on Earth, exceeded only by cellulose, employing 30% of non-fossil organic carbon, and constituting from a quarter to a third of the dry mass of wood. As a biopolymer, lignin is unusual because of its heterogeneity and lack of a defined primary structure. Lignins are energy rich molecules relative to other wood components and high lignin content increases the energy density in the wood biomass which is an advantage to exploit woods as an energy source (Tzfira *et al.* 1998; Alain *et al.* 2003). Lignin biosynthesis is mediated through the phenylpropanoid pathway and Cinnamyl Alcohol Dehydrogenase (CAD) enzyme mediates a key step in the synthesis, up regulation of the phenylpropanoid pathway could lead to enhance synthesis of lignin that will increase energy density in the CAD overexpressing plants. Its most commonly noted function is the support through strengthening of wood (xylem cells) in trees (Baucher *et al.* 1998). Lignin fills the spaces in the cell wall between cellulose, hemicellulose, and pectin components, especially in tracheid, sclereid and xylem cells. It is covalently linked to hemicellulose and, therefore, crosslinks different plant polysaccharides, conferring mechanical strength to the cell wall and by extension the plant as a whole. It is particularly abundant in compression wood but scarce in tension wood. Lignin plays a crucial part in conducting water in plant stems. The cross-linking of polysaccharides by lignin is an obstacle for water absorption to the cell wall, thereby lignin makes it possible for the plant's vascular tissue to conduct water efficiently. Lignin biosynthesis begins in the cytosol with the synthesis of glycosylated monolignols from the amino acid phenylalanine (Srivastava *et al.* 2002). These first reactions are shared with the phenylpropanoid pathway. Highly lignified wood is an excellent fuel, since lignin yields more energy when burned than cellulose.

Molecular biology advances to enhance wood synthesis in the tree

In loblolly pine genomic approach was used to identify genes and proteins involved in cell wall biosynthesis during xylogenesis (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 cDNA sequencing and from preliminary 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; Ohashi-Ito *et al.* 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 uni-gene 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 previously unknown genes. Three 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 formation 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 from wood 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; Paux *et al.* 2004, 2005; Poke *et al.* 2005; Rengel *et al.* 2009; Elissetche *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 poplar, *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 Telewski 2006; Harfouche *et al.* 2010). Breeding of forest trees is a slow process mainly because of the long generation intervals typical of most forest trees and because many traits can only be properly assessed at rotation age (Fenning and Gershenzon 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 Friml 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 been successfully used earlier 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-Uggle *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 molecular basis of this process. 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 MONOPTEROS/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 Laux 2001). Until now, transgenic

research has focused on a handful of genes involved in secondary wall formation. Over the next 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 (Désjardin *et al.* 2004). Exploitation of the corresponding genes and of genes involved in the supra-molecular organization of the cell wall (interactions between polymers) is a promising additional tool that should 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; Nieminen *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, syngas 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 photosynthate 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 developments that ultimately are regulated by genes, or 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 separated from those under some other form of regulation. In almost all biological systems, simplistic theories on the regulation of multicellular developments are being replaced by ones of greater complexity as a result of continuing research that aims ultimately to understand the development at the level 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.

ACKNOWLEDGEMENTS

The authors are grateful to Director CSIR NEERI and the juries of the evaluation panel of the DBT sponsored International Conference on Bioenergy held between 8-10 October 2010, at CSIR NEERI, Nagpur, India to adjudge this premise best presentation; third generation R&D approach towards bioenergy production, and encourage us to write this review. Finally, the authors thank Dr. Jaime A. Teixeira da Silva for improving the grammar of the manuscript.

REFERENCES

- Allen J, Browne M, Hunter A, Boyd J, Palmer H (1998) Logistics management and costs of biomass fuel supply. *International Journal of Physical Distribution and Logistics Management* **28**, 463-477
- Aloni R (2010) The induction of vascular tissue by auxin. *Plant Hormones* **E**, 485-518
- Amezaga JM, von Maltitz G, Boyes S (2010) *Assessing the Sustainability of Bioenergy Projects in Developing Countries: A Framework for Policy Evaluation*, Newcastle University, UK, 179 pp
- Anterola AM, Lewis NG (2002) Trends in lignin modification: A comprehensive analysis of the effects of genetic manipulations/mutations on lignification and vascular integrity. *Phytochemistry* **61**, 221-294
- Antonova GF, Shebeko VV (1981) Formation of xylem in conifers. 1. Formation of annual wood increment in *Larix sibirica* shoots. *Lesovedenie* **4**, 36-43
- Antonova GF, Shebeko VV, Maljutina ES (1983) Seasonal dynamics of cambial activity and tracheid differentiation in Scots pine stem. *Chemistry of Wood (USSR)* **1**, 16-22
- Antonova GF, Stasova VV (1997) Effects of environmental factors on wood formation in larch (*Larix sibirica* Ldb.) stems. *Tree Structure and Function* **11**, 462-468
- Arnaud D, Déjardin A, Lepié J, Lesage-Descauses M, Boizot N, Villar M, Bénédetti H, Pilate G (2012) Expression analysis of *LIM* gene family in poplar, toward an updated phylogenetic classification. *BMC Research Notes* **5**, 102
- Barker JE (1979) Growth and wood properties of *Pinus radiata* in relation to applied ethylene. *New Zealand Journal for Science* **9**, 15-19
- Baucher M, Monties B, Montagu MV, Boerjan W (1998) Biosynthesis and genetic engineering of lignin. *Critical Reviews in Plant Sciences* **17**, 125-197
- Beaulieu J, Doerksen T, Boyle B, Clement S, Deslauriers M, Beauseigle S, Blais S, Poulin P L, Lenz P, Caron S, Rigault P, Bicho P, Bousquet J, MacKay J (2011) Association genetics of wood physical traits in the conifer white spruce and relationships with gene expression. *Genetics* **188**, 197-214
- Bell RL, Scorza R, Srinivasan C, Webb K (1999) Transformation of ‘Beurre Bosc’ pear with the *roIC* gene. *Journal American Society of Horticulture Science* **124**, 570-574
- Berndes G, Hoogwijk M, van den Broek R (2003) The contribution of biomass in future global energy supply: a review of 17 studies. *Biomass and Bioenergy* **25**, 1-28
- Biemelt S, Tschiersch H, Sonnwald U (2004) Impact of altered gibberellin metabolism on biomass accumulation, lignin biosynthesis, and photosynthesis in transgenic tobacco plants. *Plant Physiology* **135**, 254-265
- Björklund S, Antti H, Uddestrand Ida, Moritz T, Sundberg B (2007) Crosstalk between gibberellin and auxin in development of *Populus* wood: Gibberellin stimulates polar auxin transport and has a common transcriptome with auxin. *The Plant Journal* **52**, 499-511
- Boerjan W (2005) Biotechnology and the domestication of forest trees. *Current Opinion in Biotechnology* **16**, 159-166
- Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. *Annual Review of Plant Biology* **54**, 519-546
- Bomal C, Bedon F, Caron S, Mansfield SD, Levasseur C, Cooke JEK, Blais S, Tremblay L, Morency MJ, Pavy N, Grima-Pettenati J, Séguin A, MacKay J (2008) Involvement of *Pinus taeda* MYB1 and MYB8 in phenylpropanoid metabolism and secondary cell wall biogenesis: A comparative in plant analysis. *Journal of Experimental Botany* **59**, 3925-3939
- Borjesson P (1999) Environmental effects of energy crop cultivation in Sweden-I: identification and quantification. *Biomass and Bioenergy* **16**, 137-154
- Boudet AM, Kajita S, Grima-Pettenati J, Goffner D (2003) Lignins and lignocellulosics: A better control of synthesis for new and improved uses. *Trends in Plant Science* **8**, 576-581
- Boudet AM (2003) Towards an understanding of the supramolecular organization of the lignified wall. In: Rose J (Ed) *The Plant Cell Wall*, Blackwell Publishing, pp 155-182
- Bradley MV, Crane JC (1957) Gibberellin-stimulated cambial activity in stems of apricot spur shoots. *Science* **126**, 972
- Brown CL (1979) Physiology of wood formation in conifers. *Journal of Wood Science* **3**, 8-22
- Brown KM, Leopold AC (1973) Ethylene and the regulation of growth in pine. *Canadian Journal of Forest Research* **3**, 143-145
- Brunner AM, Nilsson O (2004) Revisiting tree maturation and floral initiation in the poplar functional genomics era. *New Phytologist* **164**, 43-51
- Catesson AM (1994) Cambial ultrastructure and biochemistry: changes in relation to vascular tissue differentiation and the seasonal cycle. *International Journal of Plant Sciences* **155**, 251-261
- Che D, Meagher RB, Heaton ACP, Lima A, Rugh CL, Merkle SA (2003) Expression of mercuric ion reductase in Eastern cottonwood (*Populus deltoides*) confers mercuric ion reductase and resistance. *Plant Biotechnology Journal* **1**, 311-319
- Cleland RE (1986) The role of hormones in wall loosening and plant growth. *Australian Journal of Plant Physiology* **13**, 93-103
- Cleland RE (2004) Auxin and cell elongation. In: Davies PJ (Ed) *Plant Hormones: Biosynthesis, Signal Transduction, Action* (Vol 3), Kluwer Academic Publishers, London, pp 204-220
- Coles JP, Phillips AL, Croker SJ, Garcia-Lepe R, Lewis MJ, Hedden P (1999) Modification of gibberellin production and plant development in *Arabidopsis* by sense and antisense expression of gibberellin 20-oxidase genes. *Plant Journal* **17**, 547-556
- Déjardin A, Lepié JC, Lesage-Descauses M-C, Costa G, Pilate G (2004) Expresed sequence tags from poplar wood tissues – a comparative analysis from multiple libraries. *Plant Biology* **6**, 55-64
- Demura T and Fukuda H (2007) Transcriptional regulation in wood formation. *Trends in Plant Science* **12**, 64-70
- Dettmer J, Elo A, Helariutta Y (2009) Hormone interactions during vascular development. *Plant Molecular Biology* **69**, 347-360
- Dharmawardhana P, Brunner AM, Steven H Strauss SH (2010) Genome-wide transcriptome analysis of the transition from primary to secondary stem development in *Populus trichocarpa*. *BMC Genomics* **11**, 150
- Ecosense (2007) Klimafaktor Biokraftstoff. Experten zur Nachhaltigkeitszertifizierung [Bioenergy and its Impact on the Climate: Experts Discuss Certification Ensuring Sustainability Criteria] (V 1), Schriftenreihe zu Nachhaltigkeit und CSR PROVIDE WEB-SITE Available online: <http://hdl.handle.net/10419/29708>
- Elissetche JP, Salas-Burgos A, Garcia R, Iturra C, Teixeira R, Rodriguez J, Valenzuela S (2011) Generation and analysis of expressed sequence tags (ESTs) from cambium tissue cDNA libraries of contrasting genotypes of *Eucalyptus globulus* Labill. *BMC Proceedings* **5**, 108
- Eriksson M, Moritz T, Israelsson M, Olsson O (2006) Transgenic trees exhibiting increased growth, biomass production, and xylem fibre length, and methods for their production. U.S. patent No. 7142422 B2
- Eriksson ME, Israelsson M, Olsson O, Moritz T (2000) Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. *Nature Biotechnology* **18**, 784-788
- Fearnside PM (1999) Plantation forestry in Brazil: the potential impacts of climatic change. *Biomass and Bioenergy* **16**, 91-102
- Fenning TM, Gershenzon J (2002) Where will the wood come from? Plantation forests and the role of biotechnology. *Trends in Biotechnology* **20**, 291-296
- Funada R, Miura T, Shimizu Y, Kinase T, Nakaba S, Kubo T, Sano Y (2008) Gibberellin-induced formation of tension wood in angiosperm trees. *Planta* **227**, 1409-1414
- Gaudin V, Vrain T, Jouanin L (1994) Bacterial genes modifying hormonal balances in plants. *Plant Physiology and Biochemistry* **32**, 11-29
- Gerbens-Leenes W, Hoekstra AY, van der Meer TH (2009) The water footprint of bioenergy. *Proceedings of National Academy of Sciences USA* **106**, 10219-10223
- Gielen D, Bos AJM, Gerlagh T (1998) Biomass for greenhouse gas emission reduction (BRED). In: *Ecologizing Societal Metabolism: Designing Scenarios for Sustainable Materials Management Conference*, November 21, 1998, Amsterdam, pp 1-15
- Grima-Pettenati J, Goffner D (1999) Lignin genetic engineering revisited. *Plant Science* **145**, 51-65
- Groover AT, Nieminen K, Helariutta Y, Mansfield SD (2010) Wood formation in *Populus*. In: Jansson S, Bhalerao RP, Groover AT (Eds) *Genetics and Genomics of Populus, Plant Genetics and Genomics: Crops and Models 8 (3)*, Springer Science + Business Media, LLC, pp 201-224
- Grünwald C, Deutsch F, Eckstein D, Fladung M (2000) Wood formation in *roIC* transgenic aspen trees. *Trees* **14**, 297-304

- Haacker A, Laux T** (2001) Cell-cell signaling in the shoot meristem. *Current Opinion in Plant Biology* **4**, 441-446
- Han K-H, Ko J-H, Yang SH** (2007) Optimizing lignocellulosic feedstock for improved biofuel productivity and processing. *Biofuels, Bioproducts and Biorefining* **1**, 135-146
- Hoogwijk M, Faaij A, Broek R, van de Berndes G, Gielen D, Turkenburg W** (2003) Exploration of the ranges of the global potential of biomass for energy. *Biomass and Bioenergy* **25**, 119-133
- Howel SH, Lall S, Che P** (2003) Cytokinins and shoot development. *Trends in Plant Science* **8**, 453-459
- Harfouche A, Meilan R, Altman A** (2011) Tree genetic engineering and applications to sustainable forestry and biomass production. *Trends in Biotechnology* **29**, 9-17
- Digby J, Wareing PF** (1966) The effect of applied growth hormones on cambial division and the differentiation of the cambial derivatives. *Annals of Botany* **30**, 539-548
- Jessup WR** (2009) Development and status of dedicated energy crops in the United States. *In Vitro Cellular and Developmental Biology – Plant* **45**, 282-290
- Junghans U, Langenfeld-Heyser R, Polle A, Teichmann T** (2004) Effect of auxin transport inhibitors and ethylene on the wood anatomy of poplar. *Plant Biology (Stuttgart)* **6**, 22-29
- Kaneyoshi J, Kobayashi S** (1999) Characteristics of transgenic trifoliolate orange (*Poncirus trifoliata* Raf.) possessing the *rolC* gene of *Agrobacterium rhizogenes* Ri plasmid. *Journal Japanese Society of Horticultural Science* **68**, 734-738
- Kartha S, Larson ED** (2000) *A Bioenergy Primer: Modernized Biomass Energy for Sustainable Development* (Vol IV, No 3), United Nations Development Programme, New York, pp 1-15
- Kawaoka A, Matsunaga E, Endo S, Kondo S, Yoshida K, Shinmyo A, Ebina H** (2003) Ectopic expression of a horseradish peroxidase enhances growth rate and increases oxidative stress resistance in hybrid aspen. *Plant Physiology* **132**, 1177-1185
- Kerckow B** (2007) Competition between agricultural and renewable energy production. *Quarterly Journal of International Agriculture* **46**, 333-347
- Kirst M, Johnson AF, Baucom C, Ulbrich E, Hubbard K, Staggs R, Paule C, Retzel E, Whetten R, Sederhoff R** (2003) Apparent homology of expressed genes from wood-forming tissues of loblolly pine (*Pinus taeda* L.) with *Arabidopsis thaliana*. *Proceedings of National Academy of Sciences USA* **100**, 7383-7388
- Kirst M, Myburg AA, De León, JPG, Kirst ME, Scott J, Sederhoff R** (2004) Coordinated genetic regulation of growth and lignin revealed by quantitative trait locus analysis of cDNA microarray data in an interspecific backcross of *Eucalyptus*. *Plant Physiology* **135**, 2368-2378
- Kitin P, Funada R, Ohtani J** (2000) Analysis by confocal microscopy of the cambium in the hard-wood *Kalopanax pictus*. *Annals of Botany* **86**, 1109-1117
- Klee HJ, Romano CP** (1994) The roles of phytohormones in development as studied in transgenic plants. *Critical Review in Plant Science* **13**, 311-324
- Kleine-Vehn J, Friml J** (2008) Polar targeting and endocytic recycling in auxin-dependent plant development. *Annual Review of Cell and Developmental Biology* **24**, 447-473
- Uggle C, Moritz T, Sandberg G, Sundberg B** (1996) Auxin as a positional signal in pattern formation in plants. *Proceedings of National Academy of Sciences USA* **93**, 9282-9286
- Koehler L, Telewski FW** (2006) Biomechanics and transgenic wood. *American Journal of Botany* **93**, 1433-1438
- Koornneef M, Alonso-Blanco C, Vreugdenhil D** (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annual Review of Plant Biology* **55**, 141-172
- Kurakawa T, Ueda N, Maekawa M, Kobayashi K, Kojima M, Nagato N, Sakakibara H, Kyojuka J** (2007) Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* **445**, 652-655
- Lachaud S** (1989) Participation of auxin and abscisic acid in the regulation of seasonal variations in cambial activity and xylogenesis. *Trees - Structure and Function* **3**, 125-137
- Lal R** (2009) Challenges and opportunities in soil organic matter research. *European Journal of Soil Science* **60**, 158-169
- Larson PR** (1994) The vascular cambium: Development and structure. In: Timell TE (Ed) *Series Wood Science*, Springer-Verlag, Heidelberg, Berlin, 725 pp
- Leyser O** (2001) Auxin signaling: The beginning, the middle and the end. *Current Opinion in Plant Biology* **4**, 382-386
- Little CHA, Bonga JM** (1974) Rest in the cambium of *Abies balsamea*. *Canadian Journal of Botany* **52**, 1723-1730
- Li X, Wu HX, Dillon SK, Southerton SG** (2009) Generation and analysis of expressed sequence tags from six developing xylem libraries in *Pinus radiata* D. Don. *BMC Genomics* **10**, 41
- Li X, Wu HX, Southerton SG** (2011) Transcriptome profiling of *Pinus radiata* juvenile wood with contrasting stiffness identifies putative candidate genes involved in microfibril orientation and cell wall mechanics. *BMC Genomics* **12**, 480
- Little CHA, Savidge RA** (1987) The role of plant growth regulators in forest tree cambial growth. *Plant Growth Regulation* **6**, 137-169
- Loomis RS, JG Torrey** (1964) Chemical control of vascular cambium initiation in isolated radish roots. *Proceedings of the National Academy of Sciences USA* **52**, 3-11
- Love J, Björklund S, Vahala J, Hertzberg M, Kangasjärvi J, Sundberg B** (2009) Ethylene is an endogenous stimulator of cell division in the cambial meristem of *Populus*. *Proceedings of National Academy of Sciences USA* **106**, 5984-5989
- Mauriat M, Moritz T** (2009) Analyses of GA20ox-and GID1-over-expressing aspen suggest that gibberellins play two distinct roles in wood formation. *The Plant Journal* **58**, 989-1003
- Meilan R, Auerbach DJ, Ma C, DiFazio SP, Strauss SH** (2002) Stability of herbicide resistance and GUS expression in transgenic hybrid poplars (*Populus* sp.) during four years of field trials and vegetative propagation. *HortScience* **37**, 277-280
- Mentag R, Luckevich M, Morency M-J, Séguin A** (2003) Bacterial disease resistance of transgenic hybrid poplar expressing the synthetic antimicrobial peptide D4E1. *Tree Physiology* **23**, 405-411
- Meyermans H, Morreel K, Lapiere C, Pollet B, De Bruyn A, Busson R, Herdewijn P, Devreese B, Van Beeumen J, Jane M, Marita JM, Ralph J, Chen C, Burggraeve B, Van Montagu M, Messens E, Boerjan W** (2000) Modifications in lignin and accumulation of phenolic glucosides in poplar xylem upon down-regulation of Caffeoyl-Coenzyme A O-Methyltransferase, an enzyme involved in lignin biosynthesis. *The Journal of Biological Chemistry* **275**, 36899-36909
- Meyers BC, Galbraith DW, Nelson T, Agrawal V** (2004) Methods for transcriptional profiling in plants. Be fruitful and replicate. *Plant Physiology* **135**, 637-652
- Miyawaki K, Tarkowski P, Matsumoto-Kitano M, Kato T, Sato S, Tarkowska D, Tabata S, Sandberg G, Kakimoto T** (2006) Roles of *Arabidopsis* ATP/ADP isopentenyltransferases and tRNA isopentenyltransferases in cytokinin biosynthesis. *Proceedings of National Academy of Sciences USA* **103**, 16598-16603
- Mohri T, Igasaki T, Futamura N, Sinojima K** (1999) Morphological changes in transgenic poplar induced by expression of the rice homeobox gene *OSH1*. *Plant Cell Reports* **18**, 816-819
- Morris DA, Friml J, Začimalová E** (2010) The transport of auxins. *Plant Hormones* **E**, 451-484
- Moyle R, Schrader J, Stenberg A, Olsson O, Saxena S, Sandberg G, Bhalerao RP** (2002) Environmental and auxin regulation of wood formation involves members of the *Aux/IAA* gene family in hybrid aspen. *The Plant Journal* **31**, 675-685
- Nieminen K, Lmmanen J, Laxell M, Kauppinen L, Tarkowski P, Dolezal K, Tähtiharju S, Elo A, Decourteix M, Ljung K, Bhalerao R, Keinonen K, Albert AV, Helariutta Y** (2008) Cytokinin signaling regulates cambial development in poplar. *Proceedings of National Academy of Sciences USA* **105**, 5020032-20037
- Nilsson J, Karlberg A, Antti H, Lopez-Vernaza M, Mellerowicz E, Perrot-Rechenmann C, Sandberg G, Bhalerao RP** (2008) Dissecting the molecular basis of the regulation of wood formation by auxin in hybrid aspen. *Plant Cell* **20**, 843-855
- Offermann R, Seidenberger T, Thrän D, Kaltschmitt M, Zinoviev S, Mierstus S** (2011) Assessment of global bioenergy potentials. *Mitigation and Adaptation Strategies for Global Change* **16**, 103-115
- Ohashi-Ito K, Oda Y, Fukuda H** (2010) *Arabidopsis* VASCULAR-RELATED NAC-DOMAIN6 directly regulates the genes that govern programmed cell death and secondary wall formation during xylem differentiation. *The Plant Cell* **22**, 3461-3473
- Ohashi-Ito K, Fukuda H** (2010) Transcriptional regulation of vascular cell fates. *Current Opinion in Plant Biology* **13**, 670-676
- Patrick JW** (1986) Hormonal control of assimilate partitioning. *Plant Growth Regulator Bulletin* **14**, 7-11
- Paux E, Tamasloukht M, Ladouce N, Sivadon P, Grima-Pettenati J** (2004) Identification of genes preferentially expressed during wood formation in *Eucalyptus*. *Plant Molecular Biology* **55**, 263-80
- Paux E, Carocha V, Marques C, de Sousa AM, Borralho N, Sivadon P, Grima-Pettenati J** (2005) Transcript profiling of *Eucalyptus* xylem genes during tension wood formation. *New Phytologist* **167**, 89-100
- Pavy N, Laroche J, Bousquet J, Mackay J** (2005) Large-scale statistical analysis of secondary xylem ESTs in pine. *Plant Molecular Biology* **57**, 203-224
- Pavy N, Paule C, Parsons L, Crow J, Morency MJ, Cooke J, Johnson JE, Noumen E, Claude C, Butterfield Y, Barber S, Yang G, Liu J, Stott JK, Siddiqui A, Holt R, Marra M, Sequin A, Retzel E, Bousquet J, Mackay J** (2005a) Generation, annotation, analysis and database integration of 16,500 white spruce EST clusters. *BMC Genomics* **6**, 144
- Pavy N, Johnson JJ, Crow JA, Paule C, Kunau T, MacKay J, Retzel EF** (2007) Forest Tree DB: a database dedicated to the mining of tree transcriptomes. *Nucleic Acids Research* **35**, D888-D894
- Pavy N, Boyle B, Nelson C, Paule C, Giguère I, Caron S, Parsons LS, Dallaire N, Bedon F, Bérubé H, Cooke J, Mackay J** (2008) Identification of conserved core xylem gene sets: Conifer cDNA microarray development, transcript profiling and computational analyses. *New Phytologist* **180**, 766-786

- Pavy N, Laroche J, Bousquet J, Mackay J (2005) Large-scale statistical analysis of secondary xylem ESTs in pine. *Plant Molecular Biology* **57**, 203-224
- Peña L, Séguin A (2001) Recent advances in the genetic transformation of trees. *Trends in Biotechnology* **19**, 500-506
- Plomion C, Leprovost G, Stokes A (2001) Wood formation in trees. *Plant Physiology* **127**, 1513-1523
- Poke FS, Vaillancourt RE, Potts BM, Reid JB (2005) Genomic research in *Eucalyptus*. *Genetica* **125**, 79-101
- Reed JW (2001) Roles and activities of Aux/IAA proteins in *Arabidopsis*. *Trends in Plant Sciences* **6**, 420-425
- Rengel D, Clemente HS, Servant F, Ladouce N, Paux E, Wincker P, Couxoux A, Sivadon P and Grima-Pettenati J (2009) A new genomic resource dedicated to wood formation in *Eucalyptus*. *BMC Plant Biology* **9**, 36
- Rigault P, Boyle B, Lepage P, Cooke JEK, Bousquet J, MacKay JJ (2011) A white spruce gene catalogue for conifer genome analyses. *Plant Physiology* **157**, 14-28
- Richet N, Afif D, Huber F, Pollet B, Banvoy J, Zein RE, Lapierre C, Dizengremel P, Perré P, Mireille Cabané M (2011) Cellulose and lignin biosynthesis is altered by ozone in wood of hybrid poplar (*Populus tremula* × *alba*). *Journal of Experimental Botany* **62**, 3575-3586
- Robert HS, Friml J (2009) Auxin and other signals on the move in plants. *Nature Chemical Biology* **5**, 325-332
- Ross JJ, O'Neill DP, Rathbone DA (2003) Auxin-gibberellin interactions in pea: Integrating the old with the new. *Journal of Plant Growth Regulation* **22**, 99-108
- Sagar A, Kartha S (2007) Bioenergy and sustainable development? *Annual Review of Environment and Resource* **32**, 131-167
- Saks Y, Feigenbaum P, Aloni R (1984) Regulatory effect of cytokinin on secondary xylem fibre formation in *in vivo* system. *Plant Physiology* **76**, 638-642
- Savidge RA (1983) Prospects for manipulating vascular-cambium productivity and xylem-cell differentiation. In: Cannell MGR, Jackson JE (Eds) *Attributes of Trees as Crop Plants*, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon, UK, pp 208-227
- Savidge RA, Wareing PF (1981) A tracheid-differentiation factor from pine needles. *Planta* **153**, 395-404
- Scarpella E, Meijer AH (2004) Pattern formation in the vascular system of monocot and dicot plant species. *New Phytologist* **164**, 209-242
- Sneddon C, Howarth RB, Norgaard RB (2006) Sustainable development in a post-Brundtland World. *Ecological Economics* **57**, 253-268
- Srivastava LM (2002) *Plant Growth and Development: Hormones and Environment*, Academic Press, UK, 757 pp
- Sterky F, Bhalerao R, Unneberg P, Segerman B, Nilsson P, Brunner A, Campaa LC, Lindvall J, Tandré K, Strauss SH, Sundberg B, Gustafsson P, Uhlen M, Bhalerao RP, Nilsson O, Sandberg G, Karlsson J, Lundeberg J, Jansson S (2004) A Populus EST resource for plant functional genomics. *Proceedings of National Academy of Sciences USA* **101**, 13951-13956
- Sundberg B, Uggla C, Tuominen H (2000) Cambial growth and auxin gradients. In: Savidge R, Barnett J, Napier R (Eds) *Cell and Molecular Biology of Wood Formation*, Oxford, UK: BIOS Scientific Publisher, pp 169-188
- Tang W, Tian Y (2003) Transgenic loblolly pine (*Pinus taeda* L.) plants expressing a modified d-endotoxin of *Bacillus thuringiensis* with enhanced resistance to *Dendrolimus punctatus* Walker and *Crypyothelea formosicola* Staud. *Journal of Experimental Botany* **54**, 835-844
- Telewski FW, Jaffe MJ (1986a) Thigmomorphogenesis: The role of ethylene in the response of *Pinus taeda* and *Abies fraseri* to mechanical perturbation. *Physiologia Plantarum* **66**, 227-233
- Telewski FW, Jaffe MJ (1986b) Thigmomorphogenesis: Anatomical, morphological and mechanical analysis of genetically different sibs of *Pinus taeda* in response to mechanical perturbation. *Physiologia Plantarum* **66**, 219-226
- Telewski FW, Wakefield AH, Jaffe MJ (1983) Computer-assisted image analysis of tissues of Ethrel-treated *Pinus taeda* seedlings. *Plant Physiology* **72**, 177-181
- Torrey JG, Fosket DE, Hepler PK (1971) Xylem formation: A paradigm of cytodifferentiation in higher plants: Plant cells divide and differentiate under the control of changing hormone levels. Xylem offers a model tissue for the study of these cellular events. *American Scientist* **59**, 338-352
- Tuominen H, Puech L, Fink S, Sundberg B (1997) A radial gradient of indole-3-acetic acid is related to secondary xylem development in hybrid aspen. *Plant Physiology* **115**, 577-585
- Tzfira T, Zuker A, Altman A (1998) Forest-tree biotechnology: Genetic transformation and its application to future forests. *Trends in Biotechnology* **16**, 439-446
- UNDP/WEC (2001) World energy assessment: energy and the challenges of sustainability. United Nations development Programme. United Nations Department of Economic and Social Affairs, New York, USA: World Energy Council
- van den Broek R, van Wijk A, Turkenburg W (2002) Electricity from energy crops in different settings – a country comparison between Nicaragua, Ireland and the Netherlands. *Biomass and Bioenergy* **22**, 79-98
- Vans ML (1985) The action of auxin on plant cell elongation. *CRC Critical Reviews in Plant Sciences* **2**, 317-365
- Von Blottnitz H, Curran MA (2007) A review of assessments conducted on bio-ethanol as a transportation fuel from a net energy, greenhouse gas, and environmental life cycle perspective. *Journal of Cleaner Production* **15**, 607-619
- Wang Q, Little CA, Oden PC (1997) Control of longitudinal and cambial growth by gibberellins and indole-3-acetic acid in current-year shoots of *Pinus sylvestris*. *Tree Physiology* **17**, 715-721
- Wareing PF (1958) The physiology of cambial activity. *Journal of the Institute of Wood Science* **1**, 34-42
- Wareing PF, Hanney CE, Digby J (1964) The role of endogenous hormones in cambial activity and xylem differentiation. In: Zimmermann MH (Ed) *The Formation of Wood in Forest Trees*, Academic Press, New York, pp 323-344
- WCED World Commission on Environment and Development (1987) *Our Common Future*, Oxford University Press, Oxford, 383 pp
- Widiez T, Hartman TG, Dudai N, Yan Q, Lawton M, Havkin-Frenkel D, Belanger FC (2011) Functional characterization of two new members of the caffeoyl CoA O-methyltransferase-like gene family from *Vanilla planifolia* reveals a new class of plastid-localized O-methyltransferases. *Plant Molecular Biology* **76**, 476-488
- Whetten R, Sederoff R (1991) Genetic engineering of wood. *Forest Ecology and Management* **43**, 301-316
- Whetten R, Sun YH, Zhang Y, Sederoff R (2001) Functional genomics and cell wall in loblolly pine. *Plant Molecular Biology* **47**, 275-291
- Wodzicki TJ, Rakowski K, Starck Z, Porandowski J, Zajczkowski S (1982) Apical control of xylem formation in the pine stem. Auxin effects and distribution of assimilates. *Acta Societatis Botanicorum Poloniae* **51**, 187-201
- Yang J, Pascal Kamdem D, Keathley DE, Han K-H (2004) Seasonal changes in gene expression at the sapwood – heartwood transition zone of black locust (*Robinia pseudoacacia*) revealed by cDNA microarray analysis. *Tree Physiology* **24**, 461-474
- Yang J, Park S, Kamdem DP, Keathley DE, Retzel E, Paule C, Kapur V, Han KH (2003) Novel gene expression profiles define the metabolic and physiological processes characteristic of wood and its extractive formation in a hardwood tree species, *Robinia pseudoacacia*. *Plant Molecular Biology* **52**, 935-956
- Yang S-H, Zyl LV, No EG, Loopstra CA (2003a) Microarray analysis of genes preferentially expressed in differentiating xylem of loblolly pine (*Pinus taeda*). *Plant Science* **166**, 1185-1195
- Zajczkowski S, Wodzicki TJ, Romberger JA (1984) Auxin waves and plant morphogenesis. In: Scott TK (Ed) *Hormonal Regulation of Development II. Encyclopedia of Plant Physiology* **10**, 244-262
- Zhang Y, Brown G, Whetten R, Loopstra CA, Neale D, Kieliszewski MJ, Sederoff R (2003) An arabinogalactan protein associated with secondary cell wall formation in differentiating xylem of loblolly pine. *Plant Molecular Biology* **52**, 91-102
- Zhang J, Elo A, Helariutta Y (2011) *Arabidopsis* as a model for wood formation. *Current Opinion in Biotechnology* **22**, 293-299
- Zhong R, Ye Z-H (2007) Regulation of cell wall biosynthesis. *Current Opinion in Plant Biology* **10**, 564-572
- Zhou J, Sebastian J, Lee J-Y (2011) Signaling and gene regulatory programs in plant vascular stem cells. *Genesis* **49**, 885-904
- Zhu LH, Holfors A, Ahlman A, Xue ZT (2001) Transformation of the apple rootstock M.9/29 with the *rolB* gene and its influence on rooting and growth. *Plant Science* **160**, 433-439