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Progress on genetic modifications of pulp wood tree species relevance to India - A review

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ABSTRACT

The major tree species grown for pulp and paper industry in India are eucalypts, poplars, casuarinas, subabul and acacias. There is a growing demand for pulp and paper products with minimum adverse effect on natural forest and environment. Genetic transformation in these pulp woods are aimed at enhancing growth, wood characteristics and stress tolerance. However, genetic transformation of trees is a time consuming process because of long life cycle, recent domestication status and recalcitrance to *in vitro* procedures. Though various instances of incorporating desired trait by transformations in trees have been reported, the effect of genetically modified trees on surrounding ecosystems need further studies. Efforts towards making transgenic trees should take in to consideration of alleviation of public concerns on pollen dispersal, contamination of wild germplasm and biosafety.

Key words: Genetic modifications, Growth, Pulp woods, Stress tolerance, Wood characteristics.

The major pulp wood species grown in India under farm or agroforestry plantations are eucalypts, poplars, casuarinas, subabul (Leucaena) and acacias (*Acacia auriculiformis* and *Acacia mangium*). While eucalypts are grown in many states of India, other trees are confined to local regions like poplars in Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand and Uttar Pradesh, casuarinas in coastal India, subabul in South and Central India and acacias in Orissa and South Indian states. The vegetative propagation techniques have developed for most of these pulp woods and clones are available for various agro climatic conditions across the country. However, only in eucalyptus, clones are extensively used for plantations with the support of paper mills. Since 1989, Bhadrachalam Paperboards Limited (now ITC Limited) pioneered the development and deployment of eucalyptus clonal plantations in India (Kulkarni and Lal, 1995).

According to Indian Pulp and Paper Technical Association (IPPTA) estimates (2012), the pulp and paper industry is expected to grow by seven per cent in 2013 and likely to touch 60,000 cores mark by 2025. This means a higher requirement for pulp in coming years from the plantations which are predominantly grown on marginal lands in India. There is an acute shortage of wood products and fuel globally as a consequence of a rapidly growing human population. There are huge opportunities for forest and tree biotechnology research focused on making wood products of better quality and faster availability with fewer negative effects on native forests and environment (Boerjan, 2005). Genetic modification of tree species has made significant progress in the last two decades and was aimed at enhancing growth, wood properties and imparting biotic and abiotc stress tolerance. The unraveling of genome sequence of poplar has resulted in surge of many genetic manipulation attempts in poplar and the recently completed *Eucalyptus grandis* genome work (Myburg *et al.*, 2014) could also open up more candidates for genetic modifications. This review summarizes the recent developments in genetic modification of pulp wood species which are extensively grown for pulp and paper industry in India.

Poplar: Poplar is the most widely grown tree cash crop in north and north western parts of India. Poplar enjoys wider acceptance among farming community because of its fast growth, ease in vegetative propagation and multiple uses such as plywood, pulp, veneer, fibre board, fuel, fodder etc. Approximate 80% of plywood production in north India depends on poplar wood and the remaining wood is used for match wood and pulp purpose.

Growth: The major goal in poplar research has been primarily to increase the stem biomass through an increase in height and diameter. Salyaev *et al*. (2006) transformed poplar with

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the gene encoding uridine diphosphoglycosyl-transferase, *ugt*, which catalyses the conjugation of IAA with glucose, allowing for a larger pool of IAA for transport. The height of the resultant *ugt* transgenics was about three times that of the control plants, and root elongation was greatly enhanced. Poplars over expressing an expansin gene, *PttEXPA1* was shown to have increased stem inter node length, increased leaf expansion, and larger cells in leaf epidermis (Gray-Mitsumune *et al.*, 2007). Additionall, poplar over expressing the *Vitreoscilla* haemoglobin gene showed no significant morphological differences, but three lines had noticeably higher height and growth rates (Zhang *et al.*, 2006). Although gains have been made in the area of increased biomass, there is a need for looking in to the fine balance between fiber quality and growth while altering the plant growth in trees.

Gibberellins influence growth and development in plants, including shoot growth, leaf growth and shape, flowering and seed germination. Overproduction of GA in hybrid poplar resulted in improved height, diameter, internode length and longer and broader leaves. The xylem cells in transgenics were significantly longer than those found in control plants (Eriksson *et al.*, 2000). Han *et al*. (2011) reported that PtGA20ox7 cisgene increased rate of shoot regeneration *in vitro* and accelerated early growth. The insertion of additional copies of native genes involved in growth may provide tools to modify plant architecture, and accelerate transfer of alleles between specied which are difficult to cross. Suppression of PtGA2ox4 and PtGA2ox5 led to increased biomass growth, but had no effect on root development. The degree of gene suppression in independent events was strongly associated with phenotypes, demonstrating dose-dependent control of growth by GA2ox RNA concentrations (Gou *et al.*, 2011).

SHORT-ROOT (SHR) is a well characterized regulator of cell division and cell fate determination in the Arabidopsis primary root. Down regulation of PtSHR1 led to a strong enhancement of primary (height) and secondary growth rates in the transgenic poplars. The results suggested that the response to SHR could be dose-dependent and that a partial down-regulation of SHR could lead to enhanced meristematic activity and a coordinated acceleration of plant growth in woody species. SHR suppression has considerable potential for accelerating biomass accumulation in a variety of species (Wang *et al*., 2011). An Arabidopsis small GTPase, RabG3b, was previously characterized as a component of autophagy and as a positive regulator of xylem development in Arabidopsis. Poplars transformed with RabG3b showed increased stem growth due to enhanced xylem development. The result suggested that Arabidopsis RabG3b could be functioning to regulate xylem growth through the activation of autophagy during wood formation in Populus (Kwon *et al*., 2011).

Nucleoside diphosphate kinase 2 (NDPK2) is known to regulate the expression of antioxidant genes in plants. Transgenic poplar (*Populus alba* × *Poplus glandulosa*) expressing the AtNDPK2 gene under the control of a oxidative stress inducible SWPA2 promoter was generated to develop plants with enhanced tolerance to oxidative stress. The growth of the plants was substantially increased under field conditions including increased branch number and stem diameter (Kim *et al.*, 2011).

Another area of exploration to increase the growth rate in trees has been to enhance the availability of nutrients through biotechnological interventions. The growth and development of the trees depends not only on the inorganic nitrogen available in the soil, but also on recycling within the plant, particularly in situations with limited nitrogen. Glutamine synthetase (GS1) plays a significant role in both nitrogen uptake and recycling, as it catalyzes the incorporation of ammonium into glutamine, the precursor to glutamate. The transgenic poplar overexpressing GS1 had enhanced cellular glutamine which accounts for the enhanced growth in GS transgenic poplars (Man *et al.*, 2011). Analysis of wood samples from a three year old field trial of three independently transformed GS1 transgenic hybrid poplar lines revealed that, when compared with wild-type controls, ectopic expression of GS1 resulted significant enhancements in wood fibre length, wood density, microfibre angle, per cent syringyl lignin and elevated concentrations of wood sugars, specifically glucose, galactose, mannose and xylose (Coleman *et al*., 2012).

Sulphur is an essential element found mostly in its reduced form as the aminoacids cysteine and methionine. Given the biological significance of sulphur in plant development, it has been a key target for genetic engineering in trees, particularly glutathione (GSH) formation. Numerous strategies have been used in designing transgenics over the years (Strohm *et al.*, 1995; Noctor *et al.*, 1996; Creissen *et al.*, 1996). Similarly, the over expression of glutathione reductase (GR) in the chloroplasts of poplar led to increased GSH pools (Foyer *et al.*, 1995).

Wood characteristics: Genetic modification in trees with respect to wood formation can be broadly classified in to two categories, lignin modification and cellulose or polysaccharide modification. Nearly almost all genes in the currently accepted lignin biosynthetic pathway has been altered in trees which has resulted in wide range of changes

in tree cell wall. The modification of cellulose biosynthetic pathway for the desired wood characteristics is also an area of interest in forest biotechnology research.

Lignin modifications: Hu *et al.* (1999) was the first to demonstrate the potential of genetic engineering for modifying lignin in trees for industrial applications. *Populus tremuloides* was transformed with antisense 4-coumarate:coenzyme ligase (*4CL*) constructs that resulted in a 45% reduction in lignin content, with no concurrent changes to lignin monomer composition. Leple *et al*. (2007) reported that lignin content was reduced by 50% with a orange-brown, often patchy coloration of the outer xylem when CCR was down regulated in poplar. The evaluation of five year old transgenic trees revealed improved pulping characteristics of the trees but with compromised in growth.

The other way of increasing the pulping efficiency without compromising on plant integrity is by modifying the lignin composition without changing the total lignin content. An increase in the lignin S/G monomer ratio has been clearly shown to improve the pulping efficiency (Stewart *et al.,* 2006; Mansfield and Weiniesen, 2007). The over expression of COMT a key enzyme in syringyl pathway under the regulation of the *Eucalyptus* CAD promoter resulted in only slight increases in COMT activity in some lines, but the increased COMT activity did not result in altered S/G ratios (Jouanin *et al.,* 2000). The down-regulation of caffeoyl-coenyzme A O-methyltransferase (*CCoAOMT*), resulted in transgenic lines of hybrid poplar displaying an 11% increase in the S/G ratio (Meyermans *et al.,* 2000). Li *et al.* (2003) reported lignin content reductions as low as 52% and concomitant increases in the S/G ratio when a combinatorial approach of simultaneous reduction of 4CL and over expression of (*CAld5H)* was employed in *P. tremuloides*

Cellulose or polysaccharide modification: Genetic modification of trees with an attempt to reduce the lignin contents resulted in increased cellulose content (Hu *et al.,* 1999 ; Li *et al.,* 2003). Park *et al.* (2004) could successfully increase the cellulose and decreased xyloglucan contents in *P. alba* by expressing a fungal xyloglucanase gene. Similarly, *P. tremula* transformed with an *Arabidopsis* endoglucanase (*cel1*) were shown to have a 10% increase in cellulose content (Shani *et al.,* 2004). Coleman *et al.* (2007) reported transgenic *P. alba* × *P. grandidentata* trees expressing a bacterial UDP-glucose pyrophosphorylase (UGPase) gene had substantially increased cellulose content, and decreased lignin.

Constitutive expression of the FPF1 gene in hybrid aspen (*Populus tremula L.* × *P.tremuloides* Michx.) showed a strong effect on wood formation. Chemical screening of the wood by pyrolysis GC/MS showed that FPF1 transgenics have higher fractions of cellulose and glucomannan products as well as lower lignin content (Hoenicka *et al.,* 2012). Two members of glycosyl transferase protein families, PtrGT8D1 and PtrGT8D2, in *Populus trichocarpa* were down regulated by RNAi. The analysis of the transgenic line resulted in 29-36% reduction in stem wood xylan content without affecting cellulose quantity (Li *et al.,* 2011).The identification of functioning xyloglucan transglycosylases (XETs) in developing secondary xylem in aspen (Bourquin *et al.*, 2002) and its involvement in the formation of the G layer in tension wood (Nishikubo *et al.*, 2007) suggested that XET may be a potential target to modify cellulose properties.

Stress tolerance: The environmental stress either biotic or abiotic can affect the productivity of trees. There are various groups of plants and microbes which can with stand the stress and harsh conditions, has been explored with genomic tools for potential genes and gene products. The increased resistance to many types of stresses has already been achieved for several plant species.

Abiotic stress: Poplar transformed with a pine cytosolic GS (GS1) was shown to be more tolerant to drought than wild-type trees (El-Khatib *et al.,* 2004). At all levels of water availability, the transgenic trees had higher photosynthetic assimilation rates and stomatal conductance than the corresponding controls. The ascorbate-glutathione pathway plays an important role in protecting plants from reactive oxygen species (ROS). GSH acts as an antioxidant that can directly scavenge ROS, and also protect thiol-containing enzymes. Poplar with GR over expressed in the chloroplast recovered more quickly when exposed to high light levels and low temperatures than did wild-type trees (Foyer *et al.,* 1995; Strohm *et al.,* 1995). Hybrid aspen (*P. sieboldii* x *P. grandidentata*) over expressing the horseradish peroxidase gene (*prxC1a*) showed increased growth and elevated peroxidase activity (Kawaoka *et al.,* 2003).

The salt stress in plants is becoming a major issue which is resulted from water deficit or accumulation of ions and affecting productivity of plantations. Poplar transformed with the *E. coli* mannitol-1-phosphate dehydrogenase gene (*mt1D*) grew faster and had a higher survival rate than non-transformed controls. The up-regulation of *mt1D* in poplar led to increased mannitol levels and under salt stress, all lines had higher stomatal conductance, transpiration and photosynthetic rates (Liu *et al.,* 2000; Hu *et al.* 2005). Other species have also demonstrated gains in salt tolerance when transformed with *mt1D*, glucitol-6-phosphate dehydrogenase (*gutD*), choline dehydrogenase (*betA*) and choline oxidase (*codA*) genes.

The transcription factors assumes a special attention for imparting stress tolerance because of their potential to increase tolerance to multiple stresses via the over expression of a single transcription factor. For example, the over expression of the dehydration response element 1A (DREB1A) transcription factor in *Arabidopsis* caused increased tolerance to multiple abiotic stresses.(Kasuga *et al.*, 1999; Jaglo-Ottosen *et al.*, 1998).

Biotic stress: Damage to forest trees caused by both native and introduced pests is of global importance. These biotic stresses can affect forest growth and productivity, with substantial economic consequences.

Bacillus thuringinesis has been a source for control of lepidopteran pests with the help of Bt toxins. Genetically modified trees which can produce forms of Bt toxin offers an appealing alternative for establishing plantations that are resistant to damage from a broad range of pests. Mc Cown *et al.* (1991) were the first to report on poplars that were stably transformed with a Bt toxin gene. One example of successful engineering of poplar trees to combat an insect pest is found in the cottonwood leaf beetle (CLB), the primary insect pest in poplar plantations. Virtually all of the Bt transgenics showed very low feeding damage, whereas the non-transgenic lines sustained significantly higher levels of defoliation (Meilan *et al.*, 2000). Leaves of transgenic *Populus tremula* × *P. tremuloides* expressing a synthetic form of Bt toxin (*Cry3Aa*) proved to be highly effective in resisting damage by the phytophagous beetle *Chrysomela tremulae* (Genissel *et al.*, 2003). Davis et al (2006) evaluated the effects of a Bt transgene on wood properties in hybrid poplars, and found no significant difference in chemical composition.

A greater success was achieved in white poplar (*P. alba*) expressing an *Arabidopsis* cysteine proteinase inhibitor (*Atcys*), which resulted in up to 100% mortality of chrysomelid beetle (*Chrysomela populi*) larvae after only 16 days of feeding on transgenic leaf tissue (Delledonne *et al.*, 2001). The expression of the scorpion neurotoxin, AaIT, in hybrid poplars appeared to impart resistance against the gypsy moth (Wu *et al.*, 2000).

Genetic modifications using a variety of genes from several plants have been evaluated to improve fungal pathogen resistance, and have met with varying success. Expression of the bacterioopsin gene in tobacco suggested that defense mechanisms would be elicited by its expression leading to increased pathogen resistance (Mittler *et al.*, 1995). However, the expression of a synthetic bacterio-opsin gene in hybrid poplars did not elicit a significant increase in defense response against a variety of fungal pathogens (Mohamed *et al.*, 2001). Similarly, white poplar expressing grapevine stilbene synthase (StSy), which has been implicated in the production of resveratrol compounds, did not significantly affect the efficacy of resistance against a rust disease by *Melampsora pulcherrima* (Giorcelli *et al.*, 2004).

In contrast, transgenic poplars expressing a rabbit defensin gene (*NP-1*) Zhao *et al.*, 1999) or a chitinase (*CH5B*) appear to have increased resistance to a broad spectrum of fungal pathogens (Meng *et al.*, 2004). Hybrid poplars expressing a wheat germin-like oxalate oxidase gene, directed at metabolizing the oxalic acid produced by fungal pathogens, showed signs of delayed infection by *Davidiellapopulorum* (syn. *Septoria musiva*) (Liang *et al.*, 2001). It is evident that increasing resistance against fungal pathogens requires the use of a variety, and perhaps a combination, of transgenic products.

Reports of genetic modifications resulting in increased resistance to bacterial pathogens are less common. Transgenic poplar expressing the antimicrobial peptide, D4E1, showed mixed resistance against *Agrobacterium* and *Xanthomonas* infection (Mentag *et al.*, 2000). In particular, the transgenic line displaying the highest transgene transcript abundance showed a significant increase in resistance, as defined by reduced tumour formation after *Agrobacterium* inoculation or the development of smaller cankers following *Xanthomonas* infection.

Eucalypts: Eucalypts are widely grown in India for pulp and paper purpose and the modification of eucalyptus with respect to growth, wood properties and stress tolerance are discussed in this section.

Growth: Biomass enhancement can be achieved through manipulation of cellulose synthase genes or precursors of cellulose biosynthesis. Cellulose binding domain (Cbd) are shown to modulate the elongation of plant cells *in vitro* while the gene *Cel1* an endoglucanase is implicated in cell wall enlargement. Over expression of these genes in plants can induce elongation of cells more rapidly which in turn results in faster growth of eucalyptus trees (Shani *et al*., 2004).The gene EcHB1, isolated from *E. camaldulensis* encoding for a HD-ZIP class II protein driven by CaMV 35S promoter, has been introduced into tobacco. These transgenic plants showed greater fiber length (20%) and increased plant height (50%) when compared to the wild type (Sonoda *et al*., 2009). *Eucalyptus globulus* is one of the most economically important plantation hardwoods for paper making. However, its low transformation frequency has prevented genetic engineering of this species with useful genes. Matsunaga *et al.* (2012) reported an efficient regeneration system for

E. globulus when shoot regeneration from hypocotyls with shoot apex was employed which resulted in 12-fold higher regeneration than that from hypocotyls without shoot apex. The plants transformed with salt tolerance gene, namely a bacterial choline oxidase gene (codA) showed salt tolerance up to 300 mM NaCl

Wood characteristics: Wood characteristics of the trees are highly influenced by lignin content. However, high amount of lignin is undesirable for paper manufacturing because the residual lignin in the wood fibers results in discoloration and reduced brightness of the pulp (Chiang *et al*., 1988). Hence, it is mandatory for paper industries to remove lignin during pulping process without damaging the cellulose polysaccharides.

Regulatory sequences and genes coding for different enzymes in the monolignol biosynthetic pathways are of interest to researchers in tree genetic modifications. Chen *et al*. (2001) successfully developed two transgenic *E. camaldulensis* events harboring aspen C4H (cinnamate-4-hydroxylase) gene both in sense and anti-sense orientation with an aim to alter the quantity and quality of lignin. In an another investigation, the genome of hybrid eucalyptus (*E. grandis* × *E. urophylla*) was transformed with anti-sense DNA sequence of CAD enzyme (Cinnamyl Alcohol Dehydrogenase) from *E. gunni* using agrobacterium mediated transformation (Tournier *et al*., 2003). Among the 120 transformants which have been recovered, 58% showed significant inhibition of CAD activity. Valerio *et al*., (2003) transformed *E. camaldulensis* with antisense CAD to suppress the CAD activity. A total of 44 transgenic lines were generated of which 32% exhibited up to 83% reduction in CAD activity. However, it had been reported that after 10 months of growth in glasshouse, none of the five transgenic lines tested, showed any change in lignin profiles or CAD activity when compared to untransformed control plants.

So far in lignin modifying efforts, two different transcription factors have been used in eucalyptus transformation works. Anti-sense construct of tobacco transcription factor Ntlim1 that specifically binds to phenyl alanine ammonia lyase (PAL box sequence) and inhibit transcription of few phenylpropanoid pathway genes such as phenylalanine ammonia lyase (PAL), 4-Coumarate Ligase (4CL) and CAD, has been introduced into the genome of *E. camaldulensis*. As expected, the transgenic events showed down regulation of lignin biosynthetic genes and few of the transgenic events showed reduced lignin content by 20–29% in cell wall residues of stem xylem tissues (Kawaoka *et al*., 2006). Reduction of lignin content in *E. camaldulensis* by the suppression of gene expression using LIM domain transcription factor, Ecliml, isolated from the *E. camaldulensis* is reported by Kawaoka *et al*. (2006). These transgenic eucalyptus plants grown in the glass house showed decreased expression of several lignin biosynthesis genes such as PAL, C4H and 4CL.

CCR allelic variation has been correlated with variation in microfibrillar angle in *E. nitens* using association mapping (Thumma *et al*., 2005). Other genes of interest which are found to influence the microfibrillar orientation in the cellulose secondary cell wall are FRA1 coding for a kinesin-like protein. In Arabidopsis inflorescence stems, FRA1 is known to influence the mechanical strength of fibers and is proposed to be involved in microtubule control as well as cellulose microfibrillar order (Zhong *et al*., 2002). Recently, Fasciclin like arabinogalactan (FLAs) proteins were identified to be specialized in stem biomechanics and cell wall architecture in eucalyptus (MacMillan *et al*., 2010). The over expression of NAC family member SND2 protein resulted in increased fiber cross-sectional area in eucalyptus. The results also indicated phenotypic differences in the effect of SND2 over expression between woody and herbaceous stems like arabidopsis and emphasized the importance of expression thresholds in transcription factor studies (Hussey *et al*., 2011).

Biotic and abiotic stress resistance: Insect and herbicide resistant transgenic eucalyptus are likely to provide better pest and weed control options in plantations, particularly during the early vulnerable establishment phase. Plants conferring resistance to chrysomelid beetles and tolerance to the herbicide ammonium glufosinate were produced by Harcourt *et al*. (2000) with insecticidal cry3A and bargenes, respectively. Shao *et al*., (2002) developed transgenic *E. urophylla* events showing an increased resistance by 35% against the bacterial wilt disease caused by the pathogen, *Pseudomonas solanacearum*.

Navarro *et al*. (2010) reported that two cold induced transcription factors that specifically bind to C binding factor genes namely EguCBF1a/b were isolated from *E. gunni* and constitutively over-expressed in a cold sensitive eucalyptus hybrids. The transgenic trees showed improved freeze tolerance and opened new avenues for freeze tolerant research in eucalyptus. Enhancing soil salinization of arable land has a major impact on the global ecosystem. One approach to have more usable forest area is to develop transgenic trees with higher tolerance to salt stress. In order to impart salinity tolerance. Yu *et al*. (2012) developed salt-tolerant eucalyptus trees, by introducing mangrin gene (allene oxide cyclase homolog) and found that seven of 36 transgenic genotypes had significantly higher salt tolerance than non-transformants, and more importantly, that these plants had no significant impact on environmental biosafety.

Subabul: Subabul **(***Leucaena leucocephala*) is a fast growing multipurpose tree adapted to a variety of soils and climatic conditions and 25% raw material for paper and pulp industry comes from this tree. Limited work is been carried out in subabul by biotechnological interventions to increase the desired traits.

Removal of lignin is a major hurdle for obtaining good quality pulp and trees with reduced lignin content will be first step in this direction. Efforts were carried out to down regulate lignin biosynthesis in *Leucaena leucocephala* by using the gene encoding for an enzyme O-methyltransferase (OMT) in antisense orientation. The evaluation of total lignin content by the Klason method revealed a reduction of 28% lignin and the plants displayed a normal phenotype. Concomitantly, an increase of up to 9% in cellulose content was also observed. The results together suggested that the extent of down-regulation of OMT activity may lead to quality amelioration of Leucaena with respect to its applicability in pulp and paper manufacture (Rastogi and Dwivedi 2006). Srivastava *et al*. (2011) could correlate CCR expression pattern and quantity of lignin produced which makes it a good target for genetic engineering. Prashanth *et al*. (2011) studied the up and down regulation of CCR isolated from *Leucaena leucocephala* by using sense and antisense constructs in Tobacco. The CCR down regulated lines showed a poor lignifications of S2 and S3 wall layers and enhanced syringyl to guaiacyl ratio while the CCR unregulated lines exhibited robust growth and higher lignin content.

Jube and Borthakur (2009) developed a transformation protocol for Leucaena using phosphinothricin resistance as the plant selectable marker. Explants obtained from immature zygotic embryos were infected with *Agrobacterium tumefaciens* strain C58C1 containing the binary plasmid pCAMBIA3201 produced four putative transformed Leucaena plants with transformation efficiency of 2%. Leucaena is also used as a perennial fodder because of its fast-growing foliage, which is high in protein content. However, the use of subabul is limited as fodder due to the presence of the toxin, mimosine. Jube *et al*. (2010) used two bacterial genes pydA and pydB, encoding a meta-cleavage dioxygenase and a pyruvate hydrolase respectively for transformation. Transgenic plants expressing pydA were reduced up to 22.5% in mimosine comparison to the wild-type while there was no significant reduction for pydB-expressing lines.

Casuarina: *Casuarina equisetifolia* is a multipurpose tree, widely used in the afforestation and waste land development programmes. These trees are widely grown in coastal areas of Andhra Pradesh, Orissa, Tamil Nadu, West Bengal and Gujarat.

Transgenic calli of the tropical tree *Casuarina glauca* were produced using agrobacterium mediated gene transfer (Le *et al.,* 1996). The optimal transformation rates were obtained when explants from 45-day old seedlings were co cultivated for 3 days in presence of 25 pM acetosyringone. Obertello *et al*. (2007) studied the promoter region of a metallothionein (MT)-like gene (PcgMT1) from *C. glauca* using a beta-glucuronidase (gus) fusion approach in transgenic plants of Arabidopsis *thaliana*. Strong PcgMT1-gus expression was observed when transgenic plants were inoculated with a virulent strain of the bacterial pathogen *Xanthomonas campestris pv. campestris*. Wounding and H_2O_2 treatments led to an increase in reporter gene activity in transgenic leaves and the result suggested cgMT1 could play a role during the oxidative response linked to biotic and abiotic stresses. Satheesh kumar and Gupta (2012) studied various parameters necessary for the genetic modification of casuarinas using biolistic gene delivery system. The chimeric calli after three months of selection with appropriate antibiotic showed the transgene integration by genomic PCR.

Acacia: Very few reports are available with respect to genetic transformation work in Acacia related species. An efficient plant regeneration system was established from immature leaflet-derived callus of *Acacia confusa* Merr, through organogenesis. Rooted plantlets were hardened and successfully established in soil. The field-established plants were morphologically normal and fertile (Armugam *et al*., 2009). Transgenic herbicide tolerant *Acacia sinuata* plants were produced by transformation with the bar gene conferring phosphinothricin resistance (Vengadesan *et al.,* 2006).

Issues and concerns: The value of transgenic trees will only be realized after the completion of extensive field trials. More than 700 field trials with GM trees of 30 genera were reported in 2012, most of them were in the United States with Poplar, Pinus and Eucalyptus species. There were 84 field trials with Populus and Robinia in China and 18 trials of GM eucalyptus in Brazil. Commercial release of GM forest trees has been reported in China and approval was granted for the environmental release of two kinds of Bt trees, the European black poplar (*Populus nigra*) and the hybrid white poplar clone GM 741, together representing about 1.4 million plants on 300-500 hectares.

In the area of pest and pathogen resistance, a number of field trials have been reported, and some trials have yielded contrasting findings. For example, a three year field trial of transgenic birch expressing sugar beet chitinase revealed that although plants showed greater resistance in green house trials, in field conditions the trees were susceptible to fungal diseases such as leaf spot, caused by *Pyrenopeziza betulicola*

(Pasonen *et al.*, 2004). At the same time, a three-year trial of Bt-transgenic *Populus nigra* showed 44 to 100% mortality of larvae feeding on transgenic tree tissues from field-tested plants in comparison with control plants which showed 37% mortality (Hu *et al.*, 2001; Pasonen *et al.*, 2004). Similarly, lignin modification in trees resulted in contrasting phenotypes as in the case of CAD modification. Field trial of hybrid poplars (*P. tremula* × *P. alba*) engineered for CAD activity resulted in greater ease of delignification and superior yield in CAD suppressed lines. In contrast transgenic *Eucalyptus* with reduced CAD expression (antisense) resulted in no change in lignin quality and composition, or pulp yields (Tournier *et al.*, 2003; Pilate *et al.*, 2002).

There are limited reports on impact of genetically engineered trees on ecosystems. Although over 700 field trials exist throughout the world no reports are available on evaluation of transgenic trees over the normal rotation of a forest plantation. More recently, four year old poplar modified for CAD and COMT genes were evaluated for dynamics of microbial communities surrounding the transgenic plants. The work suggested that depending on what modifications were done, there may be little or no negative ecological impacts of growing transgenic trees (Halpin *et al.*, 2007). However, it must be emphasized that ecological studies are complicated and critically needed in order to assess the impact of transgenic trees. Furthermore, longer term trials will be required to fully appreciate the potential for unexpected changes and effects. Measuring changes in the transcriptome and metabolome of CAD and CCR modified tobacco plants had revealed that altering one gene in the lignin biosynthetic pathway affected the expression of other genes within the same pathway, as well as genes involved in detoxification, carbohydrate metabolism, and photorespiration (Dauwe *et al.*, 2007). The metabolism and transcript changes in response to CCR down-regulation in *P. tremula* × *P. alba*, which led to decrease in total lignin and an increase in G monomer units, suggested that a stress response was elicited (Leple *et al.*, 2007).

The longer life cycle and out crossing nature of trees can disperse the seed and pollen for larger distance. Pollen dissemination from transgenic trees is an area of concern and until now very limited success has been achieved in controlling pollen production in trees. Very often trees are likely to be planted within potential mating proximity of wild, compatible populations of related species leading to contamination of wild germplasm (DiFazio *et al.*, 1999). The various approaches for controlling transgenic pollen has been discussed in various reviews (Strauss *et al.*, 1995; Meilan and Strauss 1997). However, the successful cases of preventing transgenic pollen flow are low. Recently, the expression cassette of the PrMC2-barnaseH102E was found to efficiently ablate pollen in tobacco, pine and eucalyptus. The field performance of the PrMC2-barnaseH102E in representative angiosperm and gymnosperm trees indicated that this gene can be used to mitigate pollen mediated gene flow concerns associated with large scale deployment of transgenic trees (Zhang *et al*., 2012).

CONCLUSIONS

Genetic transformation of trees is a time consuming process because of various factors like long life cycle, the recent domestication status and their recalcitrance to *invitro* culture procedures. Under field conditions, trees interact differently with the surrounding ecosystem compared with any other crops of economic importance. Therefore tree crops are not generally considered for genetic modification approaches, exceptions being the fast growing trees like poplar, eucalyptus, subabul, acacia and casuarinas.

The biotechnological interventions happened in agriculture has direct applications for forestry. However, there are major differences originating from the fundamental characteristics of trees like long life cycle and out crossing nature. Efforts towards making transgenic trees should take in to consideration of alleviation of public concerns on pollen dispersal, contamination of wild germplasm and biosafety. The gap between biotechnology and gaining public acceptance is a matter of concern in tree species which can only circumvented by extensive field trials and assessing the impact on ecosystem.

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