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Review

Genetic transformation of forest trees

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In this review, the recent progress on genetic transformation of forest trees were discussed. Its described also, different applications of genetic engineering for improving forest trees or understanding the mechanisms governing genes expression in woody plants.

Key words: Genetic transformation, transgenic forest trees, gene expression.

INTRODUCTION

Forest species predominate temperate and equatorial zones and the wood produced by trees is the most abundant biological material on the earth's surface (Gammie, 1981). Wood provides fuel for most of the population of the world particularly in the developing countries, and wood is a leading industrial raw material. Despite its economic importance, the production of wood is under the threat of population growth, desertification, industries development and attack by many parasites. The classical techniques such as crossing, sexual and somatic hybridization, and breeding give a genetic blind mixture. These techniques are limited by the sterility of the descents, the genetic barrier between species and the long cycle for certain trees (for review see Sederoff, 1995). Now this genetic barrier can be overcome by introducing one or few well defined new characteristics without affecting the global architecture and the plant phenotype. Presently, only genetic transformation technology offers this possibility. Genetic transformation can be defined as a controlled introduction of exogenous genetic material into the nuclear or cytoplasmic genome of an organism in stable and inheritable manner.

This review lists the main genes introduced in forest trees species as well as the use of genetic transformation for studying genes expression in woody plants.

GENETIC TRANSFORMATION OF FOREST TREES FOR SELECTED TRAITS

Disease resistances

Forest trees can be attacked by several diseases including those caused by white pine blister rust (*Cronartium ribicola*), Dutch elm disease (*Ceratocystis ulmi*), or chestnut blight (*Endothia parasitica*). Insect pests, whether generally endemic, such as Southern pine bark beetle (*Dendroctonus frontalis*), or epidemic, such as gypsy moth (*Lymantria dispar*), are serious concern in forest ecology and management. These parasites cause much damages reducing forest tree products around the world. Genetic transformation using gene coding for Bt or proteinase inhibitors could lead to reduced damage and chemicals usage in the environment. Bt toxins bind to the epithelial glycoproteins of the intestine of insects, especially the midgut, and cause fatal leakage of fluids between the intestine and the hemocoel (Höfte and Whiteley, 1989). The most easily manipulated genotype of the pine trees, the hybrid *Populus* (*Populus alba* X *Populus grandidentata*) and Larch were transformed by *35S-Bt* (modified endotoxin gene from *Bacillus thuringiensis*). One of the regenerated plants was highly

resistant to feeding of two lepidopteran pests, the forest tent caterpillar (*Malacosoma disstria*) and the gypsy moth (*L. dispar*) (Schuler et al., 1998). For forest trees, the introduction of gene coding for antimicrobial and antifungal proteins is in the early stage of development. Clear results have only been obtained with antifungal proteins by introducing wheat oxalate oxidase gene in poplar but the test showed that the trees are not completely resistant against the disease (Liang et al., 2001).

Herbicide resistance

The first report on genetic transformation of forest trees was achieved by Fillatti et al. (1987) who introduced the *aroA* gene in the poplar by using the wild *Agrobacterium tumefaciens* strain C58/587/85. The *aroA* gene codes for 5-enolpyruvylshikimate synthase that is active in the synthesis of aromatic amino acids. Transformation was confirmed by Southern and Western blotting, and the transformed poplar were resistant to glyphosate at levels of 0.07 kg/ha (Fillatti et al., 1988). Trees transformed with *bar* gene were also tolerant to herbicide (for review see Sederoff, 1995).

Shorter cycle

The long juvenile phase of forest trees is the main constraint for their genetic improvement and delays their exploitation or mature trait analyses. For these reasons, the introduction of genes encoding products controlling plant cycle will be very helpful for forest trees improvement. In order to achieve this objective, the homeotic gene *LEAFY* (*LFY*) from *Arabidopsis thaliana*, which encodes products governing early flowering initiation was introduced in aspen (*Populus tremula* X *Populus tremuloides*). The results showed that the flowering stage was induced after 7 months instead to 8-20 years (Nilsson and Weigel, 1997) but the expression pattern varied among the interspecific *Populus* hybrids (Martín-Trillo and Martínez-Zapater, 2002) suggesting that the mechanisms controlling the expression of the homeotic genes are conserved between crops and trees and open up the possibility to improve forest trees (Rottmann et al., 2000). Recent studies show that homologs to *LFY* gene, the *PTFL* gene from *Populus* is able to induce early flowering in poplar.

Phytohormones level

The objective to modify phytohormones level in forest trees was to increase tree size, biomass production or wood quality. Introduction of the GA 20-oxidase gene from *Arabidopsis* in hybrid aspen has resulted in fast growth in diameter and height, large leaves, more numerous and longer xylem fiber and increasing biomass (Eriksson et al., 2000). This gene could be used to increase biomass production in forest trees or the use of its antisense can reduce trees size, which makes for easier harvesting. In Walnut, the expression of chalcone synthase decreases flavonoids synthetis and enhances the production of adventitious roots (El Euch et al., 1998).

Reduction of lignin

Lignins, the second most abundant compound (15-35% of the dry wood) in the biosphere after cellulose, are formed in cell walls and between cells of woody tissue by polymerization of monomeric precursors such as sinapyl and coniferyl alcohols. Its extraction from the wood is a costly process for the paper industry and generates great quantities of chemical pollutants. Lignin has also been identified as a major component limiting forage digestibility and its genetic or biotechnological modification in pasture plants is a desirable goal in the development of new forages (Waston, 1990). For these reasons, most studies were focused on the biochemical pathways in lignin biosynthesis by isolating and characterizing several genes encoding enzymes which play a key role in monolignols synthesis. These enzymes are mainly O-methyltransferase (OMT), 4-coumarate-CoA ligase (4CL) and cinnamyl alcool deshydrogenase (CAD). OMT is the enzyme catalyzing methoxylation of lignin precursors to form sinapic and ferulic acids, which are reduced respectively to sinapoylCoA and feruloylCoA by 4CL. CAD has been considered a key enzyme in the lignin biosynthesis because it catalyses the final step in the synthesis of the monolignols by converting the cinnamaldehydes to the corresponding alcohols. The genes encoding these enzymes have been isolated and characterized in different plant systems, allowing for the future modification lignin quality and quantity in forest trees. To achieve this objective the antisense of OMT gene under the control of the cauliflower mosaic virus 35S promoter was introduced in *Populus*. The generated transgenic trees showed reduction of OMT activity, modification of lignin amount and composition (Van Doorsselaere et al., 1995; Jouanin et al., 2000). Recently, reduced lignin content was obtained by down regulation of 4CL or CAD. However, increase of cellulose content and alteration of hemicellulose composition were observed in transgenic trees expressing the antisense (Baucher et al., 1996; Hu et al., 1999).

Nitrogen metabolism

Nitrogen availability is one of the main constraints for

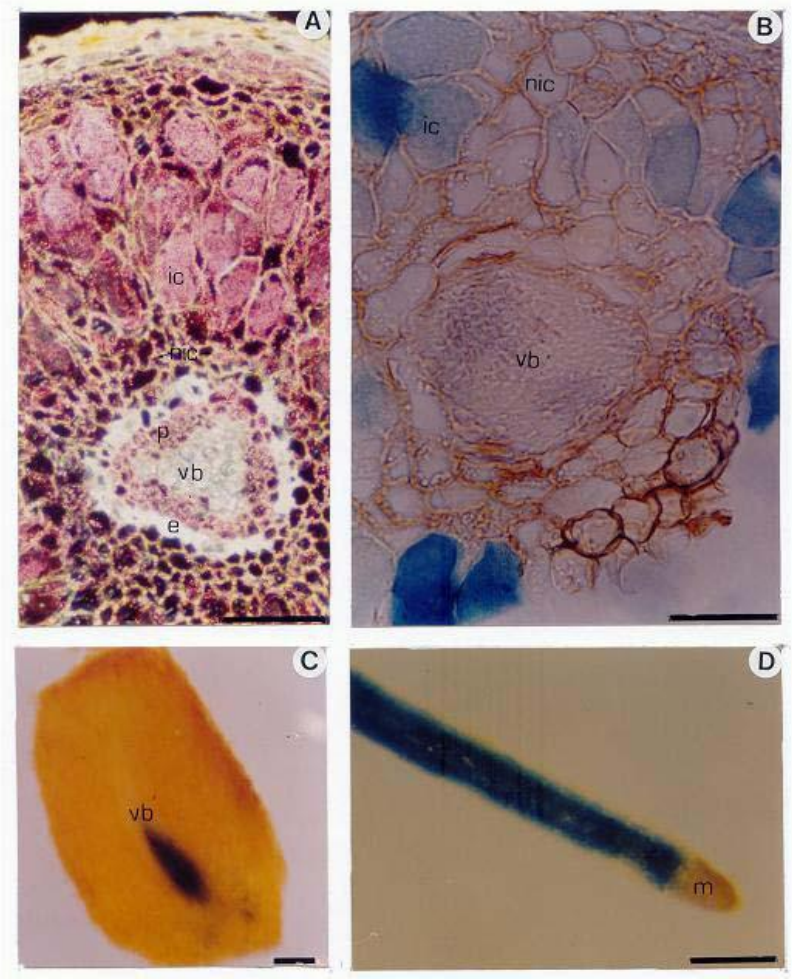

Figure 1. Histochemical analysis of β-glucuronidase (GUS) activity under the control of plant hemoglobin promoters in transgenic nodules (A, C) and roots (D) of *A. verticillata* and *C. glauca* (B). Cross section of the nodules show *P. andersonii* activity in infected cells and pericycle (A) where GUS activity is visible in pink colour after dark-field micrograph. *Lbc3* expression is confined to the infected cells (B) but *T. tomentosa* is expressed in the vascular bundle of longitudinal section of nodule lobes (C) and in the root tissues except the meristem region (D) (Diouf, 1996; Franche et al., 1998). E: Endodermis, ic: infected cells, m: meristerm, nic: noninfected cells, p: pericycle, vb: vascular bundle. Bars=200 ∝m.

plant growth and limits production without fertilizer supplies. However, many genes encoding proteins playing a key role in nitrogen fixation and assimilation have been isolated and characterized. One of these, the gene encoding glutamine synthetase under the control of 35S promoter was introduced in poplar. The generated transgenic trees showed increased protein content and better growth (Gallardo et al., 1999).

GENETIC TRANSFORMATION OF FOREST TREES FOR STUDYING GENES EXPRESSION

Expression of hemoglobin genes

Hemoglobin is present in the nitrogen fixing nodules of both legumes and nonlegumes such as *Paprasponia andersonii* (*Ulmaceae*) (For review see Appleby, 1992) and *Casuarina glauca* (For review see Appleby, 1992; Jacobsen-Lyon et al., 1995). Its role is to transport oxygen and ensure free oxygen at a low concentration in an adequate level for symbiotical bacteria respiration without damaging the oxygen-sensitive nitrogenase present in the bacteroids or *Frankia* encapsulated within the nodule. A nonsymbiotic hemoglobin gene have also been identified in many plants such as wheat, maize, rice (Taylor et al., 1994), *A. thalianna* (Trevaskis et al., 1997), *Trema tomentosa* (*Ulmaceae*) (Bogusz et al., 1988), *Physcomitrella patens* (Arredondo-Peter et al., 2001) and tomato (Wang et al., 2003). The introduction of these symbiotic or nonsymbiotic hemoglobin genes in transgenic plants has important role for a better understanding their regulation, spatio-temporal expression and evolution. To investigate this phenomenon, different techniques of transformation were used in forest trees. These techniques have been developed in two actinorhizal trees in the *Casuarinaceae* family, *C. glauca* (Diouf et al., 1995) and *Allocasuarina verticillata* (Franche et al., 1997). To further investigate the evolution of plant hemoglobins, chemeric genes consisting of the promoter region from the soybean (*lbc3*), the *P. andersonii*, and the *T. tomentosa* hemoglobin genes linked to the coding region of the reporter *gus* (*uidA*) encoding β-glucuronidase (GUS) were introduced into *C. glauca* and *A. verticillata*. The fluorimetric assays in various organs showed that the expression of soybean and *P. andersonii* promoters is active in the nodule. In contrast, the expression of *T. tomentosa* hemoglobin gene promoter is highest in roots. The histochemical analyses showed that the expression of symbiotic genes (soybean and *P. andersonii*) is mainly confined in the infected cells of the nodules of *C. glauca* and *A. verticillata* like in legume plants (Figures 1A and B). The expression of *T. tomentosa* promoter is restricted in the vascular bundle of the nodule (Figure 1C) or in all root tissues except the meristem region (Figure 1D).

These results suggest that the mechanisms governing the expression of these genes are conserved between legumes, *Casuarinaceae* and *Ulmaceae*, indicating a single origin for the predisposition to form symbiotic nodules and a close relationship of hemoglobin genes in different plants, which are phylogeneticaly distant (Diouf, 1996; Franche et al., 1998).

Expression of inducible genes

A chemeric gene consisting of the 2.8 kb bspA (bark storage protein) promoter fused to the coding region of β-glucuronidase gene was transferred into *Populus*. The transformed plants showed short day and nitrogen inducibility, and the GUS activity was localized to the bark (primary and secondary phloem, and cortex) and xylem rays. These studies showed that the short day and nitrogen inducible elements are separable (Zhu and Coleman, 2001). In contrast, the expression of *win3* gene, is localized in storage tissue of the hybrid poplar *P. trichocarpa* x *P. deltoids* and this gene possesses an element of 1.5 kb in the promoter region inducible by wounding (Hollick and Gordon, 1995b). Genetic transformation of forest trees allows also to show that the genes encoding CAD, PAL (phenylalanin ammonialyase) and Dc8 are inducible respectively by ozone and abscissic acid (Galliano et al., 1993; for review see Sederoff, 1995). Several promoters of the genes encoding enzymes for abscissic acid and auxin synthesis from soybean, *Em* from maize, Rubisco from *Arabidopsis*, show activity in conifers (for review see Sederoff, 1995). Recent studies have reported that *Em* is induced by CnABI3 in presence of abscissic acid. It was also reported that expression of *CnABI3* gene decrease during dormancy breakage. Therefore, CnABI3 plays an important role in dormancy process (Zeng et al., 2003). The *pin2* gene coding for potato proteinase inhibitor is one of the best-characterized plant defense genes, and its product inhibits animal digestive enzymes (Ryan, 1990). This gene is inducible in potato by wounding caused by insects. The *pin2* gene promoter from potato was fused to the coding region of *cat* (chloramphenicol aminotransferase) gene and introduced into the hybrid poplar (*P. alba* x *P. grandidentata*) genome, and it retained the inducibility by wound (Klopfenstein et al., 1991).

Expression of other heterologous genes

Transient expression of the promoter of the genes encoding phosphoenolpyruvate carboxylase from soybean, ubiquitin from *Arabidopsis*, alcohol deshydrogenase from corn, have been tested in conifers (for review see Sederoff, 1995). In contrast, the promoter of the gene encoding ATHB13, a transcription factor identified in *A. thaliana*, which belongs to the family of homeodomain leucine zipper (HDZip) was tested in hybrid aspen (*P. tremula P. tremuloides*). Histological analyses showed that the expression of these genes is localized in the petioles of leaves like in *Arabidopsis*. These results indicate that the transacting factors governing the expression of this gene are conserved between these plants (Hanson et al., 2002).

Foreign genes encoding detoxifying proteins

The exposure to environment stress caused by light, drought, ozone, herbicides, wounding, cold, nutrient deficiency, pathogens or sulfur dioxide are responsible for the formation of different xenobiotics such as active oxygen, superoxide radicals, and hydrogen peroxide in the plant cells. These xenobiotics are mainly detoxified by glutathione, which is synthesized by two main enzymes, γ-glutamylcysteine synthetase and glutathione synthetase. Overexpression of γ-glutamylcysteine synthetase gene in the cytosol or in the chloroplast increase foliar and root glutathione concentration in transgenic poplar (Noctor et al., 1996, 1998a; Strohm et al., 2002) and reduces the negative effect resulting from higher uptake of candmium (Rennenberg and Will, 2000). Transgenic trees can also be used to remove contaminants from soil or water, and therefore can be applied to solve pollution. In this case the overexpression of the bacterial mercuric reductase in yellow poplar induces resistance to toxic level of mercuric ion (Rugh et al., 1998).

CONCLUSION

Despite the recent progress achieved in genetic transformation of plants, foreign gene transfer in forest trees is mainly limited by two constraints. The first is related to transformation because only a few dicotyledons and conifers are sensible to *Agrobacterium*. This phenomenon can be explained by the lack of production of phenolic compounds like acetosyringone and hydroxyacetosyringone, the inducers of VirA protein which induces others *vir* genes encoding proteins playing a major role in gene transfer to the plant cell (Potrykus, 1991). The second constraint is linked to the problem of the proliferation of plant cells and their ability to support the stress from the regeneration process. We hope that the great progress achieved in the biology of *Agrobacterium* and the expression of the genes playing key role in plant development will be helpful in solving the constraint linked to the genetic transformation of forest trees. Genetic transformation of forest trees offers a great opportunity to improve production and knowledge of the

mechanism governing growth and tree development, pathways of signal transduction and the process of gene silencing in forest trees.

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