Identification and propagation of novel value-added hardwood varieties

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Jim Mattsson
Department of Biological Sciences, Simon Fraser University
8888 University Drive, Burnaby BC, V5A 1S6

Summary

The aim of this research project has been to identify and propagate novel naturally occurring hardwood varieties with decorative wood. As well, we have worked towards improving the propagation of two known hardwood species, curly birch and hybrid aspen, for future application in the plantation forestry industry. We have also embarked on generating breeding populations from silver birch and hybrid poplar to generate novel figured wood varieties in these species. In this report we outline the progress so far. Results of particular interest are (1) 2.5 year-old curly birch trees have developed visible stem swellings typical of parental curly birch plants. This finding suggests that nurseries will be able to use these swellings as a sales argument without prolongation of normal growth period before sale. (2) The generation of a breeding population of hybrid poplar is well under way, as approximately 4000 hybrid poplar shoots have been generated. Rooting and transfer to greenhouse is in process.

Introduction

High values of wood are usually due to rare features involving color, luster, grain and figure. Figured wood has been used in exclusive furniture, decorative items, knife and gun stocks, and music instruments for centuries. The rarity of figured wood can largely be attributed to the limited knowledge of its causes, in part because wood with a figure often develop in relatively old trees, which prevents a timely assessment of its cause. In many cases, attractive figure occurs only in parts of a tree, suggesting that it may be due to external factors such as abiotic and biotic stress. It is entirely possible that different stress conditions cause different types of figured wood, and that some species are more susceptible than others to stress conditions. However, little research has been done to investigate the role of genetics in the development of figured wood. Previous studies with birdseye maple, a highly valued figured wood variety, have shown to be inconclusive in determining whether or not environmental factors play a role in the birdseye figure (Bragg DC et al 1997). Other studies have demonstrated that some varieties can attribute their figured traits to genetics, such as in the case of the curly birch (*Betula pendula var. carelica*; Heikinheimo, 1951). These trees display a unique morphology compared to wild type, in that the stem shows consistent swellings/ bulges throughout (see Figure 2a).

In this study, we are looking primarily for stable genetic events that result in abnormal wood formation. Therefore we intend to test as many samples of figure forming wood cambiums as possible whether abnormal wood development is transferred by in vitro micro propagation. In this process bacteria and fungi will be removed by treatment with antibiotics and fungicides, leaving stable genetic modifications/mutations or viral infections as the most likely causes of transmitted abnormalities in wood formation The fact that figured wood usually occurs in parts of trees may be seems hard to reconcile with possible stable genetic modification. However, genetically modified sectors can be created by dominant genetic mutations, by epigenetic modification such as methylation of specific genes, by integration of transposable elements, viral or bacterial genes into the plant genome. If a propagated tree displays wood traits similar to that of its parent tree, then a genetic basis to that particular wood formation is likely. If such defective wood can be propagated *and* has a genetic cause *and* has a decorative value, then we have the prospect of generating a new variety for future forestry plantation industries.

The purpose of this project is to promote the silviculture of particularly valuable hardwoods in BC. The specific objectives are: 1. To identify and propagate novel naturally occurring hardwood varieties with figured wood, and 2. To develop efficient propagation technology for hybrid aspen (*Populus tremuloides x Populus tremula*) and the figured wood varieties commonly referred to as curly birch *(Betula pendula var. carelica)*. In the second year we have also expanded towards generating novel figured wood varieties by chemically induced mutagenesis of silver birch and hybrid poplar. While the birch population is still in its early stages, the hybrid poplar population is well under way.

Methods

Collection of woody shoots

Towards this end, there are several ways that we have used to identify trees of interest for sampling (see technical report from 2005). This year we placed an advertisement requesting assistance in locating trees with abnormal wood formation in BC Community Classifieds, a service which reaches 107 community newspapers across BC and Yukon (2.5 million readers) We have received about 30 responses, and have started collection of samples from the most promising trees. Collected samples were wrapped in moist paper towels, stored in plastic bags and were refrigerated until they could be used for propagation.

Propagation of tissue in culture:

Surface Sterilization

Surface sterilization was carried out with cambial samples using 70% ethanol as follows: wood samples (including a small amount of bark, cambium and wood) were rinsed and scrubbed as per our original technique, then cut into small squares of about 3cm long by 2cm wide and deep, so as to keep the cambium protected on both sides by either wood or bark. 3-4 pieces were then transferred to the 50mL falcon tubes and rinsed in 70% ethanol for 1min, then rinsed three times with autoclaved water under sterile conditions. A scalpel was then used to cut off bark from the sterilized samples so as to expose the live cambium tissue for callus induction.

Callus Induction

Sterilized tissue was plated onto sterile petri dishes containing callus induction media (CIM), a composition of plant growth regulators that was taken from an effective protocol previously used in the regeneration of a variety of cottonwood hybrids (Han KH et al 2000). Samples were subcultured once every 1-2 weeks in order to control and prevent fungal/bacterial contamination.

Shoot Induction

Once substantial calli were formed, calli were cut into smaller pieces and then plated onto sterile shoot induction medium (SIM). We have tested several published hormone combinations for shoot inductions (Han KH et al 2000; Wilhelm E 1999).

Hybrid aspen rooting trial

We have earlier observed that hybrid aspen lines differ in their response to shoot and root induction medium in vitro. Some lines, respond very well, whereas others grew poorly. Based on these observations, we hypothesized that the same differences may exist also in the rooting response of stem cuttings among lines of hybrid aspen. In July/August, we carried out an assessment of rooting of a large number of hybrid aspen lines in a greenhouse. Sixteen lines of hybrid aspen stem cuttings collected from Skimikin Seed Orchard (Salmon Arm, BC) were used in a rooting trial, whereby 5-8cm-long twigs (including at least 1 node) were exposed to 2 different IBA (rooting hormone) treatments and monitored for root growth in soil under controlled greenhouse conditions (~25ºC and 80% humidity). Cuttings from 5 hybrid poplar lines were also used.

Generation of mutant breeding populations

We have adapted a strategy used in fruit tree breeding to generate dominant mutants in a genetically homogenous background; a large number of rapidly growing calli cultures are generated of preferred genotype, and subjected in batches to a series of concentrations of the mutagen Ethylmethane Sulphonate (EMS). We are aiming at concentrations that cause close to 50% lethality of cells (LD50), as this level of lethality in most species correlate with high frequency (> 500 mutations/genome) of mutagenesis. LD50 is scored by a 50% reduction of growth of calli as compared to corresponding calli that have not been exposed to mutagen. Mutagenized calli are thereafter transferred to shoot induction medium to generate a mutant population. Induced shoots are thereafter transferred to hormone-free media to promote elongation, and in the second subculture, also root formation. Rooted plants are acclimatized and transferred to greenhouse.

Plants will be screened for dominant phenotypes similar to those found in curly birch (a semi-dominant mutant).

Results

Expansion of live material collection

During the 2005/2006 budget year we continued to expand our list of identified trees with potentially valuable figured wood. Some of the identified trees are shown in Figure 1 with a particularly spectacular tree shown in Figure 2. Most of trees fall in the group of trees with burls. Here we focus on trees with multiple burls as they are more likely to have a genetic rather than epigenetic basis, i.e. disease and environmental effects. We have also identified two chestnut trees with systemically wavy stem surfaces (Figure 1). Such stem deviations are likely due to a genetic deviation and therefore of particular interest.

Shoot Induction

While we have succeeded in producing shoots from hybrid aspen silver birch and curly birch using a published procedure (Han KH *et al* 2000), we have experienced poor formation of shoots in cottonwood, maple and chestnut lines. We have therefore carried out shoot induction trials using other plant hormone combinations that have shown success in previous micro-propagation studies (Wilhelm E 1999; Ďurkovič J 2003). Shoot induction has been limited using also these hormone conditions. During the end of 2005, we switched to a recently published procedure that contains in addition to the powerful cytokinin, an agent, Pluronic-F68, which aids the uptake of hormones into plant cells. With this procedure, we have had great success in the induction of shoots from hybrid poplar cultures (Figure 4), and we intend to extend its application on recalcitrant maple and chestnut material in the coming fiscal year. We have identified the exact position of the majority of trees by the global positioning system, which allows us to sample trees repeatedly until propagules have been generated.

Regeneration of new trees from collected material

We now have rooted trees in the greenhouse and in the field of hybrid aspen, curly birch, silver birch, poplars and a limited number of maples (see examples in Figures 3 and 5).

Hybrid aspen rooting trial

The reason behind our interest in developing propagation technology for hybrid aspen is that aspens are notorious for their poor rooting capacity, and therefore not widely used, despite desired traits of hybrid aspen. We have succeeded in propagation and rooting of hybrid aspen at high frequency by tissue culture propagation. This procedure is, however, generally considered technically too advanced and costly for regular nursery operations. In our tissue culture experiments, we found a considerable variation in the response to callus and shoot-inducing conditions in material from different hybrid trees. Here we tested whether these differences in regenerative capacity also translated into different rooting capacities of stem cuttings as this technology is simple and potentially cost-effective. None of the 16 aspen lines could be rooted under these conditions. In comparison, an average of 39.7% of hybrid poplars rooted when treated with 0.4% IBA and 28.3% rooted when treated with 0.8% IBA, under the same greenhouse and soil conditions as the hybrid aspen lines. Thus we can conclude that our observations from in vitro experiments do not translate to the rooting of stem cuttings in soil. The positive control, hybrid poplar, performed as expected and showed that the used conditions were appropriate.

Screen for mutants with novel figured wood traits

We have exposed a large number of rapidly growing hybrid poplar and silver birch calli to a series of concentrations of the powerful mutagen EMS, i.e. 0, 25, 50, 100 and 200 mM for six hours. Among these concentrations, 200 mM resulted in approximately 50% reduction of growth one week after mutagenesis, suggesting that this concentration results in approximately 50% lethality in exposed callus cells. As 50% lethality is a typical measure of high-frequency of mutations, we focused our attention on this material. The calli was subsequently transferred to a novel shoot-inducing medium containing the powerful synthetic cytokinin thidiazuron in combination with the wetting agent Pluronic-F68. Hybrid poplar material has responded extremely well to this combination, and we are presently focusing on this species to generate a mutant population of reasonable size. Approximately 4000 calli with shoots have been produced in the first round, and we are currently transferring shoots to medium without hormones to promote elongation of shoots. Thereafter we will transfer shoots to either hormone-free medium, or root inducing medium, for root induction, followed by transfer to soil and acclimatization. The first rooted plants have been transferred to soil. As this population continues to grow in size and as plants continue to grow, we will assess individuals for stem defects that may indicate the formation of figured wood.

Extension by publication

We are preparing a manuscript on the micro-propagation of hybrid aspen. We have found optimal conditions for shoot production and rooting of hybrid aspen in *in vitro* cultures, which is worth publishing as aspen is in general difficult to root *in vitro*. We have also found that some hybrid lines respond much better to rooting in vitro than others. After a detour to explore whether these differences in rooting capacity in vitro also translates into differences in rooting capacity of stem cuttings, we have returned our attention to our manuscript on improved micro-propagation of hybrid aspen. Final experiments will be carried out during 2006 before the manuscript can be finalized.

Discussion

The figured hardwood market is currently restricted by very limited supply and high prices. Figured wood is produced systematically only in Finland, where plantations of curly birch are relatively common. To our knowledge, this has been the first Canadian attempt to collect and propagate material from naturally occurring trees with novel figures of wood in order to generate new value-added tree varieties. We are possibly also the only laboratory in Canada that is working on micro-propagation of figured hardwoods. This work may provide an entirely new and unique market for BC forestry. Mixed plantation forestry of valuable hardwoods may be one the most ecologically sound methods of increasing the value of land as well as the final lumber products, and may also be an esthetically preferred alternative to monocultures of conifers, especially when reverting agricultural land into forests.

We are working towards identifying as many putative figured-wood trees as possible during the tenure of this project. We are testing whether abnormal wood development is transferred by in vitro micro propagation. In this process bacteria and fungi will be removed in the process, leaving stable genetic modifications/mutations or viral infections as the most likely causes of transmitted abnormalities in wood formation. Due primarily to the slow growth of trees, it may take many years before stem defects indicative of potential figured wood will appear. Thus, this is a project that will run over many years before the final results of the project can be assessed. In this situation, we are encouraged by two advancements. First, we now have a new greenhouse facility at SFU that allows us to grow forest trees around the year at optimized growth conditions, thereby increasing the rate of yearly growth in propagated trees and possibly also shortening the time to development of stem defects indicative of figured wood. Secondly, we have recorded the first stem defects in two-year-old micro-propagated curly birch trees. This time is shorter than estimates of four to five years provided by growers of curly birch trees in Sweden (Anita Wallin, Uppsala, Sweden, personal communication). Two years of growth is also within the normal time of propagation of tree nursery growers selling trees to private land and garden owners. The time may therefore have come to contact individuals within the agriforestry sector in British Columbia and provide test material for evaluation with respect to future commercialization of curly birch, and eventually other varieties identified during this project.

Literature Cited

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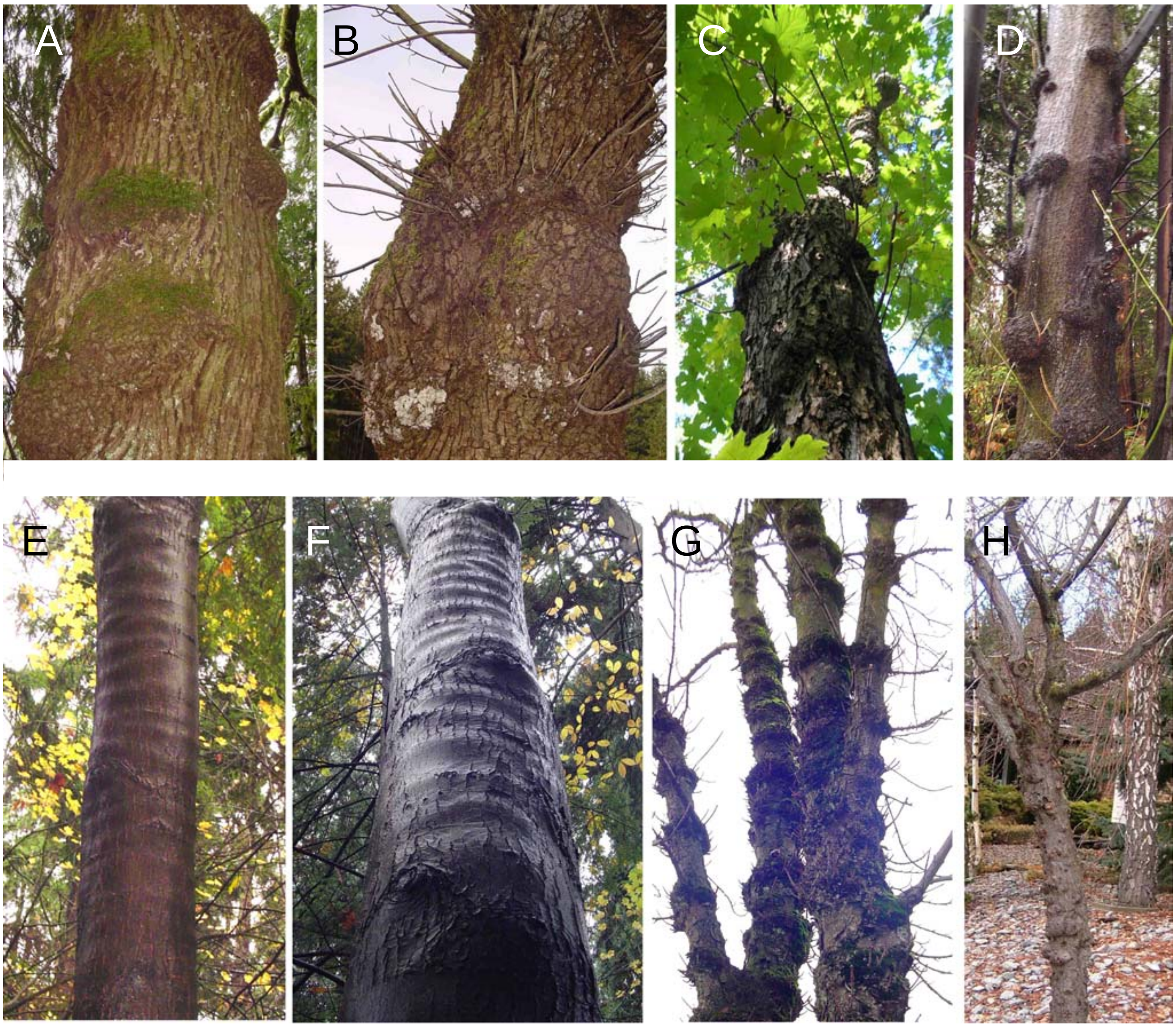

Figure 1. Some of the trees identified during the 2005/2006 fiscal year. (A-D) bigleaf maples with various degrees of systemic formation of burls. (A) maple bay, Vancouver island, (B) Cowichan lake, Vancouver island; (C) lower mainland (D) lower mainland. (E and F) chestnut with wavy stem, undisclosed provincial park; (G) chestnut with regular swellings along trunk and branches similar to curly birch; (H) cherry tree with systemic and irregular formation of burls, Vernon.

Figure 2. (A) Glen Mcleod (Duncan, BC) with his giant root/stem burl from a bigleaf maple tree. He dug it up when the tree died. Only one shoot was still alive when we became aware of this remarkable tree, and has been put into tissue culture for propagation.
(B) The figure of the burl is of outstanding quality. Should Mr Mcleod decide to cut this burl into smaller pieces and sell it at the decorative wood market, he is likely to earn many tens of thousands of dollars.

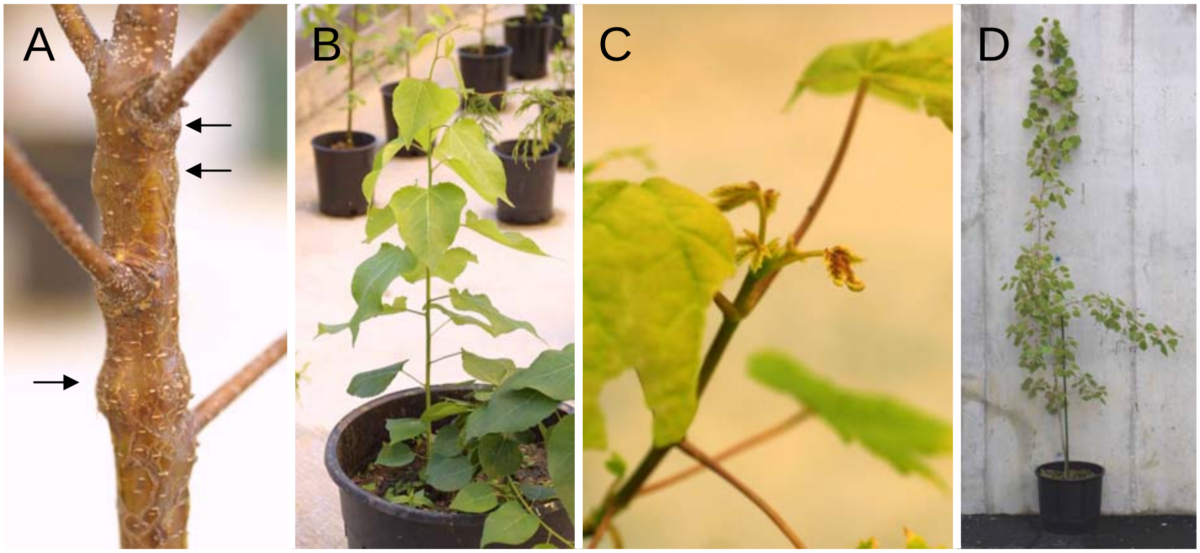

Figure 3. Regenerated trees of interest. (A) 2-year-old micropropagated curly birch has begun to show swellings indicative of figured wood formation (arrows). (B) A regenerated 1 year aspen derived from a tree with multiple burls. (C) A regenerated 1 year maple from a tree with multiple burls.
(D) A regenerated 2 year hybrid aspen.

Figure 4. Hybrid poplar shoots regenerated from mutagenized calli
(A) Mutagenized calli grown on medium supplemented with Thidiazuron and Pluronic-F68 produce shoots at high frequency. (B) Rooted shoot.

Figure 5. Curly birch, silver birch, maple and aspen trees in the new greenhouse at SFU. Automated irrigation, lights, curtains, floor-heating and cooling will allow around-the-year growth of trees, thereby shortening the time it takes for potential figured-wood defects to develop.